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DEPARTMENT OF ENVIRONMENTAL PROTECTION

Office of Water Programs
Bureau of Clean Water

Water Quality Monitoring Protocols for Surface Waters
2023

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OFFICE OF WATER PROGRAMS

BUREAU OF CLEAN WATER

WATER QUALITY MONITORING PROTOCOLS FOR SURFACE WATERS

2023

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Top left: Matthew Shank, wadeable riffle-run stream macroinvertebrate data collection; by Rebecca Whiteash; both of PA Department of Environmental Protection
Top right: Mark Hoger, continuous physicochemical data collection; by Matthew Shank; both of PA Department of Environmental Protection
Center right: Jeffrey Butt, periphyton data collection; by Matthew Shank; both of PA Department of Environmental Protection
Bottom: Timothy Wertz, fish data collection; by Matthew Shank; both of PA Department of Environmental Protection

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ABBREVIATIONS

AFDM	Ash Free Dry Mass
AIS	Aquatic Invasive Species
ALU	Aquatic Life Use
BU	Boating Use
BOL	Bureau of Laboratories
Chl-a	Chlorophyll-a
CIM	Continuous Instream Monitoring
CSO	Combined Sewer Overflow
CSS	Combined Sewer Systems
CWA	Clean Water Act
CWF	Cold Water Fishes
DELTP	Deformities, Erosions, Lesions, Tumors and Parasites
DEP	Pennsylvania Department of Environmental Protection
DCNR	Pennsylvania Department of Conservation and Natural Resources
DO	Dissolved Oxygen
DOH	Pennsylvania Department of Health
EU	Esthetics Use
EPT	Ephemeroptera, Plecoptera, and Trichoptera
EV	Exceptional Value Waters
FU	Fishing Use
FNU	Formazin Nephelometric Units
GPS	Global Positioning System
HAB	Harmful Algal Bloom
HDPE	High-Density Polyethylene
HQ	High Quality Waters
IBI	Index of Biotic Integrity
ICE	Instream Comprehensive Evaluation
IRS	Irrigation Water Supply
IWS	Industrial Water Supply
LDB	Left Descending Bank
MSDS	Material Safety Data Sheets
NELAP	National Environmental Laboratory Accreditation Program
NOAA	National Oceanic and Atmospheric Administration
NPDES	National Pollutant Discharge Elimination System
NHD	National Hydrologic Dataset
PCB	Polychlorinated Biphenyls
PFBC	Pennsylvania Fish and Boat Commission
PWS	Potable Water Supply
QA	Quality Assurance
QC	Quality Control
QMH	Qualitative Multi-Habitat
qPCR	Quantitative Polymerase Chain Reaction

RBP	Rapid Bioassessment Protocol
RDB	Right Descending Bank
RU	Recreation Use
SAC	Standard Analysis Code
SIS	Sample Information System
SRBC	Susquehanna River Basin Commission
TDS	Total Dissolved Solids
TMDL	Total Maximum Daily Loads
TSF	Trout Stocking
TSI	Trophic State Index
USEPA	United States Environmental Protection Agency
USGS	United States Geological Survey
WC	Water Contact Sports Use
WQD	Water Quality Division
WQN	Water Quality Network
WQS	Water Quality Standards
WWF	Warm Water Fishes

CHAPTER 1 INTRODUCTION

Chapter 1 Introduction

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PURPOSE

This document details the suite of monitoring and data collection protocols currently used by the Pennsylvania Department of Environmental Protection (DEP) to meet multiple surface water characterization objectives. This book is part of a larger conceptual framework (Figure 1) that DEP uses to collect quality data and make decisions on various surface water matters across Pennsylvania. Data collection protocols have been compiled in this book entitled *Water Quality Monitoring Protocols for Surface Waters* (Monitoring Book) to increase operational transparency and to facilitate the use of DEP data collection protocols. The assessment of protected water uses is one of the primary monitoring objectives of DEP staff biologists, however there are several additional and equally important monitoring objectives described throughout this document.

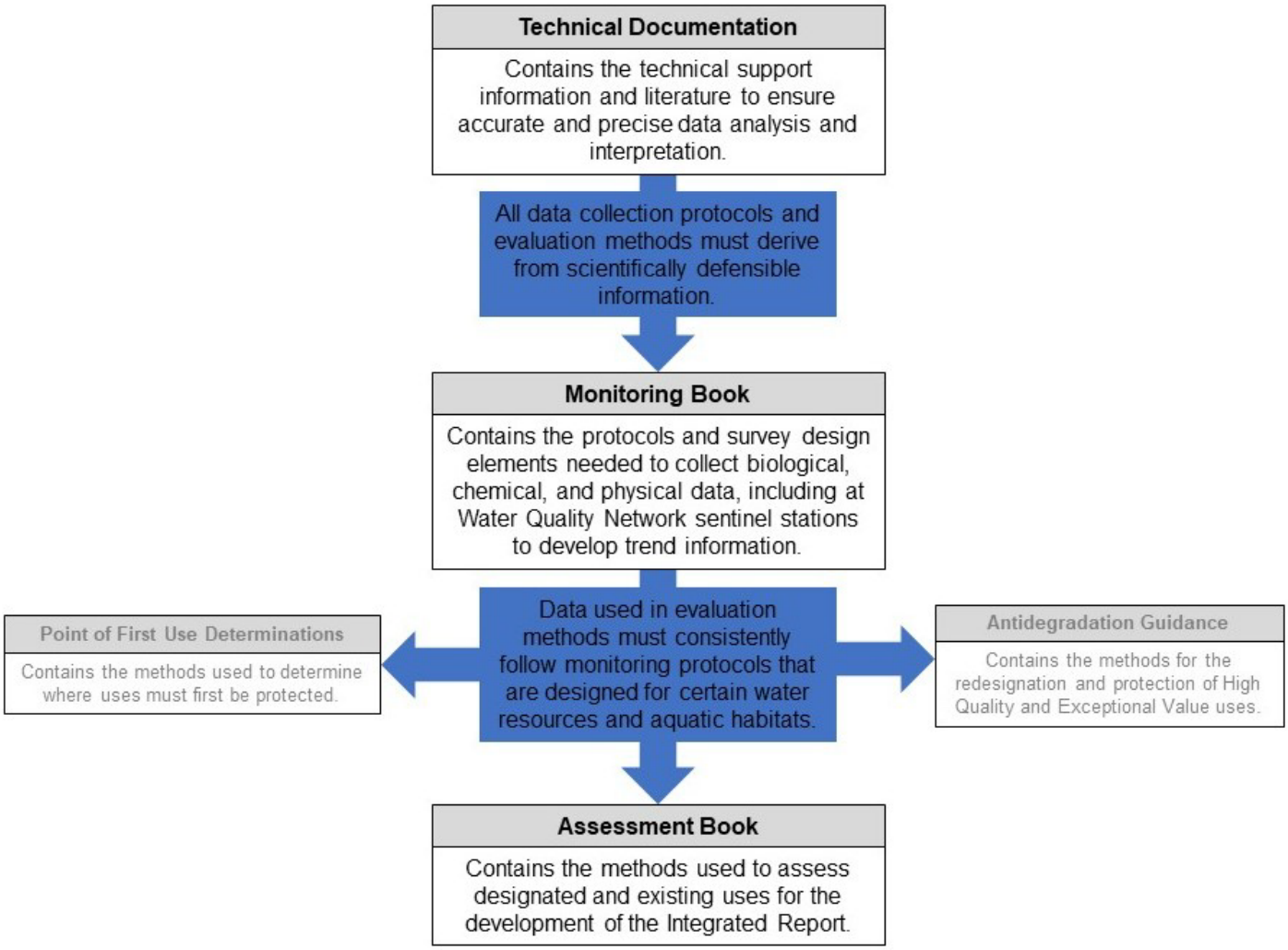

Figure 1. Conceptual framework that DEP uses to make scientifically defensible data collections and decisions.

UPDATES

This is the second update to the Monitoring Book since the initial 2018 publication that effectively consolidated DEP data collection protocols. This 2023 update includes the following updates that are worth noting:

- Three new subchapters have been added to Chapter 2 including *Chapter 2.3, Copper Biotic Ligand Model Survey Design* (Schlauderaff and Lookenbill 2023), *Chapter 2.4, Lake Assessment Survey Design* (Arnold 2023b), and *Chapter 2.5, Stream and River Assessment Survey Design* (Shull and Arnold 2023).
- Updates to the *Bacteriological Data Collection Protocol* (Miller 2023) located in Chapter 3.11 were made to account for the adoption of updated bacteriological criteria in 25 Pa. § Code 93.7 during the swimming season (May 1 through September 30).
- Data collection protocols for lakes have been consolidated into this document. Original source for lake protocols is found in Lathrop 2015. Chapters added include *Chapter 3.12, Chlorophyll-a, Phycocyanin, and Ash Free Dry Mass Data Collection Protocol* (Arnold and Butt 2023a); *Chapter 3.13, Harmful Algal Bloom (HAB) and Cyanotoxin Data Collection Protocol* (Arnold and Butt 2023b); *Chapter 3.14, Plankton Data Collection Protocol* (Arnold 2023d); *Chapter 3.15, Aquatic Macrophyte Data Collection Protocol* (Arnold 2023a); and *Chapter 5.4, Secchi Depth Data Collection Protocol* (Arnold 2023e).
- Chapter 4.1 has been renamed *In-Situ Field Meter and Transect Data Collection Protocol* is now named *Chapter 4.1, In-Situ Field Meter, Transect, and Vertical Profile Data Collection Protocol* (Hoger and Arnold 2023). A new section was added to describe in-situ vertical profile field data collection. Additional edits have been made to the *Calibration* section of this chapter.
- Chapter 4.2 has been updated to include lake sampling procedures, Potable Water Supply sampling procedures, and acid deposition sampling procedures.
- A new data collection protocol has been added to Chapter 5 of this Book which describes a trash data collection protocol. This new chapter is titled *Chapter 5.5, Trash Data Collection Protocol* (Biggs et al. 2023).
- Appendix A now includes a Lake Assessment and Field Data Sheet (Appendix A.11), a Harmful Algal Bloom (HAB) Field Data Collection Sheet (Appendix A.12), and an Aquatic Macrophyte Field Data Collection Sheet (Appendix A.13).
- Appendix E has been updated to include definitions of Quality Control sample types. Additionally, Section B has been restructured.
- Appendix F has been created to include Safety and Biosecurity Procedures. These procedures are applicable to all protocols found in this book. Specific protocols are referenced where applicable.
- Appendix G has been created to include an Undenatured Ethanol Policy.
- In some cases, heading and subheadings have been renamed and reorganized to create consistency within chapters and subchapters.

COLLECTION FRAMEWORK AND PROCESSES

Collectors will need to adhere to an established framework and process. In doing so, consistency will be maintained to produce quality data. A tiered structure to define the quality of data is as follows:

- Tier 1 – Data is generally defined as educational or environmental screening data that has known quality, but does not follow DEP or USEPA quality assurance plans. These data will not be used for assessment determination purposes, but can be used by DEP to highlight areas of interest for future monitoring efforts.
- Tier 2 – Data have clearly defined **and/or clearly follow DEP or USEPA** quality assurance plans and procedures, but may not have followed DEP monitoring protocols established in this Monitoring Book. These data may not be used for assessment determination purposes but can be used for other purposes such as trend or performance analysis.
- Tier 3 – Data are considered to be assessment level data that have approved quality assurance plans **and/or clearly follow DEP or USEPA** quality assurance plans and procedures, follow appropriate study designs, and follow protocols established in this Monitoring Book. Individuals seeking to provide DEP with Tier 3 data should also be trained and audited by DEP staff before submitting data.

All data collections conducted by DEP will follow the framework described here and throughout this book.

- IDENTIFY THE MONITORING OBJECTIVE – Why are data being collected? The primary and any secondary objectives will need to be identified before any data are collected. DEP staff will often identify the primary data collection objective and consider other, secondary objectives which may require additional data collections, which would simultaneously meet requirements of both the primary and secondary objectives. Consequently, considering both objectives would require less effort if pursued independently. As an example, DEP staff biologists will identify the primary objective as the assessment of protected water uses. Based on the information gathered during the reconnaissance process, there may be indications that the target waterbody may have an existing use that is different than the designated use. Employing additional requirements that satisfy the stream redesignation evaluation objective requirements, like the collection of reference stations and targeting additional analytical tests, could provide the opportunity to meet this secondary objective. Objectives are further described in Chapter 2 of this book.

- SAMPLING DESIGN AND PLANNING
    - Reconnaissance and Data Gathering – Reconnaissance includes the delineation of the targeted waterbody or basin and the gathering of historical and other information that will provide the best opportunity to meet data collection objectives.
        - Survey Delineation
        - Waterbody Classification

        - Activities that may affect Water Quality
            - Permitted Activities
            - Stream Restoration Efforts

    - Select a Sampling Design – Select a sampling design that will meet the monitoring objective(s). DEP typically employs a targeted sampling design.
        - Probabilistic
        - Targeted

    - Develop a Sampling Plan – A sampling plan should be developed that outlines
        - Monitoring Objectives
        - Data Collection Protocols
        - Additional Guidance
        - Quality Assurance Requirement
        - Safety and Biosecurity Procedures
        - Other important information and resources that need to be referenced and followed.

- QUALITY ASSURANCE – Water quality data that will be used by DEP will need to meet quality assurance (QA) and quality control (QC) criteria. A review of the QA/QC criteria for specific objectives will need to be performed before any collection occurs.
- SAFETY AND BIOSECURITY PROCEDURES – Safety and biosecurity procedures are to be followed at all times.

HOW TO NAVIGATE THE BOOK

This book is organized to first generally describe the planning process. Specific details are provided in Chapter 2 that describe the sample designs associated with common monitoring objectives. The purpose of detailing sampling design and planning is to give guidance regarding specific protocols that need to be implemented to meet any one objective. Chapter 3 through Chapter 5 are those protocols used by DEP to meet the various monitoring objectives. Each protocol is standalone, but often multiple protocols are used as supporting evidence to aid in data interpretation. Protocols and procedures found in these Chapters follow a standardized and structured framework to provide context and instruction to gather data that results in comparability and defensibility. Furthermore, each protocol described in this book has specific QA/QC procedures that must be incorporated into all sample designs. Specifics outlining QA/QC procedures are mentioned in each protocol and additional details are found in Appendix E. These QA/QC procedures are implemented to make collectors accountable to the adherence of the standardized protocols and will also further support comparability and defensibility of protocol implementation to meet the various monitoring objectives. The final item provided in this book is safety and biosecurity procedures. When working in and around water, it is inherently important to follow a safety plan that always keeps the collector and crew safe. Additionally, it is important biosecurity measures are followed as spread of aquatic invasive species

(AIS) is always a potential concern when conducting surface water surveys. Safety and biosecurity procedures are detailed in Appendix F.

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CHAPTER 2 SAMPLING DESIGN AND PLANNING

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INTRODUCTION

A sampling design will be consistent with the collection framework and processes described in Chapter 1 of this book. The sampling design will be chosen in order to form a data set that is appropriate for:

- The desired objective,
- The appropriate waterbody classification,
- Known and potential sources and causes affecting water quality,
- Quality Assurance (QA)/Quality Control (QC) (Appendix E), and
- Biosecurity and Safety Procedures (Appendix F)

MONITORING OBJECTIVES

Objectives further described in this section include:

- Protected Use Assessment
- Existing and Designated Use Evaluation
- Cause and Effect Survey
- Combined Sewer Overflow Compliance Monitoring
- Point of First Use Evaluations
- Protocol and Method Development
- Site-Specific Criteria or Refinement of Effluent Limits
- Water Quality Network Monitoring

Protected Use Assessment

DEP staff biologists are tasked with monitoring and assessing surface waters of Pennsylvania for the protected water uses listed at 25 Pa. Code § 93.3. Monitoring to assess protected water uses is one of the primary objectives of DEP staff biologists. To successfully complete an assessment of protected water uses, requirements should be met as described in sampling design and planning, *Chapter 2.4, Lake Assessment Survey Design* (Arnold 2023) and *Chapter 2.5, Stream and River Assessment Survey Design* (Shull and Arnold 2023), the appropriate data collection protocols, quality assurance requirements as wells as requirements described in the appropriate assessment methods established in *Water Quality Assessment Methodology for Surface Waters* (Assessment Book, Shull and Whiteash 2023). The protected water uses include Aquatic Life, Water Supply, Water Contact Sports, Boating, Esthetics, Fish Consumption, and Special Protection.

Special Protection uses include High Quality Waters (HQ) and Exceptional Value Waters (EV). See 25 Pa. Code § 93.3 for definitions and standards.

Developing a sample collection plan for special protection waters will include employing a biological data collection protocol (Chapter 3) with an assessment method. Each data collection protocol describes criteria that will need to be evaluated to determine the appropriate protocol required for a specific waterbody, waterbody reach, or sampling location. When appropriate, biological assessment

methods will include index periods, impairment thresholds, and decision matrices specific to special protection waters that will need to be addressed in the sample collection plan. Otherwise, sample collection plan development is consistent with aquatic life use monitoring.

Stream Redesignation Evaluation Monitoring

Surface water redesignation evaluations are initiated to determine the existing and designated uses of a given waterbody. The terms “existing use” and “designated use” are defined at 25 Pa. Code § 93.1. Evaluations can be initiated as part of a petition process, permitting process, or due to the results of a use assessment reconnaissance or survey that indicates an existing use may be different than the designated use. Redesignation evaluations can lead to redesignation to a more restrictive use, a less restrictive use, no change to the use, or a demonstration of “natural quality” as defined at 25 Pa. Code § 93.1.

A sample collection plan for redesignation evaluations will include and refer to information at 25 Pa. Code § 93.4b, ‘Qualifying as High Quality or Exceptional Value Waters’ and the DEP *Water Quality Antidegradation Implementation Guidance, Document Number 391-0300-002* (DEP 2003). This includes the application of biological and chemical data collection protocols. Physical habitat assessments will also be completed when employing biological data collection protocols. Thermal components of ALU (WWF, TSF, and CWF) are evaluated using fish community data and will employ the *Fish Data Collection Protocol* (Wertz and Arnold 2023) found in Chapter 3.

Point of First Surface Water Use Monitoring

Point of first use surveys are completed as part of the evaluation and permitting of wastewater discharges to intermittent and ephemeral streams, drainage channels and swales, and storm sewers. The point of first surface water use establishes where Chapter 93 Water Quality Standards (WQS) designed to protect aquatic life must be supported. WQS designed to protect human health are applicable in all surface waters, including those that exist upstream of the point of first use. Collectors will need to become familiar with DEP technical guidance document 391-2000-014, *Policy and Procedures for Evaluating Wastewater Discharges to Intermittent and Ephemeral Streams, Drainage Channels and Swales, and Storm Sewers* (DEP 2008). Point of first use surveys will employ biological data collection protocols found in Chapter 3.

Cause and Effect Monitoring

A cause and effect sampling design is employed to investigate possible relationships between point or nonpoint sources of conventional pollutants and known or suspected instream water quality problems through the collection and analysis of biological, physical, and chemical data. These surveys are designed primarily to monitor the effectiveness of permitted treatment facilities but are also used to investigate reported or suspected water quality impacts for nonpoint source and other pollution sources. *Cause and Effect Surveys* (Lookenbill and Wertz 2021) found in Chapter 2.1 includes specific survey design requirements. Cause and effect monitoring will employ biological, chemical, and physical data collection protocols found in this book.

Water Quality Network Monitoring

The Pennsylvania Water Quality Network (WQN) is a statewide, fixed station water quality sampling system operated by DEP. It is designed to assess both the quality of Pennsylvania's surface waters and the effectiveness of the water quality management program by accomplishing four basic objectives:

- Monitor temporal water quality trends in major surface streams throughout Pennsylvania.
- Monitor temporal water quality trends in selected reference waters.
- Monitor the trends of nutrient and sediment loads in the major tributaries entering the Chesapeake Bay.
- Monitor temporal water quality trends in selected Pennsylvania lakes.

Data collection is a collaborative effort between the US Geological Survey (USGS) PA Water Science Center, Susquehanna River Basin Commission (SRBC), and DEP. WQN monitoring employs routine biological data collection (primarily benthic macroinvertebrate data collection, chemical/physical data collection, and stream discharge data collection). Appendix D contains *Pennsylvania's Surface Water Quality Monitoring Network (WQN) Manual* (Pulket and Arnold 2023) which details the network's sampling design, station locations, and the water chemistry analytes collected.

Protocol, Method, and Standards Development

The DEP Water Quality Division (WQD) is responsible for developing surface water data collection protocols, water quality criteria and protected water uses, assessment methods, Pennsylvania's biannual integrated monitoring and assessment report, and Total Maximum Daily Loads (TMDLs) that allow impaired waters to meet WQS. Data collection to meet an objective of protocol, method or WQS development is unique from other data collection objectives. The ultimate purpose is to implement the Pennsylvania Clean Streams Law and the Federal Clean Water Act. To achieve this, WQS are developed to establish the criteria and protected uses that surface waters must meet. Data collection protocols are developed, at a statewide level, to collect consistent data that will be used for purposes such as assessment method and WQS development. In addition, data collection protocols also need to be applicable to other data collection objectives. Assessment methods are developed using established data collection protocols to determine if surface waters meet WQS.

Sampling design for a development objective will consider the range of natural water quality conditions across Pennsylvania. Reconnaissance will focus on a range of natural conditions and not necessarily the potential to delineate a water quality condition or the source or cause on a waterbody. The range of natural conditions are then qualified by identifying waterbody classifications. Attributes that are selected to describe a range of natural conditions generally rely on published literature. Development objectives, particularly the development of standards, may also rely on laboratory investigations.

TYPES OF SAMPLING DESIGN

The two general sample design categories are probabilistic (also called “statistical” or “random”) sampling designs and judgmental or targeted sampling designs (USEPA 2002a).

Probabilistic Sampling

A probabilistic or probability-based sampling design utilizes randomization in the selection of sample locations (USEPA 2002a). A probabilistic sampling design requires accurate information about the population being sampled to ensure that a consistent distribution of the population is sampled. The sampling location selection process will consider population characteristics (Strahler stream order, land cover, number and location of point source discharges, etc.) and will be weighted accordingly. There are various ways that sites can be picked for a probabilistic monitoring study. One approach is a Generalized Random Tessellation Stratified (GRTS) design procedure. This procedure was employed for DEP’s recreational use monitoring and statewide statistical survey requirements. GRTS provides a random sample that is balanced spatially. The sites are chosen using an R-program package called ‘spsurvey’ (Kincaid and Olsen 2011). The package ‘spsurvey’ also helps analyze the results and can give estimations of the proportion of samples in each category analyzed, along with standard error and total estimates (Kincaid 2012a, Kincaid 2012b). Other procedures may be employed to choose sites statistically; however, without a detailed characterization and classification, a probabilistic sampling design does not necessarily provide for detailed assessment delineations and is not the preferred sampling design.

Targeted Sampling

DEP’s primary monitoring objective is to collect data to assess protected water uses of specific water segments or reaches, where most other states assess larger watershed units. DEP believes the targeted “judgment-based” sampling design is the most suited procedure to assess WQS including uses of specific water segments or reaches. A targeted or judgmental sampling design provides for the selection of sample locations based on professional judgment and does not utilize randomization. This approach requires intense reconnaissance and information gathering but can result in an accurately delineated assessment and is the preferred sampling approach for DEP monitoring protocols and subsequent assessment methods.

Reconnaissance includes the delineation of the targeted waterbody or basin and the gathering of historical and other information. A review of any gathered data or information may influence the geographical extent of the survey. Aerial photography, topographic maps, and/or geographical information system (GIS) information are used to delineate the targeted waterbody, identify major tributaries, characterize land cover, and any potential sources of nonpoint source impacts (agriculture, urban areas, etc.), potential sources of point source impacts (industrial discharges, sewage treatment discharges, combined sewer overflow (CSO discharges, etc.), geology, and soil characteristics. Historical information includes past water quality data and associated use assessments. Other information can include designated and existing water uses and ongoing water

quality monitoring like the Pennsylvania WQN and US Geological Survey (USGS) stream gaging network.

SAMPLING PLANS

A sample collection plan is recommended before commencing a sampling project. This should include the primary and any secondary objectives, subsequent monitoring protocols and assessment methods that will be employed to meet objectives, tentative sampling/site location(s), timing and frequency of samples, media of interest (water, sediment, soil, macroinvertebrates, etc.), parameters to sample, QA and Quality Control (QC) plans, sampling supplies and equipment, and any other notes or comments pertaining to the project. This chapter includes general information applicable to sampling plans and additional, objective-specific information can be found in the subchapters contained within.

A sample collection plan should describe how the data will be used. Analyses and data interpretation should be anticipated in advance. A collector should consider if an objective is to defend an enforcement action or recommend regulatory changes. Also, consider what criteria may be used during its interpretation. DEP has numeric and narrative water quality criteria documented in 25 Pa. Code Chapter 93, Water Quality Standards. Numeric criteria allow for screenings during sampling. In some cases, there may be no numeric criteria for a substance or sampling matrix. For example, DEP has no numeric sediment criteria at this time. However, in order to apply these or other results that do not have numeric criteria, one can refer to the 25 Pa. Code § 93.6:

> *"(a) Water may not contain substances attributable to point or nonpoint source discharges in concentration or amounts sufficient to be inimical or harmful to the water uses to be protected or to human, animal, plant or aquatic life. and (b) In addition to other substances listed within or addressed by this Chapter, specific substances to be controlled include, but are not limited to, floating materials, oil, grease, scum and substances that produce color, tastes, odors, turbidity or settle to form deposits."*

The numeric criteria, narrative criteria, or biological standard to be measured should be described prior to study commencement.

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2.1 CAUSE AND EFFECT SURVEYS

Chapter 2 Sampling Design and Planning

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INTRODUCTION

Cause and effect surveys are designed to investigate possible relationships between point or nonpoint sources of conventional pollutants and known or suspected water quality problems through the collection and analysis of biological, physical, and chemical data. This protocol was developed to establish and standardize cause and effect survey procedures and provide guidance to DEP staff for conducting such surveys. The protocol should be used in conjunction with other DEP data collection protocols and assessment methods.

These surveys are performed primarily to monitor the effectiveness of permitted treatment facilities but are also used to investigate reported or suspected water quality impacts from nonpoint source and other pollution sources. Since such discharges exist on a wide variety of surface water habitats, the sampling design and type of sampling gear used are dependent on waterbody classification, site-specific conditions, and the nature of the discharge under investigation. DEP staff are responsible for sampling design and any modifications to sampling design due to unforeseen site-specific conditions.

SAMPLING DESIGN

The sampling design for a cause and effect survey on flowing waterbodies and, in some cases, impoundments requires a minimum of two sampling stations. One station is placed upstream from the subject discharge(s) or impact(s) to serve as a control or reference condition and at least one station is placed in a potentially impacted zone downstream. Additional sampling stations may be necessary, either placed downstream to define zones of impact and recovery; upstream to bracket multiple discharges; or across a transect in wider waterbodies. For point sources, observations in the immediate vicinity of a discharge may also be useful.

Observations in the immediate vicinity of a discharge should include:

- Floating solids, scum, sheen, or substances that result in observed deposits in the receiving water (a small amount of foam that rapidly dissipates is typical);

- Oil and grease in amounts that cause a film or sheen upon or discoloration of the surface waters or adjoining shoreline, or that exceed 15 mg/l as a daily average or 30 mg/l at any time (or lesser amounts if specified in the respective permit);

- Substances in concentration or amounts sufficient to be inimical or harmful to the water uses to be protected or to human, animal, plant, or aquatic life;

- Foam or substances that produce an observed change in the color, taste, odor, or turbidity of the receiving water, unless those conditions are otherwise controlled through effluent limitations or other requirements in the respective permit.

Any noted observations may or may not indicate impairment of WQS or a violation of associated permit conditions. Regulatory requirements and permit conditions (including 25 Pa. Code §§ 93.6, 92a.41(c), 95.2(2)(i)) should be consulted.

With the exception of observations previously described, stations sampled downstream of the discharge(s) or nonpoint sources should not be placed in the immediate vicinity of the discharge or nonpoint source, but instead should generally be located a distance downstream in a potentially impacted zone. Selecting downstream station locations is unrelated to “criteria compliance times” used to generate National Pollutant Discharge Elimination System (NPDES) permit limits. Similarly, it is not necessary to restrict the location of downstream stations to points at or downstream of the point of complete mix, as defined as the point where water quality and/or other characteristics are homogenous across a transect. If a tributary or other compromising influence is located just downstream of the discharge or source of potential impact, which would not make an upstream/downstream comparison directly applicable, DEP staff may need to modify the sampling design to account for differences in water quality that are not necessarily due to the discharge or source of potential impact.

Complete mix occurs rapidly in smaller streams at most flow conditions and it is typically appropriate to locate downstream stations in smaller streams at a point of complete mix. In larger streams or rivers, a plume of effluent could extend a significant distance laterally and longitudinally. An indicator station may be located at or downstream of this point recognizing that any chemical, physical, or biological sampling could be conducted as a composite across the transect, but effects within the plume will also be assessed if a significant portion of the receiving water is impacted. The number and placement of any in-situ collections across a transect and subsequent compositing should be completed according to *Chapter 4.1, In-situ Field Meter, Transect, and Vertical Profile Data Collection Protocol* (Hoger and Arnold 2023), or equal-width-increment or equal-discharge-increment procedures (USGS 2006). When collecting biological samples, the equal-width-increment procedure can be considered (i.e., effort physically distributed equally across the/a transect), but the sample should be collected in a manner such that the sample represents the waterbody across the transect and targets best available habitat according to the DEP protocols and methods.

If the downstream station is placed where homogenous conditions do not exist, additional downstream cross-section surveys and sampling stations may be necessary to characterize the effect. Any critical habitat of threatened or endangered species, as defined by the United States Fish and Wildlife Service, or any rare or endemic ecological community types, as defined by the Pennsylvania Natural Heritage Program, or any migration impediments must be identified within the defined plume or within the vicinity of the plume. The water quality and its effect on identified habitat or ecological communities and/or WQS will be assessed specific to identified habitat or ecological communities.

Control or reference sampling stations may be placed at any point upstream from the discharge or source where there is no potential impact from the discharge at any river flow condition. In a low-

gradient situation such as a pool, it may be most appropriate to locate the reference station far enough upstream from the discharge to preclude possible effects from pooling effluent during low flow conditions. If there is an intake structure present, it may be most appropriate to locate the reference station upstream from the intake structure to preclude possible effects from recirculating effluent during low flow conditions.

In some instances, if upstream conditions do not adequately represent control or reference conditions, it may be necessary to sample a separate waterbody for reference purposes. Ideally, this reference station would be selected within the same watershed or if no available reference station is found, an appropriate station should be in an adjacent watershed. Water quality impacts to impounded waters present at least the compounding effect the impoundment itself has on water quality and water quality indicators and may prevent traditional upstream/downstream sampling designs from detecting changes in water quality due to impacts other than the impoundment. To reduce or eliminate compounding sources of variability, physical habitat conditions should be as similar as possible, for each segment or separate waterbody selected for sampling. The following data will be collected at all sampling stations: benthic macroinvertebrates or fish (as needed), habitat assessment, field measurements or water chemistry, channel cross-section surveys and stream flow (discharge) (as needed), and bacteria (as needed).

DATA COLLECTION

Benthic Macroinvertebrates (optional)

For wadeable freestone streams, limestone-influenced streams, low gradient streams, or large semi-wadable rivers, benthic macroinvertebrate samples are collected utilizing protocols detailed in Chapter 3 of this book. Each benthic macroinvertebrate data collection protocol describes what will need to be evaluated to determine which protocol to use. Data collection protocols include semi-quantitative (population densities derived by estimation), qualitative (population densities not calculated), and quantitative (population densities derived by measurement) protocols. The survey data needs will determine which protocol is most suitable. In most instances, semi-quantitative data collection protocols are preferred and will meet most data requirements. Other optional data collection protocols include qualitative and quantitative. Qualitative protocols (Chapter 3) are appropriate Cause and Effect Survey protocols. Qualitative protocols may be more appropriate than quantitative or semi-quantitative protocols, and may be used in place of, or in conjunction with semi-quantitative data collection when the survey requires a more immediate result or the targeted surface water is not characteristic of surface waters used to develop a method or index. Qualitative surveys are also appropriate as preliminary evaluations immediately following acute pollution events where the impact to the biological community doesn’t occur for a period of one to three weeks. Qualitative surveys could be used to determine the timing of subsequent quantitative or semi-quantitative surveys.

Regardless of the data collection protocol used, sampling stations consist of a control or background station placed upstream from the discharge(s) and at least one affected or impacted station downstream from the discharge(s) in the best available riffle and run habitat. When multiple

discharges are present, sampling stations are placed between discharges to characterize the effect of each input. Physical variables of all sample stations should be matched as closely as possible between background and impacted stations to minimize or eliminate the effects of compounding variables. Sample points are placed to obtain a representative benthic sample and to avoid over sampling of clustered populations.

DEP developed an index of biotic integrity (IBI) for benthic macroinvertebrate communities collected via semi-quantitative protocols in Pennsylvania's wadeable, freestone, riffle-run streams. See Chapter 2 of the Assessment Book (Shull and Whiteash 2023). Through direct quantification of biological attributes along a gradient of ecosystem conditions, this IBI measures the extent to which anthropogenic activities compromise a stream's ability to support healthy aquatic communities (Davis and Simon 1995) and can be used to compare control or reference conditions versus impacted conditions. DEP's latest IBIs describe precision estimates for temporal variability (...whether a site's biological condition has improved or degraded over time) and intersite variability (…whether a site's biological condition is improved or degraded when compared to a nearby site). Samples collected for cause and effect surveys are to be collected on the same day to eliminate the need to consider temporal variability. Intrasite variability, as determined by the IBI for benthic macroinvertebrates applies to samples collected from a single site/station (within 100 meters). Cause and effect survey upstream and downstream stations are collected from separate sites and therefore an intersite precision estimate was developed and should be applied to compare upstream, downstream and recovery zone sites/stations. Cause and effect upstream or control versus downstream or recovery stations collected using the latest semi-quantitative protocols for wadeable, freestone, riffle-run streams with IBI scores greater than the intersite precision estimate will be considered impacted. The appropriate intersite precision estimate is determined by DEP Water Quality Division, Assessment Section. Follow-up surveys may be conducted when small IBI score differences are found between control and impact sites to confirm or to re-evaluate initial cause and effect survey results.

Fish (optional)

For most cause and effect surveys semi-quantitative fish sampling should be considered. See *Chapter 3.7, Fish Data Collection Protocol* (Wertz and Arnold 2023). The objective is to acquire a representative sample of the fish population by sampling all physical stream habitats in relative proportion to their availability.

DEP developed *Stream Fish Assemblage Assessment Method* (Wertz 2021) using a Thermal Fish Index (TFI) for fish communities collected via semi-quantitative protocols in Pennsylvania's lotic streams and rivers. The assessment method provides additional guidance for initiating and evaluating cause and effect surveys.

If fish kills are a component of a cause and effect survey investigation, quantitative procedures would be required in cases where economic damages may need to be calculated resulting from incidents causing fish kills. In these instances, to support fish/aquatic life penalties, the required fish/aquatic life data should be collected in a manner consistent with The American Fisheries Society (Southwick and

Loftus 2017) or Pennsylvania Fish and Boat Commission (PFBC) fish kill survey procedures. In many cases, it may be more practical to coordinate field sampling activities with the regional PFBC Fisheries Management staff.

Bacteria (optional)
Because of the survey and cost complexities imposed by bacterial sampling (e.g., sample frequency and short holding times), bacteria sample collection is an optional consideration limited to when sanitary impacts from discharges are suspected. Samples for bacteriological analysis are collected to define the sanitary significance of point and nonpoint sources and assess the use status (supporting or impaired) of stream segments for potable water supply and recreational uses. The samples are collected using protocols outlined in Chapter 3 of this book.

Habitat Evaluation (required)
A habitat evaluation is conducted on a measured 100-meter reach of stream at a minimum for wadeable surface waters according to *Chapter 5.1, Stream Habitat Data Collection Protocol* (Lookenbill 2017) in this Monitoring Book and *Chapter 4.1, Physical Habitat Assessment Method* (Walters 2017) in the Assessment Book. The habitat evaluation process involves rating twelve parameters for riffle/run prevalent waterbodies or nine parameters for low gradient waterbodies as optimal, suboptimal, marginal, or poor by using a numeric value (ranging from 20-0).

Field Measurements and Water Chemistry (required)
Detailed field observations on land use and potential sources of pollution are recorded on field data collection forms or in field notebooks. Dissolved oxygen, pH, specific conductance, and temperature are measured in the field using hand-held meters calibrated according to manufacturer specifications and the latest DEP protocols found in Chapter 4 of this book.

One-time grab samples are collected, at a minimum, from a control or background station upstream from the discharge, from the discharge, and from at least one downstream affected or impacted station when evaluating point source discharges. Point source discharges that do not continuously discharge or those that discharge subsurface may prevent grab samples from being collected. In these situations, Discharge Monitoring Reports (DMRs) that document the chemical violation of the NPDES permit would suffice. For nonpoint discharges, grab samples are collected upstream of the impacted segment and from within the impacted segment.

Discharge (optional)
Discharge data are collected as needed according to the latest version of USGS Techniques and Methods book 3, chapter A8, *Discharge Measurements at Gaging Stations* (Turnipseed and Sauer 2010).

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2.2 COMBINED SEWER OVERFLOW (CSO) COMPLIANCE DATA COLLECTION

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INTRODUCTION

Combined Sewer Overflows (CSOs) are unique in the way they are regulated and so require unique sampling plans to meet the demands of compliance monitoring. The Federal Register details the requirements of CSO policy and monitoring requirements. Specifically, 59 FR 18688 explains:

> *The CSO policy represents a comprehensive national strategy to ensure that municipalities, permitting authorities, water quality standards authorities and the public engage in a comprehensive and coordinated planning effort to achieve cost effective CSO controls that ultimately meet appropriate health and environmental objectives. …State water quality standards authorities will be involved in the long-term CSO control planning effort as well. The water quality standards authorities will help ensure that development of the CSO permittees' long-term CSO control plans are coordinated with the review and possible revision of water quality standards on CSO-impacted waters.*

59 FR 18696, under section 2 explains:

> *Once the permittee has completed development of the long-term CSO control plan and the selection of the controls necessary to meet CWA requirements has been coordinated with the permitting and WQS authorities, the permitting authority should include, in an appropriate enforceable mechanism, requirements for implementation of the long-term CSO control plan as soon as practicable. Where the permittee has selected controls based on the "presumption" approach described in Section II.C.4, the permitting authority must have determined that the presumption that such level of treatment will achieve water quality standards is reasonable in light of the data and analysis conducted under this Policy.*

Additionally, the Phase II permit should contain:

> *A requirement to implement, with an established schedule, the approved post-construction water quality assessment program including requirements to monitor and collect sufficient information to demonstrate compliance with WQS and protection of designated uses as well as to determine the effectiveness of CSO controls.*

The U.S. Environmental Protection Agency's (USEPA) draft CSO Post Construction Compliance Monitoring Guidance (USEPA 2011) also recommends the information to be collected for monitoring CSO compliance, consistent with the Federal Register. This guidance states that CSO Monitoring programs should include both CSO effluent and ambient in-stream monitoring. USEPA's draft guidance also suggests, where appropriate, biological assessments, toxicity testing, and sediment sampling are also implemented.

Based on the information found in the Federal Register and USEPA's guidance (USEPA 2011), designing a sampling plan for CSO compliance monitoring involves answering two important questions:

1. Does the receiving waterbody meet assessment thresholds that would support the protected use?
2. Is the CSO causing or contributing to a WQS impairment?

These two questions are not the same, because the sampling plan will be different for each question. To answer the first question, sampling must be designed in accordance with making protected use assessment decisions, which are decisions on the entire waterbody, or on defined zones as discussed in the Assessment Book (Shull and Whiteash 2023). The assessment decision process inherently avoids collecting data at or very near single discharge points, because of the possibility of using results from one discharge to make an assessment determination on the entire waterbody. Conversely, the second question requires that the sampling plan incorporates sampling at or very near single points of discharge to determine contribution to a potential Water Quality Standard (WQS) impairment. It is important to note that sampling must be conducted to answer both questions regardless of an assessment determination that shows supporting WQS upstream and downstream of the CSO.

SAMPLING DESIGN

Sampling design for CSO compliance requires a combination of use assessment and cause and effect sampling design. Both sampling designs typically include biological, physical, and chemical data collection. To evaluate any impacts from CSOs, biological and chemical evaluations will be the primary targets. Biological data collection will specifically require bacteriological monitoring. Chemical data collections will require at least discrete water chemistry monitoring. In addition, performance monitoring of the combined sewer system(s) (CSS) will also be conducted.

All monitoring plans will be submitted to DEP for approval prior to being implemented. A final report will be submitted within 90 days following the collection of the last samples for DEP review.

DATA COLLECTION

Does the receiving waterbody meet supporting thresholds?

Bacteria (required)

Bacteria data collection is conducted according to protocols outlined in Chapter 3 of this book during the swimming season (May 1st through September 30th). Assessment determinations will be conducted on upstream and downstream segments of the CSO or multiple CSOs if there is not the opportunity to sample between according to methods outlined in Chapter 2 of the Assessment Book (Shull and Whiteash 2023).

Field Measurements and Water Chemistry (required)

Data collection for other parameters identified in the CSO Control Policy are conducted according to the latest DEP protocols found in Chapter 4 of this book. Assessment determinations will be conducted on upstream and downstream segments of the CSO or multiple CSOs if there is not the opportunity to sample between according to methods outlined in Chapter 3 of the Assessment Book (Shull and Whiteash 2023).

Is the CSO causing or contributing to a WQS impairment?

Bacteria (required)

Bacteria data collection is conducted according to procedures outlined in Chapter 3 of this book for at least 1 year (4 seasons) as follows:

- During each month of the swimming season (May 1st through September 30th), samples are collected upstream and downstream of each outfall (or groups of outfalls as appropriate).
    - Samples must be analyzed for fecal coliform[1] and *E. coli*.[2]
    - 5 samples must be collected under "normal" recreating conditions.
    - 5 samples must be collected under other, critical conditions to capture high flows, storm events, etc. when CSOs are discharging.
    - Corresponding qPCR samples should also be collected for each set of samples. A minimum of 1 sample per monthly set is recommended, but additional samples are encouraged as they will better characterize the source(s) of bacteria to determine whether the CSO is contributing to the bacterial load.
- During each month of the non-swimming season (October 1st through April 30th), samples are collected at the same upstream and downstream locations established during the swimming season following the same protocol.
    - Samples must be analyzed for fecal coliform[1].
    - 5 samples must be collected in accordance with data collection protocols during normal/low flow conditions.
    - 5 samples must be collected under other, critical conditions to capture high flows, storm events, etc.
    - Corresponding qPCR samples should also be collected for each set of samples. A minimum of 1 sample per monthly set is recommended, but additional samples are encouraged as they will better characterize the source(s) of bacteria to determine whether the CSO is contributing to a use impairment or impact to the surface water.

[1] At the time of publication, fecal coliform limitations are referenced at 25 Pa. Code § 92a.47 and non-swimming season fecal coliform criteria are listed at § 93.7

[2] At the time of publication, E. coli criteria are listed at § 93.7.

Field Measurements and Water Chemistry (required)

Data collection for other parameters identified in the CSO Control Policy are conducted according to the latest DEP protocols found in Chapter 4 of this book for at least 1 year (4 seasons) as follows:

> At minimum, these parameters include BOD, TSS, ammonia-N, specific conductance, pH, temperature, and DO as well as any other parameters reasonably expected to be in the discharge.
>
> A minimum of 1 sample should be collected during each CSO discharge event throughout the 1-year sampling period unless dangerous conditions prohibit sample collection.

CSS Performance Monitoring (required)

The purpose of CSS performance monitoring is to document frequency and duration of CSO activations. A specific CSO activation event definition should be defined for each CSS. Performance monitoring includes the characterization of CSO activations and the collection of discharge data at strategic locations throughout and rainfall data within the CSS catchment area(s).

CASE STUDY

The following case study demonstrates the need to collect data using both sampling designs. Briefly, this study demonstrates adequate sampling design to answer question 2 (Is the CSO contributing to a WQS impairment?), but there is insufficient information to determine the answer to question 1 (Does the receiving waterbody meet supporting thresholds?). Therefore, more data needs to be collected to make use assessment determinations on stream segments upstream and downstream of the CSO.

> A Post-Construction Monitoring Plan and subsequent results from 2014–2017 were provided to a DEP regional office. The plan outlined routine surface water monitoring that took place at 7 sites, upstream and downstream of discharges. All samples were collected from the shoreline.
>
> Sites were identified as Site 1 through Site 8, moving upstream to downstream, respectively (Site 5 was discontinued). Site 1 was located upstream of all urbanized area and all CSO outfalls. Sites 2 through 6 were located appropriately to measure the contribution of several CSO outfalls and bracket one tributary influence. Site 7 was located downstream of all CSO outfalls, an urban influenced tributary, and a sanitary sewer outfall. Site 8 was located downstream of the entire urbanized area.
>
> Sampling frequency was five times per month during the swimming season (May 1st – September 30th). The goal of this approach was to capture both wet and dry weather events, if possible. Samples were collected on the first four Tuesdays of each month during the swimming season with a fifth “floating” sample taken each month. There was

no data collected during the non-swimming season, and no qPCR samples were collected.

A review of the data revealed that 5-base flow (0 – 0.25" rain in the previous 24-hours) samples were successfully collected within any rolling 30-day period 7 times in 2014, 4 times in 2015, 0 times in 2016, and 1 time in 2017. These periods were not well distributed throughout the swimming season, which emphasizes the need to specifically target 5 base flow events for each 30-day period. An excursion of the 5-day geometric mean criterion for fecal coliform of 200/100 mL occurred for the periods of 8/19/2014 – 9/16/2014 and 8/26/2014 – 9/23/2014 at Site 6; 8/19/2014 – 9/16/2014, 8/26/2014 – 9/23/2014, and 5/26/2015 – 6/23/2015 at Site 7; and 8/19/2014 – 9/16/2014 at Site 8. A total of 6 30-day geometric mean excursions were documented at three sites.

Exceedances of the fecal coliform criterion, "*no more than 10% of the total samples taken during a 30-day period may exceed 400 per 100 mL*," occurred at most sites throughout the four years of data. No exceedances occurred at Site 1. Site 2 and Site 7 had the most exceedances and had exceedances for each year (Table 1). In addition to base flow events, multiple wet weather events (> 0.25" within 24-hours) were sampled 7 – 9 times through the swimming season each year. The highest maximum and mean values were documented at Site 6 and Site 7 (Table 2, Figure 1).

Table 1. Number of exceedances for fecal coliform of no more than 10% > 400/100ml for rolling 30-day periods at sites for specific years.

Year	Site 1	Site 2	Site 3	Site 4	Site 6	Site 7	Site 8
2014	0	5	0	2	4	5	2
2015	0	3	0	2	3	5	3
2016	0	1	1	0	0	5	0
2017	0	1	1	1	1	1	1

Table 2. Wet weather event yearly summaries (fecal coliforms/100 mL).

Year	Statistic	Site 1	Site 2	Site 3	Site 4	Site 6	Site 7	Site 8
2014	Min	18	18	45	68	18	170	78
	Max	220	1100	330	1300	2400	**7900**	3500
	Mean	88.5	336.8	154.2	595.4	605.1	**1812.8**	894
2015	Min	55.6	42.5	22.1	410.6	290.9	613.1	365.4
	Max	782.5	776.5	2053	2419.6	**3065.5**	7068	3635
	Mean	372.5	351.2	772.4	1214.0	1037.6	**2793.1**	1324.0
2016	Min	7.5	54	21.3	19.5	36.8	1301.5	91
	Max	365.4	461.1	1046.2	1046.2	**2419.6**	12098	2442
	Mean	155.4	224.9	254.0	407.2	870.9	**3083.9**	1017.3
2017	Min	55.6	15	40.4	111.9	88	111.2	85.7
	Max	1827	3448	2735.5	2735.5	**3065.5**	2897	2586
	Mean	414.6	713.5	594.7	603.4	987.2	**1264.4**	678.2

* **Bold** values indicate the greatest maximum and mean values for a given year

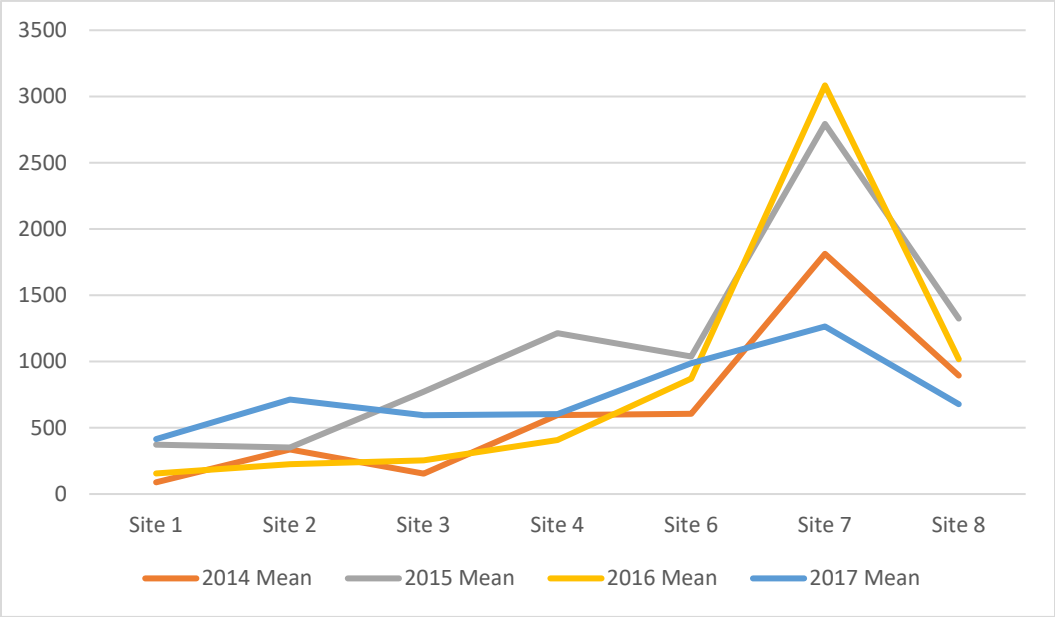

Figure 1. Wet weather event yearly means (fecal coliforms/100 mL).

Exceedances of water quality standards as implemented by the DEP's assessment methods during base flow conditions can be documented at all sites, except for Site 1, the upriver "control" site. Bacteria data collected during wet weather events (> 0.25" within 24-hours) documents that fecal coliform concentrations progressively increase from the upstream control to Site 7, where the mean concentrations were the highest each year from 2014 – 2017. Site 8 data does generally show reduced concentrations, but base flow concentrations are still above water quality standards.

This case study demonstrates that the CSO discharges were contributing to a potential WQS impairment, but since all samples were collected from the shoreline, the data cannot be used to make an assessment determination for the waterbody. It is important to note that a relatively simple modification to the sampling design at Sites 1 and 8 could be implemented to achieve an assessment determination. If data collection at Sites 1 and 8 followed the sampling design used to make assessment determinations, then both questions 1 and 2 could be answered.

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2.3 COPPER BIOTIC LIGAND MODEL SAMPLING DESIGN

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INTRODUCTION

The Pennsylvania Department of Environmental Protection (DEP) will generally follow the U.S. Environmental Protection Agency's (USEPA) guidance on using the Biotic Ligand Model (*BLM, Training materials on Copper BLM: Data Requirements & Implementation*; USEPA 2015), but this guidance offers limited information with respect to where and how often an applicant should sample for the BLM input parameters because BLM study design is very dependent on the conditions found at each location. As a result, the following guidance for copper BLM sampling design and planning was developed. If an applicant follows this guidance along with DEP monitoring protocols and assessment methods, execution of the approved plan of study should result in data collection that adequately characterizes the 10 BLM input parameters for each sampling site and receiving waterbody.

Note that all proposed plans of study will be submitted to DEP for review, consideration, and approval prior to commencement. Any revisions or modifications to a plan of study following DEP review and approval will be required to undergo additional review and approval by DEP prior to implementation. DEP also routinely shares draft plans of study with USEPA Region 3 to ensure the studies will meet the needs and intent of USEPA's BLM guidance prior to implementation.

STUDY PLANS AND FINAL REPORTS

Study plans and final reports will include an executive summary, introduction (background), sampling design, literature cited, and appendices including maps, sampling results, and any other relevant information and documentation. Final reports should also include sections for results and discussion.

Introduction

The introduction section will provide basic information about the applicant's facility and discharge, the general location and study boundaries, the receiving waterbody name, and the waterbody's protected aquatic life uses (e.g., Warm Water Fishes (WWF), Cold Water Fishes (CWF), High Quality Waters (HQ), Migratory Fishes (MF)). This section will also state the purpose of the study, which is to collect monitoring data that will support the development of an appropriate copper criterion that protects existing and designated water uses. Information will be provided for all activities in the watershed and discharges to the receiving waterbody within the study boundaries that may influence water quality in the vicinity of the applicant's discharge outfall(s). These activities include all point source discharges (e.g., National Pollutant Discharge Elimination System (NPDES)-permitted discharges and other DEP permits or approvals), nonpoint source discharges (e.g., agriculture, earth moving activities that do not require a DEP permit, certain types of stormwater discharges), and tributaries that may enter the receiving waterbody upstream, adjacent to, or immediately downstream of the discharge. The information for each discharge should include facility name, permit number, type of discharge activity (e.g., industrial, sewage, stormwater), and any other pertinent information. A map identifying all existing permitted discharges and all tributaries within the study boundaries will be included in an appendix.

Sampling Design

The sampling design section will describe the BLM input parameters to be sampled, the specific locations of the sampling events (including latitude and longitude for each site), the data collection

protocols used, the anticipated number of sampling events to be performed, and the anticipated dates of the sampling events.

The plan of study will be designed to adequately capture the spatial and temporal variability of the BLM input parameters at each sampling location to ensure that the criteria calculated from these input parameters will be adequately protective of the receiving waterbody's existing and designated use(s) under the most bioavailable conditions for copper. The water quality characteristics that determine the bioavailability of copper can vary widely in both space and time changing with biological activity, flow, weather events, human activities, land cover, and other characteristics of the watershed. The number and frequency of sampling events that will be needed to capture the temporal and spatial variability of the BLM input parameters at a sampling site are ultimately dependent upon the site's characteristics.

Results

The results section will present the sample results for each sampling event at each sampling site (addressing all parameters) including a description of any QA/QC results. All 10 BLM parameter inputs, supporting data and results are to be provided to DEP in an excel spreadsheet or other DEP-acceptable format. This section will also include information on method detection/reporting limits and potential data outliers. Any potential outlier values in the dataset will be flagged, and a description of why the value is a potential outlier value will be provided. Note, USEPA and DEP generally do not consider results from samples collected during normal expected weather conditions (e.g., a summertime 2-year, 24-hour storm event) to be outlier data. DEP will determine if any data should be removed and will describe the removal of any value in its rationale document for the development of the criteria recommendations. DEP will apply the most appropriate statistic when analyzing censored data; generally accepted approaches can be found in Helsel et al., 2020.

Discussion

The discussion section will place the results within the larger context of facility operations, including expected discharge constituents and variability. Additionally, observed seasonal and spatial variability in the sampled model parameters, stream flow, and effluent mixing will be addressed.

SAMPLING DESIGN

Sampling design will adequately capture the spatial and temporal variability of the BLM input parameters at each sampling site to ensure that the criteria calculated from these input parameters will be adequately protective of the receiving waterbody's existing and designated use(s) under the most bioavailable conditions for copper. The water quality characteristics that determine the bioavailability of copper can vary widely in both space and time changing with biological activity, flow, weather events, human activities, land cover, and other characteristics of the watershed. The number and frequency of sampling events that will be needed to capture the temporal and spatial variability of the BLM input parameters at a sampling site are ultimately dependent upon the site's characteristics.

Sampling design for copper BLM is generally consistent with cause and effect survey design with some exceptions. A minimum of two sampling stations is required. One station is placed upstream from the subject discharge to serve as a control or reference condition and at least one station is placed

downstream. Additional sampling stations may be necessary, either placed downstream to define zones of variable water quality; upstream to bracket multiple discharges; across a stream transect in wider waterbodies; or directly from the effluent.

The downstream sampling location should be representative of ambient water conditions after the receiving waterbody has completely mixed with the discharge (i.e., outside of the chronic mixing zone). The collection of data both downstream of the discharge outside of the chronic mixing zone and upstream outside of the influence of the discharge should best characterize the spatial variability of the sampling site. If a tributary or other compromising influence is located just downstream of the discharge or source of potential impact, which would not make an upstream/downstream comparison directly applicable, the sampling design may need to be modified to account for differences in water quality that are not necessarily due to the discharge.

Complete mix occurs rapidly in smaller streams at most flow conditions, and it is typically appropriate to locate downstream stations in smaller streams at a point of complete mix. In larger streams or rivers, a plume of effluent could extend a significant distance laterally and longitudinally. An indicator station may be located at or downstream of this point recognizing that any chemical sampling could be conducted as a composite across the transect, but effects within the plume will also be assessed if a significant portion of the receiving water is influenced. The number and placement of any in-situ collections across a transect and subsequent compositing should be completed according to *Chapter 4.1, In-situ Field Meter, Transect, and Vertical Profile Data Collection Protocol* (Hoger and Arnold 2023), or equal-width-increment or equal-discharge-increment methods (Turnipseed, and Sauer 2010). If the downstream station is placed where homogenous conditions do not exist, additional downstream cross-section surveys and sampling stations may be necessary to characterize the effect of the discharge(s) on the receiving waterbody.

Control or reference sampling stations may be placed at any point upstream from the discharge or source where there is no potential impact from the discharge at any flow condition. In a low-gradient situation such as a pool, it may be most appropriate to locate the reference station far enough upstream from the discharge to preclude possible effects from pooling effluent during low river flow conditions. If there is an intake structure present, it may be most appropriate to locate the reference station upstream from the intake structure to preclude possible effects from recirculating effluent during low flow conditions.

If upstream and/or downstream conditions do not adequately represent an unimpacted condition or is otherwise impaired, it may be necessary to sample a separate waterbody for reference purposes. Ideally, this reference station would be selected within the same watershed or if no available reference station is found, an appropriate station should be in an adjacent watershed. Water quality impacts to impounded waters present at least the compounding effect the impoundment itself has on water quality and water quality indicators and may prevent traditional upstream/downstream sampling designs from detecting changes in water quality due to impacts other than the impoundment. Cross-section surveys and water chemistry will be collected at all sampling stations.

In general, sampling location should not be overly influenced by specific effluent discharges or other inputs, unless the characteristics of these discharges or inputs are expected to increase the bioavailability, and thus toxicity, of copper to aquatic life. Any input (e.g., tributary, NPDES discharge, etc.) or output (e.g., water withdrawals, etc.) within the study boundaries should be carefully considered to determine its effect on ambient copper bioavailability, and whether greater sampling frequency and/or more sampling sites might be appropriate. Data collection should reflect site-specific characteristics and consider special circumstances that may affect copper bioavailability and toxicity throughout the expected range of receiving waterbody conditions.

Data Collection

Data will be collected according to DEP's Water Quality Monitoring Protocols for Surface Waters (Lookenbill and Arnold 2023).

BLM sampling guidance is as follows:

- Twelve sampling events performed over one calendar year, each conducted approximately one month apart is the minimum acceptable sampling number and frequency. Additional sampling events may be required if water quality data does not adequately characterize variability and representativeness.
- DEP recommends either 24 sampling events collected approximately every two weeks over one calendar year or 24 sampling events collected monthly over two calendar years to ensure sufficient data are collected.
- Each sampling event should include all parameters identified below.
- Each sampling event should be conducted at two locations: 1) immediately downstream of the discharge at a point of complete mix, and 2) upstream outside of the discharge's zone of influence (avoiding any backwater, etc.).
- In addition to surface water data collection, effluent samples should also be collected along with a characterization of current facility operations in an effort to describe any variability in operations and discharge characteristics. Effluent data collection would include discharge volume, permitted reporting requirements and constituents collected for the surface water monitoring aspect of the study.
- A cross-section survey should be completed with each sampling event in order to characterize representativeness of discrete samples being collected.

The plan of study will include the following 10 parameters:

- pH measured in standard units (s.u.)
- Dissolved Organic Carbon measured in mg/L
- Alkalinity measured in mg/L of $CaCO_3$
- Temperature measured in degrees Celsius
- Calcium measured in mg/L
- Magnesium measured in mg/L
- Sodium measured in mg/L

- Potassium measured in mg/L
- Sulfate measured in mg/L
- Chloride measured in mg/L

In addition to these 10 parameters, DEP recommends collecting total and dissolved copper samples at each sampling site during each sampling event. While these parameters are not required for input to the BLM to generate a copper criterion, sample data provides valuable information about the actual copper concentrations in the receiving waterbody. Thus, these samples should be collected, and the results should be provided in the final study report. DEP also recommends sampling two supplemental parameters: totals suspended solids (TSS) and total dissolved solids (TDS). These parameters serve as a general indicator of site water quality and can be used to identify potential changes in ionic composition of the receiving waterbody within the study boundaries. If these indicators change, the site-specific copper criterion may need to be reevaluated to determine if it is still protective and representative of the original conditions for which it was developed using the BLM. Finally, stream flow (discharge) measurements should be made during each sampling event at each sampling site.

The above guidance should be sufficient where the following conditions are met:

- The study reach is not impaired as determined by DEP in an assessment or is not located in a watershed with a total maximum daily load (TMDL) for copper.
- There are no regulated or unregulated pollutant discharges located upstream of the applicant's discharge point(s).
- There are no additional significant sources of stream flow (tributaries/springs/unregulated discharges/etc.), aside from the applicant's discharge point(s) between the downstream-most and upstream-most sampling sites.
- There are no water withdrawals located within the study boundaries.
- Each sampling event is conducted during a period of normal operating conditions for any upstream discharge(s) including the applicant's discharge(s).
- Sampling events do not target specific flow regimes or stream conditions, such as low flow periods.

It is important to have sufficient ambient data to accurately reflect the spatial and temporal variability of the receiving waterbody within the study boundaries. The more input data that are collected, the more accurately the water chemistry can be characterized, which will result in more confidence in the calculated acute and chronic water quality criteria values. As the size and complexity of a study area increases, the spatial and temporal variability is likely to increase. Thus, if any of the above conditions and recommendations cannot be met during the study for any reason, more sampling sites and/or a greater number of sampling events may be necessary. Alternate study designs should be considered if any of the following scenarios exist:

- The receiving waterbody is determined to be impaired by DEP in an assessment;
- Upstream discharges exist which are suspected to potentially cause fluctuations in any of the BLM parameters;

- Significant sources of stream flow or pollution besides the applicant's discharge point(s) exist within the study boundaries between any of the sampling sites.

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2.4 LAKE ASSESSMENT SAMPLING DESIGN

Chapter 2 Sampling Design and Planning

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INTRODUCTION

The purpose of this protocol is to outline the sampling design and planning associated with data collection to complete protected use assessment objectives for lakes. Specific objectives include Aquatic Life uses (ALU), Special Protection uses (SPU), Potable Water Supply (PWS), Boating (BU), Esthetics Use (EU), Water Contact Sports (WC), and Fishing (FU) assessments. Typically, an ALU assessment is the primary objective and the overarching sampling design. Meeting this objective will significantly influence how many, when, what, and where samples are collected. Additional objectives including PWS and EU assessments can be completed by collecting additional data when collecting data for ALU assessment. Other use assessments generally require a significant increase in effort and/or frequency of data collection.

As required by the U. S. Environmental Protection Agency (USEPA) and the Federal Clean Water Act (CWA) Section 314, the DEP is obligated to assess all public lakes within the Commonwealth. The Federal CWA Section 314(a)(1)(A) describes that the lakes need to be identified and classified by the lake's trophic condition and reported on a biennial basis to the EPA under CWA Section 305(b) reporting. Furthermore, 25 Pa. Code § 93.4(a) states that *"existing and designated surface water uses* [hereafter referred to as uses] *shall be protected in accordance with Chapter 93, Chapter 96 (relating to water quality standards implementation) and other applicable State and Federal laws and regulations."*

Lakes are a type of surface water where water is accumulated faster than it is released through evaporation, ground water infiltration or outflow to a continuing waterbody, and typically have a long surface water residence time. Specifically, 25 Pa. Code § 96.1 defines a lake, pond, or impoundment as;

> *A surface water with a hydraulic residence time of 14 days or more based on average annual daily stream flow. Residence time shall be determined at average annual daily stream flow and normal pool volume. In the absence of actual records, an average annual daily discharge rate of 1.5 CFS [cubic feet per second] per square mile shall be used*.

While this definition is provided for purposes of achieving and maintaining water quality standards, the ≥ 14-day residence time does not necessarily limit the use of DEP lake specific protocols and assessment methods. Lake protocols are also applicable to waterbodies with a shorter residence times, a surface area of ≥ 1 hectare (2.5 acres), and a maximum depth ≥ 1 meter. These waterbody characteristics are adopted by the DEP according to USEPA' s *National Lake Assessment Site Evaluation Guidelines* (USEPA 2011; USEPA 2016; USEPA 2021).

SAMPLING DESIGN

All assessment sampling designs for lakes will conform to the following general rules:

1. Account for major influences (e.g. potential sources) – Sampling sites are positioned to account for changes in water quality due to influences such as major tributaries, point and nonpoint source impacts, land cover changes, soil characteristics, and geology. Samples are collected at the limits of these changes to effectively "bracket" potential sources of water quality differences.
2. Ensure data are collected in accordance with assessment method scope.
3. Quality Assurance **–** Data collected will be conducted according to *Quality Assurance Project Plan (QAPP): Data Collection for the Purpose of Existing Use Evaluations, Protected Use Assessments, and Evaluations of Point and Nonpoint Source Impacts to Surface Waters* (Lookenbill and Arnold 2023).
4. Biosecurity and Safety **–** Activities will adhere to *Biosecurity and Safety Procedures* (Arnold et al. 2023).

Lakes are dynamic ecosystems where water quality is influenced by hydraulic residence time, geomorphology, depth, vertical zonation, and seasonal environmental conditions. These factors need to be considered when employing a lake assessment sample design. Tributary influences, lake size, depth, and stratification conditions also need to be considered. These factors will aid in determining the number of stations and data collection protocols used.

There are two types of lakes in Pennsylvania, dimictic lakes and polymictic lakes. Dimictic lakes are those which completely mix twice each year when the lake's water temperatures are uniformly close to 4°C - once in the spring and again in the fall. When these waters are not completely mixed thermal stratification occurs where water quality changes at intervals vertically to create zonation. Dimitic lakes have three distinct layers, the epilimnion, metalimnion, and the hypolimnion; layers are defined where 1°C water temperature change is observed. Water quality in each of the layers may be different so sampling design and plans need to reflect these changes. Sampling design should take the approach of evaluating water quality in the epilimnion and hypolimnion (where applicable). Polymictic lakes are lakes where waters never stratify and remain completely mixed throughout time. Most Pennsylvania lakes are dimictic so protocols found in this book are established for dimictic lakes; procedures will also be applicable to polymictic lakes.

It is important that data are collected frequently enough to minimize sampling error and to accurately characterize the conditions for each parameter of concern. Conditions and representativeness should be characterized over a long enough time frame to account for variations in concentrations attributable to changes to the waterbody. For instance, the following interrelated factors can contribute to spatial and temporal variation: precipitation, stream flow, geology, watershed drainage patterns, and anthropogenic influences. Knowledge and understanding of such patterns can strengthen inferences about unobserved conditions (USEPA 2005). DEP generally considers discrete samples to be representative of one day (or in the case of a lake one week or longer based on the lake's residence time) unless convincing evidence exists to suggest otherwise (e.g., a documented spill, influence of a known biological process, or supporting high-frequency monitoring data). Similarly, USEPA recommendations allow for a single sample to represent periods longer than one day when conditions are stable (USEPA 1997).

Furthermore, sampling frequency is also considered when evaluating use criteria. It is most effective to focus monitoring efforts when digressions of water quality criteria are most likely to occur, hereafter referred to as critical sampling periods. Collecting samples during critical sampling periods will require an understanding of the variables at play. Some of the variables that should be considered are discussed further in Whiteash (2021). As a result of these factors and federal requirements, DEP recommends multiple sampling events for assessing any criterion but will evaluate all existing and readily available data when making assessments. More specifically, DEP encourages at least three sampling events within the criterion duration period. For example, the iron criterion for ALU is written as a 30-day average, so DEP encourages at least three samples be collected within a 30-day period to compare conditions to the criterion.

Specific sampling events require favorable weather conditions, consideration of recent weather conditions and any other seasonal or weather variables that could affect water quality. Significant rain events can cause pollutant loadings to the lake that do not reflect normal lake water quality conditions, especially if suspended solids or turbidity remains elevated. Lake sampling should be avoided after a major storm event. To aid in determining how long after a storm event a lake will be sampled, residence time, hydrology, and basin characteristics will need to be consulted. Lake sampling should not occur under high winds as high winds tend to impact the vertical mixing of the lake. Sampling in moderate to heavy rains should also be avoided.

Reconnaissance

Prior to assessment data collection, desktop and field reconnaissance are highly recommended to characterize the waterbody and begin to identify potential sources and causes of impairment. This initial data collection also greatly assists with informing sample site placement and generally increases the efficiency and accuracy of assessment work. Reconnaissance will include the following components:

1. Desktop Data:
    a. Delineation of the targeted waterbody and land cover data
        i. National Land Cover Dataset (NLCD) watershed land cover
        ii. Urban areas GIS layers (e.g., gNet: GDC.URBAN_AREAS)
        iii. MS4 areas GIS layers (e.g., gNet: GDC.MS4)
        iv. USGS StreamStats (https://streamstats.usgs.gov/ss/)
        v. Model My Watershed (https://modelmywatershed.org/analyze)
        vi. Historical Aerial Viewer (https://datacommons.maps.arcgis.com/apps/View/index.html?appid=10af5f75f9f94f01866359ba398cb6a9)
        vii. Historical Mill Dam GIS layers (e.g., millDams_FM, available in ArcOnline)
    b. Previous assessment efforts
    c. Existing data and existing sampled locations (e.g., Water Quality Index Tool, https://fweco.shinyapps.io/padep_wqi_UI/)
    d. Identification of potential sources and causes of impairment
        i. Water pollution control facility GIS layers (e.g., gNet: GDC.EF_ WPCF_V)

   ii. Combined sewer overflow outfalls GIS layers (e.g., https://edg.epa.gov/data/PUBLIC/R3/Combined%20Sewer%20Outfalls_042412.zip)
   iii. Electronic Discharge Monitoring Report (eDMR) data
   iv. Freshwater Explorer (Cormier et al. 2021, https://arcg.is/KHb9S)
   v. Abandoned mine land GIS layer (e.g., gNet: GDC.MINE_AML_FPOLY_V)
   vi. Active mining GIS Layers (e.g., gNet: GDC.MINE_PERMIT_UG_V, BITUMINOUS_SURFACE_MINE_PERMITS, ANTHRACITE_SURFACE_MINE_PERMITS)
   vii. Oil and Gas Storage Fields GIS layers (gNet: GDC.OG_ACTIVE_STORAGE_FIELDS)
   viii. Oil and gas GIS layers (e.g., gNet: GDC.OG_WELL_PADS)
  e. Identification of PWS Sources (e.g. gnet: GDC.EF_PWS_V)
  f. Hydraulic Residence Time
  g. Lake management plans and contour maps (if available)
  h. Review of herbicide or pesticide application permits
2. Field Data:
  a. Visual observations and photographs of physical habitat and land disturbance
  b. Field meter data, including vertical profile measurements and/or cross-section surveys, if applicable
  c. Discrete water chemistry data

Once reconnaissance data are collected, sampling plans should be updated to ensure that all relevant protocols, methods, quality assurance, etc. are appropriate to satisfy the information described above and in the objective specific sections below. Data collection recommendations can be different based on the use being assessed. Data collection planning should assume that the assessed use is impaired (Shull and Pulket 2023b). Consequently, DEP will incorporate source and cause determination data collection recommendations with all initial data collection efforts. For source and cause determination guidance see Assessment Book, *Chapter 9.1 General Source and Cause Method* (Shull and Shank 2023). Similarly, if reconnaissance efforts suggest a waterbody may now be supporting a particular use due to restoration, management activities, watershed improvements, or changes to permitted activities, DEP will incorporate delisting considerations as part of the sampling plan; see Assessment Book, *Chapter 9.4, Cause Removal Method* (Shull and Pulket 2023a) for guidance. Additionally, some data are more useful than others for certain use assessments. For example, weather data can be particularly useful for plankton, cyanobacteria, and bacteria data collections. Specific data collection recommendations are provided in the respective Chapters of this book.

Hydraulic Residence Time

Calculating hydraulic residence time is required when determining trophic status classification for an ALU assessment but can also be utility when implementing other protected use assessments. Hydraulic residence time is determined at average annual daily stream flow and normal pool volume; in the absence of actual records, an average annual daily discharge rate of 1.5 CFS [cubic feet per second] per square mile shall be used (Equation 1). Historic records, dam records, and bathymetry maps can also be consulted. Lakes can either be natural or man-made and can synonymously be referred to as reservoirs, ponds, and impoundments, depending on design, purpose, and size.

Equation 1: Hydraulic residence time calculation

Residence Time $= \frac{V}{(0.969 * DA)}$; where

$V = \frac{avgD * A}{(3.07)}$; where

avgD = Average depth of the lake in feet

A = Area of the lake in acres

DA = Drainage area in square miles

0.969 = Is used to estimate the average stream flow per day for the basin. 1.5 cubic feet second (CFS) will be used where the average stream flow is unknown. The conversion to understand how many million gallons per day moves through the basin is determined through a series of multiplications to convert seconds to days and then cubic feet to million gallons. Spelled out this is 1.5 CFS * 3600 secs/hour * 24 hours/day * 1728 cubic inches/cubic feet * (1/231 gallons per cubic inches). If average stream flow is measured CFS*969,475 will be used and the resulting number will replace 0.969 in Equation 1.

Aquatic Life Assessments

Lake Trophic State

DEP's data collection protocols and ALU assessment methods for lakes have generally been developed to meet the needs of the Carlson's trophic State Index (TSI) (Carlson 1977). Carlson's TSI includes four sub-indices (Chlorophyll-a, Total Phosphorus, Secchi, and Nitrogen).

TSI data collection requires at least three, seasonal data collection efforts including a spring, a summer, and a fall effort. The TSI is designed to characterize lake productivity or eutrophication caused by environmental factors such as amount of daylight and temperature, so monitoring during each changing season is necessary. In polymictic lakes, seasons are driven solely by daylight and temperature. In dimictic lakes, seasons are more clearly indicated by monitoring stratification events (i.e. spring turnover, summer stratification and fall turnover) as is driven by wind and lake morphology. To determine sampling schedules to include the three seasons, historic temperature records, geographic location, and elevation of the lakes will be consulted. Spring sampling occurs immediately after ice off, which is between February and early May; in dimictic lakes this sampling event would represent pre-turnover conditions prior to the onset of primary production. Summer sampling generally occurs between June and early September; in dimictic lakes this sampling event would represent stratified conditions and peak primary productivity. Fall sampling generally occurs between October and December; in dimitic lakes this sampling event would represent turnover conditions and the tail end of primary production where primary production begins to cease due to limiting environmental conditions.

TSI data collection will employ a targeted sampling design and typically consist of two sampling stations, however smaller lakes may only present the opportunity for a single station while larger, more complex lakes may require more than two stations to accurately characterize variable water quality conditions that might exist. Sampling stations are positioned to account for variability in water

quality as driven by the lake's geomorphology, contour, and depth. Station 1 will be positioned at the deepest point of the lake, usually near a dam or outflow. Station 2 is located at a position generally described as 2/3 uplake and is located at a shallower depth at midchannel and/or midlake. In lakes that are small, with minimal tributary or source influences, and with uniform geomorphology, one lake station to represent the deepest point of the lake is acceptable. In instances where geomorphology creates opportunity for unique water quality conditions such as within coves or forks, additional stations may be needed.

Lake stations are located using information gathered during a desktop reconnaissance but will also require depth measurements, field meter data, and GPS data to appropriately position stations. A TSI survey typically begins by consulting reconnaissance information and GPS data to position watercraft in the general vicinity of the station to be sampled. Discrete depth measurements are collected to confirm the depth, record maximum station depth and reposition watercraft if necessary.

Once the station(s) to be sampled has been confirmed the following steps are implemented. *Lake Assessment and TSI Field Data Sheet* (Appendix A-11) may also assist in the sequence of data collection.

1. (Recommended) Complete header information on *Lake Assessment and TSI Field Data Sheet* (Appendix A-11).
2. (Recommended) A vertical profile survey is conducted with a calibrated field meter according to *In-situ Field Meter, Transect and Vertical Profile Data Collection Protocol* (Hoger and Arnold 2023b). The results of the vertical profile will inform the depths at which additional chemical and biological data collection listed below will occur.
3. (Recommended) Secchi depth measurements are made according to *Secchi Depth Data Collection Protocol* (Arnold 2023g). Secchi depth data are used for TSI calculations but may also be used to perform EU assessments.
4. (Recommended) Discrete water chemistry data and chlorophyll-a data are collected at specific depths according to *Discrete Water Chemistry Data Collection Protocol* (Shull and Arnold 2023) and *Chlorophyll-a, Phycocyanin and Ash Free Dry Mass Data Collection Protocol* (Arnold and Butt 2023a). The epilimnion or top sample aliquot should be collected from one meter below the surface first. Chlorophyll-a samples should be processed prior to proceeding with water chemistry sample processing to reduce the degradation of chlorophyll-a samples due to solar radiation. The hypolimnion or bottom sample should be collected last at each station. Optional data collection for other use assessments below may be conducted during the TSI discrete water chemistry data collection. These include:
    a. Bacteriological data (can be incorporated into a WC assessment further described below, collected from the epilimnion or top sample aliquot),
    b. Potable Water Supply data (can be incorporated into a PWS assessment further described below, collected from the epilimnion or top sample aliquot but may require additional data from additional locations or at an increased frequency),

   c. Harmful Algal Bloom and Cyanotoxin Data (informational only, can be collected from anywhere on the lake while conducting a lake survey. Follow sampling recommendations found in *Chapter 3.13, Harmful Algal Bloom (HAB) and Cyanotoxin Data Collection Protocol* (Arnold and Butt 2023b)),
5. (Optional) Continuous Instream Monitoring (CIM) data collected according to *Continuous Physicochemical Data Collection Protocol* (Hoger and Arnold 2023a) is an optional data collection effort that can be utilized to assess parameters recorded by CIM deployments (pH and DO) that have defined water quality standards in 25 Pa. Code Chapter 93. Station location and deployment depth, time, and duration would consider the information collected during reconnaissance and/or during a TSI survey. For more information regarding the use of CIM data to make assessment decisions, see Assessment Book, *Chapter 2.7, Continuous Physicochemical Assessment Method* (Hoger 2018).
6. (Optional) While plankton data are not a TSI parameter, plankton data collection is often performed with the TSI survey at stations and would be considered as part of the *Lake Biological Assessment Method* (Arnold 2023b). The depth of tow is informed by vertical profile data. Plankton data are collected according to *Plankton Data Collection Protocol* (Arnold 2023e).

For more information regarding the use of TSI data to make ALU assessment decisions, see Assessment Book, *Chapter 2.8, Lake Trophic State Assessment Method* (Arnold 2023d). In addition, discrete and continuous physicochemical data can also be used to make ALU assessment decisions, see the Assessment Book, *Chapter 2.7, Continuous Physicochemical Assessment Method* (Hoger 2018) and *Chapter 2.9, Lake Physicochemical Assessment Method* (Arnold 2023c).

Lake Biology

Plankton

Plankton data collection, performed according to *Plankton Data Collection Protocol* (Arnold 2023d), is optional at TSI stations once all other data collection has been complete and during each seasonal TSI data collection effort. TSI data collection including vertical profile data will determine the depth of tow. Plankton community composition and seasonal changes in the plankton community can aid in identifying sources and causes of impairment and can be used to interpret the results of other data collected. For example, a shift in the plankton community during specific seasons can help differentiate perceived affects associated with seasonal turnover verses those that may originate from tributaries, nonpoint sources, or other regulated activities. Additional data collections like sampling cyanotoxins and for harmful algal blooms can also be considered. For more information regarding the use of plankton data to make assessment decisions, see Assessment Book, *Chapter 2.10, Lake Biological Assessment Method* (Arnold 2023b).

Aquatic Macrophytes

Aquatic macrophyte data collection performed according to *Aquatic Macrophyte Data Collection Protocol* (Arnold 2023a) is an optional data collection effort that can be used to supplement or independently used to make ALU and BU assessment decisions.

Aquatic macrophyte data collection occurs during the height of the growing season, typically July, August and early September. An exception to this standard would be determining impacts of cool-water species such as *Potamogeton crispis* (a non-native, sometimes invasive aquatic plant) or *Hydrilla verticillata*, which can still be vigorous well into October. The *P. crispis* survey should be completed in spring because cool season species grow best in cool temperatures and senesce in warm weather, typically by July.

Aquatic macrophyte surveys use a combination of boat mounted sonar mapping and rake sampling that generally target the waterbody as a whole and can be very resource and time intensive. It is important to define the specific objectives associated with each survey and consider waterbody characteristics including waterbody size, shape, the size and shape of potentially vegetated areas, etc. when planning. For more information regarding the use of aquatic macrophyte data to make ALU assessment decisions, see Assessment Book, *Chapter 2.10, Lake Biological Assessment Method* (Arnold 2023b). See the 'Boating Assessment' section below for more information regarding BU assessments.

Special Protection Assessments

In addition to the recommendations described in the ALU sampling design section above, sampling should specifically consider the timing, location, and representativeness of existing data that likely established baseline conditions. This consideration ensures that the antidegradation component of Special Protection surface waters can be assessed. For more information regarding SPU assessment decisions, see Assessment Book, *Chapter 3.1, Special Protection Assessment Methods* (Shull and Whiteash 2023).

Potable Water Supply Assessments

DEP will conduct PWS assessments on lakes that have potable water supply withdrawals directly from the lake but may also conduct PWS assessments on lakes without potable water supply withdrawals with available PWS-related data. PWS data can be collected during seasonal TSI surveys at TSI stations when discrete water chemistry is data are collected according to *Discrete Water Chemistry Data Collection Protocol* (Shull and Arnold 2023).

To document impacts associated with the stratification process, DEP recommends additional samples be collected June through October in highly stratified lakes, where anoxic conditions may exist, to account for variability driven by wind and atmospheric temperature.

Before collecting data to assess the Potable Water Supply (PWS) Use, collectors should consider that while 25 Pa. Code § 93.4 identifies the PWS Use as applying to all surfaces waters, with the exception for which § 96.3(d) states,

> *(d) As an exception to subsection (c), the water quality criteria for total dissolved solids, nitrite-nitrate nitrogen, phenolics, chloride, sulfate and fluoride established for the protection of*

> *potable water supply* [PWS] *shall be met at least 99% of the time at the point of all existing or planned surface potable water supply withdrawals unless otherwise specified in this title*

For these parameters that are applied at the point of withdrawal, an attempt should be made to collect the samples from as near to the location and depth of the water intake as possible. If multiple withdrawals exist, all will be sampled. If the location of the water intake or water withdraw is unknown, a sample will be collected from the deepest part of lake from a 1-meter depth location; a bottom sample (1-meter from the bottom) is also recommended. If a lake does not have a PWS on it, and the biologist would like to make an assessment, the biologist will use statewide water quality criteria and apply those to the lake. In the instance of not having a PWS on the lake, the TSI assessment sampling design shall be used.

The following is an outline of data collection protocols and assessment methods that could be considered in developing an PWS assessment sampling plan:

1. (Recommended) *In-situ Field Meter, Transect and Vertical Profile Data Collection Protocol* (Hoger and Arnold 2023b).
2. (Recommended) *Discrete Water Chemistry Data Collection Protocol* (Shull and Arnold 2023).
3. (Recommended) Assessment Book, *Chapter 2.6, Discrete Physicochemical Assessment Method* (Biggs and Whiteash 2021).
4. (Recommended) Assessment Book, *Physicochemical Potable Water Supply Assessment Method* (Whiteash and Shull 2023).

Water Contact Sports Assessments

Bacteria

Bacteriological data collection, performed according to *Discrete Water Chemistry Data Collection Protocol* (Shull and Arnold 2023) and *Bacteriological Data Collection Protocol* (Miller 2023) to make Water Contact Sports (WC) assessments can be implemented at TSI stations during TSI epilimnion or top data collection but may require additional data collected at increased frequencies and additional locations to provide sufficient data that meets the needs of Assessment Book, *Chapter 5.1, Bacteriological Assessment Method* (Miller and Whiteash 2021). Additionally, during the swimming season, the Pennsylvania Department of Health (DOH) and Pennsylvania Department of Conservation and Natural Resources (DCNR) collect weekly samples for *E. coli* at public beaches for monitoring purposes (28 Pa. Code Chapter 18, §18.30). Closure notices when violations of criteria occur are also issued by DOH. In cooperation with DEP, the DOH and DCNR provide a list of closures that DEP will utilize to prioritize bacteriological monitoring and assessment.

Harmful Algal Bloom and Cyanotoxins

Currently there is no assessment method for cyanotoxins or harmful algal bloom data; however, these data can be collected as supporting information as DEP builds a dataset for assessment method development. Data collections for these biological data can be implemented at TSI stations during TSI epilimnion or top data collection. Additionally, beaches and boat launches can also be sampled (Arnold and Butt 2023b).

Boating Assessments

Aquatic macrophyte data collection performed according to *Aquatic Macrophyte Data Collection Protocol* (Arnold 2023a) is an optional data collection effort that can be used to supplement or independently used to make ALU and BU assessment decisions. For more information regarding the use of aquatic macrophyte data to make BU assessment decisions, see Assessment Book, *Chapter 6.1, Lake Biological Assessment Method* (Arnold 2023b).

Esthetic Assessments

Secchi depth measurements performed according to *Secchi Depth Data Collection Protocol* (Arnold 2023e) is recommended as part of the TSI data collection effort (see *Lake Trophic Status Assessment above*) which can also be utilized to make Esthetic assessment decisions. Sampling design and planning for Secchi depth measurements should be incorporated into and otherwise be consistent with TSI data collection. For more information regarding the use of aquatic macrophyte data to make ALU assessment decisions, see Assessment Book, *Chapter 7.1, Secchi Depth Assessment Method* (Arnold 2023f). Auxiliary to Secchi depth data, Total Dissolved Solids (TDS), Total Suspended Solids (TSS), and turbidity measurements will also be considered. To collect these parameters, *Chapter 2.4, Discrete Water Chemistry Data Collection Protocol* (Shull and Arnold 2023) will be implemented.

Fishing Assessments

Implementing fish assessments on lakes incorporates the use of the *Fish Tissue Data Collection Protocol* (Wertz 2021b). For more information regarding the use of fish tissue data to make FU assessment decisions, see Assessment Book, *Chapter 8.1, Fish Tissue Consumption Assessment Method* (Wertz 2021a).

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2.5 STREAM AND RIVER ASSESSMENT SAMPLING DESIGN

Chapter 2 Sampling Design and Planning

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INTRODUCTION

The purpose of this protocol is to outline the sampling design and planning associated with data collection to complete protected use assessment objectives for streams and rivers. Specific objectives include Aquatic Life uses (ALU), Special Protection uses (SPU), Potable Water Supply (PWS), Water Contact Sports (WC), and Fishing (FU) assessments. ALU assessment efforts have historically relied on benthic macroinvertebrate and stream habitat data collection protocols and associated assessment methods. Over the years, additional data collection protocols have been implemented at a greater frequency to produce more refined assessment decisions. More recently, updated biological data collection protocols and accompanying assessment methods, along with protocols and methods specific to SPU, PWS, WC, and FU have been developed to provide an increased ability to make and refine assessment decisions. The information that follows can generally be applied to each of the assessment objectives. Additional, use specific information is also provided where appropriate.

SAMPLING DESIGN

All assessment sampling designs for flowing surface waters will conform to the following general rules:

1. Account for major influences (e.g. potential sources) – Sampling sites are positioned to account for changes in water quality due to influences such as major tributaries, point and nonpoint source impacts, land cover changes, soil characteristics, and geology. Samples are collected at the limits of these changes to effectively “bracket” potential sources of water quality differences.
2. Ensure data are collected in accordance with assessment method scope – It is good practice to begin initial sample placement at 3rd order or higher waterbodies, because most available assessment methods cover these waterbodies. Depending on major influences it may also be necessary to collect samples on 1st and 2nd order tributaries. An example would be differences in geology that could result in acidified reaches. However, ephemeral or intermittent streams should be avoided because most assessment methods are currently not developed to account for these streams. For the same reason, sample collection should also be avoided in losing/sinking reaches.
3. Adhere to the minimum assessment unit length – The minimum length of any use assessment unit is ½ mile. Any impact delineated that is less than ½ mile is considered a localized impact, which is potentially a compliance issue, not a use impairment.
4. Quality Assurance **–** Data collected will be conducted according to *Quality Assurance Project Plan (QAPP): Data Collection for the Purpose of Existing Use Evaluations, Protected Use Assessments, and Evaluations of Point and Nonpoint Source Impacts to Surface Waters* (Lookenbill and Arnold 2023).
5. Biosecurity and Safety **–** Activities will adhere to *Biosecurity and Safety Procedures* (Arnold et al. 2023).

In addition to general assessment sampling design rules, sampling site placement will be dictated by several of the following objective-specific factors:

1. Targeted Versus Probabilistic Sampling – For assessment determination purposes, DEP utilizes both targeted and probabilistic sampling designs. DEP believes the targeted "judgment-based" sampling design is the most suited design to assess Water Quality Standards (WQS) and uses. Targeted sampling not only focuses in on sources and causes of potential impairment, it also delimits the spatial extent of the impact. This translates into more accurate and precise assessments. In addition, properly implemented targeted sampling provides information that is necessary if a Total Maximum Daily Load (TMDL) is developed. Probabilistic sampling designs can also be useful for assessment determination purposes, especially when waterbodies lack significant environmental stress or are rather homogeneous in land cover. See the Types of Sampling Design section in Chapter 2 of this book for additional details regarding targeted and probabilistic sampling designs.
2. Waterbody Representativeness – Sample placement will be different depending on the waterbody being assessed. For wadeable streams without any nearby upstream tributaries or influences, samples collected mid-stream/mid-depth are generally considered representative of the assessed reach. For larger rivers and wadeable streams with nearby upstream tributaries or influences, multiple sampling locations across a transect and along the shore (cross-section surveys) are recommended to accurately delineate an assessment. Cross-section surveys and multiple sampling locations across a transect are highly recommended to be established on rivers and streams greater than 1000 mi^2. Cross-section surveys performed using a clean and calibrated field meter that collects water temperature, specific conductance, dissolved oxygen, pH, and – preferably – turbidity are required to determine if major water quality influences exist at the sampling location described in *Chapter 4.1, In-Situ Field Meter, Transect, and Profile Data Collection Protoco*l (Hoger and Arnold 2023b).

It is also important that data are collected frequently enough to minimize sampling error and accurately characterize the conditions for each parameter of concern. Conditions and representativeness should be characterized over a long enough time frame to account for variations in concentrations attributable to changes to the waterbody. For instance, the following interrelated factors can contribute to spatial and temporal variation: precipitation, stream flow, geology, watershed drainage patterns, and anthropogenic influences. Knowledge and understanding of such patterns can strengthen inferences about unobserved conditions (USEPA 2005). DEP generally considers discrete samples to be representative of one day unless convincing evidence exists to suggest otherwise (e.g., a documented spill, influence of a known biological process, supporting high-frequency monitoring data). Similarly, USEPA recommendations allow for a single sample to represent periods longer than one day when conditions are stable (USEPA 1997).

Furthermore, sampling frequency is also considered when evaluating use criteria. It is most effective to focus monitoring efforts when digressions of water quality criteria are most likely to occur, hereafter

referred to as critical sampling periods. Collecting samples during critical sampling periods will require an understanding of the variables at play. Some of the variables that should be considered are discussed further in Whiteash (2021). As a result of these factors and federal requirements, DEP recommends multiple sampling events for assessing any criterion but will evaluate all existing and readily available data when making assessments. More specifically, DEP encourages at least three sampling events within the criterion duration period. For example, the iron criterion for ALU is written as a 30-day average, so DEP encourages at least three samples be collected within a 30-day period to compare conditions to the criterion.

Reconnaissance

Prior to assessment data collection, desktop and field reconnaissance are highly recommended to characterize the waterbody and begin to identify potential sources and causes of impairment. This initial data collection also greatly assists with informing sample site placement and generally increases the efficiency and accuracy of assessment work. Reconnaissance will include the following components:

1. Desktop Data:
    a. Delineation of the targeted waterbody and land cover data
        i. National Land Cover Dataset (NLCD) watershed land cover
        ii. Urban areas GIS layers (e.g., gNet: GDC.URBAN_AREAS)
        iii. MS4 areas GIS layers (e.g., gNet: GDC.MS4)
        iv. USGS StreamStats (https://streamstats.usgs.gov/ss/)
        v. Model My Watershed (https://modelmywatershed.org/analyze)
        vi. Historical Aerial Viewer (https://datacommons.maps.arcgis.com/apps/View/index.html?appid=10af5f75f9f94f01866359ba398cb6a9)
        vii. Historical Mill Dam GIS layers (e.g., millDams_FM, available in ArcOnline)
    b. Previous assessment efforts
    c. Existing data and existing sampled locations (e.g., Water Quality Index Tool, https://fweco.shinyapps.io/padep_wqi_UI/)
    d. Identification of potential sources and causes of impairment
        i. Water pollution control facility GIS layers (e.g., gNet: GDC.EF_ WPCF_V)
        ii. Combined sewer overflow outfalls GIS layers (e.g., https://edg.epa.gov/data/PUBLIC/R3/Combined%20Sewer%20Outfalls_042412.zip)
        iii. Electronic Discharge Monitoring Report (eDMR) data
        iv. Freshwater Explorer (Cormier et al. 2021, https://arcg.is/KHb9S)
        v. Abandoned mine land GIS layer (e.g., gNet: GDC.MINE_AML_FPOLY_V)
        vi. Active mining GIS Layers (e.g., gNet: GDC.MINE_PERMIT_UG_V, BITUMINOUS_SURFACE_MINE_PERMITS, ANTHRACITE_SURFACE_MINE_PERMITS)

vii. Oil and Gas Storage Fields GIS layers (gNet: GDC.OG_ACTIVE_STORAGE_FIELDS)
viii. Oil and gas GIS layers (e.g., gNet: GDC.OG_WELL_PADS)

e. Identification of PWS Sources (e.g. gnet: GDC.EF_PWS_V)

2. Field Data:
   a. Visual observations and photographs of physical habitat and land disturbance
   b. Field meter data, including vertical profile measurements and/or cross-section surveys, if applicable
   c. Discrete water chemistry data, biological data, and other available data

Once reconnaissance data are collected, sampling plans should be updated to ensure that all relevant protocols, methods, quality assurance, etc. are appropriate to satisfy the information described above and in the objective specific sections below. Data collection recommendations can be different based on the use being assessed. Data collection planning should assume that the assessed use is impaired (Shull and Pulket 2023b). Consequently, DEP will incorporate source and cause determination data collection recommendations with all initial data collection efforts. For source and cause determination guidance see Assessment Book, Chapter 9.1 *General Source and Cause Method* (Shull and Shank 2023). Similarly, if reconnaissance efforts suggest a waterbody may now be supporting a particular use due to restoration, management activities, watershed improvements, or changes to permitted activities, DEP will incorporate delisting considerations as part of the sampling plan; see Assessment Book, *Chapter 9.4, Cause Removal Method* (Shull and Pulket 2023a) for guidance. Additionally, some data are more useful than others for certain use assessments. For example, weather data can be particularly useful for bacteria data collection to understand how precipitation may have influenced results.

Aquatic Life Assessments

While DEP's ALU assessments for streams and rivers have historically relied on and continue to include benthic macroinvertebrate and stream habitat data collection protocols and associated assessment methods, new and updated protocols and assessment methods, that are independently applicable, create additional considerations when designing and planning an ALU assessment survey. This is particularly important considering that most ALU assessments will be updates to existing decisions, which require similar or more robust data collection effort to address or refine existing causes. For example, where Eutrophication is a suspected cause, in addition to the recommended protocols and methods outlined below, the following optional items would be considered:

1. *Continuous Physicochemical Data Collection Protocol* (Hoger and Arnold 2023),
2. Assessment Book, *Chapter 2.7, Continuous Physicochemical Assessment Method* (Hoger 2018).
3. Assessment Book, *Chapter 9.2, Eutrophication Cause Method* (McGarrell and Gocek 2023).

In another example, if Thermal Modification is a suspected cause, in addition to the recommended protocols and methods outlined below, the following optional items would be considered:

1. (Optional) *Fish Data Collection Protocol* (Wertz and Arnold).
2. (Optional) *Stream Fish Assemblage Assessment Method* (Wertz 2021).

The following is an outline of data collection protocols and assessment methods that should be considered in developing an ALU assessment sampling plan:

1. (Recommended) Field meter data are collected with a calibrated field meter according to *In-situ Field Meter, Transect and Vertical Profile Data Collection Protocol* (Hoger and Arnold 2023b). If non-mixed water quality influences are suspected, the results of the cross-section survey will determine if additional chemical and biological data collection, listed below, will occur. For more information regarding the use of discrete data to make assessment decisions, see Assessment Book, *Chapter 2.6, Discrete Physicochemical Assessment Method* (Biggs and Whiteash 2021)
2. (Recommended) Discrete water chemistry data are collected according to *Discrete Water Chemistry Data Collection Protocol* (Shull and Arnold 2023). For more information regarding the use of discrete data to make assessment decisions, see Assessment Book, *Chapter 2.6, Discrete Physicochemical Assessment Method* (Biggs and Whiteash 2021). Optional data collection for other use assessments below may be conducted during the discrete water chemistry data collection. These include:
    a. Bacteriological data (can be incorporated into a WC assessment but may require additional data from additional locations or at an increased frequency).
    b. Potable Water Supply data (can be incorporated into a PWS assessment but may require additional data from additional locations or at an increased frequency).
3. (Recommended) Biological data are collected according to the natural classification of the stream or river being assessed. The following is an outline of data collection protocols and assessment methods that should be considered:
    a. (Recommended) Macroinvertebrates
        i. *Wadeable Freestone Riffle-Run Stream Macroinvertebrate Data Collection Protocol* (Shull 2017) and *Assessment Method* (Shull 2023). In certain streams, the *Wadeable Freestone Acidification Assessment Method* (Shank 2023) is also recommended.
        ii. *Wadeable Limestone Stream Macroinvertebrate Data Collection Protocol* (Williams 2017) and *Assessment Method* (Williams 2017).
        iii. *Wadeable Multihabitat Stream Macroinvertebrate Data Collection Protocol* (Pulket 2017) and *Assessment Method* (Pulket 2017).
        iv. *Semiwadeable Large River Macroinvertebrate Data Collection Protocol* (Shull 2017) and *Assessment Method* (Shull 2018).
    b. (Optional) *Fish Data Collection Protocol* (Wertz and Arnold 2023) and the *Stream Fish Assemblage Assessment Method* (Wertz 2021).

4. (Recommended) Physical habitat data are collected according to the *Stream Habitat Data Collection Protocol* (Lookenbill 2017) and *Physical Habitat Assessment Method* (Walters 2017).
5. (Optional) Continuous Instream Monitoring (CIM) data collected according to the *Continuous Physicochemical Data Collection Protocol* (Hoger and Arnold 2023a). This is a collection effort that can be utilized to assess parameters recorded by CIM deployments (pH and DO) that have defined water quality standards in 25 Pa. Code Chapter 93. Station location and deployment depth, time and duration would consider the information collected during reconnaissance. For more information regarding the use of CIM data to make assessment decisions, see Assessment Book, *Chapter 2.7, Continuous Physicochemical Assessment Method* (Hoger 2018).

Special Protection Assessments

In addition to the recommendations described in the ALU sampling design section above, sampling should specifically consider the timing, location, and representativeness of existing data that likely established baseline conditions. This consideration ensures that the antidegradation component of Special Protection surface waters can be assessed. For more information regarding SPU assessment decisions, see Assessment Book, *Chapter 3.1, Special Protection Assessment Methods* (Shull and Whiteash 2023).

Potable Water Supply Assessments

DEP will conduct PWS assessments on surface waters that have potable water supply withdrawals directly from the surface water but may also conduct PWS assessments on waterbodies without potable water supply withdrawals with available PWS-related data.

Before collecting data to assess PWS, collectors should consider that while 25 Pa. Code § 93.4 identifies the PWS Use as applying to all surfaces waters, there is an exception where § 96.3(d) states:

> *(d) As an exception to subsection (c), the water quality criteria for total dissolved solids, nitrite-nitrate nitrogen, phenolics, chloride, sulfate and fluoride established for the protection of potable water supply* [PWS] *shall be met at least 99% of the time at the point of all existing or planned surface potable water supply withdrawals unless otherwise specified in this title*

For parameters mentioned in § 96.3(d), PWS can be assessed by collecting samples from facilities' raw water spigot. If that collection point is not available, it is recommended that samples be collected from the source waterbody as close to the intake as possible. Samples from the source waterbody should be collected upstream of the intake, no more than two miles upstream of any major influence (e.g., a tributary, land cover change), and need not be collected from downstream of the PWS withdrawal. For parameters not mentioned in § 96.3(d), PWS can be assessed by collecting samples consistent with ALU assessment sampling design described above.

The following is an outline of data collection protocols and assessment methods that should be considered when developing an PWS assessment sampling plan:

1. (Recommended) Field meter data are collected with a calibrated field meter according to *In-situ Field Meter, Transect and Vertical Profile Data Collection Protocol* (Hoger and Arnold 2023b). If non-mixed water quality influences are suspected, the results of the cross-section survey will determine if additional chemical data collection, listed below, will occur.
2. (Recommended) Discrete water chemistry data are collected according to *Discrete Water Chemistry Data Collection Protocol* (Shull and Arnold 2023). For more information regarding the use of discrete data to make assessment decisions, see Assessment Book, *Chapter 2.6, Discrete Physicochemical Assessment Method* (Whiteash and Shull 2023) and *Chapter 4.1*, *Discrete Physicochemical Potable Water Supply Assessment Method* (Whiteash and Shull 2023).

Water Contact Sports Assessments

Watersheds that have not been assessed and waters that are heavily used for recreational activities by the public or those that support public beaches are prioritized for monitoring. In addition to the recommendation in the sampling design section above, the number and distribution of docks, boating areas, residences, cabins, and access areas should be considered in locating sample sites.

Most sampling for WC assessments is conducted during the swimming season (May 1st through September 30th). This is the timeframe that the public is likely to be engaging in primary WC activities. WC activities are defined as activities where immersion and ingestion are likely and there is a high degree of bodily contact with the water, such as swimming, bathing, surfing, water skiing, tubing, skin diving, snorkeling, water play by children, or similar water-contact activities (USEPA 2012). Bacteriological sampling should not take place during times when physical conditions render WC recreation hazardous to the public. Consequently, samples collected for assessment purposes should not characterize acute or transitory situations that would be hazardous to the public, such as flooding. Additionally, it is critically important to understand the representativeness of samples collected over the course of 30 days. Bacteriological samples should be collected under flow conditions that generally represent the flow conditions experienced during recreation. For example, it would not be representative to collect all five bacteriological samples under conditions representing flow conditions greater than median flow when the stream only spends about 30% of the time under those conditions.

During the swimming season §93.7 does not specify a minimum number of samples. Consequently, sampling during the swimming season should include samples collected frequently enough and over a sufficient period to account for sampling error or variations driven by confounding variables (Biggs and Whiteash 2021). DEP has determined that 5 bacteriological samples collected within a 30-day period is typically sufficient to understand the variability in the data and target conditions that are likely to characterize impairment. This results in sampling error decreases, more confident assessment, and is consistent with USEPA's Recreational Water Quality Criteria Recommendations (USEPA 2012). For the remainder of the year, §93.7 states that the maximum fecal coliform level shall be a geometric mean of 2,000 CFU per 100 ml based on a minimum of five consecutive

samples collected on different days during a 30-day period. This minimum sampling requirement must be considered if WC assessment will be conducted outside of the swimming season.

To conduct advanced source determination and distinguish between potential fecal contamination sources, DEP collects samples to analyze for the presence of host-specific intestinal bacterial DNA from *Bacteroides fragilis* (*B. fragilis*). The DEP *Bacteriological Source Method*, defines magnitude, frequency, and duration during the swimming season and remainder of the year as at least 4 to 6 samples, each separated by at least 24 hours, spanning a maximum of 30 days and a minimum of 14 days (Miller et al. 2023). Four to six qPCR samples should be collected when preliminary land cover analysis reveals high agricultural (>30%) or developed (>15%) coverages. Sampling for qPCR is also recommended if the initial collections of the fecal indicator begin to approach criteria (e.g., qPCR samples are recommended when initial *E. coli* samples are 100 colony forming units per 100 mL (CFU/100 mL) or greater; approaching the criteria of 126 CFU/100mL in 25 Pa. Code § 93.7).

The following is an outline of data collection protocols and assessment methods that should be considered in developing an WC assessment sampling plan:

1. (Recommended) *Bacteriological Data Collection Protocol* (Miller 2023), *Assessment Method* (Miller 2021), and *Source Method* (Miller et al. 2023).
2. (Optional) Discrete physicochemical data collected according to *In-situ Field Meter, Transect and Vertical Profile Data Collection Protocol* (Hoger and Arnold 2023) can provide useful information related to water quality influences, flow conditions, and turbidity.
3. (Optional) Continuous Instream Monitoring (CIM) data collected according to the *Continuous Physicochemical Data Collection Protocol* (Hoger and Arnold 2023a). This is a collection effort that can be used to create models by comparing discrete bacteriological samples to recorded CIM data (Hoger 2018, Rasmussen et al. 2008, Stone and Graham 2014). Models may be used to collect WC data for assessment purposes, provided that CIM data collection protocols are followed (Hoger and Arnold 2023a).

Fishing Assessments

Fish tissue data collection is performed according to *Fish Tissue Data Collection Protocol* (Wertz 2021b). In addition to the recommendations described in the sampling design section above, sampling should consider impediments to fish passage. For example, a particular fish species may be found to be high in mercury just below a small impoundment; however, additional samples may be needed upstream of the impoundment to confirm that contamination extends upstream beyond that impoundment. For more information regarding the use of fish tissue data to make Fishing assessment decisions, see Assessment Book, *Chapter 8.1, Fish Tissue Consumption Assessment Method* (Wertz 2021a).

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CHAPTER 3 BIOLOGICAL DATA COLLECTION PROTOCOLS

3.1 WADEABLE RIFFLE-RUN STREAM MACROINVERTEBRATE DATA COLLECTION PROTOCOL

Chapter 3 Biological Data Collection Protocols

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INTRODUCTION

U.S. Environmental Protection Agency's (USEPA) *Rapid Bioassessment Protocols for Use in Streams and Wadeable Rivers* (Barbour et al.1999) describes two general approaches to collecting stream macroinvertebrate communities for water quality assessment purposes. These approaches are the "single, most productive habitat" approach, described here, and the "multihabitat" approach, later described in *Chapter 3.3, Wadeable Multihabitat Stream Macroinvertebrate Collection Protocol* (Pulket 2017). The single, most productive habitat approach is typically used to assess streams where cobble substrate and riffle/run habitat is predominant (generally referred to as high gradient freestone streams). This protocol identifies appropriate field collection procedures needed to evaluate macroinvertebrate communities in wadeable freestone streams where riffle-run habitat is sufficiently abundant and is a modification of the USEPA Rapid Bioassessment Protocols (Barbour et al. 1999). Samples can be collected year-round; however, certain periods of the year are required depending on the purpose of the study. Refer to *Chapter 2.1, Wadeable Freestone Riffle-Run Stream Macroinvertebrate Assessment Method* (Shull 2023) of the Assessment Book for more information on collection results and season.

DATA COLLECTION

A benthic macroinvertebrate sample collection begins with delineating a 100-meter reach along the stream where the best available representation of riffle-run habitat exists for the stream segment of interest. Within the reach, six one-minute (each kick must be at least 45 to 60 seconds in duration) kicks are conducted immediately upstream of a non-truncated D-framed net with 500 µm mesh (Figure 1). Truncated nets are shorter with less meshed surface area and are not recommended for this collection protocol.

Each kick should disturb approximately 1 m^2 (1 m x 1 m) immediately upstream of the net to an approximate depth of 10 cm (approximately 4 inches, as the substrate allows). During each kick, the net should be held stationary. Since the width of the kick area is wider than the net opening, net placement is critical to assure all kicked material flows toward the net. Avoiding areas with crosscurrents, the substrate material from within the 1 m2 area should be kicked toward the center of the square meter area. Kicks are completed in a downstream-to-upstream direction to avoid disturbing the upstream portions of the targeted reach. Collectors must ensure kicks are distributed throughout the 100-meter reach and are representative of the variety of riffle-run habitats present (e.g., slow-flowing, shallow riffles and fast-flowing, deeper riffles). It is important to note that riffle-run habitat differences will be respective of the stream segment being sampled. For example, a slow-flowing deep riffle will be different in a small stream than in a larger stream. Kicks must also be conducted throughout the width of the stream to include the left-descending, middle, and right-descending areas. The only exception to this is if a specific area of the stream is being targeted for a reason. If that is the case, then the collector must clearly document that the sample is not a complete composite and state the purpose for collection on the sample form.

Figure 1. A non-truncated D-framed net with 500 µm mesh.

When collecting same day replicate samples within the same reach, it is important not to disturb the same areas of the stream with both sets of kicks. It is also important to be cognizant of macroinvertebrate drift after kicking the first sample. For example, a kick during the replicate collection may be very close and immediately downstream of an original sample kick without being in the same location. Disturbance from the original kick just upstream of a replicate kick may have disturbed macroinvertebrates and skew the replicate sample. An example of good kick selection and incorrect kick selection is illustrated in Figure 2. It is recommended that a replicate sample be collected after every 20 samples for QA/QC purposes. Specific procedures on how to collect a QC sample is detailed in Appendix E, Section B5.

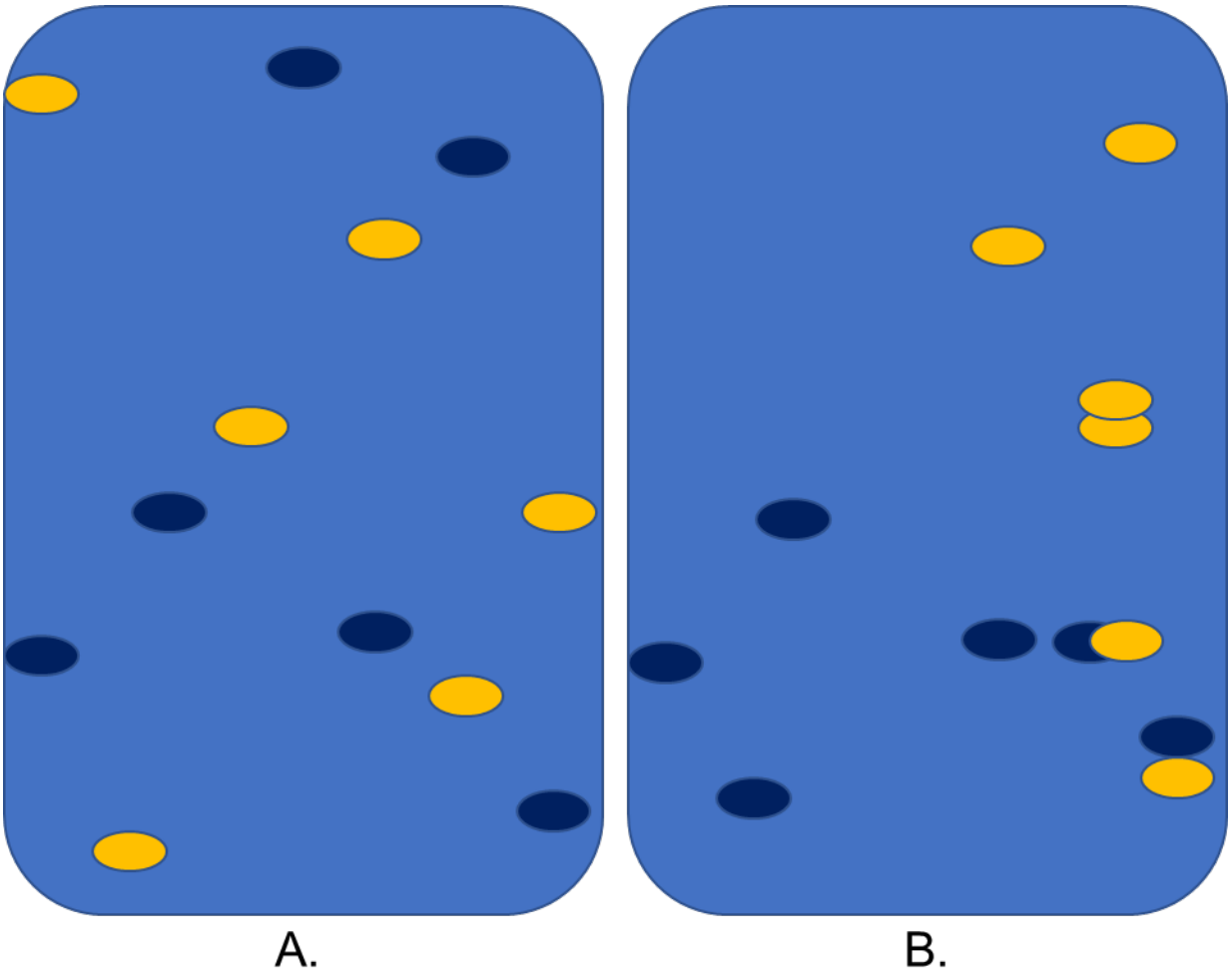

Figure 2. Blue rectangles illustrate a 100-meter reach within a stream, dark blue ellipses show the initial set of kicks during sample collection, and yellow ellipses show the replicate set of kicks during sample collection. (A) Correct sample collection with replicate collection; all kicks are collected within 100 meters and across the entire width of the stream, replicate kicks are not collected in the same location, and replicate kicks are not placed in close proximity, directly downstream of any other kicks. (B) Incorrect sample collection (with replicate); not all kicks are collected within 100 meters and across the width of the stream, replicate kicks are collected in the same location, and in close proximity, directly downstream of other kicks.

Once collected from the stream, each of the six kicks are composited into one sample jar (or as few samples jars as possible). Each jar is labeled with the date, time, collector, collectively referred to as a station ID (yyyymmdd-HHMM-Collector), and location. In addition, the sample procedure should be clearly indicated somewhere on the jar; for this protocol 6D-200 would be the appropriate label (6D stands for 6 kicks using a D-frame net; and 200 is the subsample target that is detailed in Chapter 3.6 (Lookenbill 2017)). If more than one jar is needed then each jar will also be labeled with a number (e.g., 1 of 2, 2 of 2). It is recommended that paper tags labeled using indelible ink or pencil be placed inside the sample jar(s) as well. Care must be taken to minimize damage to the collected organisms when compositing the materials. For this reason, only larger rocks, detritus, and other debris are rinsed and removed before the sample is preserved. Buckets and 500 µm mesh sieves are permitted

when compositing kicks; however, swirling the composited material in a bucket filled with water to reduce the amount of debris (elutriation) is not recommended as this action can unnecessarily damage fragile organisms, or allow for heavier organisms to be discarded unintentionally. Composited samples are preserved with at least 70% ethanol (95% ethanol is preferred) in the field. Collectors must ensure that the percentage of ethanol is high enough to properly preserve the sample and that there is ample space left in the sample jar to properly preserve organisms. See Appendix G for DEP's Ethanol Policy. Composited samples are then transported back to the laboratory for processing and all field information is recorded on a macroinvertebrate field data sheet (Appendix A.1) or mobile data collection application. Macroinvertebrate processing and identification is conducted using standard DEP protocols, which are also described in detail in *Chapter 3.6*, *Macroinvertebrate Laboratory Subsampling and Identification Protocol* (Brickner 2020).

ADDITIONAL DATA

During a DEP riffle-run macroinvertebrate survey, field meter (temperature, specific conductance, pH, dissolved oxygen, and turbidity if available), water chemistry, and habitat assessment data should also be collected. Recommended Standard Analysis Codes (SAC) for riffle-run macroinvertebrate collections are SAC 612, SAC 87, or SAC 18 (for special protection). For more information on water chemistry collection and SACs, refer to *Chapter 4.2, Discrete Water Chemistry Data Collection Protocol* (Shull and Arnold 2023). Because riffle-run surveys are designed for high gradient streams, the high gradient habitat assessment form should be used. The DEP riffle/run prevalent habitat form can be found in Appendix C.1.

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3.2 WADEABLE LIMESTONE STREAM MACROINVERTEBRATE DATA COLLECTION PROTOCOL

Chapter 3 Biological Data Collection Protocols

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INTRODUCTION

Limestone streams are unique aquatic systems that are typically very popular for recreational fishing. To properly protect the ecological integrity of limestone streams, they must be assessed correctly. This data collection protocol is intended to aid in assessing the aquatic life uses of Pennsylvania's wadable limestone streams. In the case of these limestone stream assessments, the following field procedures will apply.

A stream must meet certain criteria for this protocol to be applicable for assessments (Table 1). Limestone streams will often display low-gradient flow conditions and are almost completely void of clearly defined freestone habitat beds.

Table 1. Limestone streams definition criteria

Parameter	Criterion	Explanation
Alkalinity	Minimum 140 mg/l	Stream must maintain high alkalinity throughout the year.
Temperature	40 to 65°F, 4 to 18°C	Constant temperatures are very important. Check to see if stream is ice-free in the winter.
Known Influences	Stream originates from limestone springs or very strongly influenced by limestone springs.	N/A
Drainage Area	Maximum 20 sq. miles	There may be exception to this parameter if all other criteria are met.
Water Use	Definition of Cold Water Fishes (CWF)	Meets the definition of a CWF stream in Chapter 93.

SAMPLING CONSIDERATIONS

Net Mesh & Sieve

Many state water quality programs, federal agencies (e.g., USEPA, USGS), and other water quality monitoring organizations use net sampling devices with 500µm mesh nets. Due to the natural conditions of limestone streams, 500µm mesh size quickly clogs, preventing macroinvertebrates and vegetation from entering the net resulting in a poor sample. To ensure an accurate assessment, 800 - 900µm net mesh must be used to collect samples. A standard 500µm mesh sieve should be used to process samples.

Semi-Quantitative Procedure

The DEP Rapid Bioassessment Protocol (RBP) is a modification of the U.S. Environmental Protection Agency's (USEPA) RBP III (Plafkin et al. 1989). Modifications include: 1) the use of a non-truncated D-frame net for the collection of the riffle-run samples, 2) different laboratory sorting procedures, 3) elimination of the CPOM (coarse particulate organic matter) sampling, and 4) metrics substitutions. Unlike USEPA's RBP III methodology, no field sorting is done. Only larger rocks, detritus, and other debris are rinsed and removed while in the field before the sample is preserved. While USEPA's RBP III method was designed to compare impacted waters to reference conditions (cause and effect approach), the DEP-RBP modifications were designed for unimpacted waters, as well as impacted waters.

DATA COLLECTION

Benthic Macroinvertebrate Sampling

The handheld D-frame sampler consists of a bag non-truncated net attached to a half-circle ("D" shaped) frame that is 1 ft. wide. The net is employed by one person facing downstream and holding the net firmly on the stream bottom. One "D-frame effort" is defined as: vigorous kicks in an approximate area of 1 m^2 (1 m x 1 m) immediately upstream of the net to a depth of 10 cm (approximately 4 inches, as the substrate allows) for approximately one minute (each kick must be at least 45 to 60 seconds in duration). Kicks are completed in a downstream-to-upstream direction to avoid disturbing the upstream portions of the targeted reach. All benthic dislodgement and substrate scrubbing should be done by kicks only. Substrate handling should be limited to the removal of large rocks or debris (as needed) with no hand washing. Since the width of the kick area is wider than the net opening, net placement is critical to assure all kicked material flows toward the net. Avoiding areas with crosscurrents, the substrate material from within the 1 m^2 area should be kicked toward the center of the square meter area.

The purpose of the standardized DEP-RBP sampling procedure is to obtain representative macroinvertebrate fauna from comparable stations. The DEP-RBP assumes the riffle/run to be the most productive habitat. Riffle/run habitats are sampled using the D-frame net procedure described above. For limestone stream surveys, two D-frame efforts (kicks) are collected - one from an area of fast velocity and one from an area of slower velocity within the same riffle. Limestone streams have low gradients often making it difficult to locate well developed riffles. If there are no riffles in the sample area, a run, or the best rock substrate available is sampled. The two kicks are then composited into one sample jar (or more as necessary). Each jar is labeled with the date, time, collector, collectively referred to as a station ID (yyyymmdd-HHMM-Collector), and location. In addition, the sample procedure should be clearly indicated somewhere on the jar; for this protocol 2D-300 would be the appropriate label (2D stands for 2 kicks using a D-frame net; and 300 is the subsample target that is detailed in Chapter 3.6 (Brickner 2020)). If more than one jar is needed then each jar will also be labeled with a number (e.g., 1 of 2, 2 of 2). It is recommended that paper tags labeled using indelible ink or pencil be placed inside the sample jar(s) as well. Care must be taken to minimize damage to the collected organisms when compositing the materials. It is recommended that

the benthic material be placed in a bucket filled with water to facilitate gentle stirring and mixing as not to damage fragile organisms. Because limestone samples are very abundant in organic material, the sample is preserved with at least 70% ethanol (95% ethanol is preferred). See Appendix G for DEP's Ethanol Policy. Composited samples are then transported back to the laboratory for processing and all field information is recorded on a macroinvertebrate field data sheet (Appendix A.1) or mobile data collection application. Macroinvertebrate processing and identification is conducted using standard DEP protocols, which are also described in detail in *Chapter 3.6*, *Macroinvertebrate Laboratory Subsampling and Identification Protocol* (Brickner 2020). It is recommended that a replicate sample be collected after every 20 samples for QA/QC purposes. Specific procedures on how to collect a QC sample is detailed in Appendix E, Section B5.

Sample Collection Period

Samples must be collected from January through May. Limestone streams have a low number of sensitive taxa and only a few of these taxa are generally found in large numbers. One very important sensitive taxon is *Ephemerella*. A good population of *Ephemerella* generally indicates better water quality. The three species of *Ephemerella*: *invaria, rotunda,* and *dorothea* found in Pennsylvania limestone streams emerge in May and June and are normally difficult or impossible to collect after emergence and until nymphs mature. Collecting samples from January through May ensures these very important ecological indicator taxa will not be missed.

ADDITIONAL DATA

During a DEP limestone macroinvertebrate survey, field meter (temperature, specific conductance, pH, dissolved oxygen, and turbidity if available), water chemistry, and habitat assessment data should also be collected. Recommended Standard Analysis Codes (SAC) for limestone macroinvertebrate collections are SAC 612, SAC 87, or SAC 18 (for special protection). For more information on water chemistry collection and SACs, refer to *Chapter 4.2, Discrete Water Chemistry Data Collection Protocol* (Shull and Arnold 2023). The Riffle/Run Prevalence Habitat assessment form should be used for limestone macroinvertebrate collections. This form can be found in Appendices C.1.

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3.3 WADEABLE MULTIHABITAT STREAM MACROINVERTEBRATE DATA COLLECTION PROTOCOL

Chapter 3 Biological Data Collection Protocols

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INTRODUCTION

U.S. Environmental Protection Agency's (USEPA) *Rapid Bioassessment Protocols for Use in Streams and Wadeable Rivers* (Barbour et al.1999) describes two general approaches to assessing stream macroinvertebrate communities. These approaches are the "single, most productive habitat" approach, previously described in *Chapter 3.1, Wadeable Riffle-Run Stream Macroinvertebrate Data Collection Protocol* (Shull 2017), and the "multihabitat" approach, described here. The multihabitat approach involves sampling a variety of habitat types instead of sampling a single habitat, such as cobble substrate in riffles and/or runs. This data collection protocol identifies appropriate field collection procedures needed to evaluate macroinvertebrate communities in wadeable low-gradient Pennsylvania streams.

A low-gradient waterway in Pennsylvania is defined as having pool/glide channel morphology and naturally lacking riffles. There should be no riffles within 300 consecutive meters of the sampling reach. Cobble outcrops extending less than 1/8 of the stream width are not considered riffles. In addition, small areas with slightly increased velocities and gravel substrates are not considered riffles. Besides lack of riffle/run sequences, low-gradient streams are characterized by a gradient less than 0.5%, substrates of fine sediment or infrequent aggregations of coarse sediments, and an aggregate of the five habitat types as described in Table 1. Moderate or high gradient stream segments that have been influenced by impoundments or beaver dams are not appropriate sites for the multihabitat field sampling protocol.

DATA COLLECTION

Aquatic macroinvertebrate samples are collected using a multihabitat sample collection procedure modified from that described in Barbour et al. (1999). Sampling should occur during the months of November through May. A 100-meter length of stream is determined and set as the sample reach. The investigator then identifies which low-gradient habitat types are present within the sample reach. Table 1 describes the five habitat types and explains the different sampling techniques for each habitat. Sampling consists of 10 non-truncated D-frame net jabs, 2 in each of the five habitat types or distributed proportionally in the available habitats. A minimum surface area of approximately 0.46 m^2 is required for a given habitat type to be sampled. If the total number of jabs (10) is not evenly divisible by the number of habitat types present, the remaining jab(s) are distributed among the most abundant habitat type(s) in the reach. Each jab consists of a 30-inch-long sweep of a 0.3-meter wide area, using a 500-micron mesh D-frame dip net. The jabs are composited while the investigator works progressively upstream from the first collection site. After the 10 jabs are completed, all jabs are combined into sampling jars and preserved with at least 70% ethanol (95% ethanol is preferred) in the field. See Appendix G for DEP's Ethanol Policy. Each jar is labeled with the date, time, collector (collectively referred to as a station ID (yyyymmdd-HHMM-Collector)), and location. In addition, the sample procedure should be clearly indicated somewhere on the jar; for this protocol 10D-200 would be the appropriate label (10D stands for 10 jabs using a D-frame net; and 200 is the subsample target that is detailed in Chapter 3.6 (Brickner 2020)). If more than one jar is needed, then each jar will also

be labeled with a number (e.g., 1 of 2, 2 of 2). Jars should only be filled 2/3 full of debris to allow for adequate sample preservation (ethanol should fill jar completely). If a lot of debris or large pieces were collected, the debris can be carefully rinsed in buckets, inspected for organisms, and then discarded. The rinse water is poured through a 500 µm mesh sieve to retain the macroinvertebrates before being discarded. The net, sieve, and bucket should be thoroughly inspected for macroinvertebrates. Composited samples are then transported back to the laboratory for processing and all field information is recorded on the macroinvertebrate field data sheet (Appendix A.1) or mobile data collection application. Macroinvertebrate processing and identification is conducted using standard DEP protocols, which are also described in detail in *Chapter 3.6*, *Macroinvertebrate Laboratory Subsampling and Identification Protocol* (Brickner 2020). It is recommended that a replicate sample be collected after every 20 samples for QA/QC purposes. Specific procedures on how to collect a QC sample is detailed in Appendix E, Section B5.

Table 1. Stream Habitat Types and Field Sampling Techniques

Habitat Type	Description	Sample Technique
Coarse Particulate Organic Matter (CPOM)	Coarse particulate organic matter (CPOM) consists of a mix of plant parts (leaves, bark, twigs, seeds, etc.) that have accumulated on the stream bottom in “depositional” areas of the stream channel; In situations where there is substantial variability in the composition of CPOM deposits within a given sample reach (e.g., deposits consisting primarily of white pine needles and other deposits consisting primarily of hardwood tree leaves), a variety of CPOM deposits are sampled; However, leaf packs in higher-velocity (“erosional”) areas of the channel are not included in CPOM samples.	CPOM deposits are sampled by lightly passing the net along a 30-inch long path through the accumulated organic material to collect the material and its associated aquatic macroinvertebrate fauna; When CPOM deposits are extensive, only the upper portion of the accumulated organic matter is collected to ensure that the collected material is from the aerobic zone.
Cobble/ Gravel Substrate	Stream bottom areas consisting of mixed gravel and larger substrate particles; Cobble/gravel substrates are typically located in relatively fast-flowing, “erosional” areas of the stream channel.	Macroinvertebrates are collected by placing the net on the substrate near the downstream end of an area of gravel or larger substrate particles and simultaneously pushing down on the net while pulling it in an upstream direction with adequate force to dislodge substrate materials and the aquatic macroinvertebrate fauna associated with these materials; Large stones and organic matter contained in the net are discarded after they are carefully inspected for the presence of attached organisms which are removed and retained with the remainder of the sample; One jab consists of passing the net over approximately 30 inches of substrate.

Table 1 (cont). Stream Habitat Types and Field Sampling Techniques

Habitat Type	Description	Sample Technique
Sand/Fine Sediment	Sand/fine sediment habitat includes stream bottom areas that are composed primarily of sand, silt, and/or clay.	Sand/fine sediment areas are sampled by bumping or tapping the net along the surface of the substrate while slowly drawing the net in an upstream direction along a 30-inch long path of stream bottom; Efforts should be made to minimize the amount of debris collected in the net by penetrating only the upper-most layer of sand/silt deposits; Excess sand and silt are removed from the sample by repeatedly dipping the net into the water column and lifting it out of the stream to remove fine sediment from the sample.
Snag	Snag habitat consists of submerged sticks, branches, and other woody debris that appears to have been submerged long enough to be adequately colonized by aquatic macroinvertebrates; Preferred snags for sampling include small to medium-sized sticks and branches (preferably < ~4 inches in diameter) that have accumulated a substantial amount of organic matter (twigs, leaves, uprooted aquatic macrophytes, etc.) that is colonized by aquatic macroinvertebrates.	When possible, the net is to be placed immediately downstream of the snag, in either the water column or on the stream bottom, in an area where water is flowing through the snag at a moderate velocity; The snag is then kicked in a manner such that aquatic macroinvertebrates and organic matter are dislodged from the snag and carried by the current into the net; If the snag cannot be kicked, than it is sampled by jabbing the net into a downstream area of the snag and moving it in an upstream direction with enough force to dislodge and capture aquatic macroinvertebrates that have colonized the snag; One jab equals disturbing and capturing organisms from an area of ~0.23 m^2 (12" x 30").
Submerged Aquatic Vegetation (SAV)	Submerged aquatic vegetation (SAV) habitat consists of rooted aquatic macrophytes.	SAV is sampled by drawing the net in an upstream direction along a 30-inch long path through the vegetation; Efforts should be made to avoid collecting stream bottom sediments and organisms when sampling SAV areas.

ADDITIONAL DATA

During a DEP multihabitat macroinvertebrate survey, field meter (temperature, specific conductance, pH, dissolved oxygen, and turbidity if available), water chemistry, and habitat assessment data should also be collected. Recommended Standard Analysis Codes (SAC) for multihabitat collections are SAC 612, SAC 87, and SAC 18 (for special protection). For more information on water chemistry collection and SACs, refer to *Chapter 4.2, Discrete Water Chemistry Data Collection Protocol* (Shull and Arnold 2023). Because multihabitat surveys are designed for low gradient streams, the low gradient habitat assessment form should be used. The DEP low gradient habitat form can be found in Appendix C.2.

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3.4 SEMIWADEABLE LARGE RIVER MACROINVERTEBRATE DATA COLLECTION PROTOCOL

Chapter 3 Biological Data Collection Protocols

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INTRODUCTION

Collection of macroinvertebrate data in large semiwadeable rivers can be a daunting and complex process. To appropriately collect biological community data in large rivers and to increase efficiency, DEP separates large rivers into two categories, semiwadeable and nonwadeable. Semiwadeable rivers are defined as predominantly free-flowing systems with drainage areas > 1,000 mi^2 and have physical characteristics that allow for riffle and run sections to occur with relative frequency. These river systems tend to lack a well-defined and navigable U-shaped channel for any significant distance and frequently present difficulties for both wadeable and nonwadeable macroinvertebrate data collection protocols. In addition, large semiwadeable rivers potentially have differences in water quality across their width. Well over half of the large rivers within Pennsylvania are considered semiwadeable (Figure 1). Therefore, it is important that a standardized protocol be established for the collection of physical, chemical, and biological data in these river systems.

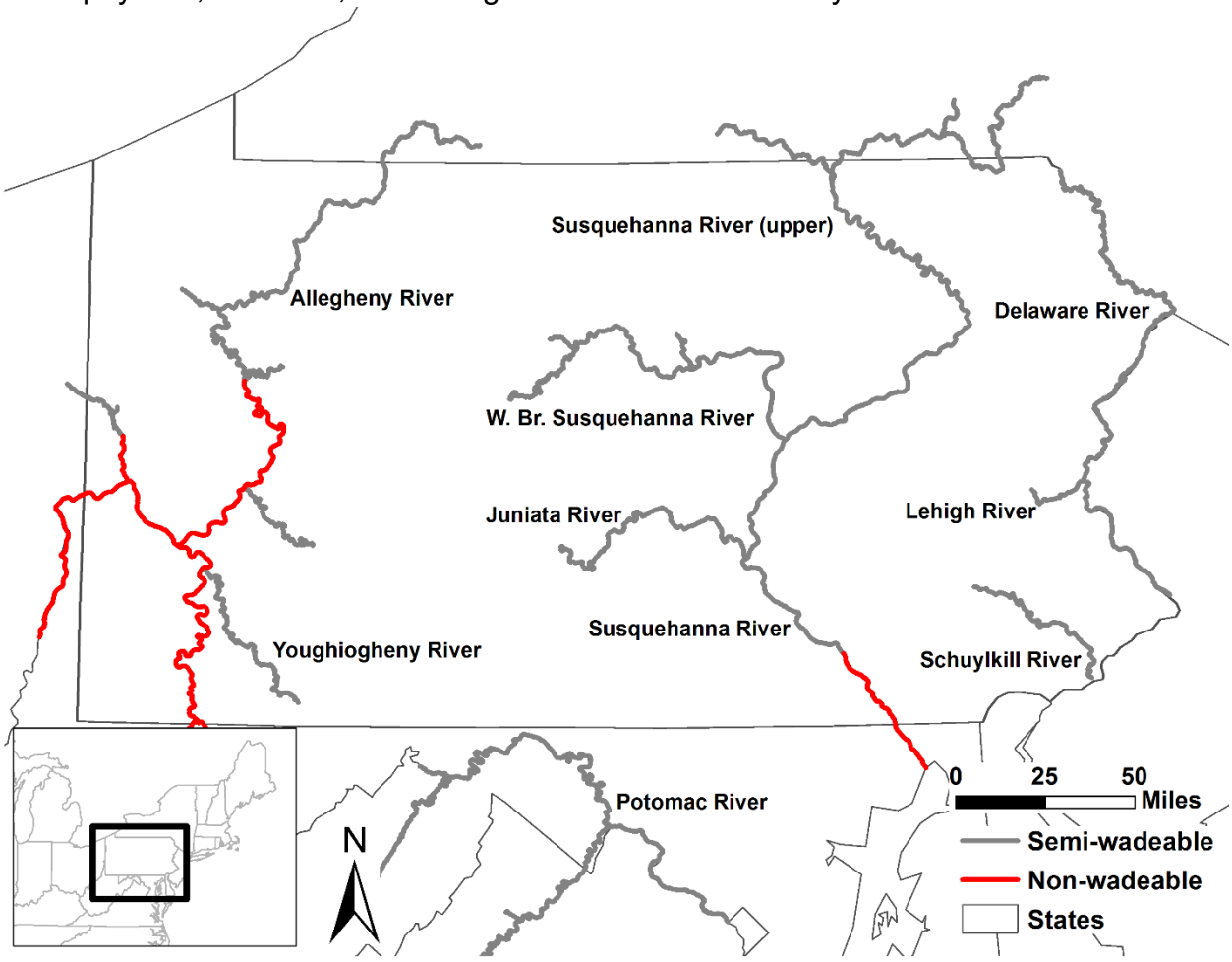

Figure 1. Semiwadeable and nonwadeable rivers throughout Pennsylvania.

The goal of this document is to lay the framework for how DEP intends on collecting macroinvertebrate and supplementary data for making Aquatic Life Use (ALU) determinations in large semiwadeable rivers within the boundaries of Pennsylvania. Working on, in, and around large semiwadeable rivers can be challenging and dangerous. This discussion seeks to provide guidance on when and how to collect these data, as well as important safety considerations that must be

considered. Safety procedures are found in Appendix F. Making accurate and defensible assessment decisions requires both enough data types (e.g., physical, chemical, and biological) and a specificity of those data within a water influence (zone) and season.

DATA COLLECTION

Water Quality

Cross-section surveys and water chemistry samples must be collected before macroinvertebrate sampling. This is needed to determine where the macroinvertebrate sample(s) are collected. All cross-section surveys are conducted using a clean and calibrated field meter that collects water temperature, specific conductance, dissolved oxygen, pH, and – preferably – turbidity (see Chapter 4). Transects (discrete points within the cross-section survey) are taken at regular intervals (each point representing approximately 5-10% of the total wetted width) across the width of the river. This is the least amount of information required to determine if major water quality influences exist at the sampling location. A major water quality influence that shows a 10% difference or greater in any field meter parameter necessitates a unique water chemistry and macroinvertebrate sample be collected. It is often helpful to create a drawing or map of the site with transect points depicted to help delimit the locations of water influence transitions across the width of the river (Figure 2). When new major water influences are identified, additional transects and water chemistry samples will be collected on upstream reaches to identify and characterize the source of variable water quality across the width of the targeted reach of river. Cross-section surveys and water chemistries will also be collected downstream, if possible, to determine the extent that the water influences do not mix. Multiple water chemistry samples over a period are recommended to provide additional information for assessments. Cross-section surveys and water chemistry data collected through the season prior to macroinvertebrate collection provides the most utility for complete and accurate assessments, but routine year-round data collection has also proven useful for ALU assessments and method development efforts. Cross-section surveys and water chemistry data collection prior to macroinvertebrate collections also provides opportunities to complete a thorough reconnaissance of the targeted semiwadable river.

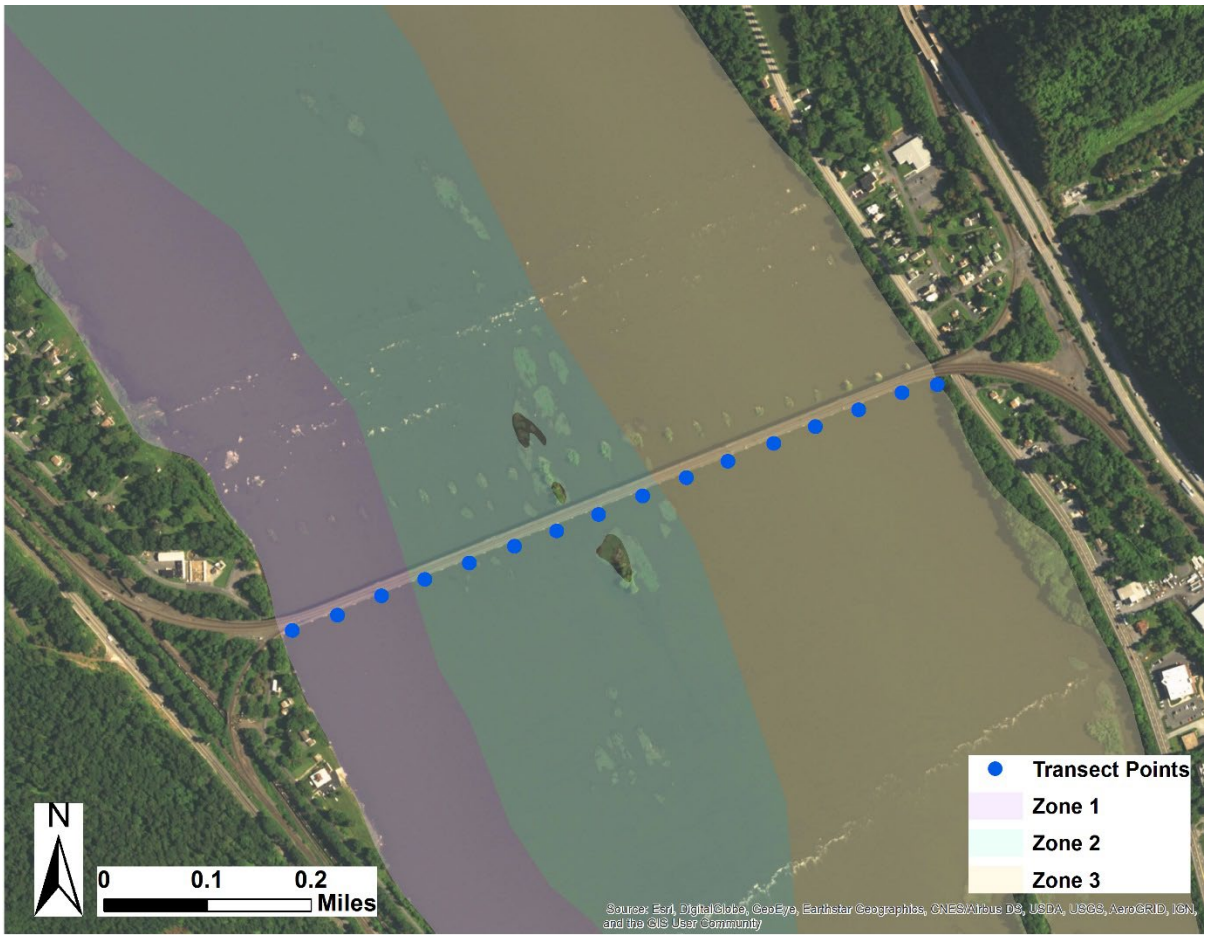

Figure 2. Example of cross-section data collection where data are collected at established transect points over time. In this example, the cross-section survey data identify three unique major water influences that create the three zones where macroinvertebrate, chemical, and physical data will be collected.

There are three water chemistry collection protocols that are acceptable when collecting chemical data on semiwadeable rivers: fixed-width sampling, isokinetic sampling (USGS 2006), and discrete mid-channel/mid-depth sampling (see *Chapter 4.2, Discrete Water Chemistry Data Collection Protocol* (Shull and Arnold 2023). Fixed width and isokinetic sampling are useful for characterizing water chemistry for the entire width of the surface water, while discrete samples are useful for characterizing unique water quality influences. Water chemistry characterization is also driven by the need to identify sources and causes of impairment. Water chemistries should include a comprehensive list of constituents including total and dissolved nitrogen and phosphorus species, total metals, and ions. For water chemistry collection, DEP routinely uses Standard Analysis Code (SAC) 612 for semiwadeable rivers. In addition, constituents such as dissolved metals, pesticides, hormones, wastewater compounds, and pharmaceuticals are added if necessary. For more information on water chemistry collection and SACs, refer to *Chapter 4.2, Discrete Water Chemistry Data Collection Protocol* (Shull and Arnold 2023).

Macroinvertebrate Sampling

Semiwadeable large river macroinvertebrate samples are collected using 6D-200 field collection described in *Chapter 3.1, Wadeable Riffle-Run Stream Macroinvertebrate Data Collection Protocol* (Shull 2017). Briefly, this consists of 6 one-minute kicks evenly distributed across the entire width of the waterbody using a 500 µm mesh D-frame net. With the transect procedure modification, additional 6D-200 samples are collected in accordance with the number of major water quality influences discovered during water quality transect data collection. For instance, if three distinctly different water influences were discovered, then three 6D-200 samples are collected, with each sample composited across the entire width of the respective water influence. Jars are labeled with the date, time, collector, collectively referred to as a station ID (yyyymmdd-HHMM-Collector), and location description. In addition, the sample procedure should be clearly indicated somewhere on the jar; for this protocol 6D-200 would be the appropriate label (6D stands for 6 kicks using a D-frame net; and 200 is the subsample target that is detailed in Chapter 3.6 (Brickner 2020). If more than one jar is needed then each jar will also be labeled with a number (e.g., 1 of 2, 2 of 2). Samples are preserved with at least 70% ethanol (95% ethanol is preferred). See Appendix G for DEP's Ethanol Policy. It is recommended that a replicate sample be collected after every 20 samples for QA/QC purposes. Specific procedures on how to collect a QC sample is detailed in Appendix E, Section B5.

Once field collections are complete, samples are taken to the laboratory for analysis. Macroinvertebrate processing and identification is conducted using standard DEP protocols, which are also described in detail in *Chapter 3.6*, *Macroinvertebrate Laboratory Subsampling and Identification Protocol* (Brickner 2020).

Macroinvertebrate samples collected at or above median flow conditions can cause a lower confidence in the results. Therefore, if collectors are not confident that the sampling was representative of the zone (e.g., collector could not collect the 6 kicks relatively evenly across the entire width of the influence) or they have collected in an inappropriate habitat type, then samples should not be used for assessments. The responsibility for this decision is left to the most experienced individual in the field during the sampling event. Individuals that are experienced with working in large rivers quickly realize when an inappropriate sample has been collected and will either discard the sample or annotate their concerns in the comments section of the macroinvertebrate data collection field form (Appendix A.1) or mobile data entry application.

ADDITIONAL DATA

Additional field meter (temperature, specific conductance, pH, dissolved oxygen, and turbidity if available) and water chemistry data does not need to be collected if the cross-sectional surveys and chemistry collection were completed as described above. A habitat assessment should also be collected for every macroinvertebrate collection. Because semiwadeable large river surveys target riffle-run habitat, the DEP Riffle-Run Prevalence Habitat Form should be used. This form is found in Appendix C.1.

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3.5 QUALITATIVE BENTHIC MACROINVERTEBRATE DATA COLLECTION PROTOCOL

Chapter 3 Biological Data Collection Protocols

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INTRODUCTION

While DEP primarily relies on semi-quantitative benthic macroinvertebrate data collection protocols described previously in this chapter, qualitative data collection protocols could also be considered. Qualitative macroinvertebrate data collection protocols may be appropriate for cause and effect surveys and point of first use surveys; however, this collection protocol is no longer used for ALU determinations.

The type of sampling gear used is dependent on waterbody type, available habitat, and other site-specific conditions. The recommended gear for sampling wadeable streams is a $1m^2$, flexible kick-screen, or a non-truncated D-framed net. The type of gear, dimensions, and mesh size must be reported for all data collections. When more than one gear type is used, the results must be recorded separately. Physical stream variables should be matched as closely as possible when comparing reference stations to candidate/impacted stations during location placement. Gear-type should also match between stations within the same survey. Matching these variables helps minimize or eliminate the effects of compounding variability.

DATA COLLECTION

Kick Screen

A common qualitative sampling protocol uses a simple hand-held kick-screen. This device is designed to be used by two persons. However, with experience, it may be used by one person. The kick-screen is constructed with a 1x1 meter piece of net material fastened to two dowel handles.

Facing up stream, one person places the net in the stream with the bottom edge of the net held firmly against the streambed. An assistant then vigorously kicks the substrate within a 1 $m^{2.}$ area immediately upstream of the net to a depth of 7-10 cm, as substrate allows. The functional depth sampled may vary due to ease of disturbance as influenced by substrate embeddedness. The amount of effort expended in collecting each sample should be approximately equivalent to make valid comparisons.

As many as four screens are collected at each site or until no new taxa are collected. Initial sampling should target riffle/run habitat. Collection in additional habitats to generate a more complete taxa list can be conducted at the discretion of the investigator.

Station data will be recorded on the macroinvertebrate data collection forms (Appendices A.1 and A.2) and habitat data will be recorded on the appropriate habitat data collection forms (Appendices C.1 and C.2) in this book. It is recommended that a replicate sample be collected after every 20 samples for QA/QC purposes. Specific procedures on how to collect a QC sample is detailed in Appendix E, Section B.2.

D-Frame Net

DEP has standardized a non-truncated D-framed net with 500µm mesh for all semi-quantitative benthic macroinvertebrate data collections in freestone streams and rivers, however it may also be appropriate to use this gear for qualitative collections. Qualitative D-framed data collection could be employed where the width of the targeted waterbody is less than 1 meter, or the targeted reach is small with very little available habitat.

In wadable, flowing waters with available riffle/run habitat, a D-framed net can be employed using a "single, most productive habitat" approach, previously described in this chapter. Facing upstream, each one-minute (each kick must be at least 45 to 60 seconds in duration) kick is conducted immediately upstream of a non-truncated D-framed net. Each kick should disturb approximately $1m^2$ immediately upstream of the net to an approximate depth of 10 cm, as substrate allows. During each kick, the net should be held stationary. With semi-quantitative protocols a prescribed, a standardized effort (number of kicks) is required. Qualitatively this gear should be used until no new taxa are collected. Kicks are completed in a downstream-to-upstream direction to avoid disturbing the other targeted portions of reach before kicks are conducted in that area. Collectors must ensure kicks are representative of the variety of riffle-run habitats present (e.g., slow-flowing, shallow riffles and fast-flowing, deeper riffles). It is important to note that riffle-run habitat differences will be respective of the stream segment being sampled. For example, a slow-flowing deep riffle will be different in a small stream than in a larger stream. Kicks must also be conducted throughout the width of the stream to include the left-descending, middle, and right-descending areas. The only exception to this is if a specific area of the stream is being targeted for a reason. If that is the case, then the collector must clearly document that the sample is not a complete composite and state the purpose for collection on the sample form.

In wadable, low-gradient waters without riffle/run habitat the "multihabitat" approach may be appropriate. The multihabitat approach involves sampling a variety of habitat types instead of sampling a single habitat, such as cobble substrate in riffles and/or runs. The investigator first identifies which low-gradient habitat types are present within the sample reach. The five habitat types and the different sampling techniques are described in *Chapter 3.3, Wadeable Multihabitat Stream Macroinvertebrate Data Collection Protocol* (Pulket 2017). Qualitatively this gear should be used until no new taxa are collected. Sampling consists of D-frame net jabs, in each of the available habitat types. A minimum surface area of approximately 0.46 m^2 is required for a given habitat type to be sampled. Each jab consists of a 30-inch-long sweep of a 0.3-meter wide area, using a 500-micron mesh non-truncated D-frame dip net. Jabs are completed in a downstream-to-upstream direction to avoid disturbing the other targeted portions of reach before jabs are conducted in that area.

It is recommended that a replicate sample be collected after every 20 samples for QA/QC purposes. Specific procedures on how to collect a QC sample is detailed in Appendix E, Section B.2.

ADDITIONAL DATA

Field meter (temperature, specific conductance, pH, dissolved oxygen, and turbidity if available), water chemistry, and habitat assessment data should also be collected. Recommended Standard

Analysis Code (SAC0 for collections are SAC 612, SAC 87, and SAC 18 (for special protection). For more information on water chemistry collection and SACs, refer to *Chapter 4.2, Discrete Water Chemistry Data Collection Protocol* (Shull and Arnold 2023). Station data will be recorded on the macroinvertebrate data collection forms (Appendices A.1 and A.2) and habitat data will be recorded on the appropriate habitat data collection forms (Appendices C.1 or C.2) in this book.

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3.6 MACROINVERTEBRATE LABORATORY SUBSAMPLING AND IDENTIFICATION PROTOCOL

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INTRODUCTION

This laboratory subsampling and identification protocol applies to the freestone riffle-run, limestone (with exception of subsample target), multihabitat, and semiwadeable macroinvertebrate collection protocols. The limestone collection protocol is currently the only macroinvertebrate collection protocol with a subsample target of 300 ± 30 organisms. All other macroinvertebrate collection protocols described above have a subsample target of 200 ± 20 organisms.

LABORATORY PROCEDURES

Laboratory Subsampling

Subsampling procedures are adapted from Barbour et al. (1999). Subsampling should take place in a controlled environment with good lighting, access to water, and plenty of workspace. Equipment and supplies needed for the benthic sample processing are:

- At least 2 large laboratory pans gridded into 28 squares. The specific size of a pan is not critical, but the number of grids (28) must be maintained if any basic density comparisons wish to be made between samples. DEP Central Office staff use Rubbermaid 2 Gallon White Durex® Container 18" x 12" x 3 1/2", because the bottom of the pan is 14" x 8", which allows for 28, 4in^2 grid cutters to be used
- A standard USGS No. 35 sieve (or 500 µm mesh sieve bucket) to remove fine materials and residual preservative prior to sub-sampling
- A random number generator or uniform objects (numbered from 1 to 28) for drawing random numbers
- Forceps (or any tools that can be used to pick organisms and debris)
- Grid cutters that approximate 1/28th of pan surface area
- A lighted magnifier (10x magnification maximum) may also be used during the sorting process but is not required. This equipment can make seeing organisms among debris easier and is good for training, but it does not make microorganisms (not visible with the naked eye) visible.
- Handheld counter
- Dissecting microscope (primarily used for identifications, but also used to confirm if organisms are identifiable).
- Storage vials for subsampled organisms
- 70-90% ethanol; See Appendix G for DEP's Ethanol Policy.
- Petri dishes

To begin subsampling, the composited sample is removed from the collection container and placed in the first gridded pan (pan 1). It is recommended to remove fine materials and residual preservative prior to subsampling by rinsing the sample through a sieve (or sieve bucket) that matches the mesh size (or smaller) of the net gear used to collect the sample. The sample is then gently distributed evenly throughout pan 1. Water may be added to the pan before distributing to reduce damage to the organisms.

At this point, there are several ways to achieve the targeted subsample of 200 ± 20 (300 ± 30 for limestone stream samples) organisms. It is important to note that this protocol is a 200 or 300 organism target; thus, collectors should avoid consistently biasing toward the lower limits of 180 organisms (270 for limestone stream samples) or upper limits of 220 (330 for limestone stream samples) organisms in the final identifiable count. Depending on the nature of the sample and the abundance of organisms, one subsampling procedure may be more efficient than another. The subsampling procedure is acceptable if it remains random/unbiased and the number of grids in pan 1 and pan 2 can be recorded. Only identifiable organisms (to the taxonomic level recommended in the identification section below) are counted during subsampling. Organisms that are not considered identifiable include pupae, larval bodies missing too many critical structures to render confident identifications, extremely small instars, empty shells or cases, and terrestrial organisms. If an organism is lying between two grids it is considered in the grid that contains the head. If the head is not easily seen, then the organism is considered in the grid containing most of the body. If a large volume sample necessitates the use of two first pans, material and organisms are removed from the same grid of both pans. For example, a large amount of debris is distributed evenly into two first pans. Then, if grid 17 is randomly selected, grid 17 must be pulled from both pans and sorted. These two grids are then counted as 1 grid from the first pan.

The following describes two potential ways of conducting randomized subsamples:

Option 1: (good for suspected high abundance samples)

The following option requires three pans (2 gridded pans and 1 viewing pan).

Randomly select 1 grid from the first gridded pan (pan 1) and remove all material and organisms. Place material and organisms in a viewing pan (pan should be as large as gridded pans to facilitate quick identification of organisms from debris). If the number of organisms (through a cursory count) appears to be much greater than 50 (e.g., 75 organisms), then move the material and organisms into the second gridded pan (pan 2). Randomly select 3 more grids from pan 1 and place all material and organisms evenly throughout pan 2. This process quickly confirms a high likelihood that there will be more than 220 (330 for limestone stream samples) organisms in the first 4 grids of pan 1 and a second gridded pan will be needed. This process also significantly reduces the amount of debris a collector must sort through before determining a second pan is needed. Once material and organisms are evenly distributed throughout pan 2, grids are randomly picked until the target number of organisms is reached. The total number of grids for each pan is recorded on the laboratory bench sheet (Appendix A-2) or annotated in the appropriate section of a mobile data entry application.

Option 2: (good for suspected low abundance samples)

The following only requires 2 pans (1 gridded pan and 1 viewing pan) if the sample has, in fact, a low abundance of organisms.

Randomly pick 1 grid at a time from the first gridded pan (pan 1) and place all material and organisms in a viewing pan to be sorted. Once all organisms are removed, the debris can be discarded. This allows for the viewing pan to be clear of debris for the next randomly selected grid from pan 1. Continue subsampling 1 grid at a time until the target number of organisms is reached. With low abundance samples, more than 4 grids from pan 1 will be selected, so there will be no need to use a second gridded pan. The total number of grids for each pan is recorded on the laboratory bench sheet (Appendix A-2) or annotated in the appropriate section of a mobile data entry application.

Once subsampling is completed, all organisms are placed in a clean vial with fresh 70%-80% ethanol. The vial is labeled using indelible ink with date, time, collector, collectively referred to as a station ID (yyyymmdd-HHMM-Collector), and location and then stored for later identification. If more than one vial is needed then each vial will also be labeled with a number (e.g., 1 of 2, 2 of 2). In addition, if identification occurred immediately after subsampling, then organisms can be placed into a petri dish instead of a labeled vial.

There will be rare occasions when subsampling results in more than 220 organisms (or 330 organisms for limestone samples) at the end of the subsampling process. If this occurs, pour the organisms out of the vial and into a clean gridded pan. Evenly distribute the organisms with fresh ethanol and then randomly select grids to remove organisms until the target number is achieved. This can be done in a backwards fashion to save time. For instance, a collector followed the procedures above, but got 244 organisms in 4 grids total. The collector then poured and evenly distributed organisms into a clean gridded pan. After removing organisms out of 4 randomly selected grids, the collector had 208 organisms in the gridded pan and 36 organisms set aside. At this point the collector simply needed to place the organisms in the gridded pan back into the vial. The 36 organisms left over were placed back into the original sample jar. Because the collector subsampled in a backwards fashion, the count of grids in pan 1 remains “4”, but the 4 grids removed during this additional process are subtracted from 28 in a “pan 2” to get 24 grids.

Digital Subsampling (Rarefaction)

Rarefaction Process

Digital subsampling or “rarefaction” is used to reduce sample size of a previously collected and processed sample. Rarefaction should not be considered a substitute for the subsampling procedures described above, but this process is acceptable when the physical sample is unavailable, or an immediate answer is needed. The concept of rarefaction was developed by Howard Sanders (Sanders 1968), modified by a number of other scientists (i.e., Hurlbert 1971, Heck et al. 1975, Simberloff 1978). Rarefaction has since been incorporated into several computer programs. Normally, rarefaction programs use the original sample numbers and, using a specified subsample amount, produce the expected number of species and a variance of this expected number of species. Depending on the program, confidence limits and plots/curves may be produced. Two important aspects of any rarefaction program are that: 1) the program can randomly reduce the number of individuals in each taxon, and 2) the program can randomly reduce sample richness. This ensures that the program functionally replicates the physical subsampling process.

For conducting rarefaction using spread sheets or designing a rarefaction program, the following steps should be followed:

1) Calculate how many macroinvertebrates would be in each grid of a 28-grid pan, assuming they are spread evenly across the pan.
2) Calculate the number of macroinvertebrates to remove based on the desired subsample (e.g., target for wadeable riffle-run streams = 200 ± 20 individuals).
3) Divide the number of macroinvertebrates to remove by the number of macroinvertebrates found per grid.
4) Round this result to the nearest whole number grid.
5) Multiply the number of macroinvertebrates per grid by the number of grids to subsample.
6) Round this result to the nearest whole number (because entire organisms are removed, not partial). This is the number of macroinvertebrates that will be removed from the sample to reach a correct subsample. The remaining macroinvertebrates will be the correct subsample.
7) Number each individual macroinvertebrate from one to the total number of individuals in the sample on a spreadsheet.
8) Using a random number generator, randomly remove the number of macroinvertebrates in step #6. What remains is the list of macroinvertebrates to keep in the new subsample.

Example Using R Script

To start the rarefication process in R, install (if needed) and load the packages in the script below. Rarefaction is needed when macroinvertebrate subsample sizes exceed the subsampling target by >10%.

- For freestone riffle/run, multihabitat, and semi-wadeable sampling protocols, the subsampling target is 200 individuals, which results in acceptable subsample sizes of 180 – 220 individuals. When using these protocols, subsamples should be rarefied when they contain >220 individuals.

- For the limestone protocols, the subsampling target is 300 individuals, which results in acceptable subsample sizes of 270 – 330 individuals. When using these protocols, subsamples should be rarefied when they contain >330 individuals.

To start the rarefication process in R, install (if needed) and load the packages in the script below. The dataset to be rarefied (saved as .csv) is typically in long format as the example dataset shows below. The Sample column indicates that data from two samples (A and B) are contained in the dataset, which contain 346 and 288 individuals, respectively. These subsample sizes are >10% of the subsampling target of 200 individuals. To rarefy the samples, the R script will change the dataset to wide format.

Table 1. Example dataset to be rarefied.

Sample	TaxaID	Count
A	Baetis	41
A	Cheumatopsyche	27
A	Chimarra	11
A	Chironomidae	170
A	Diphetor	1
A	Epeorus	2
A	Ephemerella	3
A	Heteroplectron	10
A	Hydropsyche	33
A	Leuctra	20
A	Paracapnia	2
A	Stenonema	26
B	Baetis	20
B	Cheumatopsyche	32
B	Chimarra	20
B	Chironomidae	100
B	Epeorus	1
B	Ephemerella	30
B	Heteroplectron	1
B	Hydropsyche	70
B	Paracapnia	14

```
 # Import and format data
# setwd("C:/R/rarefaction") – not needed if creating a project in RStudio

# Load packages
library(readxl)
library(writexl)
library(vegan)
library(tidyverse)
library(tidylog)

# Import data and pivot wide
df <- read_csv("Example.csv") %>%
  pivot_wider(id_cols = 'Sample', names_from = 'TaxaID', values_from = 'Count') %>% # pivot wide
  replace(is.na(.), 0)  # replace NA values with 0
df
```

The following code will loop through each sample and rarefy taxa counts to the specified range randomly. The target subsample number is also randomly selected to vary between 180 and 220. If another range in desired, simply change the numeric range in the code, as is done in the commented code below. The purrr package function map_df( ) is used to loop through each row of the wide data frame, where each row represents a site. During each iteration of the loop one site (one row) is rarefied and a random number between 180 and 220 is selected as the sample size. The output is then appended with the sample identifier column from the wide data frame, to provide identify which

site each row represents. Finally, the columns are re-ordered using dplyr::select( ) to make the sample identifier the first column of the data frame.

```
# rarefy

rarefied_df_wide <- purrr::map_df(1:nrow(df), function(row.i) {
  suppressWarnings(
    # for freestone riffle/run, multihabitat, and semi-wadeable sampling protocols
    data.frame(vegan::rrarefy(df[row.i, 2:ncol(df)], sample(180:220, 1)))

    # for limestone sampling protocol
    # data.frame(vegan::rrarefy(df[row.i, 2:ncol(df)], sample(270:330, 1)))
  )
}) %>%
  bind_cols(df[, 1]) %>%
  select(Sample, everything())

rarefied_df_wide
```

The final table will need to be reformatted into a long format and exported to complete the rarefaction process. After the data is in long format, this code checks that samples were rarefied correctly. Min (minimums) may be lower than the specified lower bound (i.e., 180), if the sample contained fewer than 180 taxa; however, Max (maximums) should never be greater than the specified upper bound (i.e., 220). The final chunk of code exports the rarefied dataset into an Excel spreadsheet. It's important to note other final tables produced from this example may not match the table below, because this is a randomized process.

```
# Wide to long format
rarefied_df <-
  rarefied_df_wide %>%
  pivot_longer(cols = 2:ncol(.), values_to = 'Count', names_to = 'TaxaID') %>% # make long
  filter(Count != 0) # remove all taxa = 0
rarefied_df

# Check subsample sizes
rarefied_df %>%
  group_by(Sample) %>%
  summarize(subsampleSize = sum(Count))

# Export taxa list to excel
write_xlsx(list(Rarefied = rarefied_df),
           paste0(Sys.Date(), '_rarified_samples.xlsx'))\
```

Table 2. Example dataset rarefied.

Sample	TaxaID	Count
A	Baetis	25
A	Cheumatopsyche	13
A	Chimarra	7
A	Chironomidae	96
A	Epeorus	2
A	Ephemerella	2
A	Heteroplectron	7
A	Hydropsyche	21
A	Leuctra	12
A	Paracapnia	1
A	Stenonema	15
B	Baetis	14
B	Cheumatopsyche	26
B	Chimarra	15
B	Chironomidae	74
B	Epeorus	1
B	Ephemerella	23
B	Hydropsyche	52
B	Paracapnia	9

TAXONOMIC IDENTIFICATION

Levels of Identification

The level of identification for most aquatic macroinvertebrates is to genus. Exceptions to genus level identification requirements are listed below (Table 3). Ephemeroptera, Plecoptera, and Trichoptera (EPT) taxa should be identified to the genus level. If an EPT taxa is collected and is unable to be identified to genus level, effort should be made to have other taxonomists assist with a positive identification. Otherwise, the taxa should be included on the enumeration sheet, separate and distinct from other identifications within the same taxonomic group. Other orders may be identified to the lowest level attainable if they are immature and do not exhibit the characteristics necessary for confident genus level identifications; however, this should be rare if the proper subsampling process is followed. Identifications with a mixture of higher and lower taxonomic levels within the same taxonomic group will skew sample scoring and may result in the sample not meeting quality assurance standards. If identifications with a mixture of higher and lower taxonomic levels within the same taxonomic group cannot be avoided, higher level identifications will not be used to calculate metrics. Eliminating these individuals from the sample avoids artificially inflating richness metrics. In addition, the sample and the identifications must be forwarded to a certified taxonomist for further evaluation, and the identified organisms should be preserved and maintained indefinitely.

Table 3. The taxonomic groups that are identified to a higher taxonomic level than genus.

Group	Identification Level
Midges	Family
Snails	Family
Clams	Family
Mussels	Family
Flatworms	Phylum (Platyhelminthes)
Aquatic Earthworms & Tubificid Worms	Class (Clitellata)
Leeches	Subclass (Lumbriculata)
Proboscis worms	Phylum (Nemertea)
Roundworms	Phylum (Nematoda)
Moss Animalcules	Phylum (Bryozoa)
Water mites	Superorder (Acariformes)

All organisms are identified and enumerated on a bench sheet (Appendix A.2) or data entry application. Organisms are then returned to the labeled vial and stored in a safe location.

Quality Control

Biologists who are involved with macroinvertebrate subsampling will need to be properly trained with documentation that tracks sorting error. Additionally, at least 10% of all samples identified by a biologist are quality assured by a certified taxonomist. DEP may also request sample vials for any results entered into internal databases for quality assurance purposes. The most common type of certification is the genus level EPT certification provided by the Society of Freshwater Science. Sorting error and identification error are further described in Appendix E.1.

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3.7 FISH DATA COLLECTION PROTOCOL

Chapter 3 Biological Data Collection Protocols

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INTRODUCTION

Fish community assessments are important as they represent a biological community that are captive to water resources during all life stages, have longer life spans, and have a greater socioeconomic status than macroinvertebrate communities alone. Standardized semi-quantitative sampling protocols can be applied to all wadeable and nonwadeable streams. The data generated from standardized protocols are vital for making ALU assessments and evaluations, developing indices and other assessment methods, promoting spatial and temporal trend analyses, and providing the foundation for a robust fish bioassessment program. The Assessment Book (Shull and Whiteash 2023), Chapter 2, includes the *Stream Fish Assemblage Assessment Method* (Wertz 2021) which requires strict adherence to the following standardized collection protocol. Other collection protocols may be considered for various assessment purposes only after rigorous analysis for similarity.

SAMPLING CONSIDERATIONS

Basic Requirements

Field sampling procedures, species identification and enumeration, and other site tasks must be applied consistently with the same level of rigor at each sample site. To maintain consistency, a field crew is directed on site by a crew leader who maintains a valid scientific collector permit (SCP) as required by Pennsylvania Fish and Boat Commission (PFBC) and has met the quality control requirements of DEP (described below and in Appendix E). Accurate species level identification of each fish collected is essential, as it provides the foundation for subsequent assessment. A minimum of one crew member must be proficient in the accurate identification of Pennsylvania fishes and should also be versed in fishes of the surrounding areas and potential exotics. Electrofishing techniques are the primary procedures adopted by DEP. Other fish collection procedures are not discussed here. Electrofishing crews will have a crew leader trained and experienced in the proper operation of electrofishing gear and appropriate electrofishing safety and strategy (described below and Appendix F).

DEP recognizes that electrofishing is an inherently hazardous activity for which safety is a primary concern. The environmental conditions under which these operations are conducted further increase the risks. Due to these important concerns for electrofishing safety, all field team members should be familiar with electrofishing techniques, safety precautions, and equipment manufacturer’s operating procedures. Electrofishing safety is described in Appendix F. Additionally, the spread of aquatic invasive species (AIS) is always a potential concern when conducting stream surveys. Biosecurity measures should be considered and implemented for all fish collections (Appendix F).

Representative Sampling

The objective of this semi-quantitative fish data collection protocol is to acquire a representative sample of the overall fish community, here referred to as the fish assemblage. Fish assemblage data are considered representative when it is collected with a standardized collection protocol that is robust enough to produce repeatable biological response(s) to environmental and/or anthropogenic

conditions. The collection protocols may or may not provide a complete inventory of all species present at a site but should be able to consistently characterize most species present and their relative abundance.

Sampling Effort

Sampling effort is measured by both distance and time and accurate measures of both are considered essential. Accumulated electrofishing time can legitimately vary over the same distance as dictated by cover, stream conditions, and the number of fish encountered. While it is understood that some individual fish will not be captured, a concerted effort by the crew members should be made to capture every fish sighted as this is fundamental to relative abundance measures. Dipnet design and mesh size will largely dictate the size range of fishes captured; frame diameter, net depth, and handle length should be of sufficient size to capture all anticipated fishes and size ranges. Net mesh should be approximately 6 mm (1/4 in). Since the ability of the netters to see stunned and immobilized fish is partly dependent on water clarity, sampling is to be conducted only during periods of “normal” water clarity and flows (e.g., baseflow conditions). Sites that are turbid should not be sampled if water clarity is under one meter unless this clarity is considered normal. The sampling period for all semi-quantitative fish collection protocols is from June 1st through September 30th and this period may provide a wide variety of stream flow conditions. Periods of high turbidity and high flows should be avoided due to their negative influence on sampling efficiency. If high flow conditions occur, sampling must be delayed until flows and water clarity return to seasonal norms. The crew leader will decide if conditions are acceptable and direct their crews accordingly. A representative fish assemblage is not only based on conditions but also on sample reach and habitat selection. A sample reach at a site is considered most representative when it;

- Contains all the best available habitat (BAH) types (geomorphic units, flow regimes, fish cover, and substrate material) within a contiguous reach.
- Is similar to that of the local area (i.e., unique habitats should be avoided).

Site Selection

Two semi-quantitative sampling procedures, wadeable and nonwadeable, are described below. Strict *a priori* designations of wadeable or nonwadeable have been avoided due to complex limitations, but see Flotemersch et al. (2006) for a detailed description of these designations used elsewhere. A stream reach at a site is considered wadeable or nonwadeable not by size, but in its ability to be efficiently surveyed during the June 1st -September 30th sampling season utilizing the following collection protocols (a stream reach may be considered for both protocols throughout the same period if both protocols can efficiently be implemented). Sites that can have both protocols efficiently implemented are referred to as “transitional zones” and are typically large wadeable sites and small nonwadeable sites (Flotemersch et al. 2006). If a site is considered within a transitional zone both protocols should be applied within a short timeframe (days-week), as feasible, to facilitate direct comparisons between collection protocols. Sites that are too large for efficient coverage using wadeable techniques (e.g., large rivers) are not considered transitional and should only be sampled using nonwadeable techniques. Duplicates (a second sample reach at a site comparable to the first, sampled the same day) and replicates (repeating the same site within the same sampling season)

should be performed as often as feasible (preferably 1 out of every 10 sites) for both collection protocols to maintain a calibration dataset for future assessments.

Final Site Determination

Specific site locations will first be determined by the type of study and its design (e.g., cause and effect, protected use assessment) secondly by access feasibility (e.g., public access, private landowner permission, distance to stream etc.) and lastly by representative habitat. Desktop reconnaissance as well as a field reconnaissance should be implemented prior to sampling to determine the appropriate gear and manpower needed to achieve a representative sample. Further consideration should be applied to the site selection process to ensure a representative sample of the conditions that are being measured. Sites located near the mouth, near an instream dam or impoundment, or near the mouth of a feeding tributary should be avoided unless these influences are inherent in the study design. General guidance for distances represents modifications from literature and DEP data exploration (Gorman 1986, Schaefer and Kerfoot 2004, Hitt and Angermeier 2008) and are provided in Table 1. In any case, potentially influencing factors should be noted on the field forms to facilitate data management and analysis. Applying a detailed reconnaissance to the site selection process will greatly reduce down-time the day of the sampling event, ensure repeatability, and provide high-quality data.

Table 1. General guidelines for site selection based on distance from potential influencing factors.

Influence	Category	Distance (km)	Description
In-stream dam and its impoundment	Minor	0.5	- Defined by dam NOT restricting fish passage under normal to high flow conditions.
In-stream dam and its impoundment	Major	1.5	-Defined by dam restricting fish passage under all flow conditions.
Proximity to mouth	Minor	0.5	- Defined as target stream within one stream order of receiving stream.
Proximity to mouth	Major	1.5	- Defined as target stream being at least two stream orders smaller than receiving stream.
Proximity to tributary	Minor	0.5	- Defined as tributaries within three stream orders of receiving stream.
Proximity to tributary	Major	1.5	- Defined as tributaries three stream orders smaller (adventitious). Should be avoided within the reach, when feasible.

DATA COLLECTION

Wadeable Protocol

For semi-quantitative fish sampling in wadeable streams, sample reach lengths will be determined by the width of the stream. The average wetted width is multiplied by 10, with a minimum reach length of 100 meters and a maximum of 400 meters (Table 2). Sample reach width is determined by measuring five wetted channel widths spaced twenty meters apart within the first 100 meters of the proposed sample reach, and averaged. Once the wetted width has been calculated, it is best to establish the stopping point before establishing the starting point. Due to the nature of this protocol, fishes will naturally be "pushed" upstream to avoid the electrical field. The stopping point should subsequently be a section that provides a natural barrier to escape (e.g., shallow riffle or cascade) and should be established first. Sample reach length can then be measured in a downstream direction to determine the starting point. For consistency, the reach should adhere to the minimum reach lengths but can be lengthened if necessary to include additional habitat types. The use of downstream blocknets should be avoided based on the size range of wadeable streams in Pennsylvania and by the bias that may be inherent in blocknetting small streams but not large.

Table 2. Sample reach lengths based on stream width.

Average Stream Width	Minimum Reach Length
<10m	100m
10 to 40m	10 X average stream width
>40m	Maximum of 400m

Before sampling, the crew leader will brief the electrofishing crew on safety and strategy (discussed below and Appendix F) and will adjust the electrofishing equipment to the desired settings (Appendix A.3). When the crew is ready to begin, the crew leader will take note of the Global Positioning System (GPS) location, will start the clock, and electrofishing will commence in a downstream to upstream direction. The electrofishing crew will be positioned along a transect, adequately covering the width of the stream, and should maintain this position while proceeding upstream to avoid gaps in the electrical field which may decrease coverage. The crew leader must be cognizant of this coverage throughout the survey and adjust as needed. For best coverage and standardization of effort, the rule-of-thumb for determining the amount of gear needed at a reach is one probe for every five meters of stream width (Figure 1). For the large wadeable streams, this will inherently require a great deal of planning to ensure the best coverage is achieved (e.g., equipment and manpower). If gaps are still present along the stream width, the crew can adapt by doing small zigzags as they proceed upstream for adequate coverage. Similar collection protocols that may employ transect zigzags across the width of the stream (see, Lazorchak 1998), multiple pass (see, Pusey et al. 1998, Meador 2003, Shank et al. 2016), or other variant, will not be considered comparable until a detailed analysis can be performed. If a stream width is too large for best coverage, nonwadeable techniques should be pursued. Each crew member with an anode probe should continuously sweep the probe side to side for best coverage. The number of nets to anode probes must be a minimum of one to one,

additional netters may be used as needed. When the crew reaches the predetermined stopping point, the crew leader will instruct the crew to stop and take note of the stoppage time and GPS location. Fish processing will then commence as described in detail below.

Figure 1. An example of “best coverage” across the width of a stream (photo by Josh Lookenbill).

Nonwadeable Protocol

For semi-quantitative fish sampling in nonwadeable streams, multiple sample reaches at a site are needed to adequately represent the size and/or complexity (e.g., water quality, habitat) inherent to large lotic systems. For smaller nonwadeable sites, two reaches representing the right and left descending banks (RDB and LDB) are applied. For large nonwadeable sites, three or more reaches; two reaches representing the left and right portion of the site are applied. If midchannel island habitat is present, the third reach will be established in the littoral zone(s) of the island; this represents the middle portion of the site. Where island habitat is not present, an additional reach(s) can be added to the banks. Additional reaches can be added to a site as needed if they represent a unique water quality zone or habitat modification (e.g., permitted discharge or area of habitat disturbance) as dictated by the study objective. A general rule-of-thumb for determining the number of reaches to apply to a nonwadeable site is one extra reach for every 30K sq/km of drainage area over 30K sq/km. For example, if a site is less than 30K sq/km, RDB and LDB would be sampled, if a site is 30-60K

sq/km a middle reach would be included. Strict definitions are avoided to provide the crew leader with discretion for best coverage.

Sample reaches are surveyed using one-pass, daytime electrofishing. Reaches are surveyed in a downstream direction for a longitudinal distance of 500 meters for a minimum of 30 minutes. Specific sample reaches should be established by desktop and field reconnaissance prior to sampling. Measures of stream width may be problematic on large rivers based on rangefinder capabilities and may have to be performed digitally during the desktop reconnaissance. To maintain strict adherence to the reach length requirements, starting and stopping locations should be adequately marked with flagging tape or other distinguishable features in the field prior to sampling. Before sampling, the crew leader will brief the electrofishing crew on safety and strategy (discussed below and in Appendix F) and will adjust the electrofishing equipment to the desired settings (Appendix A.3). When the crew is ready to begin, the crew leader will take note of the GPS location, will start the clock, and electrofishing will commence. A single boat-mounted electrofishing unit is maneuvered in a zigzag fashion from littoral shoreline areas toward the thalweg for a distance of 30 meters from the shoreline or to where stream depth reaches six meters, whichever comes first. If a mid-channel reach of the river is to be sampled, island habitat must be targeted to satisfy the littoral shoreline requirement. For nonwadeable sampling, BAH is both representative of the local conditions, and within a contiguous 500-meter reach. The habitat should be uniformly navigated and electrofished based on the proportional abundance of the habitat within the reach. Prolonged electrofishing within a single habitat type is not recommended. If a small section of the reach cannot be adequately surveyed due to navigational obstacles or flow restrictions, the area can be avoided, and the same distance added to the end of the reach (electrofishing time must be adjusted accordingly). Best effort should be made to net all fishes observed and in proportion to their abundance. The strict targeting of the largest fishes must be avoided.

Each boat crew will have one navigator (usually the crew leader), and a minimum of one netter for each anode “dropper”. Additional crew members may be utilized to aid in netting large fishes and monitoring fish stress levels. When the crew reaches the predetermined stopping point, the crew leader will instruct the crew to stop and take note of the stoppage time and GPS location. Fish processing will then commence as described in detail below.

FISH HANDLING AND PROCESSING

General Considerations

Since semi-quantitative fish surveys depend on the collection of fish for species identification, temporary retention of a significant number of fish is required. To minimize stress and lethality to the greatest extent possible, the following field procedures should be followed:

- Remove stunned fish from the electrical field as soon as possible.
- Netted fish should be transferred to containment devices as soon as possible. Containment devices vary depending on the type of survey and situations but may be a combination of:
    - Large buckets or tubs

    - Live-wells constructed of framed netting that form a cage that is submerged in the stream
- Water is replaced regularly in warm weather to maintain adequate dissolved oxygen levels in the water, reduce waste by-products, and minimize mortality.
- Aeration should be provided to further minimize stress and mortality if necessary.
- Field identifications and releases should commence immediately after collection.
- Every feasible effort will be made to minimize holding and handling times.

Special handling procedures may be necessary for species of special concern as outlined by the SCP requirements. Fish that are not retained for vouchers or other purposes are released back into the water after they are identified to species and enumerated. Invasive species will be kept and appropriately disposed of out of the water, if requested by SCP requirements. Each sample crew must have at least one person qualified as a taxonomic specialist in field fish identification and must also be trained in the identification of gross anomalies inherent to the fish health assessment.

FISH HEALTH ASSESSMENT

Incidence and prevalence of gross anomalies are important measures of fish health that have been used as an indicator of anthropogenic stress (Bauman et al. 2000) and allow for both spatial and temporal comparison. The measures of deformities, erosions, lesions, tumors, and parasites (DELTP) are completed immediately after sampling. The intent of the fish health assessment is to record the species, length, and various anomalies that may be present on each individual fish. These data can then be used to elucidate any potential issues that may be inherent to a specific species or length-class. The health assessment is not intended to be diagnostic but should be able to consistently classify specific gross anomalies by general DELTP type. Because small-bodied fishes generally have a shorter life span (or shorter exposure time) the health assessment is generally intended for fishes >100mm. It is recommended that all fish >100mm in length be assessed, however, it is understood that large surveys may have an inordinate amount of fish and may need to be subsampled. Subsampling should be random (i.e., fish with anomalies should not be targeted), should represent all species available, and should be of significant sample size relative to their abundance in the sample. The goal during this process is to record as much information about the fishes without inflicting undue stress. It is best when fishes that are to be processed for health are held in live-wells. If the fish appear to be stressed, even after all attempts have been made to reduce stress, the priority is to release and not collect health assessment data. When fish are released without health data, only species identification and enumeration are needed.

To conduct the health assessment the crew leader will assign the following three duties to the crew: inspector, handler, and recorder. The inspector (generally the crew leader) should be familiar with common fish diseases, experienced in the identification of fish and gross anomalies, and have participated in numerous fish health assessments as either handler or recorder. It is the inspector's duty to give a species identification, measure the total length (TL) of the fish (to the nearest 5 mm increment), and inspect the fish for anomalies. TL is measured from the tip of the snout to the end of

the slightly compressed caudal fin. The fish will be inspected along the right and left sides of the body, head, fins, and gills. Specific anomalies found during this inspection will be voiced to the recorder. Once the data has been collected, the inspector can release the fish. The crew leader (or inspector) should determine where fish should be released; fish should not be released near anticipated sample reaches to avoid immigration and bias in the next sample. The handler is responsible for monitoring and reducing the stress level of the fish while in retention and subsampling the fish that are then given to the inspector. The recorder observes the inspection and records the data voiced by the inspector. The recorder sets the pace of the health assessment and the inspector should adjust accordingly. The recorder should be able to anticipate the amount of space needed to record the data based on the number of fish that are in the sample, as well as write neatly to avoid error in data management. A quick reference field guide for anomalies is imbedded in the field data collection form in Appendix A.3.

FISH PRESERVATION

Most captured fish are identified to species in the field and released; however, any uncertainty about the field identification of individual fish requires their preservation for future laboratory identification (except for large specimens or species of special concern which may need to be photographed). Only the fish necessary for vouchering and laboratory confirmation will be retained. Fish are fixed for future identification in buffered 10% formalin. The container is labeled, at minimum, with date, time, waterbody, site location, and geographic coordinates. Species level identification is required and may be necessary to the subspecies level in certain instances. If a species level identification is not possible in the field, voucher(s) specimens should be retained, or properly photographed, for later laboratory analysis. Proper photographs should include a picture of the left side of the fish body in the anatomical position, a label with site information and individual identification number, and taxonomically important characters needed for species level identification. In any case, strict adherence to SCP requirements and subsequent annual updates must be considered.

The PFBC's Division of Environmental Services (DES) maintains the general SCP requirements as well as Qualified Threatened and Endangered Species (TE) Surveyor requirements. More information on SCP and TE survey requirements can be found at
http://www.fishandboat.com/Resource/EnvironmentalServices/Pages/default.aspx
DES also has guidance for vouchering, and specific species that may be important to voucher if encountered.

SITE DATA

Prior to leaving the site, the field collection sheet in Appendix A.3 must be completed, ensuring that all field-based data are recorded. Location information is critical to ensuring repeatability of a collection, and detailed descriptions are encouraged. Descriptions of location should include relative position to a stable landmark. For example, "downstream of bridge" is ambiguous, whereas "250 meters below SR144 bridge upstream of Crossfork, PA" ensures repeatability. Water quality

measures must follow guidelines as described in *Chapter 4.1, In-Situ Field Meter, Transect, and Vertical Profile Data Collection Protocol* (Hoger and Arnold 2023). Gear and effort descriptions should be as specific as the equipment allows and are considered essential to normalizing data for effort. Qualitative habitat measures (mesohabitat, velocity/depth regimes, habitat, and substrate) are recorded as a percent-occurrence within the sampled reach and should not be applied to a larger scale. Habitat measures are considered typical except for Percent of Fish in Habitat. Here, the percent of fish (the number of individuals regardless of species) that were netted out of each habitat type within the reach is recorded. This measure is atypical but provides insight into habitat utilization. This measure is not always required as it can be influenced by reduced visibility or when fish are "pushed" into another habitat by the electrical field.

LABORATORY PROCEDURES

Important Note- *All chemicals that are integral to the preservation and long-term storage of fish vouchers should be treated as hazardous. Chemical specific Material Safety Data Sheets (MSDS) forms should be referenced and be readily accessible at the laboratory. Containers should be labeled appropriately, and nitrile gloves worn at all times. General considerations for vouchering specimens come from Walsh and Meador (1998) and can be referenced for a more detailed analysis.*

Sample Preparation

Before fishes can be safely handled in the lab, they must first be transferred from 10% formalin fixative and washed. Washing includes soaking fish in fresh (tap) water for a 24-hour period per wash, for a minimum of three washes (three days). Larger samples, with more biomass, may need additional washes. After the last washing period, the sample can be gradually (over a period of days) introduced to levels of ethyl alcohol washes from 35% to 50% to 70%. The ideal concentration for long term storage is a 70% ethyl alcohol solution and should be confirmed with a hydrometer after immersion for 24 hours. Once the sample has been properly washed and preserved, laboratory identifications can be completed.

Identification and Enumeration

Taxonomic identification and enumeration are the two most important factors for quality lab data. The first step is to obtain the taxonomic identification. Fishes are identified and segregated into species groups, or taxonomic units. The second step is to lay each species out and enumerate them, this provides for a second-chance opportunity to spot any taxonomic errors. It is best to lay species out for enumeration in smaller groups, by fives or tens, to facilitate a recount. If a second taxonomic expert is available, it is best to have them confirm the identification and enumeration, which provides a third and final opportunity to catch any mistakes. Laboratory taxonomic identifications and enumerations are then added to the original field data sheet to obtain the final taxa list and enumerations. Nomenclature should follow the most recent update provided by the American Fisheries Society (Page et al. 2013). Regional taxonomic keys should be used for identification and range distribution comparisons. Notable identifications of species outside of their known range should be documented and verified before data are considered final. Fishes should be identified to the lowest taxonomic level

possible, to include species, subspecies, and hybrids (with comments on parental genus or species). If identification to this level is not possible (due to size, condition, or other variable) the use of genus or possibly family level identification may be considered. To maintain data consistency, fishes <25mm (TL) should not be included in the final identification and enumeration, but can be included as separate “comments”. For species that may have close phylogenetic “sister species” and that can occur together (sympatric), the documentation of the morphometric or meristic counts used to make the identification is encouraged.

Sample Retention

Sample retention allows for both short-term and long-term quality assurance and quality control (QA/QC) of data. In the short-term, samples can be retained for independent verification or for inclusion into a teaching/reference collection. Sample retention can also allow for long-term QA/QC of data and can be incorporated into museum records, usually at a State or Federal institution (Table 3).

Table 3. Contact information for various fish retention organizations. Contact information subject to change.

Organization	Contact	Address
Penn State University Fish Museum	Dr. Jay R. Stauffer, Jr.	432 Forest Resources Building, University Park, PA 16802
Pennsylvania Fish and Boat Commission	Doug Fischer	PFBC Centre Region Office , 595 E. Rolling Ridge Dr., Bellefonte, PA 16823
Cornell University Museum of Vertebrates	cmd7@cornell.edu	159 Sapsucker Woods Road, Ithaca, NY 14850-1923 USA
The Academy of Natural Sciences of Drexel University	http://www.ansp.org/about/staff/contacts	1900 Benjamin Franklin Parkway, Philadelphia, PA 19103
ORSANCO	Jeff Thomas	5735 Kellogg Avenue Cincinnati, Ohio 45230
National Museum of Natural History	Dr. Jeffrey T. Williams	Div. of Fishes, Museum Support Center MRC-534 4210 Silver Hill Road Suitland, MD 20746
Ohio State University	Marc Kibbey	123 Derby Hall, Columbus OH, 43210

To fulfil the requirements of this protocol, a minimum of one “Site Composite Voucher” is needed for every site, and a second “Audit Voucher” may also be needed as described below.

Important Note- *All chemicals that are integral to the preservation and long-term storage of fish vouchers should be treated as hazardous. Combustible materials used for equipment (e.g., gasoline) should also be considered hazardous. Chemical specific MSDS forms should be referenced and be readily accessible while conducting fish surveys.*

- **Site Composite Vouchers-** A site voucher is for verifying species spatiotemporal occurrence at a known site. A site voucher is comprised of a minimum of one individual representing each of the lowest taxonomic units identified in the sample. At sites with multiple reaches (e.g., nonwadeable) only one site voucher is needed, but should represent the lowest taxonomic units combined across all reaches. Not all species will necessarily need to be vouchered. Larger-bodied fishes, gamefish, or generally common species may be excluded if desired. These vouchers are labeled using waterproof, chemical-resistant paper (placed **inside** the jar) with a minimum of:
    - **Site-** Waterbody and location
    - **Date-** Either Date/Time or a DEP GIS key in the form of yyyymmdd-HHMM-Collector (Collector = Initial of first name and full last name “JSmith”)
    - **GPS Location-** Latitude, Longitude
    - **Type-** “Site Composite”
    - **Taxa list-** The taxa list should mirror the completed field and lab compilation and should include the number of specimens vouchered out of the total number identified (e.g., *Moxostoma sp*.- 5 of 5, *Percina maculata*- 1 of 11, *Micropterus dolomieu*- 0 of 25).
- **Audit Vouchers-** An audit voucher is for QA/QC of the taxonomic identification and enumeration. <u>An audit voucher is comprised of all individuals identified and enumerated at the lab</u>. All fishes identified in the lab are segregated into distinct containers (or separated within a few containers by a permeable membrane) by their respective lowest taxonomic unit and are shared with the DEP auditor. These vouchers are labeled using waterproof, chemical-resistant paper (placed **inside** the jar) with a minimum of:
    - **Site-** Waterbody and location
    - **Date-** Either Date/Time or a DEP GIS key in the form of yyyymmdd-HHMM-Collector (Collector = Initial of first name and full last name “JSmith”)
    - **GPS Location-** Latitude, Longitude
    - **Type-** “Audit Sample”
    - **Container #-** “__ of __”
    - **Species-** Lowest taxonomic unit(s) contained in the jar (enumeration is **not** needed for each species, and is described in detail below).

<u>QUALITY CONTROL</u>

As part of the collection framework and processes, QA/QC procedures need to be considered for each protocol described in this chapter. Sampling QA/QC requirements are described in Appendix E. Additionally, audits will be performed to maintain consistent application of these protocols. This will enhance the precision and accuracy of assessments. The project coordinator (DEP Quality Assurance Officer), will perform audits on both the field and laboratory data collection before these data are entered in the DEP database. For field data collection, a minimum of one audit for each protocol (e.g., wadeable and nonwadeable) will be conducted for each crew leader. For laboratory data collection, a minimum of one (but possibly as high as 10% of samples per year) “Audit Sample” will be required for each taxonomic expert.

Important Note*- It is understood that there may be some inherent subjectivity in defining the “lowest taxonomic unit”, as this is likely to vary between taxonomic experts. The goal of the laboratory audit is to make these data as consistent as possible, across the range of expertise. To this end, the project coordinator may require multiple audit samples before this consistency can be achieved. The auditor may have additional taxonomic experts perform the laboratory audit to increase consistency. Final determinations of similarity and consistency within the laboratory audits will be made by DEP.*

Original, signed audit forms will be retained by the auditor and copies will be sent to the auditee for record. Audit forms for field and laboratory data collection are available upon request.

ELECTROFISHING STRATEGY

The efficiency of electrofishing is largely determined by the user’s knowledge of the equipment. Due to the vast amount of gear types and manufacturers available, it is imperative that the user is familiar with the gear and the manufacturers recommendations specific to each unit. Here, only general guidelines are outlined to standardize capture efficiency. The nature of this protocol inherently targets fishes of all shapes, sizes, and body forms from the smallest of minnows to the largest catfishes. As body size and shape varies, so does the transfer of electricity. As size increases susceptibility to the electrical field also increases. Adjustments of waveform (AC, DC, pulsed DC), frequency (pulses per second or hertz, Hz) and duty cycle (percent “on” for a pulsed current) can all have profound effects on the electrofishing efficiency. For optimal efficiency and consistency waveform, frequency, and duty cycle must be standardized (Table 4). Environmental conditions, most notably specific conductance, may require a deviation from the standard waveform from PDC to AC. Using AC is only advised when specific conductance is very low, and PDC is not able to produce the desired response.

Table 4. Standardized electrofishing settings

Setting Category	Required Setting
Waveform	Pulsed Direct Current (PDC)
Frequency	60 Hz
Duty Cycle	25%

The desired response from fish to the electrical field is immobilization with minimal injury. By standardizing waveform, frequency and duty cycle, the output wattage (amps x volts) is the remaining adjustment needed to achieve the desired response. Adjusting the wattage is typically done by adjusting voltage (refer to the manufacturers specifications for wattage adjustments, as many of these adjustments vary across units). Testing for the desired response should be done prior to beginning the survey, outside of the survey area. To this end, output wattage is set to minimum and gradually increased until the desired response from fish is observed. It is best to use small-bodied fish for the observed response, as they are generally less susceptible to the electrical field. A quick reference guide to electrofishing adjustment is in Appendix A.3.

DEP recognizes that electrofishing is an inherently hazardous activity for which safety is a primary concern. The environmental conditions under which these operations are conducted further increase the risks. Due to these important concerns for electrofishing safety, all field team members should be familiar with electrofishing techniques, safety precautions, and equipment manufacturer's operating procedures. It is the responsibility of the project coordinator (or crew leader) to verify that crewmembers have a basic understanding of these principles prior to sampling. Specific safety requirements and considerations are further described in Appendix F, Biosecurity and Safety Procedures.

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3.8 FISH TISSUE DATA COLLECTION PROTOCOL

Chapter 3 Biological Data Collection Protocols

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INTRODUCTION

Routine monitoring for contaminants in fish tissue was initiated in 1979 as part of a nationwide network of stations encouraged by the U.S. Environmental Protection Agency (USEPA). In 1983, Pennsylvania decided the fish tissue sampling program should be designed to support public health protection. The USEPA outline for the nationwide program included a list of parameters for analysis in fish, including PCBs, pesticides, and selected heavy metals. This parameter list is used in Pennsylvania's fish tissue sampling program. The importance of the fish tissue sampling and advisory issuance program was fully recognized in May 1986 with the signing of an interagency agreement between the Department of Environmental Resources (now DEP), the Pennsylvania Department of Health (DOH), and the Pennsylvania Fish and Boat Commission (PFBC). This agreement was developed because the agencies desired to pursue a systematic approach for the detection and evaluation of fish tissue contamination and to develop procedures for informing the public that may consume such fish of possible adverse health impacts. The agreement listed the responsibilities of each agency and provided for the "timely joint issuance of a health advisory" when fish tissue contamination constituted a health risk. The first joint advisory was issued in June 1986 and included several waters throughout Pennsylvania. Fish tissue monitoring continues to this day for 303(d) impairment listings and the protection of human health through tiered meal advisories.

SAMPLING CONSIDERATIONS

Site Selection

It is important to understand fish tissue evaluations when making site selection as this is an important component of fish tissue monitoring. To facilitate this understanding, the term **site** is a monitoring term for a general area at a local scale and **delineation** is the area within the site that's being evaluated. Herein, a **sample** is collected at a site that will be within a previously assessed delineation, unless of course, it is the first sample. This relationship is important to understand because site selection is largely based on knowledge of previous assessments and their delineations as established in the Assessment Book (Shull and Whiteash 2023). The priority list for determining fish tissue sampling sites is provided in Table 1.

Table 1. Site selection priority descriptions.

PRIORITY	DESCRIPTION
1	Previously unassessed waterbody, specifically those with questionable water quality and/or elevated angling pressure
2	Previously assessed delineation, needs a verification (resample) to change a current advisory
3	Rotations, WQN stations = 5YR, Large River Stations = 2YR
4	Follow-ups, existing advisories based on historic data collections

Once a site has been prioritized, a sampling location must be identified. Sampling locations should be determined by access feasibility and must also be representative of the site. For example, if a

tributary that flows into a large river has been prioritized as a site, and the current assessment delineation is the entire sub-basin, the sample should not be collected at the mouth where riverine fish may frequently reside. In this case, samples should be collected from angler-use areas further upstream that are more representative of the site.

DATA COLLECTION

Once the site and representative sample location are determined, the next step is to determine what gear is needed to survey the area. The most common protocol is by electrofishing. Electrofishing gear should be suited to the size of the waterbody at the site and should be capable of efficient capture. While electrofishing remains the most common, seines, gill nets, trotlines, and angling may be more suitable in certain situations. Gear and nets should be kept clean and in good working condition to avoid dirt, oil, and grease from contaminating the sample.

Species are targeted by their recreational and/or consumptive importance. Generally, the most common game fishes, that are of legal harvest size, are targeted as they are the most likely to be consumed. In trout streams, samples should be wild fish or hold-overs greater than, or equal to, seven inches. The targeted size of the fish within a sample is an important component to the assessment of the data. Fish tend to bioaccumulate contaminants so older, large-bodied fish will typically have the highest contamination level. With only a few exceptions, a sample is a composite of fillets from three to five individual fish representing the same species, that are similar in size. The 75% rule is described on the back of the fish tissue field data sheet (Appendix A.4), and states that the smallest fish within a sample should be 75% the length of the largest fish within the sample. Once enough fish are collected at a site to satisfy the targeted priorities, sample preparation can begin.

If collecting a QA/QC sample, an additional three to five individual fish representing the same species, that are similar in size should be collected during the effort to be submitted as a replicate. Additional details regarding QA/QC sampling are found in Appendix E.

Field Sample Preparation

Individual fish within a sample are measured for length to the nearest tenth of an inch and weighed to the nearest ounce. A health inspection is visually conducted for Deformities, Erosions, Lesions, Tumors and Parasites (DELTP’s) using the anomaly reference guide located on the back of the field data sheet. If multiple samples are collected at the same site, the samples should be processed separately and on clean restaurant-grade aluminum foil (dull side always contacting the sample). Latex or nitrile gloves are recommended during sample preparation and should be changed between samples. Before filet preparation, the filet knife is washed and rinsed with purified hexane labeled as suitable for pesticide residue analysis. Filet preparation will vary slightly depending on the species, to mimic the most common preparation techniques for consumption by anglers. For American Eels, five one-inch cross sections are removed from a skinned and eviscerated individual. For catfishes, skinless filets are removed from each individual. For typical scaled fishes, the scales are removed and the skin remains on the filet. Two filets are usually taken from all species; however, for eels, the

sample then contains six to ten filets. If the sample is comprised of large-bodied fishes (>12in), only one filet from each fish should be included. Once a sample is filleted it is wrapped in aluminum foil and taped. To avoid processing errors, the sample needs to be labeled with a minimum of Station Name, Waterbody, Location, Date and Time, Species, Collector Number, and Sequence Number information. The wrapped and labeled sample can then be placed in a Ziploc® bag and kept on ice in a cooler until they can be transported to a deep freezer. It is helpful to double check field sheets for completeness and consistency with the labeled sample before leaving the sampling site.

DATA MANAGEMENT

Field data should be entered into the Sample Information System (SIS) database as soon as possible after the sample has been collected. General SIS data entry procedures are described in *Chapter 4.6, Sample Information System (SIS) Data Entry Protocol* (Shull 2017). Fish tissue data entry is similar to most SIS data entry, with only a few unique differences described in Figures 1-3.

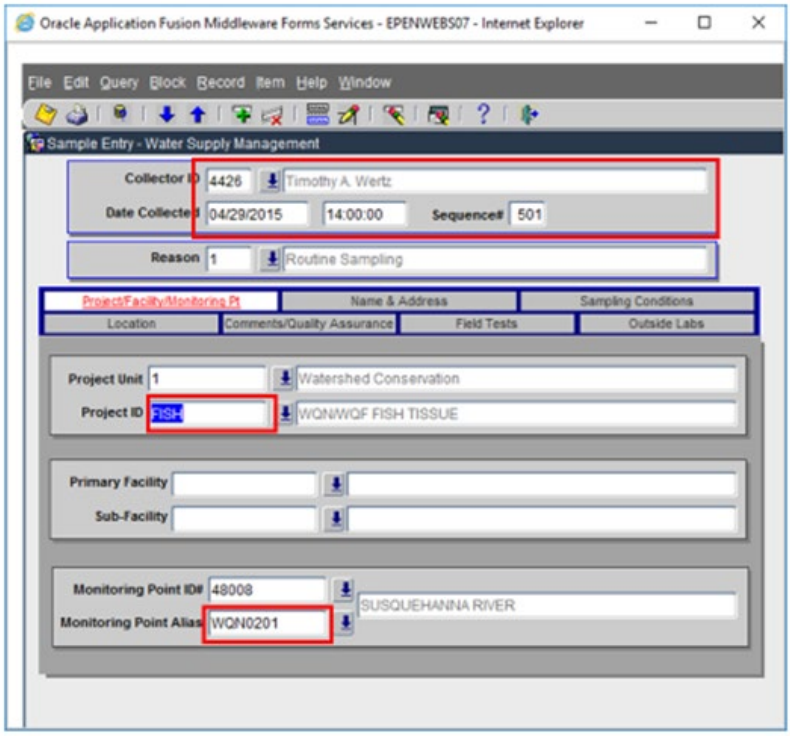

Figure 1. Important SIS data entry blocks for Project/Facility data.

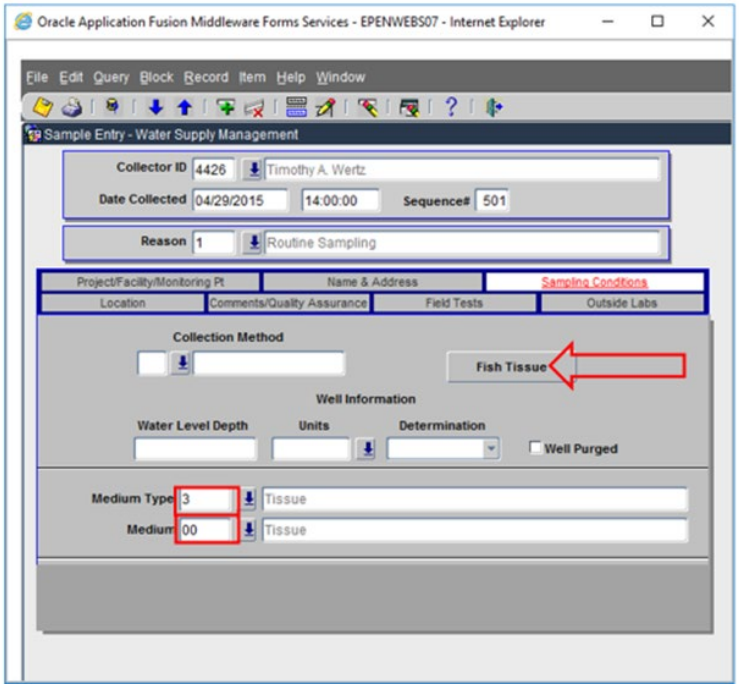

Figure 2. Important SIS data entry blocks and fish tissue button (red arrow), for Sampling Conditions data.

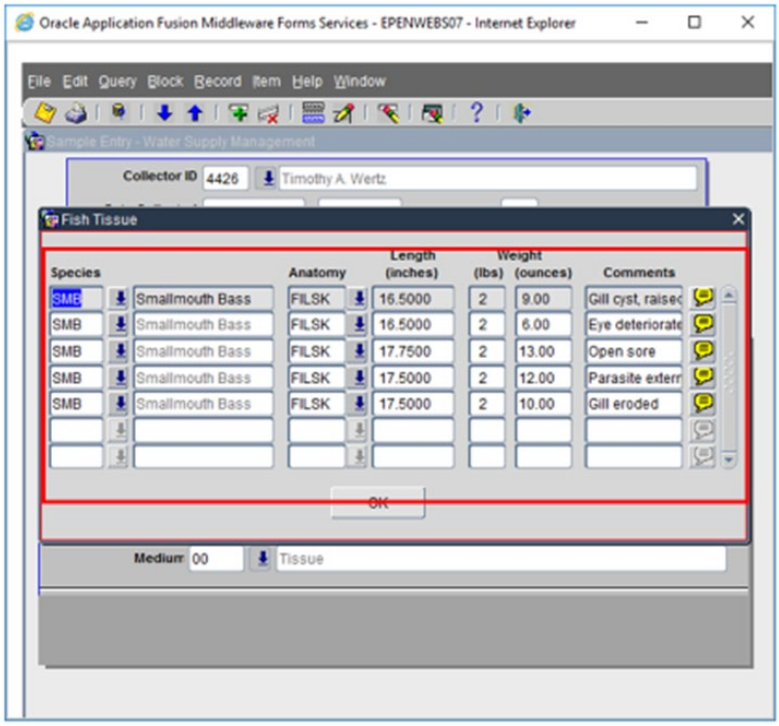

Figure 3. Important SIS data entry blocks for Fish Tissue data.

All fish tissue data are tied to the project FISH in SIS to maintain a segregated dataset for monitoring and assessment purposes. The sampling locations under the project FISH are either WQN stations or

WQF stations. If a new location has been sampled, the DEP fish tissue coordinator should be advised and can add the new location information.

SAMPLE SUBMISSION

Before samples can be delivered to the Bureau of Laboratories (BOL) for analysis, a laboratory sample submission sheet must be completed for each sample with the collection information and SAC needed. BOL Sample Submission Sheets (SSS) can be found at the e-library website (Forms – Laboratories). Collectors are to make a note that samples are “Fish Tissue Samples” in the additional information section of the laboratory sample submission sheet. A list of common fish tissue Standard Analysis Codes (SACs) are listed in Tables 2-4. SACs should be chosen based on previous results from the same species (of the same length group if there is a size range description) from a delineated assessment. If the sample is the first sample at a location, or if there is reason to believe the fish are subject to contamination, all reasonable SACs should be requested.

Table 2. SAC 059 (Metals)

Test Code	Test Description
71930	Mercury in fish, wet weight****
71936	Lead in fish, wet weight
71937	Copper in fish, wet weight
71939	Chromium in fish, wet weight
71940	Cadmium in fish, wet weight
71945	Selenium in fish, wet weight
71946	Ba in fish, wet weight
71947	SR in Fish, Wet weight
99014G	Fish Preparation and Grinding

Table 3. SAC PESTF (Pesticides in Fish Tissue)

CAS Number	Analyte (Test) Description
1024573	Heptachlor Epoxide
2385855	Mirex
26880488	Oxychlordane
309002	Aldrin
319846	Alpha-BHC
3424826	O,P-Dde
3734483	Chlordene
39765805	Trans-Nonachlor
50293	4,4'-Ddt
5103719	Alpha-Chlordane
5103731	Cis-Nonachlor
5103742	Gamma-Chlordane
53190	O,P-Ddd

Table 3 (cont). SAC PESTF (Pesticides in Fish Tissue)

CAS Number	Analyte (Test) Description
58899	gamma-BHC (Lindane)
60571	Dieldrin
72208	Endrin
72435	Methoxychlor
72548	4,4'-Ddd
72559	4,4'-DDE
76448	Heptachlor
789026	O,P-Ddt

Table 4. SAC PCBF (PCBs in Fish Tissue)

CAS Number	Analyte (Test) Description
	Lipids
	EXTRACTED DATE
11096825	Arochlor 1260
11097691	Arochlor 1254
11104282	Arochlor 1221
11141165	Arochlor 1232
12672296	Arochlor 1248
53469219	Arochlor 1242

Before shipping the sample to the lab for analysis, the lab SSSt as well as the field data sheets should be scanned and forwarded to the DEP fish tissue data coordinator and the BOL fish tissue data representative. The estimated shipment/receipt date should be described to ensure lab representatives will be available to receive the frozen samples upon arrival. Once a shipment/receipt date has been confirmed the samples are ready to send. **Completely frozen** samples that are of considerable mass can be placed in a cooler without additional ice and couriered to the BOL. Small and medium samples should always be shipped on dry ice. The completed sample submission sheet should be placed in a separate Ziploc® bag and placed inside the cooler with the sample. To ensure adequate time for lab preparation, analysis and quality control of the samples, all samples should be shipped to the lab by November 1st of the collection-year.

LABORATORY PROCEDURES

Once the sample has been received and inventoried at the BOL, the samples will need to be prepared for analysis. The processing equipment is washed and then rinsed with purified hexane labeled as suitable for pesticide residue analysis. Clean, restaurant-grade aluminum foil (dull side contacting the sample) should be placed on all surfaces the sample contacts. Each sample is ground in a meat grinder until all fillets are considered homogenous (large samples may need to be ground

multiple times). The sample is then subsampled into four 30-gram aliquots that will be used for the analysis of each SAC, with a backup for reanalysis or archival as desired. The aliquots are wrapped in foil, pressed to form a patty, labeled with sample number, and refrozen for analysis. Equipment cleaning, hexane rinses, and foil changes must be completed before the next sample can be processed. All samples should be processed for lab analysis by December 31st of the collection-year.

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3.9 MUSSEL DATA COLLECTION PROTOCOL

Chapter 3 Biological Data Collection Protocols

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INTRODUCTION

The Department's (DEP) freshwater mussel data collection protocol enables sampling in reaches where fishes and benthic macroinvertebrates could be sampled simultaneously to provide a comprehensive review of the aquatic biological community. To adequately evaluate the freshwater mussel population, two mussel sampling types were developed. The 12-dive sampling is composed of 12 five-minute dives along linear transects that are randomly spaced from both the downstream point and the shoreline within a 500-meter reach. The objective of this sampling is to build Pennsylvania's capacity to assess conditions of large rivers by providing a foundation for the development of large river assessment methods. The transect sampling type is a means to target National Pollutant Discharge Elimination System (NPDES) point-source discharges and non-point source inputs. It is composed of 16 five-minute dives within each 100-meter reach. Dives are positioned at 25-meter intervals downstream of the outfall or input. The objective of this sampling is to target pollution inputs in medium size streams to larger rivers and compare them with upstream non-impacted reaches.

Both mussel data collection sampling types then enumerate several aspects of a mussel community. All live mussels are identified to species and individuals are counted to obtain a representative description of the community in terms of relative abundance and species richness. Size measurements such as length, width, and depth can be used to approximate age classes of each species within the population. Additionally, this protocol can be used as a rapid and cost-effective way of searching for rare, threatened, endangered, and invasive mussels and evaluate population differences above and below a pollution input. Protocols such as the ones described by Smith (2006) and Clayton et al. (2013) are also recommended for this purpose.

SAMPLING CONSIDERATIONS

DEP recognizes the need to structure and oversee this activity due to the inherent hazards involved in underwater diving. Due to these important concerns for diving safety, all dive team members must be trained in mussel sampling techniques, safety precautions, and equipment manufacturers' operating procedures. Detailed safety requirements and considerations are found in Appendix F, Biosecurity and Safety Procedures. In addition, all scientific divers must have completed, at minimum, an approved SCUBA Certification Course, and have adequate training to conduct mussel surveys. It is the responsibility of the Unit Diving Officer (UDO) to verify that the team members have the appropriate training. Specific team member duties should be reviewed during each sampling event.

The safety of all personnel and the quality of the data are assured through the adequate education, training, and experience of all members of the scientific diving team. At least one UDO must be involved in each sampling event.

The safety standards which will apply and will be followed for all DEP operations are derived from the U.S. Environmental Protection Agency's (USEPA) *Diving Safety Manual Revision 1.2*, (DSM, USEPA

2010b, USEPA 2010a) and can be found in Appendix F. Additional information is found in DEP's Policy for Scientific Diving Personnel and Diving Operations (DEP 2012). Diving **with Nitrox** will be conducted in accordance with the National Oceanic and Atmospheric Administration (NOAA) Nitrox Tables for No-Decompression Limits and Repetitive Group Designation Table for No-Decompression Dives (NOAA 2016).

DATA COLLECTION

Survey Crew and Equipment

A survey crew (or dive team) consists of at least three personnel; the UDO, diver, and stand-by diver. However, more efficient crews should have one to two dive tenders. The number of crew members required will depend on the depth and type of diving at the site. For example, some sites may be surveyed by snorkel and mask, requiring fewer crew members, whereas diving to depths greater than 30 feet or in low visibility would require more personnel to safely conduct the survey. The types of dives and the number of members required are described in greater detail in the safety plan (Appendix F).

Equipment needs will greatly depend on weather conditions and the sampling environment. Most mussel surveys will be conducted from a boat. The boat needs to be large enough to accommodate 3 to 5 crew members and approximately 200 lbs. of equipment. In addition to the standard boat safety equipment, a dive flag should be added to the inventory. Weather conditions and water conditions will dictate what type of diving equipment will be necessary to conduct the survey. Survey sites are identified using a Global Positioning System (GPS) receiver and laser rangefinder. It can be more efficient to mark each location with a weighted buoy before dives are started. Equipment needed during dives and mussel processing includes numbered mesh bags, buckets, a camera, calipers, and a scale. A checklist of required and suggested equipment is provided below:

Dive Equipment
- ☐ Masks
- ☐ Snorkels
- ☐ Wetsuits
- ☐ Gloves
- ☐ Boots
- ☐ Hoods (If Applicable)
- ☐ Fins (If Applicable)
- ☐ Buoyancy Control Devices (BCDs)
- ☐ Regulators, Octos And Hoses
- ☐ Air Tanks
- ☐ Dive Weights
- ☐ Communication Gear
- ☐ Dive Flags
- ☐ Dive Knives
- ☐ Dive Lights
- ☐ First Aid Kit
- ☐ Emergency Oxygen Kit
- ☐ Other: _____

Field Meters & Related Supplies
- ☐ Dissolved Oxygen Meter
  - ☐ Replacement Membrane Kits
  - ☐ Do Probe Solution
  - ☐ Zero % Calibrating Solution (If Applicable)
- ☐ Ph Meter
  - ☐ Buffers (Ph 4, 7, 10)
  - ☐ KCl Probe Solution
- ☐ Conductivity Meter
  - ☐ Calibrating Solution (If Applicable)
- ☐ Thermometer
- ☐ Meter Field Manuals (If Applicable)
- ☐ Secchi Disk
- ☐ Other: _____

Mussel Sampling Equipment
- ☐ GPS
- ☐ Laser Rangefinder
- ☐ Numbered Mesh Bags
- ☐ Bucket
- ☐ Calipers (Mm)
- ☐ Scale (Grams)
- ☐ Camera
- ☐ Other: _____

Forms
- ☐ Field Survey Sheets
- ☐ Mussel Data Sheets
- ☐ Other: _____

Misc.
- ☐ Maps
- ☐ Markers, Pens, & Pencils
- ☐ Calculator
- ☐ Insect Repellent
- ☐ Sunscreen
- ☐ Screwdriver/Tools
- ☐ Batteries (D-Cell, Other: _____)
- ☐ Other: _____

Mussel Collection Procedure

12-Dive Sampling

Survey locations are typically situated at or near fish and macroinvertebrate sample sites and are along the left bank, right bank, or island shore. At each survey location, dives are conducted at 12 randomly selected sites in a 500-meter by 50-meter reach (Figure 1). The 12 sites will be selected to proportionally reflect shallow and deep-water habitats. This is supported by previous work by DEP showing distinct shallow and deep-water mussel communities. A minimum of four shallow water sites will be selected at each sample site to ensure adequate coverage of this habitat type. A crew member will use a GPS to identify the downstream point of the survey location. This site is the “X” site in the field survey sheet (Appendix A.5). A range finder will be used to determine the location of each of the 12 sample locations by measuring the distance from the shore and the distance from the “X” site. Once at a site, a Latitude and Longitude is taken using a GPS, and the stand-by diver will deploy the anchor.

In deeper diving conditions or in low visibility, the diver and stand-by diver will dive together and at least one of those individuals will have communications with the surface. In shallow diving conditions or if the diver is visible from the surface the stand-by diver may stay on the surface but will maintain auditory or tactile contact with the diver. In both shallow and deeper dives, the diver will record an upstream compass heading while onboard for underwater orientation. The diver will also be equipped with a 250-foot dive reel that is marked in 1-meter increments. The UDO will perform a safety check using communication gear. The diver will descend along the anchor line to the bottom of the river with the stand-by diver and clip the dive reel to the anchor.

At the beginning of the survey the diver may collect a bucket excavation sample to check for the presence of mussels in deeper sediment. The diver will communicate to the stand-by diver that the time interval should begin. The stand-by diver will keep time. For five minutes, the diver will travel upstream searching (visual and tactile) a one-meter wide area and collect live mussels and dead mussel shells in the search area. The diver will search under any large rocks that can easily be turned. The diver will also sweep across the top of the fine substrate to feel for mussels which may be partially buried. The sampling protocol recommends a surface search only; subsurface searching will not be conducted. At the end of five minutes, the stand-by diver will call to the diver via communication equipment or other diver recall system to conclude the search. While returning to the boat or shore, the diver will record or communicate the total distance traveled in meters by counting the number of marked increments on the dive reel. Distance/area searched will vary based on visibility, mussel density, and habitat. All live mussels and shell material collected for each dive are placed in a numbered bag corresponding to each dive and given to a crew member for processing.

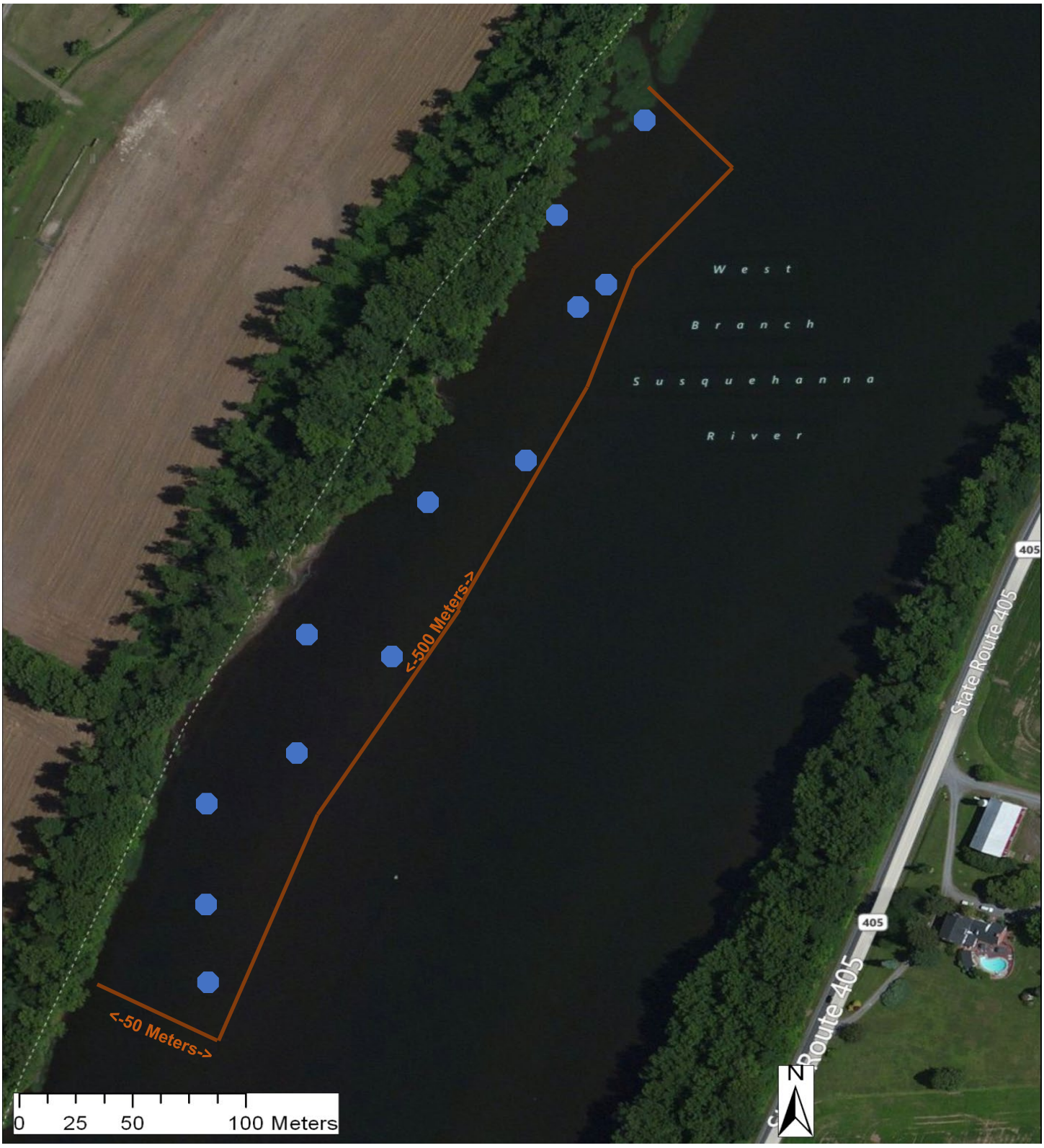

Figure 1. Twelve randomly selected sites (blue dots) within a 500 x 50 m sampling location.

Transect Sampling

Transect sampling will have 16 targeted five-minute dives per station (Figure 2). Each station occurs within a 100-meter reach. Station selection will occur by selecting an upstream or control reach that is above any potential pollution input or NPDES outfall. A second station is then selected at a downstream location below any potential pollution input or NPDES outfall. Additional 100-meter reaches can be added both upstream and downstream of these stations based on the need for examining a broader reach of the freshwater community. The targeted dives were arranged in four dive increments that were equally spaced from a point mid-channel across the river to either the left or right descending bank at each station. Each four-dive set is replicated four times separated by 25-meters from the previous set of dives. For each dive, the diver records an upstream compass heading, then enters the water with scuba or snorkel gear, and dives down to the substrate. The diver proceeds in an upstream direction for five minutes looking for mussels by searching (both visual and tactile) in one meter wide transects. The diver will search under any large rocks that can be easily turned. The diver also moves their hands across the top of the fine substrate to feel for mussels that may be partially buried. The survey includes a surface search only, subsurface searching is not conducted. For shallow water sites snorkeling will be used as the preferred method over diving. Additional targeted dives may be added within the outfall plume to examine the proximity of live freshwater mussels to the pollution input. All live mussels and shell material collected for each dive are placed in a numbered bag corresponding to each dive and given to a crew member for processing.

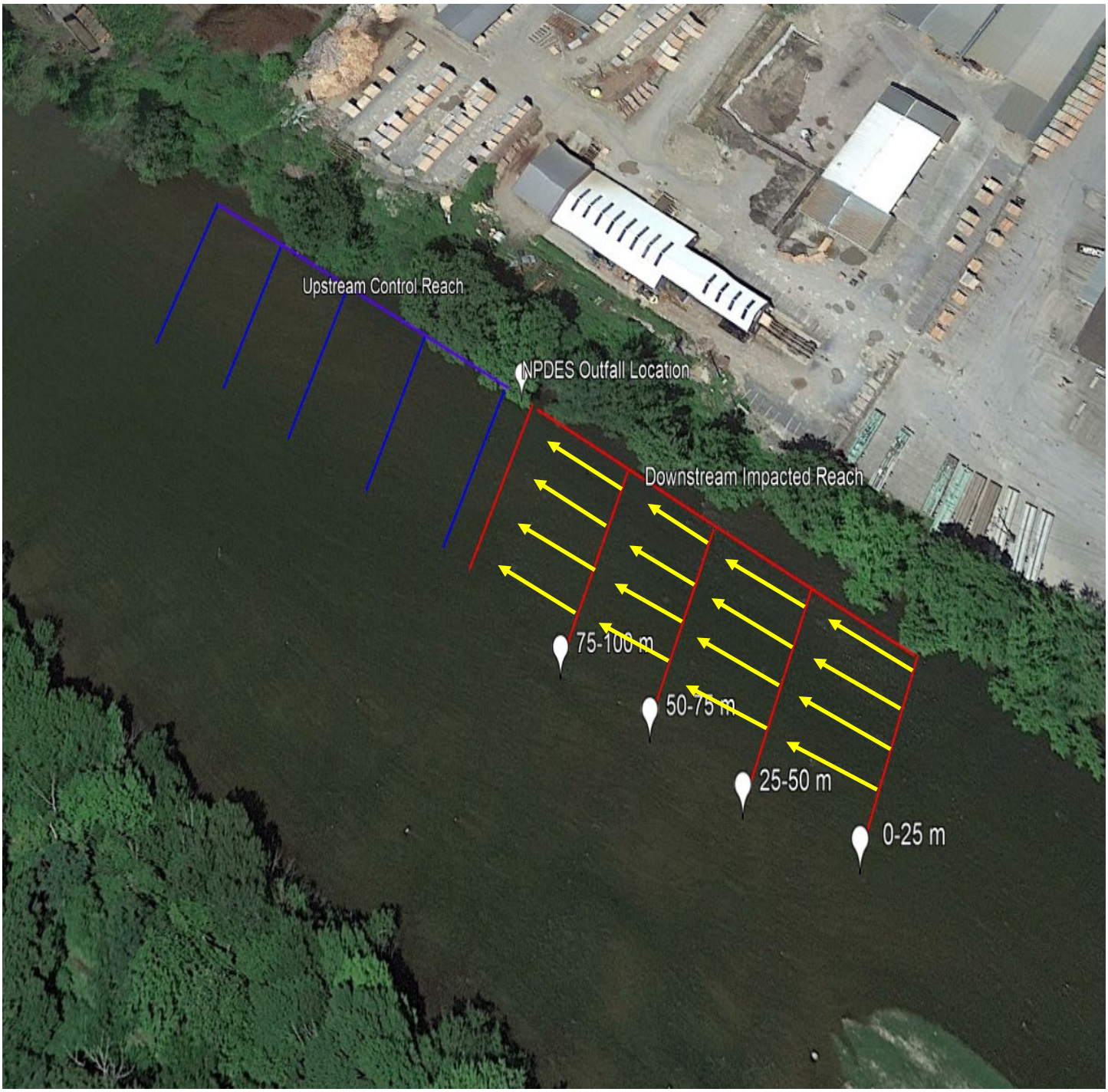

Figure 2. Depiction of sixteen targeted dives per station (yellow arrows) within a 100 x 50 m sampling location for Cause and Effect freshwater mussel sampling surveys.

Mussel Processing Procedure

Mussel processing will be the same for both 12-dive sampling and transect sampling. At the conclusion of each individual dive the diver will, either on a wrist dive slate or verbally, record or communicate the substrate composition percentages for silt, sand, gravel, cobble, boulder, and bedrock. Invasive mollusks (Zebra mussels, Corbicula, etc.) will be scored: 0 = no invasive mollusks; 1 = 1 individual; 2 = multiple individuals; 3 = small clumps of individuals; 4 = greater than 50% coverage; 5 = complete coverage of available substrate. The average depth of the searched area will be recorded. A crew member will use a cleaned and calibrated water quality field meter to record temperature, pH, dissolved oxygen, percent dissolved oxygen, turbidity, and specific conductivity according to *Chapter 4.1, In-Situ Field Meter, Transect, and Vertical Profile Data Collection Protocol* (Hoger and Arnold 2023). Water chemistry measurements will be recorded at each station at a minimum. All live mussels are identified to species level on-site. Some representatives may be taken back to the laboratory for verification if a photograph is insufficient (State and/or Federal permits required). Live mussels are then weighed (in grams) on a scale, measured (length, width, and depth in millimeters) using a caliper, photographed, and returned to the water in suitable habitat. Measurements of length, width, and depth are described in Figure 3. When returning live mussels, care should be taken to orient the mussel posterior side up. All mussel data are recorded on mussel data sheets provided in Appendix A.5. Mussel shells will be identified if both valves are present and noted as “dead” on the data sheet. Mussels and shells found near the sampling sites can be identified, measured if live, and recorded on mussel data sheets, noted as “incidental.”

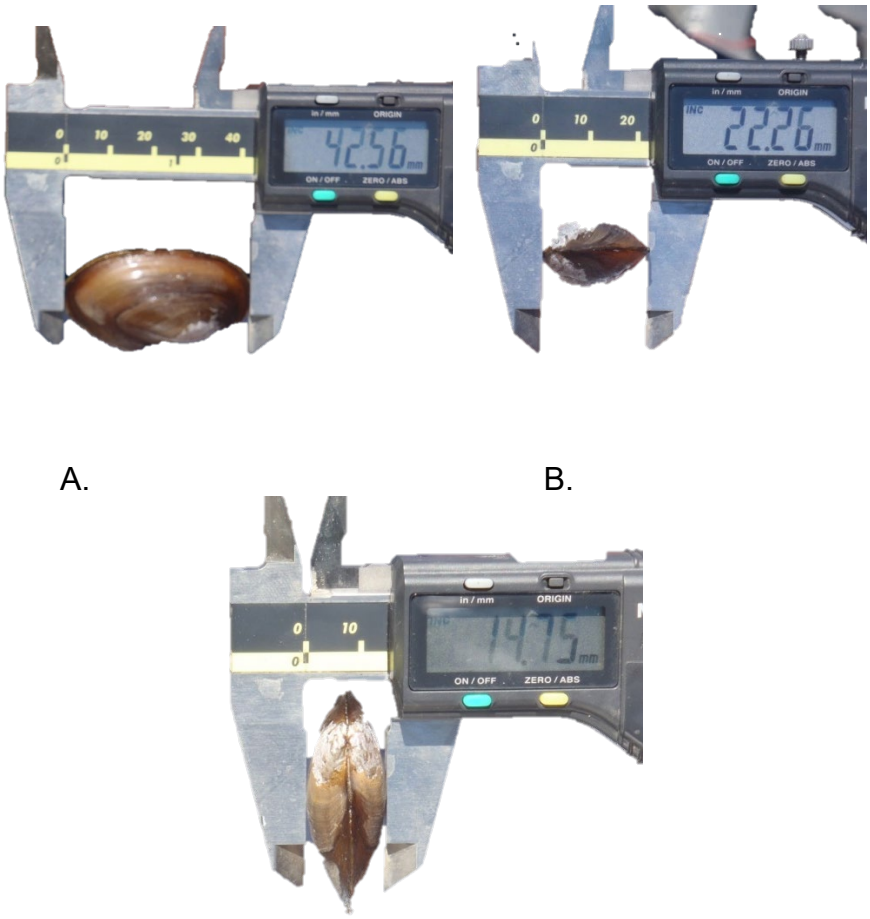

Figure 3. A. Measurement of length is the maximum distanced between the anterior and posterior margins. B. Measurement of width is the maximum distance between dorsal and ventral margins. C. Measurement of depth is the maximum distance between left and right valves (shell halves).

Quality Assurance / Quality Control

Since mussel surveys take a significant amount of time and require many moving parts, these surveys require one site per season per crew be QC'd. Additional QA/QC requirements are detailed in Appendix E.

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3.10 PERIPHYTON DATA COLLECTION PROTOCOL

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INTRODUCTION

Characterizing lotic benthic algae communities is an important tool used by DEP in determining the quality of its natural flowing waters. This is because, as is pointed out by authors R. Jan Stevenson and Loren L Bahls,

> *Benthic algae (periphyton or phytobenthos) are primary producers and an important foundation of many stream food webs. These organisms also stabilize substrata and serve as habitat for many other organisms. Because benthic algal assemblages are attached to substrate, their characteristics are affected by physical, chemical, and biological disturbances that occur in the stream reach during the time which the assemblage developed* (Barbour et al. 1999).

In short, benthic algae and its analysis provides important information concerning the condition of streams and rivers.

Algae, in this document, is a term used to describe a group of aquatic organisms that do not represent a single formal taxonomic group. Rather, algae are defined as, in the words of authors John D. Wehr and Robert G. Sheath, "… *a loose group of organisms that*… [are] *aquatic, photosynthetic*, [and with] *simple vegetative structures without a vascular system*…" (Wehr and Sheath 2003). Lotic periphyton includes several major taxonomic groups which, in Pennsylvania, are dominated by cyanobacteria, diatoms, and green algae.

Benthic algae, in lotic systems, is collected by DEP as part of its on-going monitoring responsibilities needed to evaluate protection of surface water quality. Certain benthic algae organisms, such as diatoms, are very useful because they have a very strong relationship with nutrient concentrations and have been used as trophic indicators (Potapova and Charles 2007, Porter et al. 2008, Hausmann et al. 2016). Consequentially, collecting algae samples provides DEP with a tool that facilitates the determination of trophic status and evaluate protection of surface water quality of rivers and streams.

DEP biologists currently use two data collection procedures to collect periphyton from lotic systems. These procedures are:

- Qualitative Multi-Habitat (QMH) Periphyton Data Collection Procedures
- Quantitative Benthic Epilithic (QBE) Periphyton Data Collection Procedures

The field procedures employed to collect both the QMH Periphyton and the QBE Periphyton samples are appreciably derived from those described in the U.S. Geological Survey (USGS) document *Revised Protocols for Sampling Algal, Invertebrate, and Fish Communities* as *Part of the National Water-Quality Assessment Program* (USGS OFR 02-150, Moulton et al. 2002). Many of the terms used in this DEP protocol are also found in this USGS protocol and it is there that the reader may refer for definitions of these terms.

This document will also stipulate procedures and requirements that participating laboratories must follow to consistently process and report results for the various subsample types. Quality control practices needed to validate the identification and enumeration of algal, cyanobacteria, and diatom taxa will also be discussed.

DEP biologists also collect water chemistry samples and discrete water parameters with a properly calibrated field meter during algae sampling. Although a variety of chemical analytes are often collected, which frequently depend upon the monitoring needs to serve other objectives, the water chemistry that must be collected to support a comprehensive analysis of the algae community should include total nitrate & nitrite nitrogen, total nitrogen as N, Total Organic Carbon (TOC), total ortho phosphorus as P, total phosphorus as P, dissolved nitrate & nitrite nitrogen, dissolved ortho phosphorus, and dissolved phosphorus as P. Discrete water parameters collected with a field meter should include water temperature, specific conductivity, pH, and dissolved oxygen (both mg/l and % saturation).

This document does not address the data protocols for the collection and processing of water chemistry samples or those for the collection of discrete water chemistry parameters with a field meter. For these protocols, the reader is referred to *Chapter 4.2 Discrete Water Chemistry Data Collection Protocol* (Shull and Arnold 2023).

SAMPLING CONSIDERATIONS

Many different aquatic algae sampling options are available for the biologist to consider. For example, the USGS OFR 02-150 lists four separate data collection protocols. These include three quantitative protocols and one qualitative protocol, each of which are designed to target different aquatic algae habitats. These USGS data collection protocols include:

- Richest Targeted Habitat (RTH) – quantitative, represents a riffle or hard substrate "… where the taxonomically richest algal or invertebrate community is theoretically located."
- Depositional Targeted Habitat (DTH) – quantitative, represents a depositional habitat where fine sediments such as silt and sand accumulate.
- Phytoplankton (PHY) – quantitative, represents the water column habitat.
- Qualitative Multi-Habitat (QMH) – qualitative, whereas each of the three quantitative sampling protocols defined above, in general, targets a single habitat type, this sampling protocol is intended to represent the several different habitats that may be present in a reach.

Currently DEP collects algae samples from lotic waters by using two different procedures. These two procedures include:

- QMH Periphyton Sampling Procedure (Similar to the USGS QMH procedure)
- QBE Periphyton Sampling Procedure (Similar to the USGS RTH procedure).

For each of the sampling procedures described below, every 20th site sampled should be replicated to as part of the data collection quality assurance (QA) and quality control (QC) (Appendix E). In addition, QA/QC procedures described throughout this protocol should be followed accordingly.

The timing of algae sample collection is another important consideration for the biologist. Algae growing in streams and rivers are readily influenced by high flow events, temperature, and sunlight. Consequentially, the question of how long the biologist should wait before sampling a stream or river after a high flow event must be considered. Various researchers have studied the recovery of benthic algae communities following a scouring flow and have made suggestions. For example, Peterson and Stevenson (1990) suggested a three-week postponement in sampling after a scour event to allow for recolonization (Peterson and Stevenson 1990, Barbour 1999). DEP biologists have considered the three-week "wait" period and, based on the experience gained from observing the recovery of many streams in Pennsylvania, as well as considering other logistical issues, have decided to use, in general, a two-week "wait" period to resume sampling after a scour event. However, each stream or river possesses its own scour response to flow which is based upon its own geomorphic characteristic. The biologist must use knowledge of the watershed and best professional judgement, as well as stream gauge data available on the USGS website (available at https://waterwatch.usgs.gov/new/index.php?r=pa&id=ww_current) to make decisions regarding sampling delay after a scour event.

Temperature and sunlight availability also play a major role in the growth of algae in rivers and streams. Typically, DEP biologists will begin sampling benthic algae in the early spring in those streams that will later be shaded by the surrounding forest when leaf-out occurs. Obviously, stream water temperatures are relatively cool in this early spring-time period and will influence the composition of the algae community. Later, biologists may return to the same stream to sample the algae community in mid-summer and/or early fall when the stream is fully shaded and water temperatures are more elevated so that they may characterize an algae community that may differ appreciably from that observed in the spring. Sampling these streams across seasons allows the biologist to better understand the transition in algae community composition that occur with temperature and shading changes.

QMH PERIPHYTON SAMPLING PROCEDURE

The biologist may need to collect a QMH periphyton sample of microalgae and/or macroalgae so that they may better understand the algal community present in any of the multiple habitats available within a stream reach or area but not require area-based (for area-based sampling, refer to the QBE Procedure discussed below) community data, photopigment, or ash-free dry mass (AFDM) measures. This type of sampling aligns, in part, with the USGS OFR 02-150 QMH sampling procedure. Because this procedure may sample many types of algae habitats, there may be a cross over into the sampling situations referred to as DTH (Depositional Targeted Habitats) and RTH (Richest Targeted Habitats) in the USGS OFR 02-150.

This sampling procedure provides DEP biologists with a tool to investigate special monitoring questions. Such questions, for example, may address monitoring concerns related to the habitat of early life phases of fish species or perhaps the algae community composition that may lead to the production of cyanotoxin in non-thalweg stream environments. In these examples, the sample location might be better described as a specific habitat or area, such as a Smallmouth Bass young-of-the-year nursery habitat or that of a small area where cyanobacteria are observed to be growing. Deciding to delineate a sample reach versus a sample area is in part dictated by the question or concern that the DEP biologist is attempting to address. Ultimately, the biologist must characterize the habitat they sample and does so as described below in the data collection section of this protocol.

With this protocol, the biologist should submit a subsample to a laboratory for algae identification and enumeration. Also, the biologist should submit another subsample to a laboratory to test for the presence of cyanotoxins.

This protocol is intended for use in Pennsylvania wadeable riffle-run streams and semiwadeable large rivers. Refer also to *Chapter 3.1, Wadeable Riffle-Run Stream Macroinvertebrate Data Collection Protocol* (Shull 2017b) and *Chapter 3.4, Semiwadeable Large River Macroinvertebrate Data Collection Protocol* (Shull 2017a) for terms and definitions that also apply to this section.

Field Data Sheet

The biologist will also complete the DEP QMH Periphyton Field Data Sheet (Appendix A.7) at each monitoring site. To complete this field data sheet, the biologist must provide information pertaining to the following (refer to the QMH Periphyton Field Data Sheet in Appendix A.7 for more specific requirements for each data record):

- Site/sample information including water chemistry collection information and personnel involved
- Latitude and longitude of the site
- Watershed Area – determined prior to making the field visit
- In-situ Field Meter Values – refer to *Chapter 4.1*, *In-Situ Field Meter, Transect, and Field Meter Data Collection Protocol* (Hoger and Arnold 2023) for field meter use and calibration requirements
- Total algae slurry volume (mL, minimum volume rules apply – refer to Table 1 in the Subsampling Procedure section)
- Densiometer and Compass Readings (refer to the Densiometer and Compass Data Collection section – located near the end of the periphyton protocol - for instructions in using these devices)
- Percent relative qualification of the periphyton habitat
- Notes pertaining to the three Algae ID Bottles collected
- Other notes (including any pertaining to cyanotoxin testing, if collected).

Habitat Types

To collect a QMH periphyton sample the biologist will need to employ several approaches to sample across multiple habitats. The multiple habitats that may be sampled will include any one or more of the following:

- Epilithic (Rock)
- Epiphytic (Plant)
- Epipelic (Silt)
- Epidendritic (Wood)
- Episammic (Sand)
- Gravel (subset of Epilithic material but listed independently here due to difference in sampling protocol).

Data Collection

When sampling across multiple habitats, the biologist should proportionally represent each habitat type in the targeted reach or area when making a composite sample. For example, if after inspecting the targeted stream area, the biologist observes that the multiple habitats present include about 45% Epiphytic, 10% Epipelic, 30% Epidendritic, and 15% Episammic and no rock, cobble, or gravel (these percentages are recorded on the field data sheet in the Periphyton Habitats section), then the final composited algae slurry should be accumulated from a quantity of materials proportionally collected to represent this habitat observation as best as possible. Because this protocol is qualitative in nature the biologist must select sample material in a fashion that, in their professional opinion, best reflects this observed habitat characterization. In other words, based upon the above habitat characterization, the biologist would attempt to sample material surfaces that roughly represents 45% Epiphytic, 10% Epipelic, 30% Epidendritic, and 15% Episammic.

The multiple habitat sampling procedures, by type, will be performed as follows:

- For each sampling procedure described below, use de-ionized water to rinse the scrubbed material into the algae slurry pan. The collector must remain aware of the total amount of rinsate generated for the complete composited sample. In short, the final accumulated algal slurry should be kept to a minimum by conserving rinse water so that the total algae slurry (from all substrate) isn't greater than that needed for lab analysis by a factor of two or three. This is intended to maintain total slurry concentrations that better enable algae identification and enumeration and cyanotoxin tests as well as facilitating handling during sub-sampling homogenization.
- Epilithic (Rock) sampling performed by DEP is restricted to rock that is in the size range of cobble (generally 2.5 to 10 inches) and is thus more easily moved by the biologist. Boulders or bedrock are not readily transportable and are not considered here. Gravel, which is a subset of epilithic habitat, is treated separately in this protocol because it is sampled differently. Cobble sampling is best accomplished by removing cobble substrate from the benthos and transporting it to the bank where the sampled surfaces may be scrubbed by a plastic or steel brush while collecting the rinsate into the algae slurry pan. The sampled surface should include scrubbing all that surface which is exposed to the aquatic environment above the benthos.

- Epiphytic (Plant) sampling is best accomplished by clipping the aquatic plant, using pruning shears or scissors, just above the sediment level (allowing roots to remain in the sediment) and at the water line (retaining only the submerged portion) and transporting the material to the bank where the total plant surface may be scrubbed by a plastic brush. Collect the rinsate from this scrubbing into the algae slurry pan. When scrubbing the plant surface, care should be taken so that plant material is not inadvertently pulled off the plant and collected in the pan. For plants that are less physically robust, such as grasses, the plants may be placed in a capped plastic container, along with some de-ionized water, and agitated vigorously. After agitating, the de-ionized water may be poured into the collection pan and the plant material discarded. On the field data sheet, record notes regarding the plant taxa from which samples were collected.
- Epipelic (Silt) sampling may be accomplished by using a silt/sand/gravel PVC sampler and a kitchen spatula (see Figure 1). The silt/sand/gravel PVC sampler may be constructed from a short section (about 3 or 4-inch length) of PVC pipe (4 or 5-inch diameter) and capped at one end. After placing this PVC sampler in the water, rotate it up-side-down to remove air. Once the air is removed, push the open end of the PVC sampler a few millimeters into the silt. Without moving the PVC sampler, slide the plastic kitchen spatula underneath the PVC sampler to secure the upper layer of silt inside the PVC sampler. Carefully extract the PVC sampler and spatula from the stream without spilling the contents. Pour the contents of the PVC sampler into the collection pan. As with all collecting strategies used for this QMH procedure, the final accumulated algal slurry should be kept to a minimum so that the total algae slurry (from all substrate) isn't greater than that needed for lab analysis by a factor of two or three. Quantities of homogenized algal slurry needed to perform each laboratory test are indicated in Table 1.

Figure 1. Silt/sand/gravel PVC and spatula sampling apparatus. This apparatus may consist of a section of capped 4 or 5-inch diameter PVC pipe and a kitchen spatula. The spatula must be large enough so that when it is slid under the PVC pipe it will prevent the loss of silt, sand, or gravel from the sample as it is pulled from the benthos.

- Epidendritic (Wood) sampling may be best accomplished by collecting submerged wood and transporting it to the bank where the surfaces may be scrubbed with a plastic or metal brush while collecting the rinsate into the algae slurry pan. Use de-ionized water to wash the

scrubbed material into the algae slurry pan. When scrubbing the wood surface, care should be taken so that wood material is not inadvertently pulled off and collected in the pan. The biologist may find it useful to use a small saw to cut portions of larger submerged branches for removal to the scrubbing area. As with all collecting strategies used for this QMH procedure, the final accumulated algal slurry should be kept to a minimum by conserving rinse water so that the total algae slurry (from all substrate) isn't greater than that needed for lab analysis by a factor of two or three. Quantities of homogenized algal slurry needed to perform each laboratory test are indicated in Table 1.

- Episammic (Sand) sampling is accomplished in much the same way as that described for Epipelic (Silt) by using a silt/sand/gravel PVC sampler and a plastic kitchen spatula (see Figure 1). The silt/sand/gravel PVC sampler may be constructed from a short section (about 3 or 4-inch length) of PVC pipe (4 or 5-inch diameter) and capped at one end. After placing this PVC sampler in the water, rotate it up-side-down to remove air. Once the air is removed push the open end of the PVC sampler a few millimeters into the sand. Without moving the PVC sampler, slide the plastic kitchen spatula underneath the PVC sampler to secure the upper layer of sand inside the PVC sampler. Carefully extract the PVC sampler and spatula from the stream without spilling the contents. Pour the contents of the PVC sampler into a capped plastic bottle and agitate vigorously. After allowing the sand to settle to the bottom of the container pour the agitate (with algae entrained) into the collection pan. As with all collecting strategies used for this QMH procedure, the final accumulated algal slurry should be kept to a minimum so that the total algae slurry (from all substrate) isn't greater than that needed for lab analysis by a factor of two or three. Quantities of homogenized algal slurry needed to perform each laboratory test are indicated in Table 1.
- Gravel sampling is accomplished in the same way as that described for Episammic (Sand). Refer to the Episammic (Sand) sampling procedure provided above.

Subsampling Procedure

QMH periphyton samples must always be subsampled for algae identification and enumeration and sometimes, depending on the requirements of the project, cyanotoxin analysis. Subsampling may be performed in the field if electric is available to operate a blender and a magnetic stirrer. If subsampling can't be performed in the field the sample may be temporarily stored in labeled 500mL bottles that are capped and stored in a wet ice cooler to await later treatment.

Note: This is an important step needed to prevent cross-contamination between samples. Prior to performing any subsampling procedures on a site sample, thoroughly clean all equipment that may come in contact with the algae slurry sample and, at the conclusion of this cleaning, rinse with de-ionized water. Because site samples are often accumulated through the field day and subsampled at the conclusion of that day's work, all equipment must be appropriated cleaned between samples.

Table 1. QMH Periphyton Subsample Tests: Homogenized Volume (mL), Bottleware, Preservation, Transportation, and Storage

Subsample Test	Homogenized Volume (mL)	Bottleware	Preservation	Transportation	Storage
Algae ID Bottle #1	100	120mL Plastic	7mL Formaldehyde	Cool, dry, dark	Cool, dry, dark
Algae ID Bottle #2	100	120mL Plastic	7mL Formaldehyde	Cool, dry, dark	Cool, dry, dark
Algae ID Bottle #3	100	120mLPlastic	7mL Formaldehyde	Cool, dry, dark	Cool, dry, dark
Minimum Algae Slurry Volume (plus excess) without Cyanotoxin Test	400				
Cyanotoxin Test	250	Whirl-Pak®	None	Dry-ice	-80°C
Minimum Algae Slurry Volume (plus excess) with Cyanotoxin Test	700				

Subsampling consists of the following steps:

Slurry Preparation

Once all algae slurry is collected, pour the contents of the collection pan into a large plastic beaker (beaker should be large enough to contain at least 2000mL of algae slurry). Dilute the algae slurry with de-ionized water to provide enough material for all subsample tests (refer to Table 1 for minimum quantities required). Since this procedure is intended to be qualitative in nature, sample surface area is not determined and the ID/enumeration laboratory will not report results in the same units as that provided for quantitative samples (see below). Consequentially, the quantity of surface area sampled or quantity of algae slurry collected is not reported to the laboratory.

Further, the identification/enumeration QA process requires the submission of blind duplicates to laboratories. Typically, identification/enumeration blind duplicates are submitted at a quantity approximately equal to 10% of the total quantity of samples submitted to the laboratory. Consequentially, multiple ID/Enumeration bottles are collected from each sample.

Subsampling for Algae Identification/Enumeration (ID/Enumeration) and Cyanotoxin

Once the dilution process (if required to achieve minimum quantities stipulated in Table 1) is complete and the final algae slurry sample volume is recorded, the sample must be homogenized. Often a

handheld low-speed blender is applied to the contents of the beaker to break apart the larger pieces of algae. This blending also helps to better homogenize the algae slurry. A high-speed blender should not be used at this point because at high speeds the blender may lyse the algae cells, making the identifications more difficult.

Once blended, the algae slurry beaker may then be placed on a magnetic stirrer to be maintained in a homogenized state as subsamples are extracted (see Figure 4). Pour 100mL of well homogenized algae slurry into a graduated cylinder to ensure accuracy of volume measurement – then pour this volume into a labeled 120mL algae ID/enumeration sample bottle being careful to leave no algae residue in the graduated cylinder. Return the slurry beaker to the magnetic stirrer to resume homogenization prior to pouring the second and third 100mL algae ID/enumeration subsamples. Preserve each identification and enumerations subsample with 7mL of formaldehyde (volume of formaldehyde preservative must be reported to the ID/enumeration laboratory). Refer also to Table 1 for subsample and preservative volumes as well as for transportation and storage requirements.

To subsample for cyanotoxin return the algae slurry beaker to the magnetic stirrer to re-homogenize. Pour 250mL of homogenized sample into a Whirl-Pak® bag and seal. Then transport and store as prescribed in Table 1. Additional information of subsampling for Cyanotoxins are found in *Chapter 3.13, Harmful Algal Bloom (HAB) and Cyanotoxin Data Collection Protocol* (Arnold and Butt 2023b).

Labels and Submission

Algae Identification and Enumeration bottles will be labeled with a GIS Key (e.g., 20170516-1115-jbutt, indicating the date, time, and collector), DEP algae sampling site ID number, stream/river name, and location description.

An example of an Algae Identification and Enumeration Laboratory Submission Data sheet can be found in Appendix A.8. This data sheet provides essential information required by the laboratory performing identifications and is used to determine final reporting values to DEP. Also, location information is provided on this submission sheet. This location information may assist the laboratory performing identifications in providing more accurate algae taxa identifications, especially as they may pertain to species or variation level identifications of diatoms.

QBE PERIPHYTON SAMPLING PROCEDURE

Benthic epilithic periphyton is the preferred sampling substrate targeted by DEP to collect data pertaining to lotic algal communities for use in its monitoring program. One reason for this preference is that many streams and rivers in Pennsylvania are relatively fast-flowing and have a geomorphology that creates riffles where cobble substrate is plentiful. These cobbled riffles present the DEP biologist with a consistent substrate from which to extract samples across many streams in Pennsylvania. Consistency in sample substrate is needed to support the diatom Multi-Metric Index (MMI) development and implementation. As noted by Barbour et al., "*For comparability of results, the same substrate/habitat combination should be sampled in all reference and test streams*" (Barbour et al.

1999). Also, as described by Kelly et al., "*Under many circumstances (particularly at fast-flowing sites), the epilithon is the most abundant substratum, sampling procedures are relatively straight-forward and ecological preferences of most common species are well understood*" (Kelly et al. 1998). Kelly et al. also points out that, regarding the epilithon, "*The performance of major diatom-based indices on this substratum is well understood*" (Kelly et al. 1998).

In 2005, Marina Potapova and Donald Charles analyzed the large algae data set created by the USGS National Water-Quality Assessment Program (USGS NAWQA, Potapova and Charles 2005) and demonstrated several important findings regarding the type of substrate sampled. The USGS NAWQA program collected as many as four different sample types and compared results from paired samples from hard and soft substrates. From this analysis, those findings that support the use of the benthic epilithic periphyton, given the typically good availability of cobble substrate in many Pennsylvania streams, are:

- "*Ordinations of assemblages from hard and soft substrates were highly concordant and provided similar information on environmental gradients underlying species patterns*."
- "…*only one sample has to be collected at each site for water quality assessment surveys, and that sample can come from whatever substrate is available*."

Consequentially, based upon the recommendations from the scientific literature, the nature of the monitoring activity pursued by DEP, and the characteristic of the streams and rivers typically being sampled in Pennsylvania, DEP chose and continues to use a benthic epilithic periphyton sampling protocol. This protocol is intended for use in Pennsylvania wadeable riffle-run streams and semiwadeable large rivers.

Site Selection

Within the area where the stream is to be sampled, select a reach in which a riffle is located and which is, in general, representative of the stream in that locale. Representative feature considerations should include wetted width, substrate type and size, shading, and standing periphyton crop.

Transects and Rock Selection

Rocks must be extracted randomly from the stream or river along three transects at each sampling site. The term transects, as it is used here, should not be confused with the same term as it is used in *Chapter 4.1, In-Situ Field Meter, Transect, and Vertical Profile Data Collection Protocol* (Hoger and Arnold 2023). Regarding periphyton sample collection, these transects are only used to facilitate the random selection of rocks as demonstrated in Figure 3 and to measure wetted width of the stream for data recording on the field data sheet.

Rocks (sometimes referred to as cobble) to be extracted for sampling must be of the size and shape to facilitate the rock scrub. A suitable sample rock is one that allows the foam ring on the Pennsylvania Epilithic Periphyton (PEP) Sampler to seal properly (refer to the section "Collecting the Periphyton Slurry – The Rock Scrub," located below, for a description of the PEP Sampler and refer to Figure 4). For the foam ring to seal properly, the sample rock must be small enough to fit in the

PEP Sampler but be larger than the approximately 3-inch diameter foam gasket. Also, the rock must be relatively smooth (flat) so that ridges or other irregularities in the scrub surface area do not interfere with the foam gasket seal and allow the use of the scrubbing and slurry collection tools. If the first rock picked at each location will likely not facilitate the scrub process, choose the next suitable rock located immediately upstream at that same transect location. As with all other aspects of this protocol, experience will be the best guide for the biologist in making appropriate choices in selecting appropriate sample rocks.

The quantity of rocks that must be sampled at each site varies based upon the size of the watershed located upstream of the site location. Watersheds with areas equal to or greater than 1,000 square miles (2,590 square kilometers) are to be considered as a semiwadeable large river and will require a 27-rock QBE periphyton sample to properly represent the habitat and species variability in that sample reach. Watersheds smaller than 1,000 square miles in area should be considered as a wadeable riffle-run stream that will require a 9-rock sample. Watershed area is an attribute that must be determined through a GIS based inquiry which must be performed prior to sampling in the field. For example, Pine Creek in Lycoming County, immediately upstream of the confluence with Ramsey Run at N41.283650 W77.320917, has an upstream watershed area of 940 square miles and is classified as a wadeable riffle-run stream thus requiring a 9-rock sample. Whereas, the West Branch Susquehanna River at Lewisburg, downstream of the Route 45 bridge at N40.9653 W76.8782, has an upstream watershed area of 6,820 square miles and is considered a semiwadeable large river requiring a 27-rock sample. Refer to *Chapter 3.1, Wadeable Riffle-Run Stream Macroinvertebrate Data Collection Protocol* (Shull 2017b) and *Chapter 3.4, Semiwadeable Large River Macroinvertebrate Data Collection Protocol* (Shull 2017a) for a more complete definition of these types of surface water characterizations as well as important safety procedures that must be considered by field staff before working in these waterbodies.

Setting up Transects and Making the Sample Rock Selections in Wadeable Riffle-Run Streams

To facilitate the random rock selection in wadeable riffle-run streams, the biologist must choose a sample reach that longitudinally (parallel to the stream thalweg) measures no more than about 100 meters in length and in which is located enough riffle to deploy the three sample transects – each transect must be deployed over a riffle. An example of such a sample reach is shown in Figure 2.

Figure 2. An example of a periphyton sample reach in a wadeable riffle-run stream. Three transect measuring tapes are pulled across the riffle section of the stream to facilitate random rock selections.

The step-by-step process for setting the three transect lines, computing the random rock sample pick locations, and collecting the rocks is as follows:

1. Three measuring tapes must be pulled across the stream to create three transects. Each tape is pulled across the stream so that it is oriented perpendicular to the direction of flow in the stream in a manner like that shown in Figure 2.
2. The biologist must exercise care when pulling the transect tapes across the stream so that they do not step on, or interfere with, the substrate that will be targeted for sampling. In general, the collector should remain within a few feet of the tape on the downstream side, thereby leaving substrate on the upstream side of the tape undisturbed and available for sampling. Also, to lessen the chance of disturbing the substrate along any transect, the biologist should not closely situate any of the transect tapes to one-another. Widely spacing the transect tapes over the sample reach helps to prevent biologists from disturbing substrate in adjacent transects and additionally helps the biologist to better represent the potential habitat variation in that stream.
3. Transect tapes should be pulled beyond the wetted margins of the stream so that wetted width may be accurately measured. The wetted stream width parameter is used to calculate random rock selection locations.
4. Record the wetted margin tape measurements for each transect at the right descending bank (RDB) and the left descending bank (LDB) locations on the QBE Periphyton Field Data Sheet. In streams too wide for pulling a tape, the biologist should use a laser range finder. An example of this record, along with a transect random rock pick calculation, is provided in Figure 3. This figure is an excerpt from the DEP QBE Periphyton Field Data Sheet (Appendix A.6).
5. Determine the wetted width for each transect and record this width in the QBE Periphyton Field Data Sheet.

6. Divide the wetted width for each transect by 3. The result of this mathematical operation is referred to as the 1/3rd transect distance. Record this 1/3rd distance for each transect on the data sheet. Essentially, each transect is divided into equal thirds and, for a 9-rock sample (wadeable riffle-run stream), a single rock is extracted from a randomly determined location in each of the 1/3rd transect sections. In a 27-rock semiwadeable sample, three rocks will be extracted from randomly determined locations in each of the 1/3rd transect sections.
7. Random percentage numbers are used to compute sample locations along the tape in each of the 1/3rd transect sections. Random percentage numbers may be taken from a random number table or from the roll of a 10-sided die (the first die roll represents the tens place of a percentage use in the location calculation – for example, a roll of 1 represents 10. The second die roll represents the units place – for example, a roll of 5, when combined with the previous die roll, will be recorded as the random percentage of 15%). Record three random percentages – which may range from 0% to 99% - for each 1/3rd transect section in the designated spaces of the field data sheet.
8. Compute the tape locations on each transect to determine where random picks are to occur. For example, as shown in Figure 3 below, the rock pick location on Transect 1 in the first 1/3rd location would be computed as: (15% x 16.3ft) + 3.8 = 6.2ft on the tape measure. In this example, 16.3ft is the 1/3rd distance on transect 1 tape and 3.8ft is the RDB location. The second rock pick location on Transect 1, located in the second 1/3rd section would be computed as: (78% x 16.3ft) + 16.3ft (first 1/3rd section) + 3.8ft (the RDB) = 32.8ft on the tape measure. The remaining rock pick locations for this 9-rock sample are similarly computed. Locations for a 27-rock semi-wadable sample are computed in a similar fashion except each 1/3rd transect section will use three random percentage numbers instead of one random percentage number.
9. Once the random sample locations along each transect are computed, the biologist must collect suitable sample rocks from each location. Rock samples must be collected from upstream of the transect tape and the biologist should collect the first suitable sample rock encountered when moving upstream from the transect tape location. As previously discussed, a suitable sample rock is one with the characteristics that allows the foam ring on the PEP Sampler to seal properly. Refer to the section “Collecting the Periphyton Slurry – The Rock Scrub,” located below, for a description of the PEP Sampler and refer to Figure 4. If the first rock picked at each location will likely not facilitate the scrub process, choose the next suitable rock located immediately upstream at that same transect location.
10. When collecting the sample rocks, the biologist should prevent any contact with the surface of the rock exposed to the stream water – in other words, lift the rock by touching it only from the embedded surface (bottom) side. Collect the sample rocks into a plastic bin, being careful to avoid any contact of the upper surface of the rock with the container or other rocks. This care is needed because even the slightest disturbance to the sample area on the rock will skew the results generated during the laboratory subsample analyses (refer to the section “Collecting the Periphyton Slurry” for a description of that sample area).
11. Transport the sample rocks to the field sample processing station and prepare for the collection of the Periphyton Slurry. Collection of the Periphyton Slurry is described below. The

field sample processing station, as shown in Figure 4, should be setup in a fashion that allows for the positioning of all field sampling hardware on a horizontal surface, such as on a table or the foredeck of a boat, and located where biologists may process the sample in a manner that minimizes the likelihood of fouling or loss of sample material due to environmental elements such as rain or wind, for example.

Transect Random Rock Pick Calculation					
Transect 1	**(Upstream)**		Random #'s: 15	Random #'s: 78	Random #'s: 25
RDB	**LDB**	**W / 1/3**	6.2 ft	32.8 ft	40.5 ft
3.8 ft	52.7 ft	48.9 / 16.3			
Transect 2	**(Middle)**		Random #'s: 33	Random #'s: 42	Random #'s: 11
RDB	**LDB**	**W / 1/3**	12.4 ft	27.5 ft	37.1 ft
7.8 ft	49.6 ft	41.8 / 13.9			
Transect 3	**(Downstream)**		Random #'s: 5	Random #'s: 89	Random #'s: 72
RDB	**LDB**	**W / 1/3**	15.0 ft	45.0 ft	58.5 ft
14.2 ft	63.1 ft	48.9 / 16.3			

Figure 3. Excerpt from the DEP QBE Periphyton Datasheet demonstrating the random rock pick calculation made for a hypothetical small stream (wadeable riffle-run) that requires a 9-rock sample.

Setting up Transects and Making the Sample Rock Selections in Semiwadeable Large Rivers

To facilitate the random rock selection in semiwadeable large rivers, the biologist must choose a sample reach that longitudinally (parallel to the river thalweg) measures no more than about 100 meters in length and in which is located enough riffle to deploy the three sample transects – each transect must be deployed over a riffle.

Sampling in semi-wadable large rivers often differs from that of sampling in wadeable riffle-run streams in four important ways. One difference may be the wetted width of the river – it may be too wide for the positioning of a measuring tape. In these wide wetted-width locations a laser range finder should be used instead of a tape measure. A second important difference relates to depth – in such rivers, the biologist may encounter sections of the transect that are too deep to sample. A section of river is too deep to sample if the biologist is unable to safely grab substrate from the benthos while wearing waders. If an area of the transect is too deep for sampling, the biologist must bypass that area and collect additional sample rocks from those sections of the transect that may be safely sampled and note this adjustment on the QBE Periphyton Field Data Sheet. A third difference is that the biologist should expect to facilitate the sampling by using a power boat, canoe, or kayak. The last difference relates to the possibility of multiple sampling zones, as defined by water influence transitions, occurring at a river site. Situations involving multiple sampling zones, and how to handle them, are discussed in the next paragraph.

Multiple sampling zones may need to be created to properly sample periphyton in semiwadeable large rivers. This occurs when field meter transect data indicates a relative lack of homogeneity in parameters such as temperature, pH, specific conductivity, dissolved oxygen, or turbidity when

measured along a river transect and is characteristic of water influences originating from un-mixed tributary influences. When such water quality heterogeneity is observed, a semiwadeable river will need to be subdivided into separate sampling zones and a 27-rock periphyton sample be independently collected from each zone of influence. For example, the Susquehanna River near Harrisburg, based on transect data, shows three distinct zones of water influence, these being the “East” zone, the “Middle” zone, and the “West” zone. When a river must be subdivided into multiple zones, transect sets in each zone must be restricted to the width of river section that defines that zone.

Once these differences are accounted for, the step-by-step process for setting the three transect lines, computing the random rock pick locations, and collecting the rocks is identical to that defined above for a 9-rock wadeable riffle-run stream except that a 27-rock sample is used.

Regarding the use of boats, the biologist should familiarize themselves with the targeted site prior to going to the field to sample. The target sample locations may be located some distance from points of access and will thus require the use of a power boat to facilitate the field work. In some instances, all that is needed is a canoe or a kayak. The biologist is reminded that all Pennsylvania Fish and Boat Commission boating laws and safe boater guidance must be observed if sampling with a boat.

Collecting the Periphyton Slurry – The Rock Scrub

After the rocks are transported to the field sample processing station location, the biologist will scrub the rocks to collect the periphyton slurry. This slurry collection is made using a PEP Sampler, de-ionized water, scrub brushes, pipets, squirt bottles, and collection containers (500ml plastic bottles, refer to Figure 4). The PEP Sampler is a clamping device that secures a rock sample and provides a fixed circular sample area (equal to 20.6cm^2) and a foam gasket seal (toilet seal) that is clamped to rock (cobble) substrates using partially flexible clamping boards that can be tightened with quick release clamps. The following procedure steps will be used to conduct the rock scrubs:

1. After clamping the rock in the PEP Sampler, with the top face of the rock facing up, verify that the foam ring seal is secure by squirting an adequate amount of de-ionized water into the scrub chamber and observing that no water leaks out. If no leaks are observed, continue to step 2, otherwise re-clamp the rock in the PEP Sampler and re-verify that the seal does not leak. As previously discussed, for the foam ring to seal properly the rock must be small enough to fit in the PEP Sampler but be larger than the approximately 3-inch diameter foam gasket. Also, the rock must be relatively smooth (flat) so that ridges or other irregularities in the scrub surface area do not interfere with the foam gasket seal and allow the use of the scrubbing and slurry collection tools.
2. Using the scrub brushes and a small amount of de-ionized water, scrub the rock area within the PEP sampler. After a liberal amount of scrubbing, pipet the algae slurry out of the PEP sampler and into the 500mL sample collection bottle(s). Repeat this process until the rock surface area within the PEP sampler is clean – the rock surface is clean when the de-ionized water is relatively clear after scrubbing. Determining when this process is complete and the

scrub water is clear, is a matter of professional judgement and experience because, depending on the composition of the rock, continued scrubbing may displace mineral rendering the scrub water unclear. When scrubbing is complete, unclamp the rock and dispose of the rock. However, if stream site is to be repeatedly sampled and cobble is scarce, the biologist may choose to return the cobble to the riffle so that sampleable substrate is not depleted.

3. At this point, it is important to note that because the periphyton community is a three-dimensional arrangement of often microscopic organisms, sometimes stratified into layers, all of which contribute to the base of the food web within the stream and may strongly influence the cascade of energy and nutrients through all trophic levels in the stream, it is important for the biologist to accurately represent that community through the diligent collection of algae material.
4. Repeat steps 1 and 2 until all rocks have been scrubbed and all algae slurry is collected into the 500mL collection bottles. Sometimes multiple 500mL collection bottles will need to be utilized for a single sample. All 500mL bottles used to collect the algae slurry at a site should be labeled and the quantity of bottles noted in a comments field for the QBE Periphyton Field Data Sheet – this is to ensure that the biologist, when processing subsamples at the end of the sampling day, doesn't forget to include all sample bottles when compositing the algae slurry.
5. Cap the 500mL collection bottles, being careful not to spill any of the slurry. Label the bottles with the date and site information and place in a dark cooler with wet ice to await the subsampling procedure – do not freeze.
6. Clean all equipment including the PEP sampler, brushes, etc. First scrub all equipment in the stream water followed by a rinse with de-ionized water. Throrough cleaning is required to prevent the contamination of samples with residue from other sites.
7. Organize and stow all gear for safe transport to the next work site.

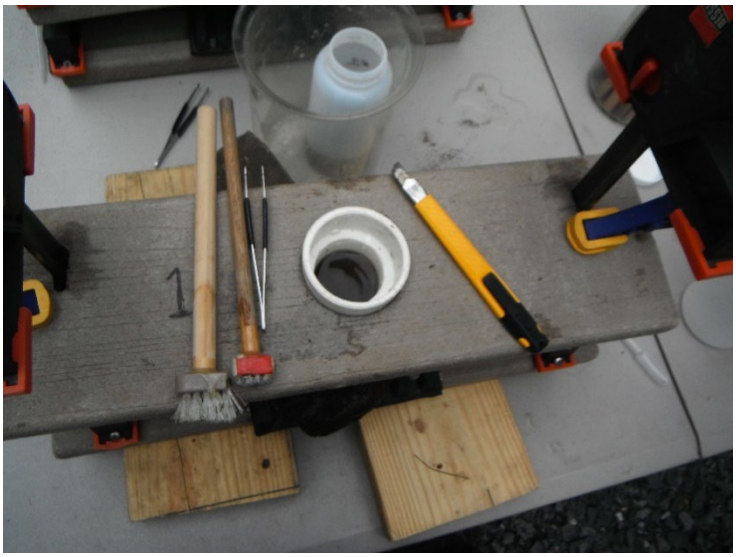

Figure 4. The Pennsylvania Epilithic Periphyton (PEP) Sampler with various tools used to sample algae from rock substrate. Rocks suited for periphyton scrubbing must be of size and shape to fit properly in the PEP sampler – large enough and flat enough for the sampler circular foam gasket to form a leak-proof seal around the area to be scrubbed.

Field Data Sheet

The biologist will also complete the DEP QBE Periphyton Field Data Sheet at each sampling location (Appendix A.6). In addition to performing the Transect Random Rock Pick Calculation as discussed above, the biologist must provide information pertaining to the following (refer to the QBE Periphyton Field Data Sheet for more specific requirements for each data record):

- Site/Sample Information including water chemistry collection information and personnel involved
- Latitude and longitude of the site
- Watershed Area – determined prior to making the field visit
- Representation of the Inorganic Substrate
- In-situ Field Meter Values – refer to *Chapter 4.1*, *In-Situ Field Meter, Transect, and Field Meter Data Collection Protocol* (Hoger and Arnold 2023) for field meter use and calibration requirements
- Densiometer and Compass Readings (refer to the Densiometer and Compass section of this document for instructions for use these devices)
- Quantity of rocks sampled (equates to surface area scrubbed – each rock = 20.6cm^2)
- Transect random rock pick calculations
- Total algae slurry volume (mL, minimum volume rules apply – refer to Subsampling Procedure section)
- Notes pertaining to the Algae ID Bottles collected
- Notes, including collector and sequence numbers, pertaining to the Phycocyanin sample bottle
- Notes, including collector and sequence numbers, pertaining to the Acidified Chlorophyll-a filters
- Notes, including collector and sequence numbers, pertaining to the AFDM filters
- Other notes (including any pertaining to cyanotoxin testing, if collected).

Subsampling Procedure

The QBE Periphyton Sampling procedure requires subsampling for the identification and enumeration of algal taxa, the quantitative determination of chlorophyll-a and phycocyanin photopigments, the quantitative determination of AFDM, and in some cases for the characterization of cyanotoxins. This subsampling may be performed in the field at the sample site (if electricity is available for operating a blender and a magnetic stirrer) or the sample may be temporarily stored in labeled 500mL bottles that are capped and stored in a wet ice cooler to await later (same day) subsampling treatment. Table 2 provides information regarding test volumes, bottle, filter requirements, preservation procedure, as well as transportation and storage needs for the QBE subsamples. Subsampling procedures for chlorophyll-a, phycocyanin, and AFDM are briefly described here and additional details are found in *Chapter 3.12, Chlorophyll-a, Phycocyanin, and Ash Free Dry Mass Data Collection Protocol* (Arnold and Butt 2023a). Subsampling procedures for cyanotoxins are briefly described here and additional details are found in *Chapter 3.13, Harmful Algal Bloom (HAB) and Cyanotoxin Data Collection* (Arnold and Butt 2023b).

Note: This is an important step needed to prevent cross-contamination between samples. Prior to performing any subsampling procedures on a site sample, thoroughly clean all equipment that may come in contact with the algae slurry sample and, at the conclusion of this cleaning, rinse with de-ionized water. Because site samples are often accumulated through the field day and subsampled at the conclusion of that day's work, all equipment must be appropriated cleaned between samples.

Subsampling consists of the following procedures and must be done in the order listed**.**

Initial Slurry Preparation

Composite the contents of all the 500ml collection bottle(s) for a site into a large plastic beaker (beaker should be large enough to contain at least 2000 mL of algae slurry, or more, and is able to be used with the magnetic stirrer and low-speed blender). Once all slurry is collected into the beaker, apply a low-speed hand-held blender to this slurry. This blending helps to homogenize the slurry by breaking apart the larger pieces of algae. It is important that a high-speed blender with razor-sharp blades **not** be applied at this point. The algae identification/enumeration (hereafter referred to Algae ID) bottles must be poured off prior to the application of high-speed blending with razor-sharp blades – such blending will lyse algae cells, making identifications and counting more difficult.

Table 2. QBE Periphyton Subsample Tests: Homogenized Volume (ml), Bottleware, Preservation, Transportation, and Storage

Subsample Tests	Homogenized Volume (mL)	Bottle or Filter	Preservation	Transportation	Storage
Algae ID Bottle #1	100	120ml Plastic	7mL Formaldehyde	Cool, dry, dark	Cool, dry, dark
Algae ID Bottle #2	100	120ml Plastic	7mL Formaldehyde	Cool, dry, dark	Cool, dry, dark
Algae ID Bottle #3	100	120ml Plastic	7mL Formaldehyde	Cool, dry, dark	Cool, dry, dark
Chl-a Filter #1 (Acidified)	2	Whatman™ GF/F	1mL MgCO3 - Fold-Foil wrap	Dry-ice	-80°C
Chl-a Filter #2 (Acidified)	2	Whatman™ GF/F	1mL MgCO3 - Fold-Foil wrap	Dry-ice	-80°C
Chl-a Filter #3 (Acidified)	2	Whatman™ GF/F	1mL MgCO3 - Fold-Foil wrap	Dry-ice	-80°C
Phycocyanin Bottle	30	120ml plastic	None	Wet-ice	Refrigerate
AFDM Filter #1	2	Whatman™ GF/F	Fold-Foil wrap	Dry-ice	-80°C
AFDM Filter #2	2	Whatman™ GF/F	Fold-Foil wrap	Dry-ice	-80°C
AFDM Filter #3	2	Whatman™ GF/F	Fold-Foil wrap	Dry-ice	-80°C
Minimum Algae Slurry Volume (plus excess) without Cyanotoxin Test	400				
Cyanotoxin Test	250	Whirl-Pak®	None	Dry-ice	-80°C
Minimum Algae Slurry Volume (plus excess) with Cyanotoxin Test	700				

Once this initial blending is complete in the 2000mL beaker, pour the complete algae sample into multiple 1000mL graduated cylinders – as many as is required to contain the entire sample. Next, dilute the algae slurry with de-ionized water to provide enough material for all subsample tests (refer to Table 2 for minimum quantities required). For example, if the biologist is electing not to subsample for the cyanotoxin test, but is intending to subsample for ID/Enumeration, chlorophyll-a, phycocyanin, and AFDM only, then the minimum quantity of algae slurry needed is 400mL. However, if the biologist is electing to subsample for cyanotoxin, in addition to all the other subsamples, then the minimum quantity of algae slurry needed is 700mL. These minimum quantities will ensure that enough algae slurry is available for all subsamples plus a little extra (in case of small spills and enough material being available for the magnetic stirrer to work and the Hensen-Stemple extractions to be made). The biologist should not over dilute the algae slurry – the laboratory tests are best facilitated by providing as concentrated a subsample as possible. If after compositing all the 500mL collection bottles for a site, the total algae slurry is greater than the minimum quantity required, do not further dilute beyond that needed to move the volume to the next highest 100mL increment level – for example, if after compositing all bottles, the total volume is found to be about 1,244mL, then dilute to 1,300mL. Doing this helps to ensure accuracy of volume measurement in a 1000mL graduated cylinder which are often incremented in units of 100mL.

The total algae slurry volume must be recorded on the field data sheet and, eventually, reported to the laboratory performing the identifications/enumerations. Since the ID/Enumeration laboratory must provide results to DEP that enable the biologist to convert results to cells/cm^2 and report biovolume (unit of $\mu m^3/cm^2$) estimates, accurate reporting of algae slurry volume and surface area scrubbed is essential. Also note that the ID/Enumeration QA process requires the submission of blind duplicates to laboratories. Typically, ID/Enumeration blind duplicates are submitted at a quantity approximately equal to 10% of the total quantity of samples submitted to the laboratory. This is why multiple Algea ID bottles are collected from each sample. This QA process is discussed in detail later in this protocol.

Subsampling for Algae Identification/Enumeration

Once the Initial Slurry Preparation process is complete, the first subsampling that must be completed is that needed for the Algae Identification and Enumeration.

Pour all graduated cylinders from the Initial Slurry Preparation Process back into the 2000mL beaker. This total sample is then homogenized on a magnetic stirrer (see Figure 5). Once the sample is well homogenized (this may take approximately 30 seconds on the magnetic stirrer and is evident when a good vortex is observed to be established in the beaker), pour 100mL of the homogenized algae slurry into a 100mL graduated cylinder to ensure accuracy of volume measurement. Thereafter, pour this volume into a labeled 120mL Algae ID sample bottle while being careful to leave no algae residue in the graduated cylinder. Do not use de-ionized water to rinse this residue into the Algae ID bottles – adding de-ionized water at this point will further dilute the sample and introduce volume related discrepancies into the final results reporting. A swirling motion on the 100mL graduated cylinder followed by a quick pour into the sample bottle will usually prevent sample material from being

retained in the graduated cylinder and will ensure that all material is transferred. Sometimes a small quantity of sample material must be returned to the graduated cylinder from the sample bottle and a re-pour used to transfer all algae material. After collecting an Algae ID bottle, return the slurry beaker to the magnetic stirrer to re-establish homogenization prior to pouring the second mL100mL Algae ID subsample and later, the third 100mL Algae ID subsample.

Preserve each 100mL Algae ID subsample with 7mL of formaldehyde (volume of formaldehyde preservative must also be reported to the ID/enumeration laboratory). Refer also to Table 2 for preservative volumes as well as for transportation and storage requirements.

Algae Identification and Enumeration bottles will be labeled with a GIS Key (e.g., 20170516-1115-jbutt, indicating the date, time, and collector name), stream/river name, and location description.

Prior to submitting the Algae ID samples to a laboratory, an Algae Identification and Enumeration Laboratory Submission Data sheet must be completed and submitted to the laboratory along with the samples. An example of such a submission sheet can be found in Appendix A.8. This data sheet provides essential information required by the laboratory performing identifications and is used to determine final reporting values to DEP. Also, sample site location information is provided on this submission sheet. This location information may assist the laboratory performing identifications in providing more accurate algae taxa identifications, especially as they may pertain to species or variation level identifications of diatoms.

Note that Algae ID sample bottles, once preserved, may be stored in a cool, dry, and dark location (refer to Table 2), for upwards of several months prior to sending to the lab. Often, DEP will retain these samples until the end of a collection season and ship all samples together.

When ID/Enumeration subsampling is complete, the biologist may then proceed to subsampling for phycocyanin, acidified chlorophyll-a, AFDM, and cyanotoxins.

Subsampling for Phycocyanin, Acidified Chlorophyll-a, and AFDM

After the Algae ID subsamples have been obtained, the slurry may be further blended in a highspeed blender. Highspeed blending in a food-grade processing type of blender (those with stainless steel “razor sharp” blender blades) is needed to properly prepare the algae for phycocyanin, acidified chlorophyll-a, and AFDM subsampling in samples with robust filamentous or other forms of algae that resisted the low-speed blending. When highspeed blending is complete, return the sample to the 2000mL beaker and magnetic stirrer to provide continuous homogenization.

Hereafter, the order of subsampling will first be phycocyanin, followed by AFDM, then acidified chlorophyll-a, and lastly, if needed, cyanotoxin. Filtration procedures for chlorophyll-a, phycocyanin, and AFDM are found in *Chapter 3.12, Chlorophyll-a, Phycocyanin, and Ash Free Dry Mass Data Collection Protocol* (Arnold and Butt 2023a).

Subsampling for Cyanotoxins

After the acidified chlorophyll-a subsamples are collected, continue homogenizing the algae slurry on the magnetic stirrer. Pour 250ml of this homogenized slurry into a 500mL graduated cylinder. Swirl this slurry around the graduated cylinder and quickly pour it into a Whirl-Pak® bag to prevent algae material from adhering to the sides of the graduated cylinder. Again, don't rinse down with additional de-ionized water as that would dilute the sample and introduce error into the final results. Seal the Whirl-Pak® bag then transport and store as prescribed in Table 2. Additional details are found in *Chapter 3.13, Harmful Algal Bloom (HAB) and Cyanotoxin Data Collection Protocol* (Arnold and Butt 202b2).

DENSIOMETER AND COMPASS DATA COLLECTION

In smaller streams, the riparian vegetative cover may intercept much of the incident solar radiation that may otherwise be available for supporting primary production in the lotic habitat. Trees situated along the riparian corridor are very effective at appreciably reducing the quantity of light reaching the stream (Hill and Roberts 2009). There is great potential for a combination of both light and nutrients to colimit algal growth (Hill 1996). Additionally, the East/West or North/South orientation of the stream reach being sampled will also seasonally contribute to the quantity of incident sunlight on the stream reach. Consequentially, a measurement of canopy closure (thus inferring intercepted solar radiation) over the stream reach, and stream orientation, should be made by the biologist and considered, in conjunction with water chemistry measures, when analyzing the results of algae sampling.

Stream orientation is measured with a compass. Facing downstream at the Densiometer "1/2 Downstream" location (refer to either the QBE or QMH Field Data Sheets in Appendices A.6 and A.7), site the compass along the thalweg in the downstream direction and orient the compass with magnetic north. Record this sighted azimuth direction clockwise from magnetic north (in degrees) on the data sheet.

Stream canopy closure is measured with a Concave Spherical Densiometer (for example, part number 43888 available from Forestry Suppliers, Inc at www.forestry-suppliers.com). Procedures for estimating canopy closure follow Platts et al. (1987). Measuring canopy closure is suggested over measuring canopy density since canopy closure measures are less affected by seasonality (Fitzpatrick et al. 1998). Vegetative canopy closure is the area of the sky bracketed by vegetation. The densiometer's concave mirror surface has 37 grid intersections forming 24 squares. To eliminate bias from overlap, only 17 of the 37 grid intersections are used as recording points. The 17 grid intersections to be used are delimited by taping a right angle on the densiometer (see Figure 6).

For streams measuring less than 25 meters across (82 feet), densiometer readings are recorded at four locations along the mid-reach transect – these being, facing the right-descending bank, facing mid-channel downstream, facing mid-channel upstream, and facing left-descending bank. This is accomplished, for example at the right-descending bank, by facing the right wetted edge of the stream and holding the densiometer about 1 foot from the shoreline and about 1 foot above the water

surface (see Figure 7). With the leveled (bubble level) densiometer now pointed toward the bank (taped right-angle points toward the recorder) and with the observer's head reflection near the top grid line (see Figure 6) then determining canopy closure at that location. Thereafter, canopy closure is counted at each of the remaining mid-reach transect locations in a similar fashion. Canopy closure count is equal to the number of densiometer line intersections (maximum of 17) that are surrounded by vegetation (representing canopy closure) or intercepted. Finally, the count for each location is then summed together and divided by 68 (4 x 17) to calculate a percent closure. For example, if the canopy closure count at the right-descending bank is 15, the count at mid-channel facing upstream is 3, the count at mid-channel facing downstream is 5, and the left-descending bank count is 16, then the canopy closure value for the site is determined by the following: [(15 + 3 + 5 + 16)/68] x 100 = 57% (round to nearest whole percent).

For streams larger than 25 meters across the same procedure is used except eight recordings are made. In addition to right bank, left bank, and mid channel (upstream and downstream) locations, mid-reach upstream and downstream densiometer measurements are additionally made at the ¼ and ¾ intervals on the mid-reach transect. Canopy closure or density scores are summed and divided by 136 (8 x 17) to calculate a percent closure.

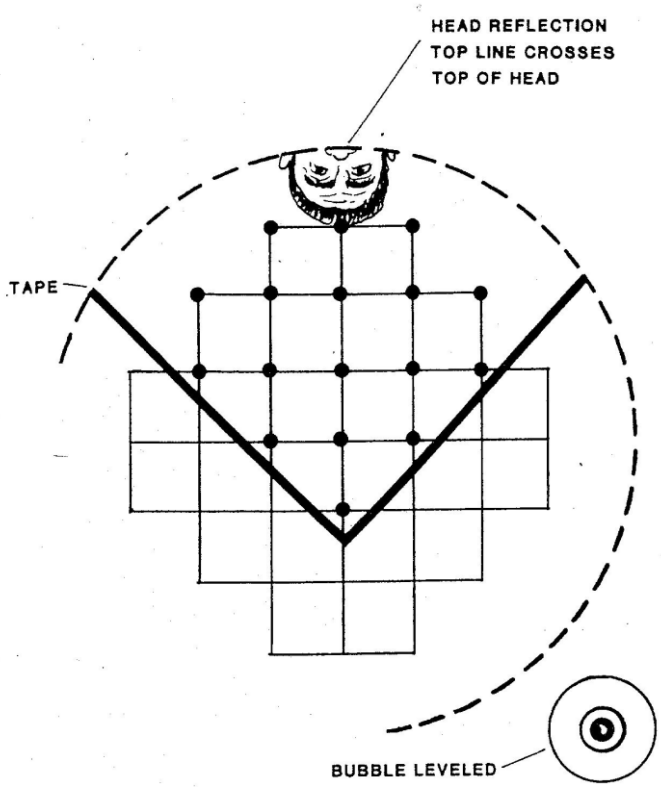

Figure 6. Concave spherical densiometer with placement of head reflection, and 17 observation points. From Platts et al. (1987).

Figure 7. Proper use of the densitometer at the right descending bank location.

LABORATORY PROCEDURES

Although strict adherence to the field data collection procedures is necessary to achieve comparability between samples, field data collection represents only a portion of that which is needed to ensure robust periphyton data results. Laboratory procedures are also extremely important and must also be defined and strictly adhered to if sample comparability is to be achieved. The purpose of this section is to define those laboratory procedures needed to process periphyton related samples. These laboratory procedures include those applied by BOL as well as those that must be applied by contracted labs. These protocols are as follows:

- Phycocyanin by Fluorometry
- Acidified Chlorophyll-a by Fluorometry with Pheophytin
- Ash-Free Dry Mass by SM10200-I
- Algae Identification and Enumeration
- Quality Assurance (QA) Audit of Algae Identification and Enumeration.

At present, DEP's Algae Monitoring Program processes phycocyanin, acidified chlorophyll-a (which includes pheophytin reporting), and AFDM samples at its BOL facility. The laboratory procedures used to process these samples are extensively defined in BOL documentation and may be obtained. BOL methods currently used include:

- Phycocyanin by Fluorometry Rev 003, effective December 2024
- [Acidified] Chlorophyll A by SM10200-H; Rev 005, effective February 2023
- Ash Free Dry Mass; Rev 006, effective July 2024
- Plankton Identification and Enumeration Rev 006, effective December 2024

Algae Identification and Enumeration samples collected by DEP are processed through private companies, academic institutions, as well as through DEP BOL. Because these identification samples are processed through several different facilities, the reader is directed to both the BOL Plankton Identification and Enumeration document as well as to the Algae identification and Enumeration Protocol description provided below. Further, the use of multiple identification and enumeration

processing facilities obligates DEP to employ a rigorous QA Audit process designed to ensure consistency, and thus comparability, between the results produced by the various labs. This QA Audit process is also discussed below.

General Description of Algae Identification and Enumeration Procedure

The identification and enumeration of periphyton samples collected by either the QMH or the QBE periphyton field procedures requires that two separate procedures be performed to completely characterize the sample. These include a Palmer-Maloney Chamber procedure as well as a Diatom Slide procedure.

The Palmer-Maloney Chamber procedure, sometimes referred to by lab technicians as the “wet count,” or “soft algae count,” identifies and counts all algae taxa to the genera level, at a minimum, at moderate magnification (e.g., 100x or 400x). However, if certain morphological structures are observable during this procedure, identifications may be made to the species, or sometimes variety, taxonomic level. It is important to recognize that during the Palmer-Maloney procedure, diatom counts are also made. Diatoms, however, are only characterized as living or as dead and are not taxonomically identified in this procedure. Palmer-Maloney Chambers used in this process must be calibrated.

As previously mentioned, the Palmer-Maloney procedure is sometimes referred to as the “wet count” or the “soft algae count” as a way of distinguishing it from the Diatom Slide count. The “wet count” name originates from the fact that the submitted identification subsample is further subsampled by placing a small homogenized quantity of liquid (hence the term “wet”) into a Palmer-Maloney chamber for microscopic inspection. The term soft algae is also often applied to this procedure because the algae taxa identified and counted in this procedure frequently contain those possessing only soft cell tissues that would otherwise be destroyed as part of the nitric acid digestion used to create slides for the diatom slide procedure. Examples of such “soft algae” commonly identified in samples collected from Pennsylvania streams and rivers include cyanobacteria or green algae taxa. However, Palmer-Maloney results do sometimes include other “soft algae” taxa, which are less commonly found in Pennsylvanian freshwater periphyton samples.

In contrast to the Palmer-Maloney procedure, diatoms are identified to the species, and often to variety, taxonomic level in the Diatom Slide procedure. In this procedure, liquid from the subsample is processed with nitric acid to dissolve away all soft tissues in the sample thereby exposing the glass frustules of the diatoms. A detailed microscopic analysis of the diatom glass frustule morphology is then performed to make the species or variety identification at high magnification (1000x oil immersion). The identifications and counts obtained during this procedure produce what is often referred to as the “permanent slide” result. Permanent slides are a product of the procedure referred to as the Preparation of Diatom Slides Using Naphrax^{TM} Mounting Medium, as listed below. DEP requires the identification laboratory to produce three permanent slides per submitted algae identification subsample. These three permanent slides are maintained by DEP as voucher specimens and are also used in the QA audit.

As a separate option, the BOL Algae Identification and Enumeration procedure may be used to produce genera level taxonomic identifications and enumerations. Also, the BOL procedure only provides results corresponding to the Palmer-Maloney procedure described above. These results, though less informative than those produced by the complete procedure, which combines results from both the Palmer-Maloney and Diatom Slide procedures, are used when an algae community result is needed from a laboratory in a very short period of time (historically, DEP has experienced that complete sample results from laboratories are often obtained only after many months of processing time at those facilities). If this BOL option is to be used, modifications to the standard BOL Algae Identification and Enumeration procedure are needed. These modifications include counts of living and dead diatoms during the Palmer-Maloney count phase and the fact that the BOL method specifies “phytoplankton… samples” whereas a periphyton sample is submitted. [Note: living diatoms are those that are observed to contain soft tissue within the frustule during the Palmer-Maloney procedure whereas those that contain no such soft tissue are considered to be dead at the time of sampling. Also, the QA procedures expected of Participating Labs (see below) must be required of samples processed by DEP BOL.]

Algae Identifications that use both the Palmer-Maloney soft algae analysis as well as the diatom slide analysis will rely, in general, upon the *Protocols for the Analysis of Algal Samples Collected as Part of the U.S. Geological Survey National Water-Quality Assessment Program, Report No. 02-06* by Charles et al. 2002 (available at https://water.usgs.gov/nawqa/protocols/algprotocol/algprotocol.pdf). The protocols from Report No 02-06 that are routinely applied, in part or in whole, by DEP include:

P-13-58: Tracking of Algae Sample Analysis – Figure 1 (Sample process and analytical flow)
P-13-48: Subsampling Procedures for USGS NAWQA Program Periphyton Samples
P-13-42: Diatom Cleaning by Nitric Acid Digestion with Microwave Apparatus
P-13-49: Preparation of Diatom Slides Using Naphrax™ Mounting Medium
P-13-50: Preparation of Algal Samples for Analysis Using Palmer-Maloney Cells
P-13-39: Analysis of Diatoms on Microscope Slides Prepared from USGS NAWQA Program Algae Samples
P-13-63: Analysis of Soft Algae and Enumeration of Total Number of Diatoms in USGS NAWQA Program Quantitative Targeted-Habitat (RTH and DTH) Samples.

The rationale for performing the QBE and QMH Periphyton Sampling Procedures as well as the Algae Identification and Enumeration Procedures, as based upon the USGS NAWQA protocols, is to produce results of the quality needed for the development of a trophic index, that needed for a diatom based Multi-Metric Index (MMI), as well as that needed to provide information that contributes to aquatic life use considerations. These procedures provide community-level data as well as the identification of pollution sensitive or insensitive diatoms. To achieve segregation between potential levels of pollution tolerance within the diatom group, identification to the species or variety taxonomic level is required in the Diatom Slide Procedure. In short, diatoms, when identified to species or a variety of taxonomic levels, provide information concerning the nutrient and organic enrichment

condition of natural waters (Porter et al. 2008) and will also serve as indicators for determining biological condition gradients and developing nutrient criteria (Hausmann et al. 2016).

Detailed Description of Analytic Services for Algae Identification and Enumeration

DEP requires that algae identification and enumeration of benthic algae samples be done in a way that produces results "comparable" to those provided by The Academy of Natural Sciences (ANS). ANS produces results as described by the previously mentioned protocols for the analysis of algal samples collected as part of the USGS NAWQA Protocol (Charles et al. 2002).

The word "comparable," in the above paragraph, is emphasized because DEP is primarily interested that the results produced by the identification and enumeration process completely harmonize with those produced by the ANS. Consequentially, there may be a limited opportunity for flexibility regarding the exact procedures by which the identification and enumeration laboratory results are produced. This creates the possibility for the processing laboratory to slightly modify the procedures of performing identification and enumeration as detailed by the USGS NAWQA Protocol so long as that laboratory does so in close consultation with, and approval by, DEP.

Algae identification and enumeration results will be achieved by performing the following process steps. It is important to note that this process requires a close collaboration between DEP and the Participating Laboratory (PL). To facilitate this collaboration, the areas of responsibility are indicated in each step. Also, the applicable USGS NAWQA protocol is also indicated, where appropriate. The process steps will include:

1. Collection of periphyton sample and division into 100mL subsample bottles preserved with 5% to 10% formaldehyde. Service provided by DEP as described above.
2. Preparation of permanent diatom microscope slides via acid digestion to produce "clean" identification and enumeration slides as well as three permanent archive (voucher) slides for each sample – refer to USGS NAWQA Protocols P-13-58 Figure 1, P-13-48, P-13-42, and P-13-49. In general, a permanent slide diatom specimen density should be constructed so that between 5 and 10 diatom specimens are observable in a single microscope field at 1000x magnification (recommendation by Charles et al. 2002).
3. 300 Natural Unit ID/Count Analysis of soft algae and diatoms in a calibrated Palmer-Maloney chamber for each sample – refer to USGS NAWQA Protocol P-13-58 Figure 1, P-13-48, P-13-50 and P-13-63. Identification of soft algae to lowest possible taxonomic level (at discretion of analyst but minimum of at least general level expected). Differentiate diatoms into living (containing cytoplasm) and dead (containing no cytoplasm). Identifications and counts must be harmonized. Identifications and counts provided by the PL. Harmonization coordinated between DEP and PL as defined in the harmonization section below.
4. 600 Valve ID/Count Analysis of diatom microscope slides for each sample –refer to USGS NAWQA Protocol P-13-39. Identification to species or variety taxonomic level. Identifications and names must be harmonized. Identifications and counts provided by the PL. Details on coordinating harmonization between DEP and PL is defined in the harmonization section below.

5. Blind Duplicate and QA Audit. DEP will provide blind duplicates samples to the PL. The QA Audit process for these blind duplicates is defined below. Also included in this QA process is the collecting of taxa specific photomicrographs by the PL, as defined in the QA Audit Process below. The PL will also complete its own internal QA Audit process to help ensure its own method quality, particularly as it relates to the processing of the Palmer-Maloney slides and the diatom slides. The PL will design and implement its internal QA Audit process in consultation with DEP. The PL will share the results of their internal QA Audit process with DEP.
6. Reporting Identification and Enumeration Results to DEP. The PL will make a final identification and enumeration results report (preliminary reports are made prior to the completion of the QA Audit process) to DEP in the format discussed below. The final results report will include all necessary changes to preliminary results reports provided by the PL as required by the outcome of the Blind Duplicate and QA Audit processes. This is a collaborative process between the PL and DEP.

Blind Duplicates and Other QA Audit Process:

Blind Duplicate QA Audit processing success will be determined with reference to published and peer reviewed scientific literature. The scientific literature referenced and currently consulted by DEP for this QA Audit process is included in the Literature Cited section of this document and is summarized below. However, the rapid pace at which the algae science is progressing will require DEP and the PL to frequently review and discuss the current body of applicable scientific literature and accepted procedures. Both the PL and DEP will be expected to make on-going adjustments to incorporate the recent changes and advances in the applicable scientific literature. Scientific literature for performing the QA Audit process includes:

- Barbour et al. 1999
- Kahlert et al. 2009
- Kelly 2001
- Whittaker and Fairbanks 1958
- Wolda 1981
- PCER 1998

The body of scientific literature consulted suggested that the appropriate procedure for determining the quality of reported diatom identifications and enumerations is to use indices of similarity to compare the results of a sample with those from a corresponding blind duplicate sample, also processed by the PL, as well as a comparison with results independently produced by an auditor. Whereas the blind duplicate is intended to demonstrate the ability of the PL to replicate their own results, the comparison to the auditor is intended to confirm the accuracy of identification and enumerations. Deciding who in DEP or what outside laboratory will serve as the auditor will be decided by DEP at the beginning of each project. DEP typically submits blind duplicates at a rate of 10% of the regular sample load. If blind duplicate or auditor similarity comparisons fail to pass (see below for pass scores), DEP must consult with the PL to resolve this quality issue before incorporating data into the DEP Algae Database.

The QA process will also require the PL to provide photomicrographs (in a digital format agreed upon by both the PL and DEP) to DEP at the time of data submission. These photomicrographs will include an image example of each taxa type identified in the DEP sample set and must be photographed from the DEP sample set. Diatom identification images must be photographed at 1000x and must clearly show all structures needed to confirm the identification to the appropriate species or taxonomic level. Images collected during the Palmer-Maloney 300 Natural Unit process must be photographed at the appropriate magnification needed to make the identification and must also clearly show all structures needed to confirm the identification. Photomicrographs must include a calibrated scale bar. Photomicrograph files must be entitled to include the taxon name, magnification, date of photograph, and DEP sample from which it was obtained. Again, the PL is required to provide only one type image for each taxon identified in the entirety of the DEP sample set (per contract or Purchase Order), regardless of how many times the taxa is re-identified throughout the sample set.

The indices of similarity that DEP uses in the QA Audit process will include the following:

- *Percent Community Similarity of Diatoms* (PS) – this is also referred to as the Whittaker and Fairbanks (1958) similarity and is recommended by USEPA RBP. The USEPA RBP suggests a percent similarity score of > 75% to pass. This index is based upon relative abundance and places more emphasis upon the more abundant taxa while de-emphasizing the rare taxa. Computed as:

  $$\mathbf{PS = 100 - (0.5\Sigma \left| a_i - b_i \right|)}$$

  where:
  a_i = percent abundance of species i in sample A
  b_i = percent abundance of species i in sample B.

- *Bray-Curtis Similarity Index* (D) – This index is also based upon relative abundance. Note that Whitaker-Fairbanks Percent Community Similarity and the Bray-Curtis Similarity Index are numerically equivalent. The scientific literature (Kelly 2001) suggests that a Bray-Curtis score of > 60% is generally considered to be a good agreement between the taxonomist and the auditor, especially when taxa diversity is relatively high. However, this pass score should be increased to 70% when taxa diversity is relatively low. Computed as:

  $$\mathbf{D = \Sigma \min (a_i , b_i)}$$

  where:
  a_i = percent abundance of species i in sample A
  b_i = percent abundance of species i in sample B.

Harmonization:

The harmonization of algae sample identification and enumeration procedures between DEP and the PL, and between processing laboratories, is essential to the success of DEP's algae monitoring and

method development effort. DEP has experienced inconsistencies between laboratories regarding their reported identifications and counts. Such inconsistencies were also noted by USEPA during the analysis of their 2008/2009 National Rivers and Streams Assessment (NRSA) diatom data (Lee et al. 2017). There are several possible reasons for these inconsistencies, many of which are described by Kahlert et al. (2009). Kahlert et al. (2009) suggests that harmonization is likely imperative to achieve consistency between laboratories and taxonomists. Kahlert et al. (2016) suggests methods by which this is may be achieved. Currently, DEP recognizes that to achieve proper harmonization, the following operational considerations and activities must be continuously reviewed and coordinated between both DEP and the PL:

- The body of current taxonomic references and literature to which the taxonomist refers to properly identify algae taxa
- Defining appropriate species complexes into which are grouped problematic identification OTU's (Operational Taxonomic Units) (Refer to Lee et al. 2017) as well as summarizing taxa that may easily be misidentified (Refer to Kahlert et al 2016)
- All laboratory procedures involved with the processes of subsampling, and identifying/enumerating algae samples
- Possible use of taxonomic intercalibration exercises and other harmonization procedures (Kahlert et al. 2009, Kahlert et al. 2016)

Because of the importance of diatom related data to DEP, the proper harmonization of diatom taxa is especially emphasized. In demonstration of the importance of harmonization, refer to Kahlert et al. 2009 in which the authors suggest that proper diatom taxa harmonization, rather than long experience with diatoms, is more important for achieving similarity between samples (and between taxonomist and auditor).

Initial steps for achieving harmonization between DEP and the PL will involve the following at the beginning of the project:

- DEP will provide to the PL the currently acceptable taxa list. This taxa list will include reference codes for each taxa along with authorship information.
- DEP and the PL will review the PL's identification and enumeration procedures.

Notes regarding taxa lists, DEP naming conventions, and identifications:

- The taxa list to which DEP refers, in general, is typically that offered by The Academy of Natural Sciences in their current list of Diatom and Non-Diatom (soft algae). This current downloadable Academy taxa list is available at http://diatom.ansp.org/Taxa.aspx. DEP also frequently refers to Diatoms of the United States (DOTUS) for taxa nomenclatural details (available at https://westerndiatoms.colorado.edu/). The Academy list is preferred because it is downloadable and is easily incorporated into a database.
- By DEP naming conventions, taxa names must include the source of authorship as part of the name. For example, *Achnanthidium minutissimum* (Kützing) Czarnecki, *Achnanthidium minutissimum* var. *gracillima* (Meister) Bukhtiyarova, or *Phormidium autumnale* (Agardh)

Trevisan ex Gomont. This convention is used by The Academy and must be rigidly adhered to by the PL, thus facilitating compatibility with the DEP database.

- For identifications, DEP also consults DOTUS (available at https://westerndiatoms.colorado.edu/), AlgaeBase (available at http://www.algaebase.org/), the Diatom New Taxon File maintained by The Academy of Natural Sciences (available at http://dh.ansp.org/dntf), the Catalogue of Diatom Names maintained by the California Academy of Sciences (available at http://researcharchive.calacademy.org/research/diatoms/names/index.asp). And USGS Biodata (available at https://aquatic.biodata.usgs.gov).
- The above notes, and institutional references, are not exhaustive and may include others when deemed appropriate.

The above represents initial harmonization steps that must be performed at the beginning of each project. Bear in mind that changes in algae taxonomy occur frequently and issues with problematic identifications will always exist. Consequentially, DEP and the PL will continuously confer so that they together may contend with taxonomy changes and problematic identifications as frequently as is deemed necessary by either of the two parties.

DEP will appoint an Auditing Laboratory at the beginning of each project. DEP will advise the PL as to the identity of the Auditing Laboratory at the beginning of the project.

Algae Identification and Enumeration Results Reporting, Data Formatting, and PL Internal Diatom and Palmer-Maloney Slide Processing QA Audit Report Requirements:

DEP will require that certain types of data be generated by the PL when processing Algae ID and enumeration samples. Data transfer between DEP and the PL must follow a specific format, as demonstrated by the tables presented in this section. This specific table format is necessary to efficiently support data transfer into DEP's Microsoft® Access based Algae Database. Three data table transfers are needed to properly exchange data between DEP and the PL. These tables include:

- Algae Identification and Enumeration Laboratory Submission Data Sheet, provided by DEP to the PL upon submission of samples.
- Palmer-Maloney and Diatom Slide Applied Factors Table, provided by the PL to DEP.
- Identification/Enumeration Results Table, provided by the PL to DEP.

Results will not be fully incorporated into the DEP Algae Database until all PL processing quality issues (as per blind duplicate results and auditor comparison results) are resolved and corrected data are received from the PL.

Each of the Results Reporting Tables will be defined and formatted as follows:

1. Algae Identification and Enumeration Laboratory Submission Data Table. DEP will transfer samples and the Algae Identification and Enumeration Laboratory Submission Data Table to the PL at the beginning of the laboratory processing phase of the project. This data table will

exist in the form of a Microsoft® Access data table (or Excel data table if necessary). Refer to Appendix A.8 for the format of this table. The following fields are required in this table to fully describe each sample to the PL (origin of information from the DEP Algae Database are included in parenthesis):

- DEP Algae Sample GIS Key ID. Access Foreign Key that references the corresponding Primary Access Key in the DEP Algae Database Samples Table. Configuration of this GIS KEY ID is yyyymmdd-HHMM-Collector. For example, Algae Sample GIS Key ID's have, in the past, included 20140813-0800-jbutt, and 20140821-0800-ggocek.
- Date sample was collected. (Duplication from the DEP Algae Database Samples Table).
- Sample Type. QMH (Qualitative Multi-Habitat) or QBE (Quantitative Benthic Epilithic) periphyton sample.
- DEP Algae Sampling Site ID number. Access Foreign Key that references the corresponding Primary Access Key in the DEP Algae Database Sites Table.
- Stream Name. (Duplication from the DEP Algae Database Sites table).
- Location Description. (Duplication from the DEP Algae Database Sites Table).
- County. (Duplication from the DEP Algae Database Sites Table).
- Latitude and Longitude. (Duplication from the DEP Algae Database Sites Table).
- Benthic Surface Area (cm^2). Applies only to QBE Periphyton samples (Duplication from the DEP Algae Database Samples Table).
- Algae Slurry Total Sample Volume (mL). Applies only to QBE Periphyton samples (Duplication from the DEP Algae Database Samples Table).
- ID Bottle SubSample Volume (mL). Typically 100mL. (Duplication from the DEP Algae Database Samples Table).
- Formaldehyde Added to ID SubSample Bottle (mL). (Duplication from the DEP Algae Database Samples Table).
- Note: Transfer of all this data to the PL is necessary. Location of sample may help the algae taxonomist in deciding species or variety level taxa ID due to possible geographic floristic variations. Surface areas and volumes are required by the PL to perform sample specific calculations that effect values of the Palmer-Maloney Correction Factors and the Diatom Slide Applied Factors.

2. Palmer-Maloney and Diatom Slide Applied Factors Table. The PL will transfer to DEP sample specific values as determined during the analysis of the Palmer-Maloney slide and reported in the Palmer-Maloney and Diatom Slide Applied Factors Table (as a reminder, any Palmer-Maloney Chambers used to produce for DEP must be calibrated). To facilitate this data transfer process, DEP will provide a Microsoft® Access data table (or Excel data table if necessary) template to the PL at the beginning of the ID/Enumeration project, which the PL will then populate and return to DEP. An abbreviated example of this table is provided in Table 3 and demonstrates real data provided by The Academy of Natural Sciences for samples collected in 2013. This table will include the following fields to fully characterize each sample to DEP:

- DEP Algae Sample GIS Key ID. Access Foreign Key that references corresponding Primary Access Key in the DEP Algae Database Samples Table. Obtained by PL from the Algae Identification and Enumeration Laboratory Submission Data Table
- Field Dilution Correction Factor (FDCF). DEP submits to the PL a 100ml algae ID/enumeration subsample bottle to which is added a formaldehyde preservative. This correction factor is determined by the PL from values provided on the Algae Identification and Enumeration Laboratory Submission Data Table and is computed as: (Volume of Algae ID subsample (ml) + Volume of Formaldehyde added (ml)) / Volume of Algae ID subsample (ml). For example, if volume of Algae ID subsample = 100ml and the amount of formaldehyde added to that subsample is 5 mL, then this correction factor = (100ml + 5ml)/100ml = 1.05.
- Subsample Dilution Correction Factor (SDCF). If the PL needs to make a dilution to the submitted ID/enumeration subsample bottle the SDCF is recorded in this field by the PL. SDCF is computed as: (Volume of original Algae ID subsample (ml) + Volume of distilled water added (ml)) / Volume of original Algae ID subsample (ml)).
- Palmer-Maloney Dilution Correction Factor (PMDCF). If the PL needs to make a dilution during the Palmer-Maloney analysis, the PMDCF will be recorded in this field by the PL. Computation is similar to those field dilution correction factors demonstrated above.
- Palmer-Maloney Microscope Field of View Volume, and Palmer-Maloney Number of Fields Observed. Each field of view in the microscope will contain a certain volume, and is dependent upon the Palmer-Maloney chamber depth and the diameter of the field of view of the microscope. For example, from DEP sample 20130424-0800-jbutt, the PL microscope's field of view volume in the Palmer-Maloney was 0.000048497ml/field of view. The number of fields of view is a record of the number of fields of view that must be completely counted to reach, or slightly surpassed, the 300-natural units threshold required by the Palmer-Maloney analysis.
- Palmer-Maloney Total Volume of all Fields Observed. This value is calculated by the PL by multiplying the field of view volume by the quantity of fields observed. For example, from the DEP sample 20130424-0800-jbutt, this value was (0.000048497ml/field of view) x (10 fields of view) = 0.0004897ml.
- Algal Slurry Total Sample Volume (ml). Duplicated from the Algae Identification and Enumeration Laboratory Submission Data Sheet.
- Benthic Surface Area of sample (cm^2). Applies only to QBE Periphyton samples Duplicated from the Algae Identification and Enumeration Laboratory Submission Data Sheet.
- Cell Density Factor (CDF). This factor is computed by CDF = (FDCF * SDCF * PMDCF * Algae Slurry Total Sample Volume) / (Total Volume of all Fields observed in the Palmer-Maloney * Benthic Area Sampled).

Table 3. Example of the Palmer-Maloney and Diatom Slide Applied Factors Table for 2013 DEP algae Samples processed by The Academy of Natural Sciences.

PADEP Algae Sample GIS Key ID (PADEP)	Algae Slurrry Total Sample Volume (ml)	Benthic Area Sampled (cm^2)	FDCF - Field Dilution Correction Factor	SDCF - SubSample Dillution Correction Factor	PMDCF - Palmer-Maloney Dilution Correction Factor	Microscope Field of View Volume in Palmer-Maloney (ml)	Number of Fields Observed in Palmer-Maloney	Total Volume of all Fields Observed in Palmer-Maloney	CDF - Cell Density Factor in Palmer-Maloney
20130424-1100-jbutt	700	185.4	1.05	1.00	2.00	0.000048497	10	0.00048497	16349.1
20130424-1400-jbutt	700	185.4	1.05	1.00	2.00	0.000048497	13	0.00063046	12576.2
20130424-0800-jbutt	700	185.4	1.05	1.00	1.00	0.000048497	10	0.00048497	8174.5
20130916-0800-jbutt	1784	556.2	1.05	1.00	2.00	0.000048497	43	0.00208537	3230.0
20130916-1400-jbutt	1099	556.2	1.05	1.00	1.00	0.000048497	47	0.00227936	910.2
20130916-1100-jbutt	1109	556.2	1.05	1.00	1.00	0.000048497	66	0.00320080	654.1
20130917-1100-jbutt	1214	556.2	1.05	1.00	1.00	0.000048497	47	0.00227936	1005.5
20130917-1400-jbutt	1834	556.2	1.05	1.00	4.00	0.000048497	37	0.00179439	7717.9
20130917-0800-jbutt	1014	556.2	1.05	1.00	2.00	0.000048497	39	0.00189138	2024.2

3. Identification/Enumeration Results Table. The PL will transfer to DEP, taxa specific values for each sample as determined during the analysis of the Palmer-Maloney chamber and diatom slides and reported in the Identification/Enumeration Results Table. To facilitate this data transfer process, DEP will provide a Microsoft® Access data table (or Excel data table if necessary) template to the PL at the beginning of the ID/Enumeration project, which the PL will then populate and return to DEP. An abbreviated example (shows only the results from a single sample and not those generated from all samples) of this table is provided in Table 4. This table will include the following fields to fully characterize each sample to DEP:
    - DEP Algae Sample GIS Key ID. Access Foreign Key that references corresponding Primary Access Key in the DEP Algae Database Samples Table.
    - Taxonomic NADED ID Number. Access Foreign Key that references corresponding Primary Key in the DEP Algae Database Taxa List Table. North American Diatom Ecological Database (NADED) ID numbers, unique for each taxa name-taxa author, are available from The Academy of Natural Science current taxa list at http://diatom.ansp.org/Taxa.aspx and are also found in the Diatoms of the United States website available at https://westerndiatoms.colorado.edu/ . For example, *Achnanthidium minutissimum* (Kützing) Czarnecki is listed as NADED 1010, *Achnanthidium minutissimum var. gracillima* (Meister) Bukhtiyarova as NADED 1063, and *Phormidium autumnale* (Agardh) Trevisan ex Gomont as NADED 890028 in the Academy current taxa list.
    - Algae Taxon Name with Author Reference. Refer to the Harmonization section, above, for requirements.
    - Natural Units Counted in Palmer-Maloney. Natural Units are counted for each sample during the soft algae analysis in a Palmer-Maloney counting chamber. Natural Units are natural groupings of algae such as an individual filament, colony, or isolated cell and represent the usual grouping of cells that occur in a natural setting. Each such natural grouping is counted as one natural unit. For example, a colony of microcystis, which may contain dozens or perhaps hundreds of cells, is counted in a soft algae sample as one natural unit. This counting procedure is used for all non-diatom taxa. Living and dead diatoms, regardless of their colonial grouping, are counted so that each cell is a natural unit, even when they are observed in colony form. Living diatoms must be

distinguished from dead diatoms. This unit exists to prevent colonies or filaments from dominating the result of the 300 Natural Unit ID/count, thereby enabling a good representation of the taxa diversity in the soft algae sample.

- Number of Cells Counted in Palmer-Maloney. The number of cells are counted during the soft algae analysis in a Palmer-Maloney counting chamber. In soft algae samples, an attempt to count (or estimate, if a count of a colony is impractical) the number of cells will be performed. Each diatom frustule or valve is counted as a cell in a soft algae sample. Care needs to be exercised in producing this result, especially when colony estimation is performed since the cell count is used to derive cell abundance and biovolume in the permanent slide results.
- Number of Valves Counted in Diatom Slide for each Taxa. These are the number of diatom valves counted for each taxa in the microscope fields of view during the analysis of the diatom slide. Diatoms contained on these slides have been treated to remove soft tissues from the sample, thereby rendering the silicate structure of the diatom frustules highly visible in a microscopic study.
- Total Number Valves Counted on Diatom Slide. Equal to the sum of all valves for each taxa counted in the microscope fields of view during the analysis of the diatom slide. Microscope fields of view on the diatom slide are completely counted until a threshold quantity of 600 total valves is reached or slightly surpassed.
- Diatom Valve Relative Abundance. This computed value is equal to the number of diatom valves counted in each taxa on the diatom slide divided by total number of valves counted on the diatom slide.
- Number of Living Diatoms Counted in Palmer-Maloney Analysis. This is the quantity of living diatom cells counted as part of the Palmer-Maloney identification and Enumeration Process. This value is derived for the entire sample and consequentially is the same for each taxa in the sample. *[Special Note: At this point it is necessary to describe, in detail, the difference between living and dead diatoms. By definition, Undifferentiated Diatoms - Living (NADED 249999) are the number of diatoms containing cytoplasm counted during the Palmer-Maloney analysis. Because of the presence of cytoplasm, these diatoms are to be considered as having been alive at the time the sample was collected in the field. Note that the cytoplasm in a living cell, along with all the cell's organelles, may be reduced into a small packet, of sorts, due to the formaldehyde preservation and/or the storage time. Whereas, by definition, Undifferentiated Diatoms – Dead (NADED 249998) are the number of diatoms or diatom valves counted during the Palmer-Maloney analysis that don't contain any evidence of cytoplasm. Because these diatoms or valves do not contain cytoplasm these diatoms are to be considered as having been dead at the time the sample was collected.]* Living and dead diatoms are identified and counted as taxa types in the Palmer-Maloney Identification and Enumeration process. Because living and dead diatoms contribute to the Natural Unit count, this distinction must be made to correct the diatom slide count to eliminate that proportion of diatoms presumed to have been dead at the time the sample was collected in the field (in other words, the diatom slide result must be adjusted to estimate only the quantity of diatoms that were alive at the time of sampling). This data field is needed because the nitric acid treatment used to prepare the diatom slide dissolves all soft tissues making it impossible to distinguish between diatoms that were

living versus those that were dead at the time the sample was collected. Consequentially, the taxonomist must ID and count all observed diatoms then make the correction discussed in the Proportioned Diatoms field.

- Proportioned Living Diatom Valves. This computed value is equal to Diatom Valve Relative Abundance value multiplied by the number of Undifferentiated Diatoms – Living (NADED 249999). This value is the number of diatoms valves of each taxa estimated to have originated from living diatoms in the sample.
- Cell Density Factor. This is a duplication of the CDF from the Palmer-Maloney and Diatom Slide Applied Factors Table – see above. This value is derived for the sample as a whole and consequentially is the same for each taxa in the sample.
- Benthic Cell Density. This computed value reflects the quantity of cells for each taxa estimated to occur per cm^2 of benthic surface. For each taxa identified from the diatom slide, the Benthic Cell Density is equal to that taxa's Proportioned Living Diatom Valves multiplied by the sample CDF. Whereas, for each taxa identified in the Palmer-Maloney slide, the Benthic Cell Density is equal to Number of cells counted in the Palmer-Maloney slide multiplied by the sample CDF.
- Biovolume per Cell – Applied Constants. This value will be incorporated by DEP as part of its data upload into the DEP Algae Database. Estimated biovolumes per cell for each taxa type may be determined by two procedures. One procedure is to make measurements on each algae cell observed in the diatom slide or in the Palmer-Maloney chamber and compute an average biovolume per cell for each taxon. The procedure for calculation may follow that offered by the Academy of Natural Sciences, which is available at http://diatom.ansp.org/nawqa/Biovol2001.aspx. The benefit of this procedure is that the biovolumes used are more reflective of the sample. However, the difficulty with the process of making measurements of every cell observed, or even a subsampling of those observed, is that a large amount of time may be expended to obtain such measurements. A less time consumptive procedure is to use archived taxa-level biovolume data from the Academy of Natural Sciences. Archived data are available from https://diatom.ansp.org/autecology/download.asp?file_id=4 and provide various biovolume statistics for each taxon. These biovolume statistics are compiled from the data the Academy of Natural Sciences has accumulated over many years of research and would provide a reliable representation of the sampled taxa population. When relying upon this later procedure, DEP would typically use the average biovolume data statistic for each taxon, although other values such as minimum and maximum biovolumes are also available. The problems with this alternative procedure are that it is not sample specific, and the data offered on the Academy website was last updated in 2001. These Academy of Natural Sciences provided biovolume data are further complicated by the fact that many taxa names have changed since 2001 and many new taxa may have since be added to this now dated list. Because of possible taxa name changes, synonyms may have to be researched to determine the biovolume of a taxon from this dated Academy of Natural Sciences list. However, these problems could be partially solved by simply referring to that biovolume data already existing as part of

DEP Algae Database from samples collected and processed by the Academy of Natural Sciences in 2013, 2014, 2015, and 2016. At the beginning of each project, DEP will stipulate to the PL which of these two procedures are to be used. [Note: connections to both of the mentioned web sources may also be found at the Academy Autecology site found at https://diatom.ansp.org/autecology/#browse.]

- Benthic Cell Biovolume. This historically PL reported value is presently not included in this table exchanged between DEP and the PL. This value will be incorporated by DEP as part of its data upload into the DEP Algae Database. This computed value is equal to the Benthic Cell Density multiplied by Biovolume per Cell-Applied Constant.

Table 4. Example of the Identification/Enumeration Results Table, limited to a single sample collected in 2013 (table submitted by PL to DEP would contain results for all samples). The left-most column is not part of the actual Access or Excel based Result Table but is provided here to demonstrate the analysis process used to generate the data.

	PADEP Algae Sample GIS Key ID	Taxonomic NADED ID Number	Algae Taxon Name with Author Reference	Natuaral Units Counted in Palmer-Maloney	Number Cells Counted in Palmer-Maloney	Number Valves Counted in Diatom Slide	Total Number Valves Counted on Datom Slide	Diatom Valve Relative Abundance	Number Living Diatoms Counted in Palmer-Maloney	Proportioned Living Diatom Valves	CDF - Cell Density Factor	Benthic Cell Density (cells per cm2 of sampled benthic surface area)
Diatom Slide ID/Count Result	20130424-0800-jbutt	1023	Achnanthidium pyrenaicum (Hustedt) Kobayashi			435	631	0.6894	291	200.61	8174.50	1639887.61
	20130424-0800-jbutt	7043	Amphora pediculus (Kützing) Grunow			46	631	0.0729	291	21.21	8174.50	173413.40
	20130424-0800-jbutt	16004	Cocconeis placentula Ehrenberg			2	631	0.0032	291	0.92	8174.50	7539.71
	20130424-0800-jbutt	16005	Cocconeis placentula var. euglypta (Ehrenberg) Grunow			1	631	0.0016	291	0.46	8174.50	3769.86
	20130424-0800-jbutt	16011	Cocconeis pediculus Ehrenberg			12	631	0.0190	291	5.53	8174.50	45238.28
	20130424-0800-jbutt	27008	Diatoma moniliformis Kützing			1	631	0.0016	291	0.46	8174.50	3769.86
	20130424-0800-jbutt	27013	Diatoma vulgaris Bory			7	631	0.0111	291	3.23	8174.50	26389.00
	20130424-0800-jbutt	34030	Fragilaria vaucheriae (Kützing) Petersen			4	631	0.0063	291	1.84	8174.50	15079.43
	20130424-0800-jbutt	37990	Gomphonema sp. 1 ?			6	631	0.0095	291	2.77	8174.50	22619.14
	20130424-0800-jbutt	44073	Melosira varians Agardh			9	631	0.0143	291	4.15	8174.50	33928.71
	20130424-0800-jbutt	45001	Meridion circulare (Greville) Agardh			1	631	0.0016	291	0.46	8174.50	3769.86
	20130424-0800-jbutt	46023	Navicula gregaria Donkin			5	631	0.0079	291	2.31	8174.50	18849.28
	20130424-0800-jbutt	46104	Navicula tripunctata (Müller) Bory			1	631	0.0016	291	0.46	8174.50	3769.86
	20130424-0800-jbutt	46154	Navicula rhynchocephala Kützing			2	631	0.0032	291	0.92	8174.50	7539.71
	20130424-0800-jbutt	46646	Navicula caterva Hohn et Hellerman			2	631	0.0032	291	0.92	8174.50	7539.71
	20130424-0800-jbutt	46661	Navicula capitatoradiata Germain			2	631	0.0032	291	0.92	8174.50	7539.71
	20130424-0800-jbutt	46859	Navicula lanceolata (Agardh) Kützing			10	631	0.0158	291	4.61	8174.50	37698.57
	20130424-0800-jbutt	46893	Navicula antonii Lange-Bertalot			4	631	0.0063	291	1.84	8174.50	15079.43
	20130424-0800-jbutt	46896	Navicula rostellata Kützing			2	631	0.0032	291	0.92	8174.50	7539.71
	20130424-0800-jbutt	48002	Nitzschia acicularis (Kützing) Smith			2	631	0.0032	291	0.92	8174.50	7539.71
	20130424-0800-jbutt	48004	Nitzschia amphibia Grunow			1	631	0.0016	291	0.46	8174.50	3769.86
	20130424-0800-jbutt	48008	Nitzschia dissipata (Kützing) Grunow			22	631	0.0349	291	10.15	8174.50	82936.84
	20130424-0800-jbutt	48013	Nitzschia frustulum (Kützing) Grunow			21	631	0.0333	291	9.68	8174.50	79166.99
	20130424-0800-jbutt	48025	Nitzschia palea (Kützing) Smith			2	631	0.0032	291	0.92	8174.50	7539.71
	20130424-0800-jbutt	48123	Nitzschia pusilla Grunow			1	631	0.0016	291	0.46	8174.50	3769.86
	20130424-0800-jbutt	55002	Reimeria sinuata (Gregory) Kociolek et Stoermer			11	631	0.0174	291	5.07	8174.50	41468.42
	20130424-0800-jbutt	57002	Rhoicosphenia abbreviata (Agardh) Lange-Bertalot			9	631	0.0143	291	4.15	8174.50	33928.71
	20130424-0800-jbutt	65048	Surirella minuta Brébisson			3	631	0.0048	291	1.38	8174.50	11309.57
	20130424-0800-jbutt	110004	Encyonema minutum (Hilse) Mann			4	631	0.0063	291	1.84	8174.50	15079.43
	20130424-0800-jbutt	155003	Planothidium lanceolatum (Brébisson ex Kützing) Lange-Bertalot			2	631	0.0032	291	0.92	8174.50	7539.71
	20130424-0800-jbutt	155017	Planothidium frequentissimum (Lange-Bertalot) Lange-Bertalot			1	631	0.0016	291	0.46	8174.50	3769.86
Palmer-Maloney ID/Count Result	20130424-0800-jbutt	249998	(undifferentiated) diatoms> (dead)	53	53						8174.50	433248.50
	20130424-0800-jbutt	249999	(undifferentiated) (diatoms)>(living)	291	291						8174.50	2378779.50
	20130424-0800-jbutt	309000	Cladophora glomerata (Linnaeus) Kützing	3	11						8174.50	89919.50
	20130424-0800-jbutt	510001	Scenedesmus quadricauda (Turpin) Brébisson	1	4						8174.50	32698.00
	20130424-0800-jbutt	533000	Spirogyra sp.	1	4						8174.50	32698.00
	20130424-0800-jbutt	569000	Ulothrix sp.	5	52						8174.50	425074.00
	20130424-0800-jbutt	893002	Pleurocapsa minor Hansgirg	5	95						8174.50	776577.50

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3.11 BACTERIOLOGICAL DATA COLLECTION PROTOCOL

Chapter 3 Biological Data Collection Protocols

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INTRODUCTION

All surface waters of Pennsylvania are evaluated for Water Contact Sports (WC) according to the criteria for bacteria in §93.7 of 25 Pa. Code Chapter 93. This protocol identifies appropriate sample collection procedures needed to assess for WC in applicable waters. For sampling design considerations for lakes refer to *Chapter 2.4, Lake Assessment Survey Design* (Arnold 2023) and for rivers and streams refer to *Chapter 2.5, Stream and River Assessment Survey Design* (Shull and Arnold 2023b).

DATA COLLECTION

Bacteriological data collection protocols follow the same procedures described in *Chapter 4.2, Discrete Water Chemistry Data Collection Protocol* (Shull and Arnold 2023a). A list of sampling supplies and equipment is outlined below:

Sampling Supplies and Equipment

- BOL Sample Submission Sheet (Submission Sheet)
- DEP Bacteria Monitoring Field and Site Data Sheets (Appendix A.10)
- List of sites, maps, and/or GPS
- 125-mL screw-capped, polypropylene or polystyrene bottles with sodium thiosulfate
- 1-L screw-capped, polypropylene bottles (MST qPCR)
- Sample blanks containing sterile (autoclaved) water obtained from BOL
- Disposable, powderless gloves (such as nitrile)
- Coolers and loose ice
- Courier shipping labels
- Van Dorn or Kemmerer (Lakes)
- Field meter (required for rivers and streams greater than 1000 mi^2)
- Sampling pole and zip-ties (optional)

DEP uses 125mL screw-capped bottles treated with sodium thiosulfate to neutralize the effects of residual chlorine or 1L (MST qPCR) screw-capped bottles. DO NOT RINSE THE BOTTLE BEFORE SAMPLING.

Lake Sampling

Bacteriological samples will be collected using disposable, powderless gloves (such as nitrile, elbow length preferred) from lakes. Samples from a boat are collected with motor off. Samples are collected between a minimum depth of 0.3-meters and maximum depth of 1 meter. Samples collected at a depth of one meter can be collected using a sampling pole or from depth specific water sampling equipment (e.g., Van Dorn, Kemmerer). Instruction on how to use a Van Dorn or Kemmerer are described in *Chapter 4.2, Discrete Water Chemistry Data Collection Protocol* (Shull and Arnold 2023a).

Preservation
The collector should vertically insert bottles into a cooler, cap-side up. The samples should be cooled with loose ice. Enough ice should be added to the cooler to ensure samples remain at ≤6°C during overnight shipping. Samples submitted to BOL follow the same procedures described in *Chapter 4.2, Discrete Water Chemistry Data Collection Protocol* (Shull and Arnold 2023a).

Quality Assurance
Quality assurance requirements follow the same procedures described in Appendix E, DEP's Quality Assurance Procedures.

Field Meters
Collection of field meter data is recommended for bacteriological sampling and required for rivers and streams greater than 1000 mi^2. Field meter procedures are described in *Chapter 4.1, In-Situ Field Meter, Transect, and Profile Data Collection Protocol* (Hoger and Arnold 2023).

SAMPLE SUBMISSION

Bacteriological sample submission requirements follow the same procedures described in *Chapter 4.2, Discrete Water Chemistry Data Collection Protocol* (Shull and Arnold 2023a). A Standard Analysis Code (SAC) is a unique code that details analytical tests to be applied to a specific sample. Each DEP program uses specific SACs for specific projects or purposes. For example, SAC B022 is used by "Water Management" when submitting bacteriological samples for *E. coli* with a 24-hour hold time. Table 1 lists other bacteriological test descriptions and SACs.

Table 1. BOL Bacteriological Test Descriptions and SACs

Organism	Test Description	SAC
Fecal coliforms	Membrane Filtration (8-hr. Hold Time)	B002
Fecal coliforms	Membrane Filtration (24-hr hold time)	B032
Fecal coliforms + Strep	Membrane Filtration	B003
Fecal coliforms + *E. coli*	Membrane Filtration (24-hr hold time)	B021
E. coli	Membrane Filtration (24-hr hold time)	B022
Enterococci	Membrane Filtration	B015
Fecal coliforms + Total/Human/Avian/Bovine/Swine Bacteroides	Membrane Filtration + MST qPCR	B035
Fecal coliforms + *E. coli* + Total/Human/Avian/Bovine/Swine Bacteroides	Membrane Filtration + MST qPCR	B036
E. coli + Total/Human/Avian/Bovine/Swine/Horse/Dog/Deer Bacteroides	Membrane Filtration + MST qPCR	B037
Total/Human/Avian/Bovine/Swine Bacteroides	MST qPCR	B038

DATA MANAGEMENT

General guidelines for data entry are described in *Chapter 4.6, Sample Information System (SIS) Data Entry Protocol* (Shull 2017). Step-by-step processes of SIS data entry are also provided in Appendix B.3. However, the following includes recommendations specific to managing projects, monitoring points, and samples using bacteriological data.

Projects

Create a project specific to each recreational use monitoring and assessment effort. This will include delineation of subbasin targeted for a specific project. 'Project ID' should be formatted to include the letters 'BAC', four alpha characters, followed by two-digit year. 'Description' should include four-digit year, "Bacteria Monitoring Project", followed by the targeted subbasin or watershed. Include in the 'Comments' a general description of the purpose and scope of the project along with any pertinent specific information (Figure 1).

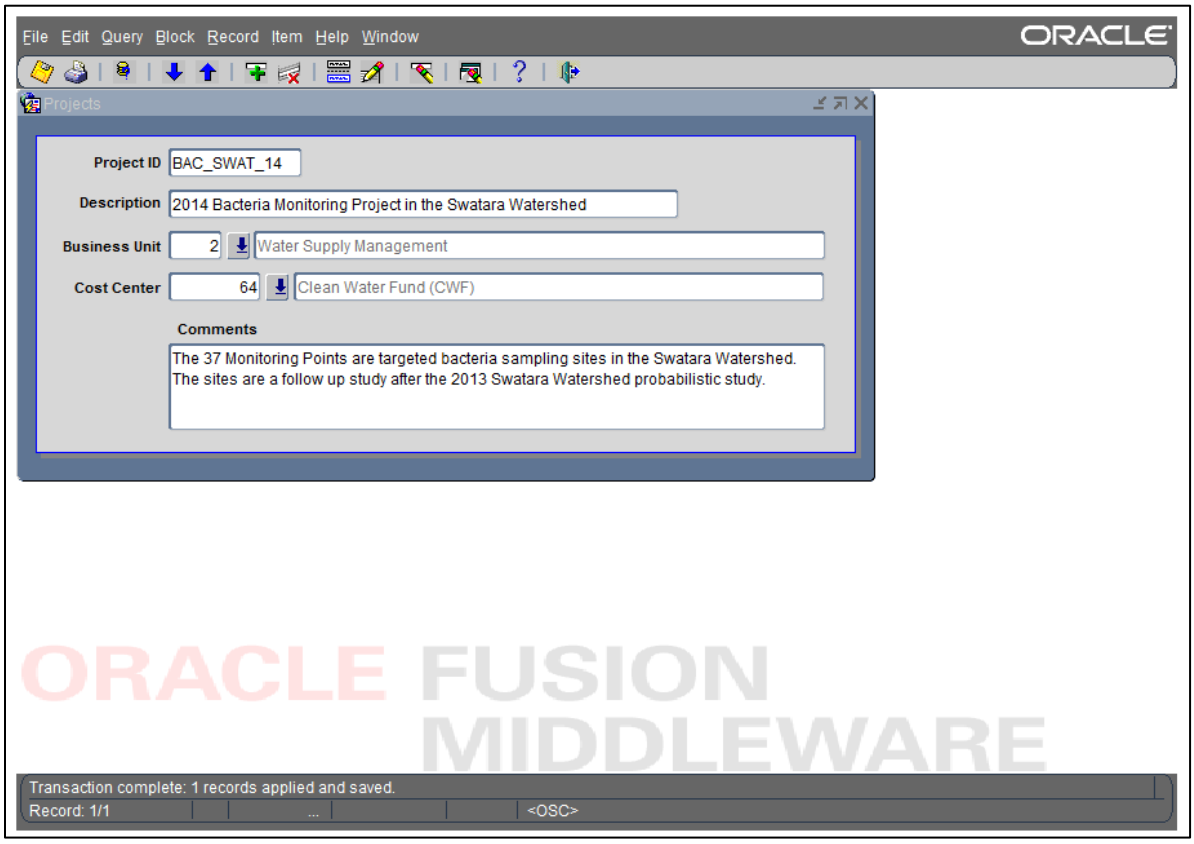

Figure 1. SIS Project entry and edit screen with template information.

Monitoring Points

Create a monitoring point for each sampling location. The 'Name' of each monitoring point will begin with the same four alpha characters followed by sequential numbers. Within the location tab populate 'State', 'County', 'Latitude', 'Longitude', 'Datum' (NAD83), and 'Location'. Include a general location description in 'Location' (Figure 2). Within the Function/Administration tab 'Medium Type' should be "Water" and for surface water samples 'Sample Medium' will be "Surface Water" (Figure 3). Within the Aliases tab 'Alias ID' will begin with the same four alpha characters followed by sequential numbers as 'Name'. Description will include the watershed, year, and targeted site number (Figure 4). Within

the National Hydrologic Dataset (NHD) tab click ‘Launch NHD Locator, snap the monitoring point to an NHD reach, and ‘Get NHD. Lastly, link the Monitoring Point to the Project by clicking ‘Projects’, populating the ‘Project ID’, ‘Alias ID’, and ‘Effective Date’ as the date the monitoring point was first sampled (Figure 5).

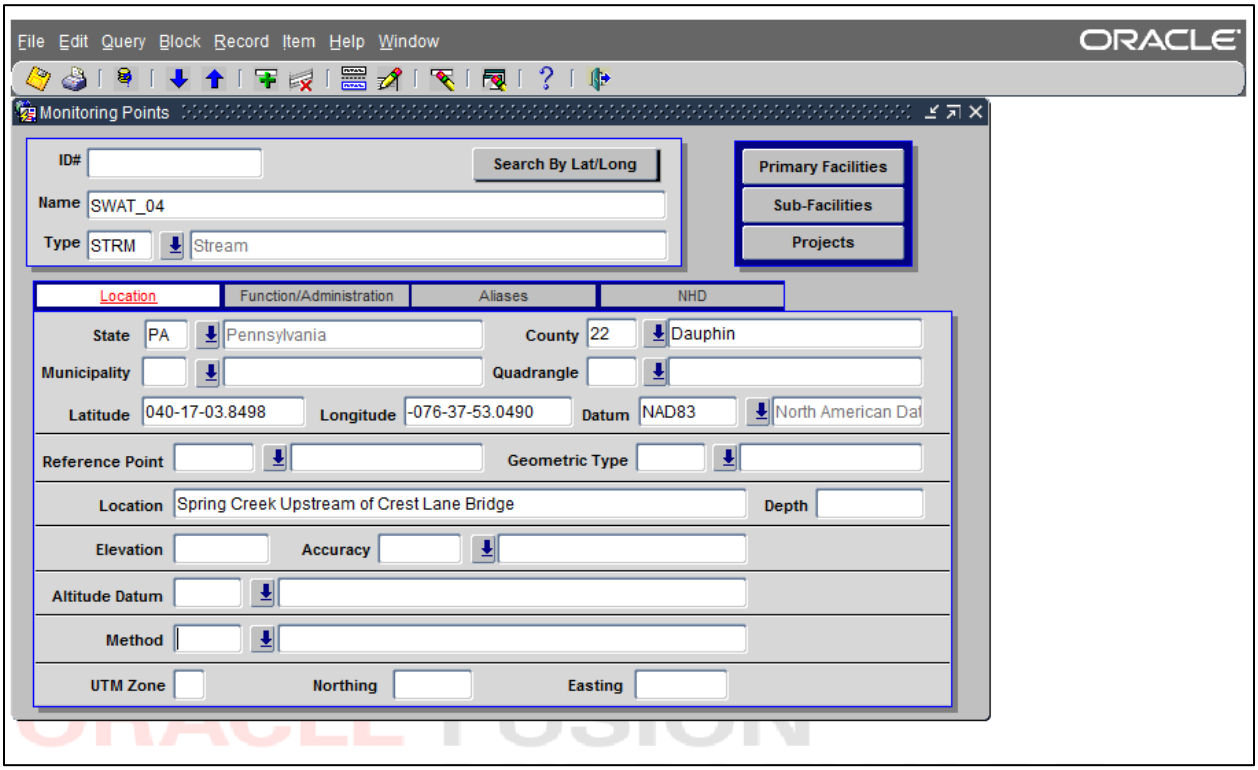

Figure 2. SIS Monitoring Point, Location tab entry and edit screen with template information.

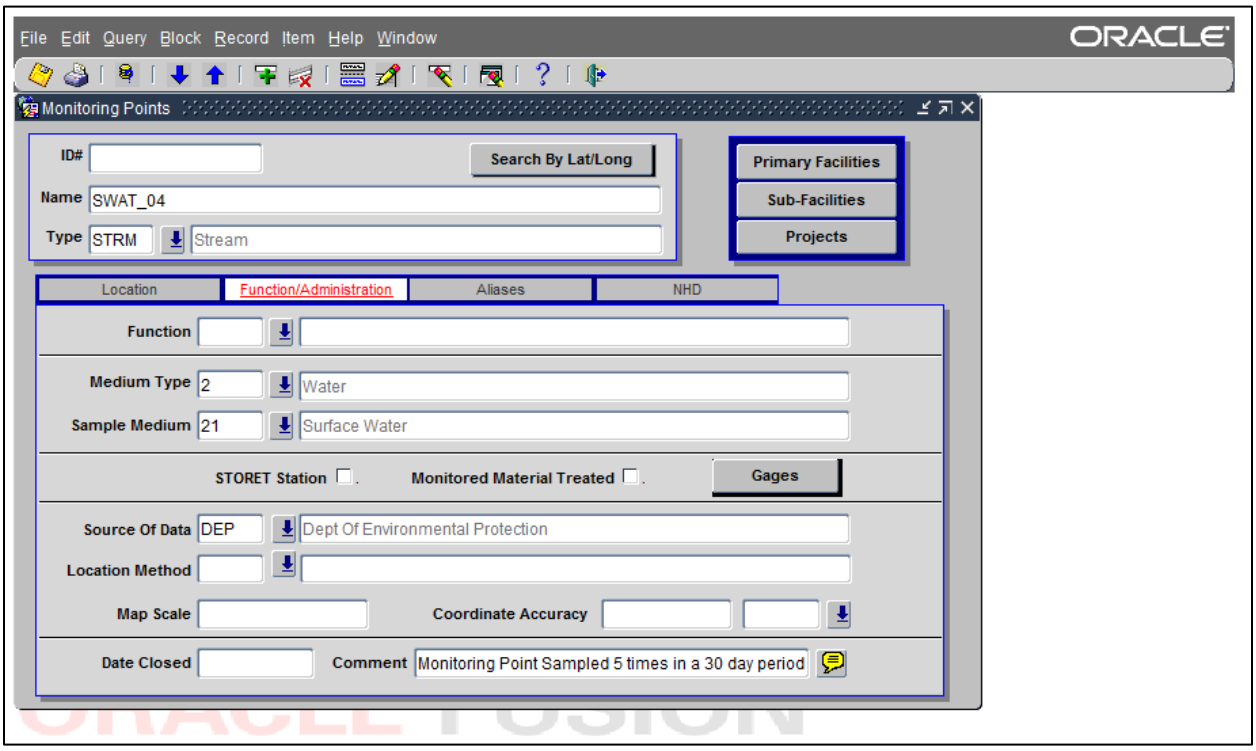

Figure 3. SIS Monitoring Point, Function/Administration tab entry and edit screen with template information.

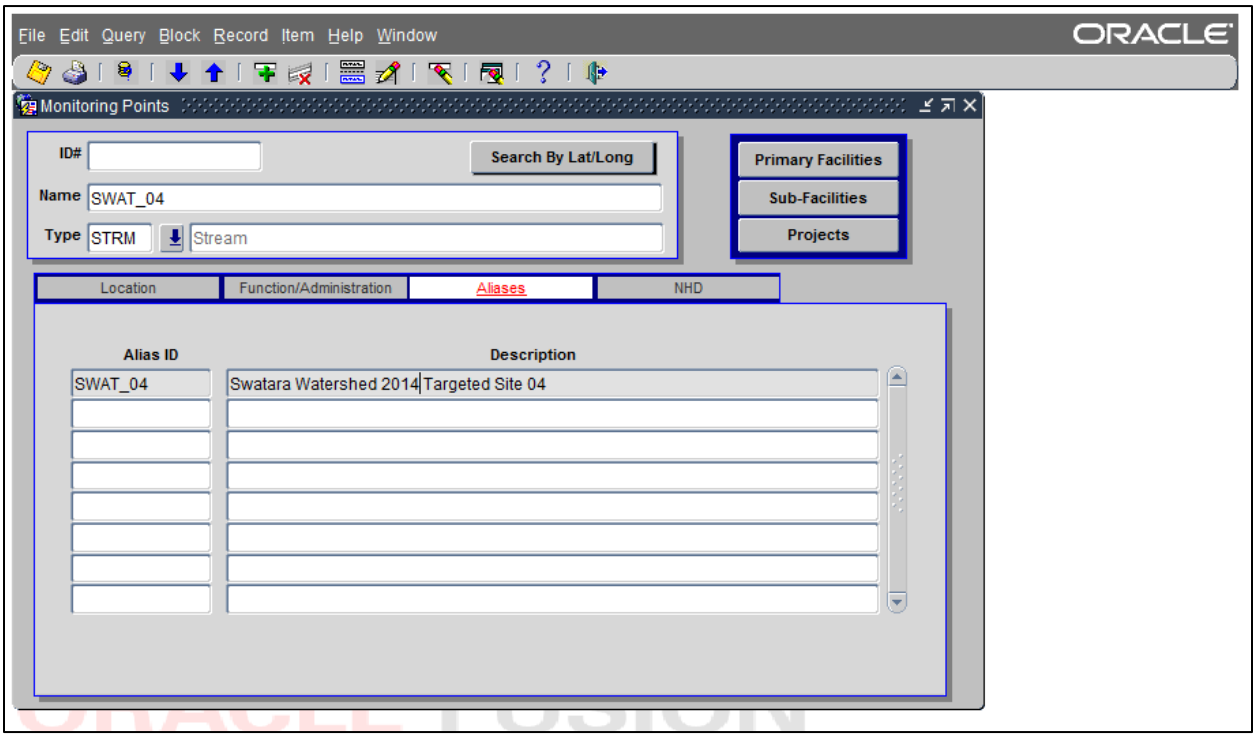

Figure 4. SIS Monitoring Point, Aliases tab entry and edit screen with template information.

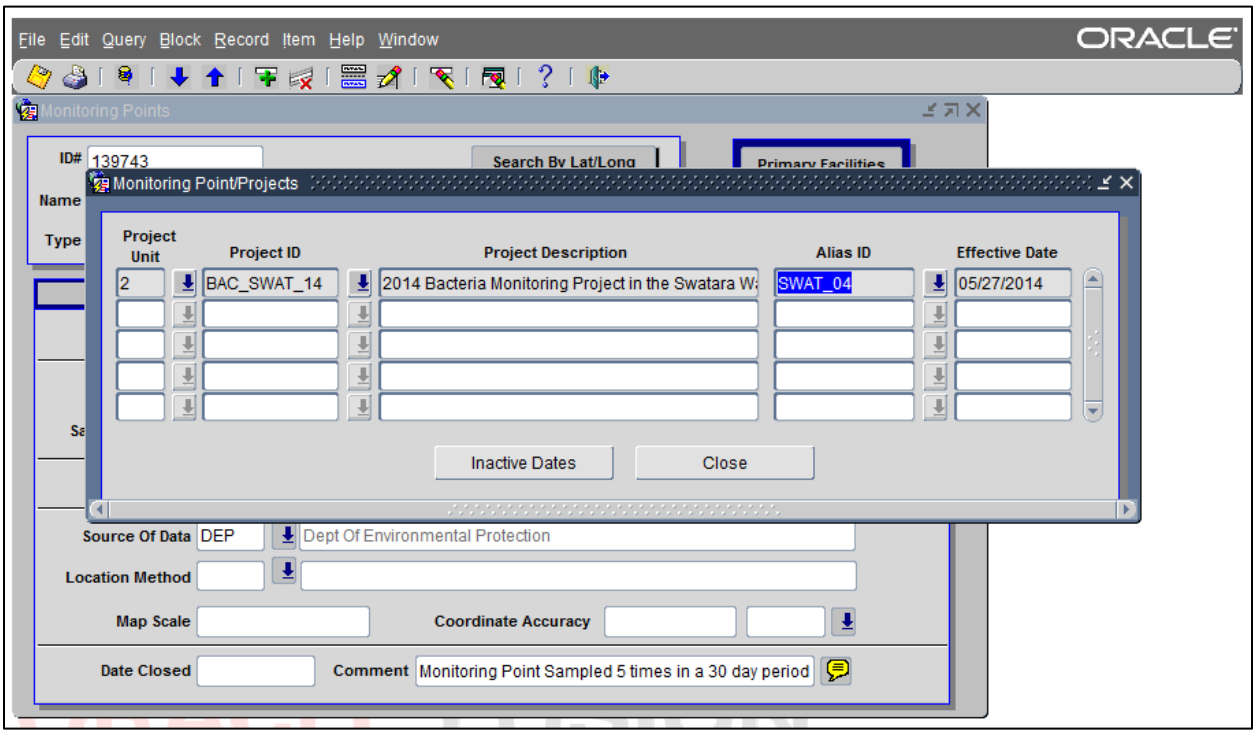

Figure 5. SIS Monitoring Point, Project link and edit screen with template information.

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3.12 CHLOROPHYLL-A, PHYCOCYANIN, AND ASH-FREE DRY MASS DATA COLLECTION PROTOCOL

Chapter 3 Biological Data Collection Protocols

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INTRODUCTION

DEP implements chlorophyll-a (Chl-a) sampling as part of *Chapter 2.4, Lake Assessment Survey Design* (Arnold 2023). Additionally, Chl-a, phycocyanin, and ash free dry mass (AFDM) sampling is part of the *Chapter 3.10, Periphyton Data Collection Protocol (Butt 2017)*. Chlorophyll-a is the primary pigment produced by most algae in freshwater and an important water quality parameter by which to assess algal productivity. Phycocyanin is a photopigment that, in Pennsylvania, is nearly exclusively descriptive of cyanobacteria.

Chlorophyll-a is an important water quality parameter in streams and lakes. Chlorophyll-a can impact water quality, is often correlated with elevated nutrient concentrations and is a direct measurement of algal biomass. Since Chl-a is a direct measurement of algal biomass, it is a useful assessment tool to determine the trophic state of surface waters. Additionally, phycocyanin and AFDM are used by DEP to help explain the algal biomass in a stream. This algal biomass, in turn, may be used to infer characteristics regarding the nutrient status of a stream and provide information used to judge the biological condition status of that stream.

DATA COLLECTION

There are two steps to data collection. The first is to either obtain a benthic epilithic slurry (benthic slurry) or surface water sample. Next the benthic slurry or water sample will be filtered to obtain a small volume of slurry or disk filter of the algal materials to be analyzed. Techniques to obtain a benthic slurry or surface water sample are briefly described here; full details on sampling protocols for benthic and water sampling are found in *Chapter 3.10, Periphyton Data Collection Protocol* (Butt 2017) and *Chapter 4.2, Discrete Water Chemistry Data Collection Protocol* (Shull and Arnold 2023). Field data and field notes for chlorophyll-a, AFDM, and/or phycocyanin samples will be entered on the appropriate data collection sheet (Appendix A.6 or Appendix A.11).

For each of the sampling procedures described below, every 20th sample should be replicated as part of the data collection quality control (Appendix E). QA/QC procedures described throughout this protocol should also be followed accordingly. The U.S. Environmental Protection Agency (USEPA) recommends that quality control measures for chlorophyll-a include a laboratory duplicate, a field duplicate, and a field blank, each done at a rate of once per basin (USEPA 2013). Field blanks will utilize “ultra-pure water” instead of the periphyton slurry and will use the same acidified chlorophyll-a subsampling procedures as those described above. Because the term “basin” could include a broad geographic area and thus include much of the sampling that occurs in Pennsylvania, for example the Susquehanna River basin, it will be left to the field biologist to decide the extent that the term “once per basin” will imply – DEP recommends a conservative approach, preferring to submit QA samples regularly (once per week per designated basin, for example). USEPA suggests an acceptance criteria of 25% relative percent difference for the laboratory and field duplicates, and 0.00µg/L ±0.11µg/L for the field blank (USEPA 2013).

Field Data Collection

Benthic Sampling

For benthic sampling, field collection procedures will follow those described in *Chapter 3.10, Periphyton Data Collection Protocol (Butt 2017).* Briefly, rocks are collected from a series of transects and are scrubbed to collect the attached algal matter. This algal matter is composited into a slurry, which is then emulsified and blended at high speed to homogenize the slurry. The homogenized slurry will be used to obtain subsamples and aliquots for filtering.

Although appreciable effort is made by DEP biologists to thoroughly homogenize the algae slurry, variation in duplicate subsample results have been noted in nearly all reported lab results. This variation is, in part, due to robust forms of filamentous algae that resist complete homogenization, even after the use of a highspeed food-grade blender. The best way to deal with this unavoidable variation in photopigment and AFDM results is to run replicate subsamples. What has proven to be effective is collecting three replicate subsamples each for phycocyanin, acidified chlorophyll-a, and AFDM tests. The biologist may then utilize the average, median, minimum, and maximum values generated by the three replicate subsamples to better estimate the true values of each parameter.

Surface Water Sampling

In a stream, water is typically obtained through a grab water sample collected below the surface. To collect a water sample 500mL of water is collected into an HDPE bottle. The bottle is to be rinsed three times before filling the bottle completely.

In a lake, water is to be collected from one meter below the surface. Samples collected at a depth of one meter can be collected using a sampling pole or dispensed from depth specific water sampling equipment (e.g., Van Dorn, Kemmerer). Instruction on how to use a Van Dorn or Kemmerer are described in *Chapter 4.2, Discrete Water Chemistry Data Collection Protocol* (Shull and Arnold 2023).

Subsampling Procedures

Subsampling for Phycocyanin (benthic)

Phycocyanin is subsampled by measuring 30mL of homogenized algae slurry in a 100mL graduated cylinder then transferring that quantity to a 120mL plastic sample bottle. Swirl the sample in the 100mL graduated cylinder then pour rapidly into the sample bottle to help prevent residue from sticking to the graduated cylinder.

Note: Do not use de-ionized water to rinse the graduated cylinder as doing that would introduce error into the final results. A swirling motion on the 100mL graduated cylinder followed by a quick pour into the sample bottle will usually prevent sample material from being retained in the graduated cylinder and will ensure that all material is transferred. Sometimes a small quantity of sample material must be returned to the graduated cylinder from the sample bottle and a re-pour used to transfer all algae material.

Phycocyanin subsamples require no preservation additives but does require appropriate low temperature transportation and storage. The phycocyanin bottle will be labeled with a GIS Key (yyyymmdd-HHMM-Collector), if an Algae ID sample was also collected the same GIS Key can be used. The word “Phycocyanin” and the stream name will also be written on the bottle. In addition, three unique collector-sequence numbers will be assigned to the subsamples.

Since periphyton algae slurry solution is usually quite concentrated, with regard to phycocyanin photopigments, BOL will be able to extract three separate phycocyanin samples from this single 30 mL submission – BOL typically does this by re-homogenizing the 30mL sample and then extracting three ~5mL subsamples. Triplicating the sample will allow the biologist to utilize the average, median, minimum, and maximum values generated by the three replicate subsamples to better estimate the true values of each parameter.

On the field data sheet, record the three collector-sequence numbers that are to be used for phycocyanin submissions to Bureau of Laboratory (BOL). Transfer these collector-sequence numbers, along with an accurate date and time, the GIS Key, and stream name to the BOL) Microbiology Sample Submission Sheet (Refer to Appendix A.9 for BOL Microbiology Sample Submission Sheet). Indicate the Standard Analysis Code (SAC) of B031 for the phycocyanin test on the Sample Submission Sheet. Also, indicate on the Sample Submission Sheet that 30mL of sample is supplied and that three 5mL subsamples are drawn from this single submission.

Prepare the bottled phycocyanin sample for transportation. Sample transport is on wet-ice and thereafter refrigerated. Phycocyanin samples have a short pre-processing holding time (~24 hours), which requires the BOL microbiologists to preform preliminary processing procedures to stabilize the sample and protect against degradation of the phycocyanin photopigment. Due to the short holding times for pre-processing at BOL, phycocyanin subsamples should either be hand-delivered at the end of each field day or transported to BOL via overnight courier at the end of each field day.

Subsampling for AFDM (benthic)

AFDM is subsampled by extracting three 2mL replicates of homogenized algae slurry. This is performed using a 2mL spring loaded Hensen-Stemple Pipette (Wildco part number 1806-D52) that extracts a subsample from the algae slurry beaker while the slurry remains on the magnetic stirrer and is continuously homogenized during subsample extraction.

The contents of the 2mL Hensen-Stemple Pipette is then deposited on a Whatman™ GF/F type filter. This is facilitated by a vacuum-draw filtering device (see Figure 1) which includes a cup-like design that holds the GF/F filter and into which the 2mL Hensen-Stemple Pipette algae slurry subsample is deposited. Filtration assembly should consist of a 47mm magnetic filter funnel, a plastic vacuum flask, a magnetic funnel cup, a vacuum hand pump, vacuum tubing, and a 47mm Whatman™ GF/F filter. To set up for filtration, the magnetic funnel will be placed atop the plastic vacuum flask. The hand vacuum pump and tubing will then be attached to the flask. With tweezers, preferably plastic tweezers, remove a GF/F filter from its packaging and gently place the filter on the filter funnel, rough

side up. Place the magnetic cup atop the funnel to create a seal. While maintaining the Hensen-Stemple Pipette in an open position, and inside the vacuum-draw filtering device, rinse the sampler with de-ionized water, also depositing this onto the GF/F Filter, to be sure that all algae slurry material from the sampler has been deposited on the filter. After depositing the algae slurry into the filtering device, draw a vacuum on the filtering device. The vacuum should not exceed 3psi or persist for longer than 10 minutes, as greater pressure and/or duration may damage cells resulting the loss of photopigments (Arar and Collins 1997). With the vacuum still applied, rinse the side walls of the filter apparatus cup with deionized (DI) water to make sure that all algae material is deposited on the GF/F filter. Allow this rinse water to thoroughly drain prior to releasing the vacuum on the filter apparatus.

Remove the GF/F filter from the filter apparatus with forceps in a manner that doesn't touch or disturb the algae material deposited on the filter. Be sure to handle the filter only by the outer edges and do not touch the inner circle where the algal matter is contained. Using two forceps, one in each hand, carefully fold the round GF/F filter in half so that the algae material is "sandwiched" inside this now folded half-circle. Continuing to use forceps, perform one more fold, creating a quartered circle (see Figure 2). Place this folded filter on a small piece of aluminum foil. Fold the aluminum foil over the filter to contain the filter entirely within the foil while allowing ample overlap of the foil. Fold the foil edges over several times to create a sealed envelope around the filter. Each filter replicate is to be folded into its own foil envelope. Place this foil envelop into a small plastic Ziploc® bag. Each AFDM filter is to be placed into its own small plastic Ziploc® bag. Place the three plastic bags into a small paper envelop and seal this paper envelope. This paper envelope will be labeled with a GIS Key (yyyymmdd-HHMM-Collector), if an Algae ID sample was also collected the same GIS Key can be used. The word "AFMD" and the stream name will also be written on the bottle. In addition, three unique collector-sequence numbers will be assigned to the subsamples. This envelope is then sealed inside a Ziploc® plastic bag - multiple paper envelopes may be sealed into a single Ziploc® plastic bag. Record the three collector-sequence numbers for this subsample on the field data sheet.

Transfer the three collector-sequence numbers, along with date and time, the GIS Key, and stream name to the BOL Microbiology Sample Submission Sheet (Appendix A.9). The SAC for AFDM-a is B027.

Prepare the AFDM sample for transportation. Sample transport is on dry-ice and thereafter frozen at -80°C. Because of relatively long holding times, AFDM samples may be held for a few days on dry-ice in coolers and delivered to BOL at the end of the field week.

Quality control measures for AFDM include a laboratory duplicate, a field duplicate, and a field blank, each done at a rate of once per basin. Field blanks will utilize "ultra-pure water" instead of the periphyton slurry and will use the same AFDM subsampling procedures as those described above. Because the term "basin" could cover a broad geographic area and thus include much of the sampling that occurs in Pennsylvania, for example the Susquehanna River basin, it will be left to the field biologist to decide the extent that the term "once per basin" will imply – DEP recommends a

conservative approach, preferring to submit QA samples regularly (once per week per designated basin, for example).

Figure 1. Vacuum-draw filtering devices on left and algal slurry beaker on a magnetic stirrer on the right.

Figure 2. The proper procedure for folding the chlorophyll-a and AFDM filters.

Subsampling for Chl-a (surface water and benthic)

Filtration equipment should be setup prior to collecting the water sample aliquot from the water sampling equipment or benthic slurry. All equipment should be pre-cleaned with deionized water. Filtration assembly should consist of a 47mm magnetic filter funnel, a plastic vacuum flask, a magnetic funnel cup, a vacuum hand pump, vacuum tubing, and a 47mm Whatman™ GF/F filter. To set up for filtration, the magnetic funnel will be placed atop the plastic vacuum flask. The hand vacuum pump and tubing will then be attached to the flask. With tweezers, preferably plastic tweezers, remove a GF/F filter from its packaging and gently place the filter on the filter funnel, rough side up. Place the magnetic cup atop the funnel to create a seal. The magnetic cup should be at least

200mL capacity for water samples, a smaller capacity can be used for benthic slurry subsampling. At this point a water aliquot can be obtained from the water or benthic slurry sample.

For the surface water samples a graduated cylinder will be used to obtain a 50mL aliquot. Rinse the graduated cylinder three times with the sample water. Fill the graduated cylinder to 50mL with the sample water. If subsampling from a benthic slurry, a 2mL aliquot will be obtained using a Hensen-Stemple Pipette. Next, carefully, add the contents of the graduated cylinder or Hensen-Stemple Pipette to the filtration apparatus. Next, add 1mL of $MgCO_3$ directly to the pooled water or slurry. Apply vacuum using the hand-pump to draw the water through the filter to collect the algal matter. The vacuum should not exceed 3psi or persist for longer than 10 minutes, as greater pressure and/or duration may damage cells resulting the loss of photopigments (Arar and Collins 1997). Rinse the filtration funnel cup with DI water to wash down any remaining algal cells. Once all liquid is filtered, release the pressure on the hand vacuum pump. Obtain a small square piece of aluminum foil (~ 4 inches by 4 inches) in which the filtered sample will be wrapped. Alternatively, a small petri dish wrapped in foil or a 50mL plastic centrifuge tube wrapped in foil can also be used to contain the filter. Remove the magnetic cup and carefully remove the filter. To remove the filter, use tweezers and gently lift the filter off of the filter funnel. Be sure to handle the filter only by the outer edges and do not touch the inner circle where the algal matter is contained. Place the filter on the piece of aluminum foil and fold in half and in half again. Manipulate the folded filter so that it only takes up half of the foil (Figure 2). Fold the foil over to cover the filter; fold up the sides of the foil to form a sealed pillow. Place the wrapped filter in a small plastic bag. For benthic samples, this process is to be repeated two more times, for a total of three chl-a subsamples per slurry. If using a small petri dish or centrifuge tube the filter can be folded in half placed inside of the container and the container can be wrapped in foil as to not allow light penetration to any part of the filter.

The sample should be labelled with waterbody name, date collected, time collected, collector number, sequence number. Collector numbers and sequence numbers are determined according to instructions found in *Chapter 4.2, Discrete Water Chemistry Data Collection Protocol* (Shull and Arnold 2023). If using the plastic bag, place the plastic bag in a manila envelope. (Note: Multiple subsamples for the day can go into one manila envelope). The manila envelope will be marked with waterbody name, date collected, collector numbers and sequence numbers for all samples contained within the envelope, additionally “Chlorophyll-a samples” should be clearly indicated on the manila envelope. Specifically, surface water samples will be labelled “Chl-a Non-Acidified (SAC = B029)” and benthic samples will be labelled “Chl-a Acidified (SAC = B059).” Finally, place the labeled envelope in a whirl-pack bag, and seal well.

Samples are to be immediately placed on ice for transport from the field to the laboratory, where samples will be held at -80 °C freezer until samples are analyzed. If transport from the field to a freezer will be more than a few hours, dry ice should be used for transport instead of wet ice. Samples will be submitted to BOL for analysis. A Sample Submission Sheet will be submitted with the samples; the collector is to report total volume filtered in the ‘Comments’ section of the form so the

lab can perform calculations. Surface water samples will be submitted for non-acidified Chl-a under SAC B029; benthic samples will be submitted for acidified Chl-a under SAC B059.

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3.13 HARMFUL ALGAL BLOOM (HAB) AND CYANOTOXIN DATA COLLECTION PROTOCOL

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2023

INTRODUCTION

Cyanobacteria, which are also referred to as blue-green algae, are a group of organisms that naturally occur in the environment. Cyanobacteria are able to produce toxins known as cyanotoxins. Cyanotoxins are found within the cells of the organisms and are either released during cell die off or are lysed when cells become permeable. Under certain environmental conditions, excessive colony growth can cause blooms where there is potential for large die offs and/or cellular stress. These blooms are known as Harmful Algal Blooms (HABs). Due to the nature of cyanotoxin release during a HAB, human, animal, and environmental health are of concern. Water samples and testing for cyanotoxins are needed to ultimately confirm a harmful bloom. As part of DEP's monitoring strategy, the protocols for both surface water collections and benthic algal collections are described herein.

Furthermore, due to the human, animal, and other environmental health impacts, response to HAB events will be guided by the Pennsylvania HABs Interagency Policy Task Force and will follow a strategic data management workflow; this workflow is also described herein.

DATA COLLECTION

Sampling Season

Sampling for HABs should occur during the summer recreation season (June through October) or as needed, as described in the 'Aquatic Life Assessments' section of *Chapter 2.4, Lake Assessment Survey Design* (Arnold 2023).

Benthic Sampling

For benthic sampling, field collection procedures will follow those described in *Chapter 3.10, Periphyton Data Collection Protocol* (Butt 2017). Briefly, algal matter is collected from various substrate types depending on the stream and composited into a slurry. The slurry is then emulsified and blended at high speed to homogenize. The homogenized slurry will be used to obtain a cyanotoxin sample.

To obtain a cyanotoxin sample, pour 250mL of the homogenized slurry into a 500mL graduated cylinder. Swirl this slurry around the graduated cylinder and quickly pour it into a Whirl-Pak® bag to prevent algae material from adhering to the sides of the graduated cylinder. Do not rinse down with additional deionized water as that would dilute the sample and introduce error into the final results. Seal the Whirl-Pak® bag, then transport and store on dry ice at -80 °C.

The benthic cyanotoxin sample will be labeled with a GIS Key, yyyymmdd-HHMM-Collector (e.g., 20170516-1115-Collector, indicating the date, time, and collector name), DEP algae sampling site ID number, stream/river name, and location description. Field data and field notes will be recorded on the appropriate Field Data Collection Sheet (Appendix A.6 or A.7)

Surface Water

HABS are initially characterized by field observation. Common characteristics include spilled paint or pea soup appearances, bubbling scum, hairy/silky strands, green dots/clumps, and/or green streaks (Figure 1).

Figure 1. Common HAB observations.

Depending on the HAB target area, the collector will select one of four procedures; scum, transect, surface, or depth sampling. Transect sampling occurs when a beach or boat launch is the focus of study. Scum sampling occurs where pocketed accumulation of a HAB exists. Surface sampling occurs typically in open water but can occur near the shoreline as well. Depth sampling occurs where a visual bloom is somewhere other than the surface.

In all cases protective equipment is required for sampling. Personal protective equipment includes protective gloves, waders where body emersion is necessary, and a floatation device when entering the water or while on a boat. Direct skin contact should be avoided being cautious particularly to open cuts or sores.

Prior to water sample collection, observational and sample information is to be recorded on the HAB Field Data Collection Sheet. The HAB Field Data Collection Sheet and instructions on how to fill out the sheet are provided in Appendix A.12. Techniques to collect samples are described below. Protocols described are similar to those used by the Ohio EPA. A demonstration video can be found on the Ohio Environmental Protection Agency's YouTube Channel.

The water sample should be collected into one 500mL HDPE bottles. Bottles are to be rinsed three times and filled to the top allowing no head space. Sample bottles are to be labeled with collector identification number (collector ID) and sequence number according to those details provided in *Chapter 4.2, Discrete Water Chemistry Data Collection Protocol* (Shull and Arnold 2023), waterbody name, location description (i.e. east shore), date collected, and SAC B049 + B041; additionally, HABS is to be written on the cap of all bottles. All samples are to immediately be placed on ice. A HAB Sample Submission Sheet (SSS) shall also be filled out (Appendix A.13). The HAB SSS is unique to this data collection protocol. Due to the nature in urgency of sample analysis, samples submitted need to be ranked to help the lab prioritize sample processing. The ranking system is based off fields on the HAB Field Data Collection Sheet (Appendix A.12). The ranking system is on a scale from 1 (highest priority) to 4 (lowest priority). A rank of one indicates that water was collected from a primary recreation location (swimming beach) where with the presence of visual bloom characteristics were observed. The lowest priority rank indicates that water was collected from a secondary location with no visual bloom observations.

Transect (Composite) Sampling

Composite samples are intended to represent an average of the recreational area. The recreational area may be the designated swimming area or the area in and around a boat launch. Transect sampling requires wading out into a defined area to collect a composite sample that is representative of the designated recreational area. The transect technique is employed in swimming beach areas, and boat launches. To complete a transect, a water composite churn or food grade bucket and a small water collection container is needed. The collection container could be a pitcher or an extra sample bottle. Both pieces of equipment should be rinsed three times with surface water prior to obtaining a sample.

Three transects will be set up, spaced evenly across the recreational area to include the left perimeter, center, and right perimeter of the recreational area. Three water samples will be collected and composited from each transect; so, a total of nine water samples will be composited together. To perform the transects, the collector will wade into the water to where the water is ankle deep; at this point a sample of water from just below the surface will be collected into the churn. The collector will then wade in a straight line from the first sampling point to a point where the water is knee deep; a sample of water from 15 cm below the surface will be collected into the churn. The transect will be continued to where the water is hip deep where a sample of water from 15 cm below the surface will be collected into the churn. The collector is to return to the shoreline along the same line that was just collected. Once back to shore the collector will proceed to the second and third transect lines. Three samples will be collected from each transect at the same depths as the first transect, ankle deep, knee deep, and hip deep. Once all transect water is composited, the cyanobacteria sample can be aliquoted from the churn and into one 500mL plastic HDPE bottles, making sure to aggregate the water with the churn press or stirrer. The bottle should be rinsed three times prior to obtaining the sample. Once the sample is obtained, churn equipment should be rinsed with deionized water.

Scum Sampling

Scum sampling typically targets a small localized bloom or a bloom with unique characteristics such as a bubbling foam or clumps. Sometimes HABs will occur in pockets of scum or growth along the shoreline or near boat launches and beaches. Although a scum may be located anywhere in a roped beach or boat ramp area, the scums are frequently pushed by the wind against the shoreline, ropes, or docks. Scums are of concern for all persons and pets recreating in the water due to their elevated concentrations of cyanobacteria and cyanotoxins compared to the surrounding water. Because of their smaller body mass and behaviors, scums pose an exceptionally severe toxigenic risk to children and pets. Consequentially, if a scum is observed, grabs should be collected with care and diligence.

The objective in this sampling approach is to accumulate the surface scum into the sample bottles in a way that neither concentrates nor dilutes the scum material from its existing condition in the lake. To collect the sample, bottles will be inverted and submerged to just below the surface. The bottle will be reverted to a position where the opening is facing up to allow the water to flow into the bottle. Once the bottle is filled slowly bring the bottle to the surface and cap. Another technique that can be used is to situate the bottle where half of the bottle opening is in the water and half is out of the water; the bottle is then tilted to allow for natural flow into the bottle without skimming the surface.

Surface Sampling

Typically, surface sampling is used in situations where a uniform bloom is notated and is determined to be at a large localized or widespread scale. Additionally, surface sampling may occur as part of routine monitoring where bloom characteristics may not be observed but data are needed for development or comparison. The technique used is similar to the scum grab technique where the bottle is inverted upside-down and submerged but instead of just below the surface the sample is collected from a depth of 15 cm or about an arm’s length; the bottle will then be reverted, allowed to fill and slowly brought back up to the surface for capping.

Depth Sampling

Depth sampling occurs where a visual bloom is somewhere other than the surface and/or a specific depth is being targeted for a particular reason. For example, during routine water quality monitoring or where a drinking water supply at a known depth is of interest.

Generally, the photic zone is the primary focus for depth sampling. To determine the extent of the photic zone a Secchi Disk reading is required. *Chapter 5.4, Secchi Depth Data Collection Protocol* (Arnold 2023b) describes procedures for obtaining a Secchi Disk measurement. The photic zone can then be calculated by taking the Secchi Disk reading and multiplying by 2. For example, if the Secchi Disk reading is 1.5 meters, the photic zone extent would be from the surface to 3 meters. Depth sampling will also be used when a collector can visually see a HAB floating at a specific depth in which the collector is interested in targeting.

To obtain a depth sample a Van Dorn or Kemmerer grab sampler will be used to obtain a water sample at the specified depth. To collect water with a grab sampler, the procedure should follow that

outlined in *Chapter 4.2, Discrete Water Chemistry Data Collection Protocol* (Shull and Arnold 2023). Summarized, the sampler will be set in the open position and rinsed with native water. Once rinsed the sampler is to be lowered to the depth of interest and the messenger will be sent down to trip the sampler shut. The sampler will be towed up to the boat and brought on board. Water will be aliquoted into one 500mL plastic HDPE bottles. The bottle should be rinsed three times with lake water prior to filling. Once the sample is obtained, sampling equipment should be rinsed with deionized water.

Water Quality Field Measurements (Optional)

If able, a discrete field measurement can also be obtained at the same depth in which water was collected; for transect samples the discrete field measurement comes from the middle transect knee deep location. Parameters typically recorded include water temperature, specific conductance, pH, and dissolved oxygen (mg/L and % Saturation). Other parameters could include turbidity, chlorophyll, phycocyanin, and Secchi Disk readings. Field meters should be properly calibrated to the unit specifications prior to use and users should follow that protocol described in *Chapter 4.1*, *In-situ Field Meter, Transect, and Vertical Profile Data Collection Protocol* (Hoger and Arnold 2023). To collect a Secchi Disk reading refer to *Chapter 5.4, Secchi Depth Data Collection Protocol* (Arnold 2023b).

Sample Submission

Samples are to be submitted to DEP's Bureau of Laboratory (BOL). A chain of custody form, which is referred to as the Sample Submission Sheet (SSS), is to be submitted with all samples. To submit a sample or a series of samples, the following procedure should be carried out. Once a sample is collected, sample bottles should be placed in a sealable plastic bag and placed in a cooler with ice. Pertinent sample information will be logged on the HAB Field Data Collection Sheet (Appendix A.12) or on a smart device field application (Survey 123). Once a cooler is ready to be delivered to BOL, the SSS is to be included in the cooler intended for delivery. The SSS is to be placed inside a sealable plastic bag and taped to the inside of the cooler lid. The cooler then can either be hand delivered to BOL, or if hand delivery is not an option, a courier service will be used. The DEP contracted courier service has numerous drop-off/pick-up locations located throughout the state. If using the courier service, the cooler pick-up needs to be scheduled by 1400 (military time) on the day of sampling; and coolers need to be dropped off to the courier location by 1630 (military time).

Survey 123 and Sample Information System Documentation

Instructions for Survey123 data entry is guided by the fields contained in the HAB Field Data Collection Sheet (Appendix A.12). The link to Survey123 will only be provided to trained field collectors and will only be supplied through a specific request submitted to DEP or by attending a training event.

DEP staff are additionally required to enter field data and associated sample information into the Sample Information System (SIS). Instructions for SIS data entry can be found in Appendix B.3.

QUALITY CONTROL

Samples for QA/QC will follow that described in Appendix E, Section B5. For every 20 environmental samples a blank and replicate will be collected. It is also recommended that in instances where high concentrations are anticipated that additional QA/QC samples be collected.

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3.14 PLANKTON DATA COLLECTION PROTOCOL

Chapter 3 Biological Data Collection Protocols

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INTRODUCTION

Plankton are microscopic free-floating plants and animals of mainly pelagic and limnetic waters with dynamic population changes that respond to environmental conditions such as temperature, sunlight, wind, and water quality and can, themselves, contribute to water quality changes in lakes. Lake plankton composition is comprised of two types of plankton, phytoplankton and zooplankton. Phytoplankton are those organisms that perform photosynthesis and are considered the primary producers in a lake's food web. Another name for phytoplankton is planktonic algae. Monitoring phytoplankton communities is particularly important as they are used as water quality indicators of nutrient enrichment. Evaluating phytoplankton can give insight into the water quality conditions of a lake as the photosynthesis performed by the planktonic algae influences the physical and chemical components of the waterbody such as pH, oxygen and carbon dioxide concentration, clarity, color, and odor. Phytoplankton blooms can also impact recreation and aquatic health, where some species of phytoplankton produce toxins which can cause fish kills and illness to those who recreate. Phytoplankton types and abundance also influence the zooplankton community, and both comprise most of the food base of the lake with implications for fisheries.

Zooplankton are secondary producers within the plankton community. Zooplankton are free-living animals that are suspended in the lake's water column; they generally have limited mobility; movement of zooplankton within the water column is limited to water circulation and movement. Four major groups dominate zooplankton communities; the four groups include protists, rotifers, cladocerans, and copepods. Zooplankton are either herbivores that feed upon the phytoplankton or are predators that feed on other zooplankton; they provide a food source for the lake's fishery and the continuation of the lake's food chain.

Since plankton populations can vary throughout a lake system and changes in community composition can vary seasonally, collection of plankton data and interpretation of data helps provide context to the physiochemical and biological interaction of the lake. DEP uses plankton data to support assessment determinations to describe the ecosystem's food chain and as a screening tool for presence of nuisance cyanobacteria.

DATA COLLECTION

DEP plankton collections are for both phytoplankton and zooplankton. As part of the *Chapter 2.4, Lake Assessment Survey Design* (Arnold 2023), plankton are collected seasonally, once during the spring sampling season, once during the summer sampling season, and once during the fall sampling season. As part of the WQN data collection, plankton are collected once during the summer sampling event.

To collect plankton samples, a plankton tow is performed. A Wisconsin style net with a 5 inch diameter opening rigged with a 80 micron nylon mesh net and collection reservoir with funnel tubing attached to assist with transferring to a sample bottle is to be used (Figure 1). The net should be

attached to a rope which is marked off in meters. Prior to conducting a plankton tow, the tubbing will be clamped shut with a plastic clamp or heavy-duty paper clip so that a seal is created as to prevent loss of sample water. A sample bottle (125mL or 500 mL HDPE plastic bottle) will be labeled for submission to BOL according to instructions provided in *Chapter 4.2, Discrete Water Chemistry Data Collection Protocol* (Shull and Arnold 2023). The label will include a collector number, sequence number, lake name, location description, and SAC B048, which is the Standard Analysis Code (SAC) for phytoplankton and zooplankton identification.

Figure 1. Wisconsin plankton sampler; 5 inch opening diameter and 80 micron mesh.

The tow will be performed on the opposite side of the boat where all other data collection was conducted as the collection of profile data or measuring Secchi depth will generate water movement dispersal and could impact the plankton sample. A sample will be comprised of water sampled from two consecutive tows with the same sampling device. Prior to collecting a plankton sample, a vertical profile will need to be performed according to Chapter 4.1, *In-situ Field Meter, Transect, and Vertical Profile Data Collection* (Hoger and Arnold 2023). Profile data will be used to determine the depth of the tow. To perform the first tow, the net should be lowered to 1 meter above the lake's bottom, unless anoxic conditions exist. If anoxic conditions exist as determined by profile readings, the start of the tow will begin where dissolved oxygen is ≥2 mg/L. Once at the desired depth, the collector will immediately begin the tow where the net is traversed, hand over hand, up through the water column at a rate of approximately 0.3 meters/second until the net is raised out of the water. For this first tow, the sides of the conical portion of the net will be carefully rinsed by splashing lake water against the outside of the net; be sure to not introduce water into the collection cone as this would impact final volume calculations. Alternatively, one can use a squirt bottle to rinse the sides of the net. Once the net is rinsed, hold the end tube over the collection bottle, any bottle size will work as long as the volume is large enough to capture the anticipated sample amount typically a 500 mL HDPE is used, and unclamp to release the water sample. Re-clamp the tubing and perform a second tow. The second tow will follow the same procedure as before and should be performed away from where the first tow was conducted. To perform the second tow, lower the net to the desired depth and traverse

through the water column until the net is raised out of the water. The net will be rinsed again by splashing lake water onto the sides to wash down the net. The collector alternatively can use a squirt bottle to rinse the sides of the net. Once the conical portion of the net is rinsed, place the tubing over the sample collection bottle, unclamp and collect the water sample. The tube should be re-clamped and the collection reservoir should undergo a final rinse using the same technique as described above. Situate the tubing over the collection bottle to collect the remaining sample water. Record the approximate volume collected. Knowing the approximate volume will help determine how much preservative is needed. The plankton sample should be preserved with a 37 percent formaldehyde solution, where 3mL of solution would be added to every 100mL of water sample (USGS, 2018). Other permissible preservation would be Lugols solution where 1mL would be added to every 100mL of sample.

On the *Lake Assessment and TSI Field Data Sheet* (Appendix A.11), record type of preservation and final amount used to preserve; also include this information on the sample bottle. On the *Lake Assessment and TSI Field Data Sheet* also record the depth in which the tow was started and the diameter of the plankton net opening to facilitate the calculation of total volume of lake water filtered. To calculate multiply sample depth by 2 and then by the area of plankton net opening in meters. Multiply this value by 1000 to convert value to liters; this is your total volume of lake water filtered in liters (Equation 1). Volume of lake water is needed to calculate plankton density per liter of lake water (n/L) for standardization once results are obtained from BOL.

Equation 1: Volume of lake water filtered

V= $\pi r^2(dx2)x1000$; where

r = Radius of the net opening in meters. If a 5 inch net opening is used, the radius would equal 2.5 inches which converts to 0.0635 meters

d = Depth of the tow in meters.

1000 = Conversion from 1 cubic meter to litters.

If the lake is shallow, perform 2 tows to a depth that will accommodate the net without collecting bottom sediment or debris. If net sampling is not feasible, collect a grab sample in a 500mL sample container, indicate as a grab sample on the field data sheet (Appendix A.11). Record depth of sample; 0.5 meter depth is preferred if available. Grab samples should be preserved according to instructions provided above.

Plankton samples will be delivered to BOL and analyzed under SAC B048. Samples do not need to be transported on ice but can be placed in a cooler to be maintained with all samples collected during the sampling event. Long-term storage will be in a cool dark place, like a refrigerator. Samples for QA/QC should be collected for every 20 environmental samples or once per project and will follow details found in Appendix E, Section B5.

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3.15 AQUATIC MACROPHYTE DATA COLLECTION PROTOCOL

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INTRODUCTION

Aquatic macrophyte data can be used to supplement trophic status index (TSI) based Aquatic Life Use (ALU) assessment decisions (Canfield et al. 1993) and to also make independent ALU, Boating (BU), and Esthetics (EU) assessment decisions.

Aquatic macrophytes play an important role in lake ecosystem functions. The density, diversity, and growth patterns of aquatic plants are unique to each lake. Microorganisms living on the plant material form a food base for macroinvertebrates, which in turn support other lake-dwelling species through a diverse food web. Aquatic plants provide cover, nesting areas, building materials, and food for fish, amphibians, reptiles, birds, and aquatic mammals. Aquatic plant beds are important fish spawning and nursery areas and play a major role in the status of a lake's fishery. Emergent vegetation provides shoreline habitat and cover for reptiles, amphibians, birds, and small mammals. Aquatic plants, both in-lake and shoreline, also function as buffers from runoff and destructive wave action. Lack of vegetation increases the vulnerability of lakeshore stability, reduces available habitat, and results in lower biodiversity and overall biomass of the lake ecosystem.

Dense populations of aquatic macrophytes, especially invasive or non-native species can impact recreational uses like BU and EU. Boating includes the use of a surface water for power boating, sail boating, canoeing, and rowing for recreational purposes. Esthetics includes the use of a surface water as an esthetic setting to recreational pursuits such as photography, art, picnicking, meditation, etc. To determine the degree to which the presence or paucity of aquatic macrophytes influences a lake system and its uses, it is advisable to quantify the non-algal plant coverage. This information can then be applied to the overall assessment of lake use determinations.

DATA COLLECTION

Aquatic macrophyte surveys are designed to characterize percent area coverage, percent biovolume, plant community composition and general abundance through a combination of boat mounted sonar mapping and rake sampling. Plant species are identified using appropriate keys and references and/or by collecting representative specimens for expert verification.

Sonar mapping is performed to develop a vegetation heat map of the submerged aquatic vegetation. Rake sampling is performed to confirm densities characterized by sonar mapping and to characterize plant community composition and general abundance.

Sonar Mapping

DEP uses an onboard Lowrance® sonar unit to collect bathymetry and plant biovolume data used to develop vegetation and bathymetry heat maps (Figure 1). The Lowrance® sonar unit require at minimum a battery to operate the unit, an attachment point to the boat, this could be a permanent setup or a removable setup, and a mounting system for the navigation screen, again this could be a permanent setup or removable setup. Additionally, it is recommended a security digital (SD) card be

used to save and store data being collected by the unit. Data can be downloaded from the SD card to a computer and are saved, stored, and post-processed through an annual contract with the BioBase, LLC company. BioBase, LLC post-processes sonar data into bathymetry maps, vegetation heat maps, and bottom composition heat maps.

To complete a sonar survey, a boat is used to navigate around the lake (safety considerations for boat use are found in Appendix F). The Lowrance® sonar unit uses down-scanning and side-scanning sonar to record point data that includes latitude, longitude, and depth. Surveys will be performed using evenly spaced tracks. Tracks can either be performed in a circular manner, paralleling the shoreline, or as transects that snake perpendicularly from the shoreline (Figure 2). BioBase, Lowrance, and a number of other scientific groups to include state agencies, like the Minnesota Department of Natural Resources, and the Texas Parks and Wildlife Department use perpendicular tracks to complete a plant mapping survey (©Navico, Inc. 2021, Perleberg et al. 2016, Bennett et al. 2020). Depending on lake size, one track method may be better than the other or both track methods may be used. For instance, if the lake is deep and conical in its geomorphology the circular tracks may be implemented as the circular path will better represent the lake's contour. In another instance, the lake may have various geomorphologic characteristics, where one section is shallow and uniform in depth and another section is conical and deep, in these situations the collector may choose to use perpendicular transects in the uniform section of the lake and circular tracks where the lake is deep quickly changing depths.

Regardless of the track method selected, tracks should be evenly spaced 20 to 40 meters apart. Tracks should be closer together (i.e. 20 meters apart) along the shoreline and in the photic zone where vegetation growth is most dense. In open water where little to no vegetation is present, tracks can be spread out more (i.e. 40 meters apart). Where dense vegetation exists, the boat should be operated at lower speed between 3 and 10 miles per hour (mph) depending on density and bottom contour and substrate variability. In open water where little vegetation exists, the boat can navigate up to 20 mph (©Navico, Inc., 2021).

Data will be collected continuously once the Lowrance unit is set to record. To set the unit to log follow the device's instruction manual. Briefly, to set the unit to record, navigate to the menu and select 'Settings.' In settings select 'Sonar.' From the Sonar menu, select 'Log Sonar….' Give the file a name under 'File Name', save data to an external drive, and push 'Record.' the Lowrance unit is now in logging mode. Depending on the lake size, the lake may need to be divided into mapping zones because of file size limitations. It is recommended that data file recording is no longer than one hour. Files should be named according to a lake and date and if necessary zone number (e.g. LAURLK_210628 or LAURLK_Z1_210628, LAURLK_Z2_210628, etc.) and saved on an external SD card so that they can later be saved and uploaded to BioBase.

Once uploaded to BioBase a series of manual post-processing of data is necessary. First, the data needs to be offset by 0.2 meters or 8 inches to account for placement of the transducers. (Note: This offset can vary depending on where and how the transducer is mounted to the boat. Also depending

on the sonar mapping device being used this offset can happen in the mapping device itself). This will offset all depth readings by 0.2 meters. To conduct the offset, select ‘Analyze and Edit’ for the sonar file of interest. Select ‘Data Offset’ and set to 0.2 meters. Describe the offset if desired and push ‘Apply Offset and Reprocess.’ The second manual post-processing would be a trip re-process if necessary. BioBase will process all data points with a 25 meter buffer interpolation but in some cases where track spacing was not close enough in the field for side-scan to fill in the gaps, trip re-processing at a greater buffer may be required. To re-process to give a wider interpolation, select ‘Trip Reprocessing’ set interpolation buffer to no more than 40 meters, leave all other values default, and push ‘Submit.’ There is a delay in this type of re-processing of data, the collector should allow for a day to see changes. If 40 meters did not fill in gaps these data should be flagged, and field attempts to get better coverage with the sonar will need to be considered. Perform these two manual post processes for each of your sonar files uploaded to BioBase.

Once satisfied, merge data files to get a complete map. To merge data files, navigate to one of the sonar files and select ‘Analyze and Edit.’ Under the tools menu push ‘Merge Trips.’ Select the sonar files you want to merge. Push ‘Merge Trips.’ The merge will be the final map, where the percent of waterbody surveyed (POWS) needs to be ≥ 90%. If POWS is ≤ 90% after all post-processing, additional field data collection will be needed to complete the map; it is recommended that the additional data are to be collected within two weeks of the initial data collection effort. The final map will contain the calculated biovolumes and percent surface area coverage values.

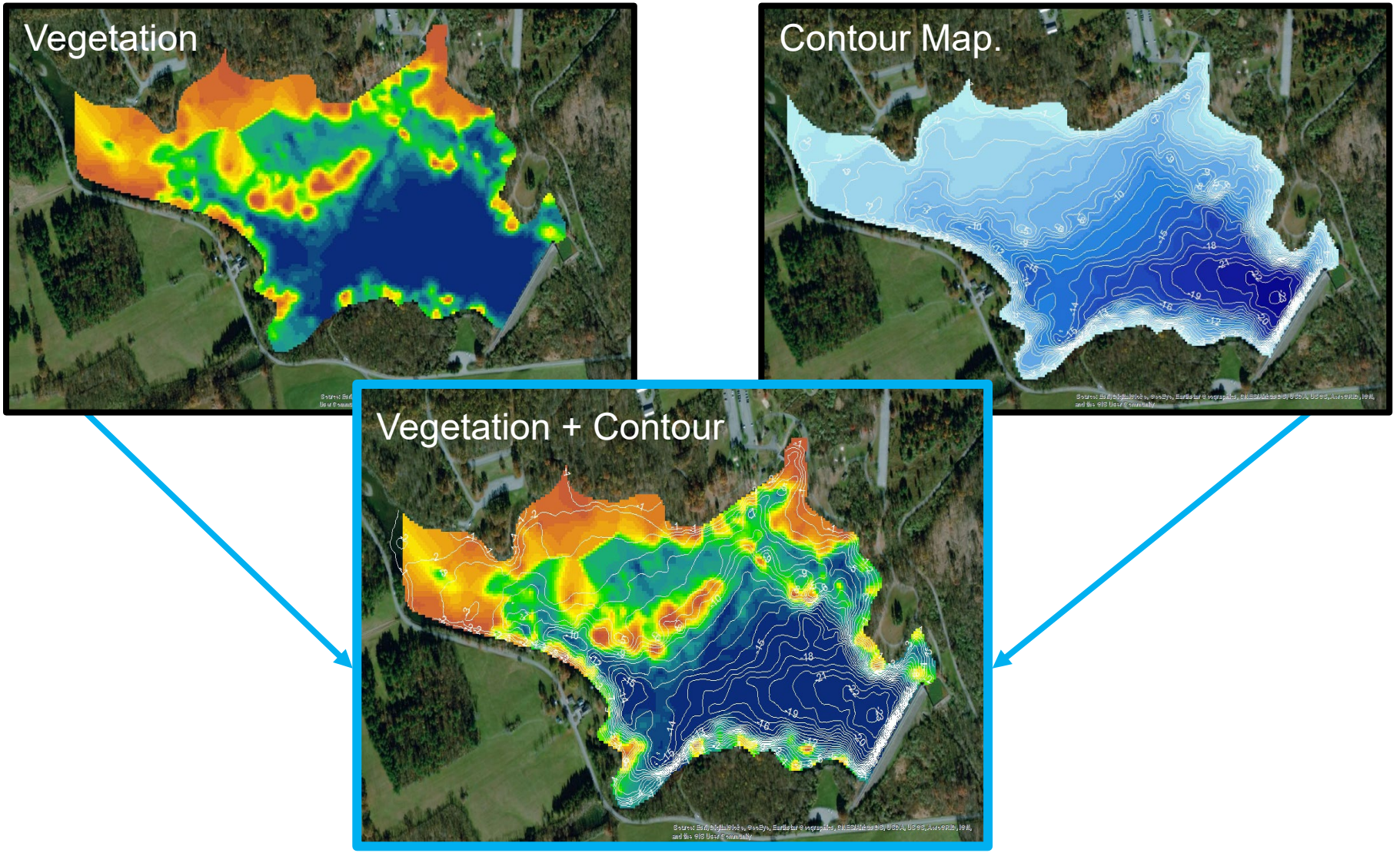

Figure 1. Example of lake vegetation mapping results. GIS maps produced from BioBase.

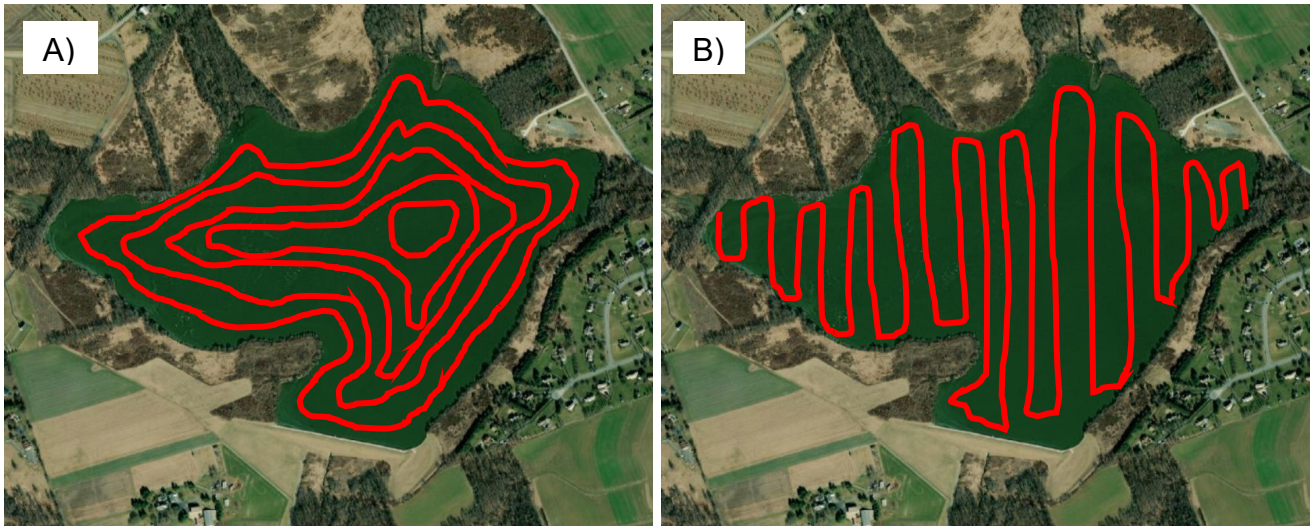

Figure 2. Example of sonar mapping tracks. A) Circular Track; B) Transect Track.

Density and Composition Rakes

In addition to collecting sonar data to develop a vegetation map of a lake, ground truthing data and species composition and abundance are important supporting data which will help in the interpretation of sonar data. DEP uses sonar mapping to describe vegetation biovolume and lake bathometry through heat map visualization and interpolation calculations. Producing biovolume vegetation heatmaps using sonar equipment allows one to collect refined data over a large area in a very short amount of time. While sonar provides a nice visual tool of overall conditions, sonar fails to describe unique characteristics of a lake like vegetation types, species, and species abundance. Because of this, DEP recognizes that is important to collect truthing and characterizing data to help describe a condition to help inform assessment determinations.

Past DEP protocols lean on field reconnaissance and professional judgement to determine locations in which truthing data and composition data are collected. There are many pros and cons to use on the spot decision making and best professional judgement. One major con is that this approach is not standardized which could create biasness in data comparability and interpretation. Standardized approaches are much more desirable for data management, analysis, and defensibility. For instance, the U.S. Environmental Protection Agency's (USEPA) National Lake Assessment survey recommends evenly spaced stations for physical habitat lake stations (USEPA 2017). Minnesota Department of Natural Resources additionally have adopted a number of standardized techniques to include plant inventories along transects evenly spaced around the lake's shoreline or a point-intersect approach (Perleberg et al. 2016); these are standardized approaches that allow for a repeatable and unbiased data collections. Additionally, Texas Parks and Wildlife Department has adopted a quantitative approach to describing submerged aquatic vegetation (Bennett et al. 2020); their approach includes the use of sonar and random-point surveys to make accurate assessments.

Site Selection

Prior to the start of a DEP aquatic plant survey where density, composition and abundance are of interest, pre-determined sampling sites should be identified through a desktop reconnaissance exercise where approximate rake toss locations are selected. Procedures for the desktop reconnaissance, using ArcGIS software, specifically ArcGIS Pro 2.2.0 version, is described below. Generally, this guidance will provide a standard stepwise process to select sampling points for aquatic plant surveys.

The stepwise process outlined herein incorporates a combination of the approaches used by those organizations noted above. To begin the collector should select a lake of interest using the NHD_Waterbody layer. The lake of interest should be extracted, and a new project should be created. Using the NHD_Waterbody layer determine the shape length by referencing the attribute table column labelled SHAPELEN. The value given in SHAPELEN is the length of the polygon boundaries in meters. With this length, the number of rake tosses will be determined. To determine the number of sampling points at least 8% of the total shoreline will be assessed and each point will represent a 30.5-meter shoreline segment (Perleberg et al 2016). Under this criteria, the number of points will be determined using Equation 1. The minimum number of sampling points is 20, so if NRT in Equation 1 is less than less than 20, the number of sampling points will need to be adjusted to 20 and spaced accordingly.

Equation 1: Rake toss sampling point spacing distance

NRT = (0.08 * d) / 30.5 ; where

NRT = number of rake tosses and

d = the total length of the shoreline in meters.

Once the number of rake tosses are determined, the shoreline length will be divided by NRT to determine the rake spacing distance (RSD) for each rake toss location. In ArcGIS, use the polygon to line tool to convert the lake polygon to a line feature class (Figure 3). Next, use the generate points along lines tool to determine rake toss locations where point placement will be set to “by distance” and the RSD will be manually entered in the distance field (Figure 4). Once points along a line are generated, calculate geometry to determine latitude and longitude of each point. These will be the approximate locations in which rake tosses and species abundance will be collected. The resulting layer containing your waypoints should be saved and uploaded to a GPS unit to help navigate to your provisional rake toss sampling locations.

Other procedures for site selection include taking a hardcopy map of the lake, measuring the perimeter of the lake, and placing the desired number points at equal distance along the line representing the shore. To do this use a string to outline the lake. Take a measurement of the string and divide by the number of points needed. Space points and determine the coordinates of those points using the map grid.

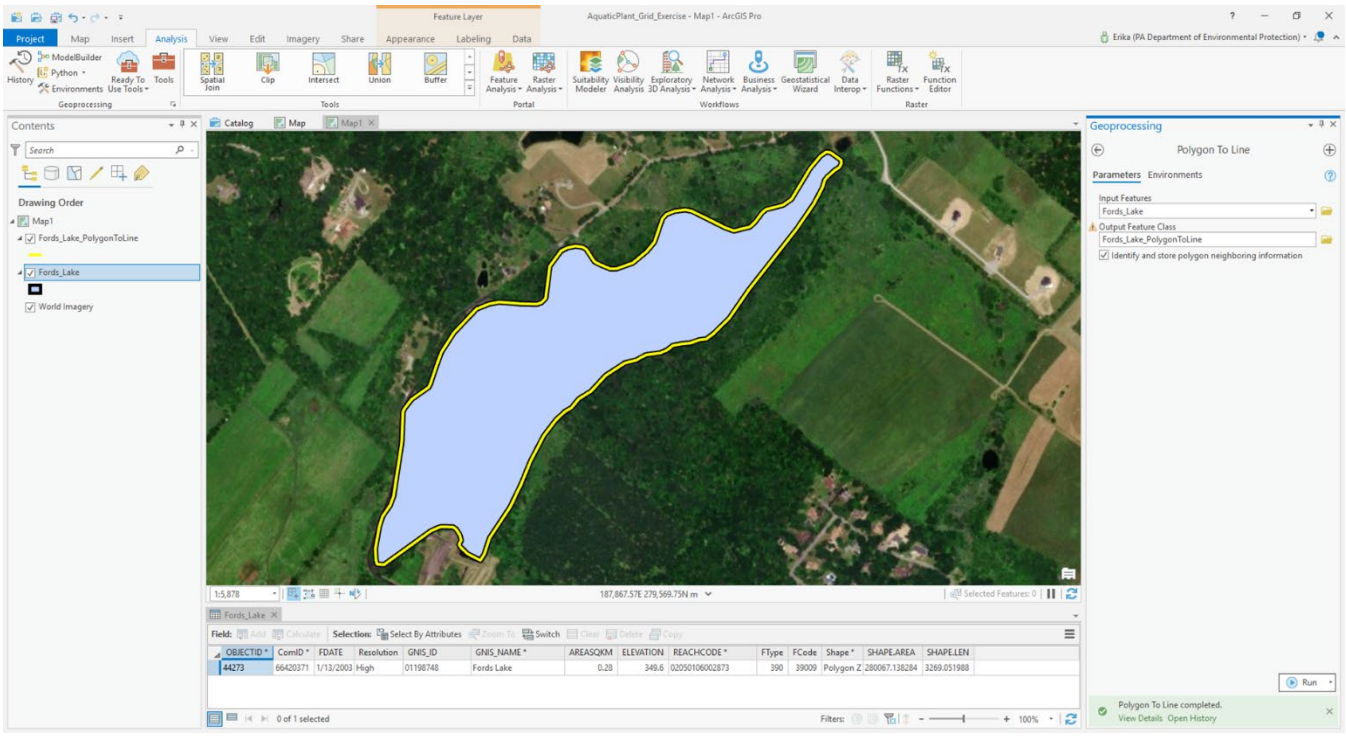

Figure 3. Polygon to line line feature class.

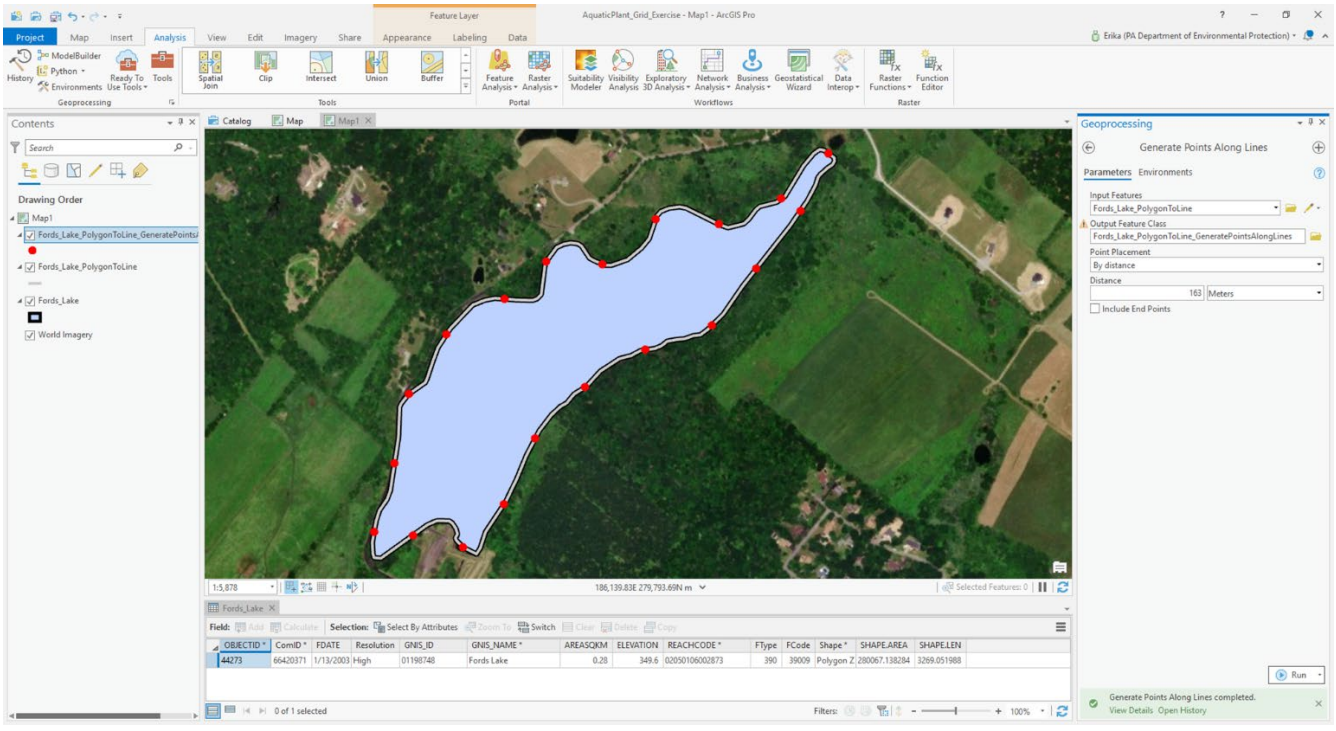

Figure 4. Generate points along lines point feature class.

Field Procedures

In the field determine the light attenuation zone by taking a Secchi Disk reading, approximately at the middle of the lake, and multiplying the Secchi Disk reading by 2; this is the maximum depth extent to

expect plant growth. Knowing this number will aid in defining the exact rake toss sampling location. Secchi Disk data collection is described in *Chapter 5.4, Secchi Depth Data Collection Protocol* (Arnold 2023).

Once the waypoints have been imported to your GPS unit, navigate to each waypoint. Determine the extent of the light attenuation zone to define the littoral zone to be sampled. Use a range finder to approximate the distance from the attenuation zone extent to the shoreline. If the attenuation zone is the full extent of the lake use a centroid point to define the outer extent of the littoral zone to be sampled. Use a random number generator or a 10-sided die to obtain a percent from shore distance a sample will be collected. Navigate to the sampling point; this is the centroid of the 30.5-meter parallel to shoreline segment. Once at a sampling point, anchor and record the latitude and longitude (Figure 5). Observe for aquatic vegetation off both sides of the boat. Indicate the dominant bed type(s) as floating (F), emergent (E), and/or submerged (S) on the field data sheet (Appendix A.13); indicate 'NA' if no bed was observed. Select the largest plant bed and target it for one rake toss. The targeted plant bed can come from any paralleled direction up to 15 meters from either side of the centroid sampling point. Placing buoys to define the horizontal extent may be helpful. A one-sided or two-sided prong rake head attached to a long line will be thrown off the boat. Throw the rake past the targeted plant bed. Allow the rake to seat and rake the bottom using a pulling motion towards the boat. The rake should then be brought on board in an upward motion. Rate the plant density of each rake toss first (Table 1, Figure 6) and record on the Aquatic Plant Survey Field Data Sheet (Appendix A.13).

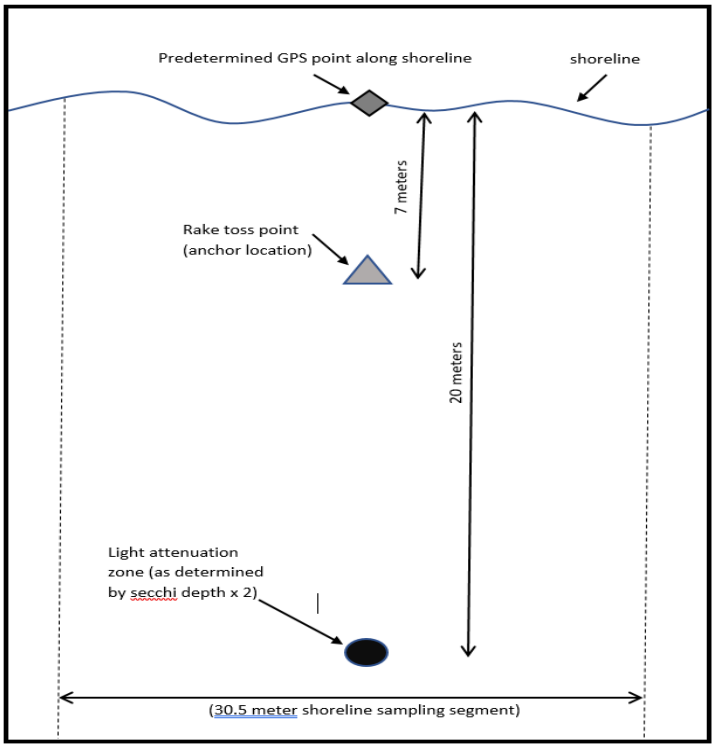

Figure 5. Determining rake toss sampling location. In this example the distance from the light attenuation zone to the shoreline is 20 meters. The random percentage as would be determined by a 10-sided die or random number generator was determined to be 30% from the shoreline. As a result, the rake toss sampling location would be 7 meters (rounded up from 6.67 meters).

Table 1. Rake Toss Density Rating Table.

Description	None	Sparse	Light	Moderate	Dense	Very Dense
Range	0%	1 - 20%	21 - 40%	41 - 60%	61 - 80%	>81%
Rating	0	1	2	3	4	5

After plant density is rated, plant species presence will be identified to the lowest taxonomic classification possible. Additionally, for each rake toss, species abundance will also be scored to describe the proportional percent composition where the total composition for the plants listed will be 100%. Species and species abundance will be recorded on the Aquatic Plant Survey (Appendix A.13) for all rake tosses.

Vouchering Specimens

Representative specimens of each plant species, at every lake are to be collected and vouchered either through specimen preservation or photo documentation. Specimens that are unknown and cannot be identified in the field require a vouchered specimen preserved using a blotter paper and a plant press. Known plants can either be vouchered as a preserved specimen or as photo documentation. If the same unknown is collected at multiple rakes, each occurrence will need documented by at least a photo or photos that capture all identifying characteristics; specimen preservation is optional.

Specimens being preserved will be placed in a sealable plastic bag labeled with date, lake name or alias, rake number, and collector and formatted as ‘yyyymmdd-LakeName-R#-Collector.’ Specimens being preserved are to be kept moist and out of the sun until they can be processed. To process, specimens are pressed using a plant press where specimens are sandwiched between newspaper (labeled with ‘yyyymmdd-LakeName-R#-Collector’), blotter paper, and cardboard spacers to help the drying process. Plants should remain in the plant press until they are flat and dry or for no more than one week. After the specimens are dry and flat, remove and store plants in their labeled newspapers in a safe dry place until an experienced taxonomist can perform identifications.

Equipment Care

Be cognizant of transporting plant fragments to other lakes. Boats and trailers are major transporters of both plant and animal aquatic invasive species. The following steps should be completed at the end of each sampling day: Drain and wash the inside and outside of your boat, the motor, oars, anchor, and any equipment used during the survey, as well as the entire trailer. The recommended procedure is to use a high-pressure washer without soap and preferably hot water. Whenever possible, allow the boat/trailer to dry out completely for several days before launching in another lake. When it is not possible to dry out the boat for several days a 2% bleach solution should be sprayed on all surfaces. Additional biosecurity considerations are detailed in Appendix F.

Rake Rating – 1
Sparse, barely any, plants, 1 to 20% coverage

Rake Rating – 2
Light, about 21 to 40% coverage

Rake Rating – 3
Moderate, about 41 to 60% coverage

Rake Rating – 4
Dense, about 61% to 80% coverage

Rake Rating – 5
Very Dense, >81% coverage

Figure 6. Examples of how rakes would be rated in the field, based off relative visual comparisons.

Quality Control

Since aquatic macrophyte surveys take a significant amount of time and require many moving parts, crews only have to perform a replicated effort every 5 years. Additional QA/QC requirements are described in Appendix E.

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CHAPTER 4 CHEMICAL DATA COLLECTION PROTOCOLS

4.1 IN-SITU FIELD METER, TRANSECT, AND VERTICAL PROFILE DATA COLLECTION PROTOCOL

Chapter 4 Chemical Data Collection Protocols

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INTRODUCTION

Water quality instruments provide the opportunity to quickly gather current water quality data but proper care and calibration of the equipment, and QA/QC documentation are required for these data to be accurate and defensible. Many water quality meters have interchangeable probes to record a wide variety of parameters. The most common parameters are temperature, conductivity, pH, dissolved oxygen, turbidity, and depth. A full discussion of probe technology and considerations for their selection and use is provided in *Chapter 4.3, Continuous Physiochemical Data Collection Protocol* (Hoger and Arnold 2023).

CALIBRATION

Proper calibration will follow manufacturers' guidelines. In addition to these guidelines, DEP requires several procedures to further ensure reliability and defensibility of data. Field meter calibration will be checked before each day of use to ensure that calibration is within the guidelines found in Table 1. In most circumstances, it is recommended that the sensors are calibrated at this time, even if a sensor is within the calibration guidelines. All calibration activity, including checks, need to be logged and records maintained for quality assurance. Additional record keeping and QA/QC requirements are detailed in Appendix E. A calibration form is provided in Appendix B.1. This form contains the required fields for a proper calibration record. Paper copies of the calibration log will need to be periodically scanned for backed-up digital storage.

Table 1. Calibration criteria for measured parameters.

Field Parameter	Calibration Criteria
Temperature	± 0.2°C
Specific Conductance	± 5 µS/cm or 3%, whichever is greater
pH	± 0.2 units
Dissolved Oxygen	± 0.3 mg/L
Turbidity	± 0.5 FNU or ± 5%, whichever is greater

Calibration for all parameters should bracket any expected values. A summary of calibration recommendations is provided in Table 2. Before calibration, the sensors should be rinsed three times with the calibration solution. While other parameters can be calibrated before going out into the field, dissolved oxygen (DO) sensors should be calibrated onsite to avoid any influence of changes in barometric pressure from changes in altitude, weather, or pressurization in buildings. It is also recommended that DO calibration be checked periodically throughout the day. Accurate turbidity calibration can be more challenging than other parameters as the sensors are very sensitive to standard contamination and light infiltration. Clean, dedicated sensor guards and calibration cups should be used. In addition, when calibrating in zero standard, or any other very low standard, it can be necessary to have the guard in place and use an opaque container to submerge the sensor in the standard. This procedure more closely replicates the deployed environment than calibrating in just the calibration cup, and therefore, can provide more consistent and accurate calibration.

CLEANING AND MAINTENANCE

Water quality instruments dedicated to periodic field meter use generally do not require an intensive cleaning regiment. Sensors should be visually inspected during calibration and any deposits or biological growth removed following manufacturers' recommendations. An occasional cleaning with mild soap and water is recommended. With proper maintenance and care, many sensors have a lifespan of several years. One exception is pH probes that have a limited supply of reference solution. Some manufacturers provide a refillable reservoir of reference solution to prolong the life of their pH probes. Those that do not have a refillable reservoir often need to be replaced yearly. The pH millivolt readings should be recorded during calibration to track the life of the probe. The pH mV values should be within the following criteria:

- Buffer 7: readings should be -50 to 50 mV, 0 mV is ideal
- Buffer 4: readings should be +165 to +180 mV from the buffer 7 reading
- Buffer 10: readings should be -165 to -180 mV from the buffer 7 reading.

Additional information on cleaning and maintenance is provided by Hoger and Arnold (2023) and the equipment's manufacturer.

STORAGE

Most probes require being stored in a moist environment. A small amount of fluid in the bottom of the calibration cup is sufficient. Submerging pH probes or other sensors with a reference solution will deplete that solution and reduce the life of the sensor.

RECORDING

Care should be taken to allow meters to stabilize before any readings are recorded. Temperature and conductivity will often stabilize within seconds. Depending on the environment and life of the probe, other sensors like pH or DO may take a couple minutes to fully stabilize. If necessary, leave the meter in situ while other tasks are completed, so all parameters have plenty of time to stabilize.

The Water Quality Division (WQD) stores field meter readings in the DEP's Samples Information System (SIS). Discrete readings at continuous instream monitoring (CIM) stations are additionally stored with the CIM data as field visits in Aquarius.

Table 2. Summary of calibration recommendations

Parameter	Calibration	Notes
Temperature	Check against NIST certified equipment	
Specific Conductance	One-point calibration with checks • Calibrate at a high value (1000 µS/cm) • Check at a low value (100 µS/cm) • Rinse with Deionized (DI) water and check in air (should be 0 µS/cm)	If values above 1000 µS/cm are expected, also check in a higher standard.
pH	Three-point calibration (4,7,10)	Two-point calibration acceptable if conditions are known.
Dissolved Oxygen	100% water-saturated air: • Water in bottom of calibration cup (do not submerge sensor) • Calibration cup on loosely to prevent pressure build-up • Run a wipe cycle, if possible, to remove water droplets on sensor	Calibration **onsite** to avoid influence of barometric pressure differences. Check throughout the day. Allow plenty of time in cold weather.
Turbidity	Three-point calibration (0, ~100, ~1000)	• If only two-point calibration available, calibrate at 0 and high value, check in low value. • Pour standards slowly to avoid bubbles. • Use opaque calibration cup or shield from direct light.

CROSS-SECTION SURVEYS

Waterbodies are dynamic systems changing throughout time and space. Regular cross-section surveys, transects, help determine how homogenous a system is, allow for better understanding of the system as a whole, and lead to better study design and management decisions. The confluence of a tributary, groundwater infiltration, point-source discharges, and differences in flow, depth, and growth of photosynthetic organisms can all result in inconsistent water quality. In general, wide, flat, slow-moving streams are more heterogeneous. As stream channels narrow and slope increases, better mixing often occurs. DEP frequently utilizes cross-section surveys to determine proper deployment of continuous monitoring equipment and direct the collection of biological and chemical samples.

As with any water quality meter work, DEP requires daily calibration (or calibration checks) of the meter before performing cross-section surveys. In addition, post-survey calibration checks are completed to confirm that any variation in the survey was not due to calibration drift of the instrument.

Cross-section surveys should be conducted multiple times to document differences (or similarities) over time. DEP refers to these repeated survey points as a water quality transect. Established transects are often repeated monthly, but more importantly will target various flow levels and other events that may lead to variation in the survey.

The number of measurement points in a transect is influenced by the stream size and heterogeneity. In the smallest streams (width < 15m), surveys usually consist of three points: left descending bank (LDB), mid-channel, and right descending bank (RDB). Streams 15m to 50m wide often have five points: LDB, ¼ across, mid-channel, ¾ across, and RDB. As streams become wider than 50m, surveys are established at 10-20m intervals. However, in Pennsylvania's largest rivers, widths can reach over one mile across; and in these cases, intervals may be in excess of 100m. These large-river transects frequently use bridge piers or other reference points to facilitate consistency of subsequent surveys. In the absence of stable reference points, a range finder is used to measure distance to the bank.

Evaluation of Transect Data

When evaluating water quality transect data, compare each point to a reference point in the transect. The reference point can be a point of interest in the transect (e.g., CIM location, grab sample location) or just a point from the transect that is likely to be well representative of the waterbody (e.g., mid-point). The difference between each point and the reference point is then evaluated for significance. For temperature and pH, the difference in readings between the reference point and a transect point is considered significant if the difference is greater than 0.5 units. For specific conductance, DO, and turbidity, the difference is considered significant if it is greater than 10% of the reference point reading. If transects are conducted when turbidity is low (less than 10 FNU), a difference of one FNU is equivalent to a 10% difference.

It is important to evaluate transects over a range of conditions to best understand shifts in mixing patterns under various conditions. Most streams will show significant differences in water quality in at least small sections of a transect for short periods of time (e.g., along the bank, during a rain event). Transect evaluations should characterize the extent of these spatial and temporal fluctuations.

Display and Storage of Transect Data

Properly collected transects, under a variety of water quality conditions, are likely to encompass a wide range of values. Because of this, charting the raw values together often leads to difficulty in discerning meaningful differences. For example, it would be difficult to see a 0.5°C difference in temperature when charting together a summer transect with water temperatures around 30°C and a winter transect with water temperatures around 3°C. Therefore, transect data are best displayed by charting the comparisons of transect points and the reference point. The thresholds of significance should be included in the chart to facilitate a quick evaluation of the data.

All transect survey data are recorded and archived along with meter calibration documentation. A sample transect form is provided in Appendix B.2. Measurement data from established water quality transects are entered and stored in the SIS database. More information on SIS is found in *Chapter 4.6, Sample Information System (SIS) Data Entry Protocol* (Shull 2017).

VERTICAL PROFILE SURVEYS

To characterize water quality through the water column, such as in lakes or pooled sections of a river, a vertical profile is to be conducted. Vertical profile surveys are particularly important in lakes. Since lakes have large depths, long retention times, and dynamic mixing characterizations, it is important to understand water quality vertically through the water column. Most Pennsylvania lakes are dimictic, where lakes will show distinct zonation and mixing which is important to understand prior to collecting chemistry and biological data on a lake. Collecting profile data will allow the collector to determine the mixing conditions at time of sampling. Profile data will include the collection of *in-situ* water temperature, specific conductance, pH, and DO at a minimum; other parameters are optional.

Profiles will be collected through the water column at incremental depths and will be recorded on the *Lake Assessment and TSI Field Data Sheet* (Appendix A.11). To start, a sonar depth finder or a weighted line marked in meter increments will be used to determine maximum depth. Knowing the maximum depth will allow the collector to proceed with caution as the field meter approaches the bottom so that substrate disturbance is minimal during the bottom data sample point. Once the maximum depth is determined, profile measurements can commence. The first measurement will be collected at the surface, where the water quality probes are to be situated just under the water's surface. Where maximum depth is ≤5 meters, measurements will be taken every 0.5 meters. If the maximum depth is between 5 and 20 meters, increments should be a maximum of 1 meter apart; where maximum depth is >20 meters, data will be collected at 1 meter increments to 20 meters and then adjusted to 2 meter increments until reaching the bottom. At each increment the collector is to allow enough time for field meter readings to stabilize before recording. As benthos are approached,

the collector is to pay attention to resistance in the field meter as it hits bottom. When resistance is felt, the field meter will immediately be raised to 2 inches off the bottom and a final recording at this maximum depth will be recorded; the depth is also to be noted. After the last measurement is taken, the field meter will be brought up to the surface and an additional measurement at 1 meter shall be recorded. This measurement ensures the field meter has not drifted out of calibration during the profile. The difference between the first surface reading and the final surface reading should be no greater than 10% for dissolved oxygen and specific conductance and no greater than 0.5 units for pH and temperature. In the event readings surpass difference thresholds, this should be noted, and data will need to be recollected. If the 1 meter check is still greater than 10% or 0.5 units after recollection, the field meter's calibration will need to be checked and documented.

Evaluation of Profile Data

When evaluating water quality profile data, two considerations will be given. First is consideration as to whether the lake is stratified. Stratification ensues as the surface waters rapidly warm, causing the upper layer to become less dense than the cooler bottom layer. Stratification typically involves the formation of three stable layers. The hypolimnion (bottom layer) has the coldest (often near 4°C), densest water. The epilimnion (surface layer) is comprised of warm, circulating, less-dense water that floats upon the cold and relatively undisturbed hypolimnion. The boundary layer between the two is the metalimnion, characterized by a steep thermal gradient, or thermocline, defined as a thermal change of at least 1 degree C per meter of depth. If the lake is stratified, the two thermocline boundaries are to be noted on the *Lake Assessment and TSI Field Data Sheet* (Appendix A.11).

Where a station has a maximum depth of <3 meters, profile data will be further scrutinized to determine if and where a bottom chemistry sample would be warranted. To make a determination, comparisons of water quality are made between the 1 meter below the surface profile measurement and all points approaching the bottom. The difference between the points are then evaluated for significance. For temperature and pH, the difference in readings between the reference point and a transect point is considered significant if the difference is greater than 0.5 units. For specific conductance and DO the difference is considered significant if it is greater than 10% of the reference point reading. If significance is noted a bottom chemistry sample would be warranted at the depth where the most significance occurs.

Display and Storage of Profile Data

All profile survey data are recorded and archived along with meter calibration documentation. A vertical profile form is provided in Appendix A.11. Measurement data from established vertical profiles are entered and stored in the SIS database. More information on SIS is found in *Chapter 4.6, Sample Information System (SIS) Data Entry Protocol* (Shull 2017).

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4.2 DISCRETE WATER CHEMISTRY DATA COLLECTION PROTOCOL

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INTRODUCTION

As part of its water quality monitoring programs, DEP collects water chemistry data to assess the quality of Pennsylvania's surface water resources. This information is used to detect or confirm pollution sources and causes, for routine water quality monitoring, and to establish abiotic-biotic relationships. This document provides guidelines for standardized water chemistry collection from surface waters. The procedures described here are adapted from scientific, peer-reviewed procedures, and were developed by technical experts. This protocol does not attempt to describe the entire spectrum of water quality data-collection techniques (such as continuous instream monitors) but does describe the surface water sampling procedures appropriate for typical DEP investigations and may require modification as situations dictate.

Variations to this protocol will be dependent upon specific data collection objectives, site conditions, equipment limitations, or limitations imposed by the procedure. Investigators should document modifications and report the final procedures employed. Investigators should be aware of, and work to mitigate, the potential for sample contamination at all phases of the sample collection process by observing proper sample collection, handling, and preservation procedures described here. The most common sources of error (also known as "interference") are cross-contamination, poor sampling locations, and incorrect preservation.

COLLECTION REQUIREMENTS

Collector Identification Number

Field staff required to collect surface water samples must have an assigned four-digit collector identification number (e.g., 0925). This number along with a sequential three-digit sample number (e.g., 0925-001), and date/time of sample are used to identify individual samples. Supervisory staff will request collector identification numbers for their field staff with the "Collector ID Request Form" found at the eLibrary website (ID Request Form).

DEP Laboratory Submission Sheet

Field staff will need to have at least Querying and Sample Entry security roles for the program or business unit that they are collecting samples for. The program or business unit will be consistent with the Program Code entered on the "DEP Laboratory Submission Sheet". Collectors must submit samples to the DEP Bureau of Labs (BOL) using the laboratory submission sheet". BOL Sample Sample Submission Sheets (SSS) can be found at the e-library website (Forms – Laboratories). Field staff are required to document collector identification number, reason code, cost center, program code, sequence number, date collected, time collected (in military time format), fixative(s), Standard Analysis Code (SAC), legal seal numbers for each sample collected (if required), the number of bottles submitted per test suite, facility information (if required), collector name, date, phone number, supervisor information, and any additional comments that lab analysts will use to properly handle samples. Entries should be made with indelible ink or typed electronically. Entries must be legible. Any information that is not applicable to the sample(s) needs to have an 'N/A' in the entry area. Compliance or SDWA sample check boxes must be checked either 'yes' or 'no'. Relinquishing

signature, date, time, and delivery method must be indicated on the SSS. If a collector makes a mistake on the SSS, it is recommended that a new SSS be completed with the correct information and the errored SSS be destroyed. If the collector does not have extra copies of SSS with them, the collector is to use a strikeout with a single line through the error and include initials and date somewhere near the strikeout. The corrected information can then be added to the SSS near the strikeout; if no room is near the strikeout an asterisk can be used and the change can be noted in open space on the SSS form.

As described previously, collector identification numbers are unique to each field staff collecting samples. Reason codes, cost centers, and program codes are program specific and should be obtained from the program responsible for coordinating sampling efforts. Sample sequence numbers are three-digit sequential numbers (001-999) unique to a sample collected on a given day generated by field staff collecting samples. Date and time collected should be accurately documented, especially if field parameters with specific diurnal fluctuations (temperature, dissolved oxygen) will accompany analytical results. To avoid complications with daylight savings time, collection time should be recorded using eastern standard or UTC-5 time throughout the year. However, sample holding times will need to be considered when there are time differentials.

A SAC is a unique code that details analytical tests to be applied to a specific sample. Each DEP program uses specific SACs for specific projects or purposes. For example, SAC 018 is used by collectors when submitting water chemistry samples for Special Protection Surveys. The analytes/tests listed under SAC 018 are those specifically identified by regulations that surface water must meet and therefore be assessed for, if a special protection determination is warranted. Other programs have developed unique SACs for their specific purposes, and the DEP BOL encourages programs to create SACs tailored to a program's specific needs. New SACs can be requested by filling out the Request Form for Standard Analysis Code. An example of a commonly used SAC is provided below (Table 1).

Table 1. Chemical parameters per SAC 612 collected for semiwadeable river surveys.

Test Code	Test Description	Reporting Limit	Units	Reference
00095	Specific Conductance at 25C	1	UMH/CM	SM 2510B
00403	pH	0	pH UNITS	SM 4500H-B
00410	Alkalinity, pH 4.5	0	MG/L	SM 2320B
00530	Total Suspended Solids	2	MG/L	USGS-I-3765
00600A	Total Nitrogen	0.064	MG/L	SM 4500N-ORG
00602A	Dissolved Nitrogen	0.064	MG/L	SM 4500N-ORG
00608A	Dissolved Ammonia	0.02	MG/L	EPA 350.1
00610A	Total Ammonia	0.02	MG/L	EPA 350.1
00630A	Total Nitrite and Nitrate	0.04	MG/L	EPA 353.2
00631A	Dissolved Nitrite and Nitrate	0.04	MG/L	EPA 353.2
00665A	Total Phosphorous	0.01	MG/L	EPA 365.1
00666A	Dissolved Phosphorous	0.01	MG/L	EPA 365.1

00671A	Dissolved Orthophosphate	0.01	MG/L	EPA 365.1
00680	Total Organic Carbon	0.5	MG/L	SM 5310C
00900	Total Hardness	0.11	MG/L	SM 2340A+B and EPA 200.7 revision 4.4
00916A	Total Calcium	30	UG/L	EPA 200.7 Revision 4.4
00927A	Total Magnesium	10	UG/L	EPA 200.7 Revision 4.4
00929A	Total Sodium	200	UG/L	EPA 200.7 Revision 4.4
00937A	Total Potassium	1	MG/L	EPA 200.7 Revision 4.4
00940	Total Chloride	0.5	MG/L	EPA 300.0
00945	Total Sulfate	1	MG/L	EPA 300.0
01007A	Total Barium	10	UG/L	EPA 200.7 Revision 4.4
01022K	Total Boron	200	UG/L	EPA 200.7 Revision 4.4

Table 1. Chemical parameters per SAC 612 collected for semiwadeable river surveys.

Test Code	Test Description	Reporting Limit	Units	Reference
01042H	Total Copper	4	UG/L	EPA 200.8 Revision 5.4
01045A	Total Iron	20	UG/L	EPA 200.7 Revision 4.4
01051H	Total Lead	1	UG/L	EPA 200.8 Revision 5.4
01055A	Total Manganese	10	UG/L	EPA 200.7 Revision 4.4
01067H	Total Nickle	4	UG/L	EPA 200.8 Revision 5.4
01082A	Total Strontium	10	UG/L	EPA 200.7 Revision 4.4
01092A	Total Zinc	10	UG/L	EPA 200.7 Revision 4.4
01105A	Total Aluminum	200	UG/L	EPA 200.7 Revision 4.4
01132A	Total Lithium	25	UG/L	EPA 200.7 Revision 4.4
01147H	Total Selenium	7	UG/L	EPA 200.8 Revision 5.4
70300	Total Dissolved Solids	2	MG/L	USGS-I-1750
70507A	Total Orthophosphate	0.01	MG/L	EPA 365.1
82550	Osmotic Pressure	1	MOSM/KG	DEP BOL
99020	Total Bromide	50	UG/L	EPA 300.1

Legal seals and associated legal seal numbers are required under circumstances where it is imperative to document the integrity of samples from sample collection to sample analysis. Legal seals are not always required and should be used per a program's specific requirements. Legal seal numbers must be singly listed (include letter and number) for each sample. Legal seals can be obtained from BOL. Refer to BOL for more information concerning legal seals.

Collector name, date, phone number, and # of bottles submitted were added to the laboratory submission sheet to meet NELAP chain-of-custody requirements. Using the area at the bottom of the form, each bottle submitted for the samples identified must be accounted for by enumerating the number of bottles per category listed for inorganic and organic analyses/tests. Each submitted form is also required to have printed the collector's name, the date, collector's signature (Relinquished by:), and collector's phone number. There are also spaces to document a facility name, facility identification number, and an alternate contact. These three pieces of information are not required.

The last piece of information to be documented is additional comments that lab analysts will use to properly handle samples. This information is documented in the 'Comment' field at the bottom of the

form. The most common use of this field is to add or delete tests to or from a specified SAC. For example, SAC 018 does not include a test for turbidity; however, a sample collector may want BOL to document turbidity for a sample so they would indicate in the ‘Comment’ field to add the turbidity test to the sample. If many samples will be submitted with consistent modifications of a SAC, the BOL prefers a new SAC be created for those samples. Other important comments to consider include identifying potentially toxic or otherwise dangerous samples, samples submitted for individual tests, and other important information that lab analysts will need to know to handle the samples correctly. The BOL recommends contacting the appropriate BOL staff before submitting samples requesting organic tests, potentially dangerous samples, or samples that need to be handled differently.

Sampling Supplies and Equipment

DEP programs can and do employ a multitude of program specific surface water sampling techniques that will require standard supplies (e.g., sampling bottles, preservatives, etc.), and specialized supplies (e.g., 0.10µm filters) or equipment (e.g., Van Dorn sampler). This section describes the equipment and supplies required to collect the most commonly used surface water sampling techniques that may be employed. Additional techniques are added as they become applicable and as standard procedures are formalized.

Typically, a 500mL High-Density Polyethylene (HDPE) bottle is used for storing an unfiltered, non-chemically preserved sample for inorganic constituent analyses and 125mL HDPE bottles are used for storing filtered or unfiltered samples such as metals and nutrients. Water samples collected for total or dissolved organic carbon or volatile organic compound analyses are stored in two 40mL Volatile Organic Analysis (VOA) amber glass vials. Additional analyses may require other specialized containers, so it is important to check the BOL site when working with new analyses. A suggested check list of recommended supplies and equipment is provided below:

SAMPLE CONTAINERS
☐ 500 mL sample bottles - inorganic, total metals, cyanides, phenolics, other
☐ 125 mL sample bottles - dissolved metals
☐ 1000 mL amber glass bottles - organics: semi-volatiles, pesticides, PCBs
☐ 40 mL glass vials - organics: VOAs
☐ 125 mL bac-t' bottles - bacteriological analysis (fecal coliform & *E.coli*)
☐ 1 L bac-t' bottles - bacteriological analysis (fecal indicators & qPCR)
☐ other: _____

FIXATIVES
☐ HNO_3
☐ H_2SO_4
☐ pH test strips
☐ other: NaOH, HCl

FIELD METERS & RELATED SUPPLIES
☐ dissolved oxygen meter
- ☐ DO probe solution
- ☐ zero % calibrating solution (if applicable)

☐ pH meter
- ☐ buffers (pH 4, 7, 10)
- ☐ KCl probe storage solution

☐ conductivity meter
- ☐ calibrating solution (if applicable)

☐ thermometer
☐ meter field manuals (if applicable)

OTHER
☐ 0.45µm groundwater filters or appropriate filtration apparatus with filters
☐ alkalinity test kit
☐ blank water (lab tested)
☐ extra bottles for duplicates
☐ pipetter & pipettes
☐ depth integrated or depth specific sampling equipment
☐ rinse squirt bottle
☐ eyewash bottle

FLOW
☐ flow meter
☐ rods (for anchoring tape bank-to-bank)
☐ tape measure
☐ wading rod

FORMS
☐ laboratory water chem. sheets
☐ bac-t' forms
☐ physical data field forms
☐ flow field form
☐ habitat assessment forms
☐ chlorine demand forms
☐ other:_____

SHIPPING
☐ courier shipping forms
☐ tape & dispenser
☐ shipping coolers
☐ ice

MISC.
☐ maps
☐ GPS
☐ waders
☐ gloves
☐ markers, pens, & pencils
☐ calculator
☐ insect repellent
☐ screwdriver/tools
☐ batteries (D-cell, other:_____)
☐ other: _____

DATA COLLECTION

Many surface water samples are manually collected instantaneous discrete samples. Water samples should be collected before conducting other types of in-stream field work in the study reach, such as discharge measurements or benthic macroinvertebrate collection to avoid disturbing the water column. Water samples are normally collected in HDPE, VOA vials, or amber glass bottles (for organic compound analysis). Samples should be preserved immediately upon collection, if necessary, with the proper type and amount of chemical fixative (usually an acid to an amount where the matrix pH is less than 2.0 pH units). All sample bottles should be cooled and held at ≤6°C until receipt by the laboratory. The sample bottles should be labeled in accordance with the procedure described later in this document. The bottles used for a sample are determined by the analyses required for that sample and the different types of preservation required for the analyses of interest.

Some chemical analyses require laboratory technicians to calibrate specialized laboratory equipment, prepare specialized reagents, or otherwise perform pre-analytical preparation before samples can be analyzed. If a collector is going to submit several samples involving specialized preparation (such as for bacteriological analyses, which involve agar plating), they should contact the appropriate section at the laboratory to ensure enough time is allocated for the pre-test procedures. If the laboratory is not notified to expect a large volume of these samples, holding times may be surpassed and the sample may be voided.

Field personnel must ensure that the samples collected will be representative of the aquatic system of interest. A discrete sample temporally and spatially represents the part of the surface water system being investigated. The data collection protocol and constituents chosen for analysis is critically dependent on the purpose and scope of the survey being conducted. Obtaining representative samples is of primary importance for a relevant description of the aquatic environment. Interference of the sampling process must be minimized; collectors must be alert to conditions that could compromise the integrity of a water sample. The most common causes of sample interference during collection include poor sample-handling and preservation techniques, input from atmospheric sources, and contaminated equipment or reagents. Each sampling site needs to be selected and sampled in a manner that minimizes bias caused by the collection process and that best represents the intended environmental conditions at the time of sampling.

Before handling sample bottles, the collector should ensure his or her hands are clean and not contaminated from sources such as food, coins, fuels, mud, insect repellent, sunscreen, sweat, nicotine, etc. Alternatively, collectors should wear disposable, powderless gloves (such as nitrile).

Labeling Bottles

While the minimum required information is the collector number and sequential sample number, collectors may add additional information such as date and time collected, general test(s) description (total metals, TOC, etc.), “filtered”, and preservation type. This will help prevent confusing what

bottles are for which tests and to ensure the sample is properly preserved. Labeling should be done so that at least 1” of space is left at the top of the bottle to allow BOL to apply lab labels.

Permanent marker will rub off HDPE bottles during collection and transport. So, clear packing tape is wrapped around the bottle to protect the hand-written labels. Using ball-point and other non-permanent ink pens must be avoided. BOL discourages the use of masking tape. Collectors should keep a log book of all samples they collect for later reference. The sample log entry should annotate the unique collector identification and sequence number, date and time, the waterbody name, sample location, SAC code, and any additional analytical tests performed or excluded. Additional information on labeling samples can be found on the BOL website.

Direct Surface Water Sampling of Wadeable, Flowing Waterbodies

The most common type of water sampling is conducted in wadeable, flowing waterbodies, where water is collected directly into the sample bottle. This procedure is not generally used in situations where contact with contaminants is a concern.

The collector should face upstream, taking care to not alter flow patterns or disturb substrate sediments upstream of where they will collect the sample. Collection bottles should be inserted into the water column vertically, facing down to avoid inadvertently collecting surface debris/films. In most situations, samples are collected at mid-depth in the approximate thalweg (the line defining the points along the length of a stream bed with the greatest depth). The collector should remove the stopper/lid from the sampling container just before sampling, taking care not to contaminate the cap, neck, or the inside of the bottle with his or her fingers, wind-blown particles, precipitation, clothes, body, or overhanging structures. All bottles are rinsed three times instream before filling the bottle. Once the sample is collected and capped, the collector should rinse any dirt or debris from the outside of the container.

Vertical Depth Sampling in Lakes

For dimictic lakes, DEP employs the use of horizontal Van Dorn or Kemmerer grab sampling equipment (Figure 1). These grab samplers allow for the collection of large volumes of water at a specified depth. Samplers should be attached to a rope that is marked in 1-meter increments to assist in lowering to specific depths. The sampler should be set according to Figure 1. The rubber stoppers will be set to the open position using the pin on top of the sampler. Once set the sampler should be rinsed three times with lake water to remove any particles or contaminants obtained during transit. Once rinsed move the sampler to a location that has not been influenced by the rinsing (this could be on the opposite side of the boat or towards the front or rear). The sampler will be lowered to the desired depth. Once at the desired depth wait a few seconds to allow sampler to fill as to acquire the maximum volume. The collector will then send the messenger down to trip the sampler closed; raise the sampler back to the surface to obtain sample aliquots. As the sampler comes to the surface the collector will carefully reach over the boat to bring the sampler onboard. A small amount of water should be discarded from the drain nozzle to rinse.

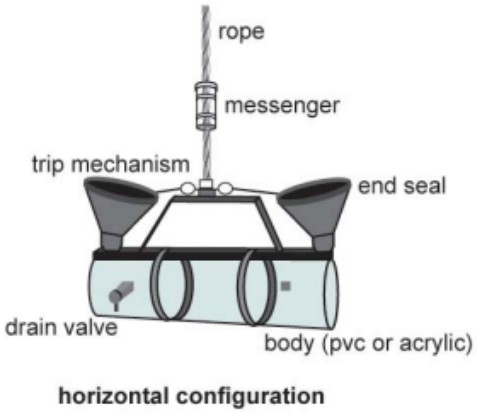

Figure 1. Van Dorn sampler configurations (Image from: Zalewsky 2015).

Aliquots can now be obtained. Samples to be collected for general chemistry, metals, ions, and nutrients will be collected in HDPE grade plastic bottleware; size and volume requirements vary depending an analysis to be performed. The Standard Analysis Code used for most lake work is SAC 083. For SAC 083, two 500 milliliter (mL) HDPE bottle will be needed for general chemistry analyses, one 125 mL HDPE bottle will be needed for metal analyses, and one 125 mL HDPE bottle will be needed for nutrient analyses. All plastic bottleware, including the lid, will be rinsed three times with sampler water prior to obtaining a final aliquot. In instances where organic compounds are to be analyzed for instance, organic carbon, methane, or pesticide glass amber glass bottleware should be used. The collector is to consult BOL prior to any data collection to ensure proper bottleware is used and adequate volumes are obtained.

In dimictic lakes depths to be sampled are a location 1 meter below the water's surface and 1 meter above the lake's bottom. The 1 meter above the lake's bottom location will be predetermined with the maximum depth obtained during profile data collection; this exact depth in which the "Bottom" grab sample was obtained will be recorded. The exact depth of the "Top" sample (1 meter below the surface) will also be recorded. In shallow dimictic lakes where depths are less than 3 meters, one sample collected from 1 meter below the surface will suffice. In polymictic lakes where complete mixing occurs year-round, one grab sample with the Van Dorn or Kemmerer sampler will suffice. The grab sample should be collected at a depth that represents mid-depth. After completing collections at a site location, equipment will be cleaned using deionized water, where the equipment will be rinsed three times and allowed some time to air dry.

Field Meters

Field meters provide the ability to collect in-situ data that is not available through discrete samples submitted to BOL. Standard field parameters include dissolved oxygen (DO), temperature, specific conductance, pH, and turbidity. While BOL does report pH, it is understood that the laboratory reported pH of a grab sample can and will migrate. Therefore, the lab result may not reflect the actual instream pH. Collection of field meter data is highly recommended and required depending on the

specific field survey protocol being used. Field meter data will be collected per *Chapter 4.1, In-Situ Field Meter, Transect, and Vertical Profile Data Collection Protocol* (Hoger and Arnold 2023).

Collecting Unfiltered Samples

For unfiltered samples, the collection bottle is rinsed at least three times with the water to be sampled. The collector removes the lid from the bottle and partially fills the bottle under water or from the depth integrated or depth specific sampler. The bottle is then removed from the water, capped, shaken vigorously, uncapped, and inverted. Rinsing waste is discarded downstream of the collector to ensure no contamination reenters the sample bottle. Unfiltered, inorganic samples will be filled to the neck of the bottle, allowing for “head space” as requested by the BOL. Specialized bottles, such as bacteriological bottles, VOAs, and 1L amber glass jars, are not rinsed. VOAs used to collect volatile samples, however, are filled to the top and capped so that no air remains in the bottle. 1L Amber glass jars are submerged and filled only to the neck. 4L cube containers (Cubitainers) are rinsed three times and filled. Regardless the bottle type, it is imperative that the bottle is filled with water the same way every time to maintain consistency. The proper amount of chemical preservative is then added (Table 2); the bottle is recapped and then inverted several times to mix the reagent with the sample.

Collecting Dissolved (Filtered) Samples

Dissolved samples must be filtered and fixed immediately after sample collection using a 0.45µm disposable (single-use, metals free) filter (e.g., AquaPrep 600 Groundwater Filters, VWR: #28145-142 or equivalent). Filters should be rinsed with trace-metal free deionized (“ultrapure”) water before sample collection begins. Rinsing removes trace contaminants (if any) from the manufacturing process.

A 1000mL squeeze-type bottle or churn is typically used to collect the raw water to be filtered. This container is rinsed three times in the same manner as for an unfiltered sample. The filtering device (e.g., syringe) is also rinsed three times using the raw water in the container before filtration begins. Raw water is then aliquoted from the container into the filtering device and rinsed through the filter. Once all equipment is rinsed, raw water may pass through the filter into the sample bottle. Again, the sample bottle must be rinsed three times with filtered water before filling the sample bottle. As with the unfiltered 125ml sample, water is filled to the neck of the bottle. It is imperative that the collector accurately fills water to this line to ensure consistent dilution of the fixative between samples. If the collector is not consistent in performing this quality assurance step, his or her dissolved constituent concentrations may be greater than the equivalent total constituent concentration in the unfiltered sample. The reagent preservative is then added and mixed.

Preservation

Without preservation, water sample constituents will continue chemical interactions or undergo other physical processes, such as metals precipitation. Moreover, laboratory pH measurements are usually higher than field measurements because of carbon dioxide degassing from the matrix. Keeping the water samples ≤6°C minimizes these processes. Most chemical preservatives function by decreasing

the matrix's pH below 2.0 (or above pH 12 for cyanide - fixed with NaOH), which limits constituent reaction. For example, 125mL bottles require 2mL of preservative to achieve a pH threshold <2.0 (Table 2). Most water samples measuring metals concentrations use 1:1 HNO_3; however, it is important to note that ferrous iron is preserved with 1:1 HCl, not HNO_3. The 10% H_2SO_4 solution is used for storing water samples measuring phosphorus and nitrogen species such as ammonia. However, for VOA samples such as TOC, the 10% H_2SO_4 solution should be obtained from the organics laboratory, because it is not the same as standard 10% H_2SO_4 solution. If the recommended amount of preservative is added then pH does not need to be checked after preservation. If a pH check is still desired, then the testing device (pH test strip) is held below the sample bottle and a small amount of the sample is poured on the device. The testing device should not contact with the sample bottle.

Table 2. Recommended chemical preservative amount for commonly used sample bottle sizes.

Bottle Size	Preservative Amount
40 mL VOA	≈ 1.0 mL
125 mL HDPE	2.0 mL
500 mLHDPE	8.0 mL

Reusing graduated pipettes to dispense preservatives is permitted, but the collector should understand that doing so introduces potential for contamination at several points in the preservation process. The pipette must be carefully guarded against contacting any surface and should be replaced often. Additionally, the preservative contained in the bulk storage container can become contaminated from constant opening and insertion of the pipette. Therefore, it is recommended that preservatives and bulk containers are refreshed regularly. Most preservatives are highly reactive acids that can cause chemical burns to skin, reactive equipment, and clothing, so collectors should exercise caution when handling, transporting and storing these chemicals. Refer to Material Safety Data Sheets (MSDS) for proper safety precautionary procedures.

Sample location considerations

In general, collectors should avoid sampling in eddies, pools, side-channels, or in tributary mixing zones unless necessary due to site-specific or other environmental considerations. Collectors must also be aware of potential point-source and non-point sources of water quality influence.

For slow moving streams with easily disturbed sediment, the collector should sample from the stream bank, boat, or bridge using a sampling extension pole. If sampling from a boat, the collector should sample near the bow as the boat moves upstream or faces upwind.

For point source surveys and characterizing other influences, representative water samples are collected from the discharge pipe/influence, from upstream (control), and downstream locations at a minimum. Sampling stations located upstream of the discharge pipe should be in a non-impacted zone to serve as a control. If there are multiple discharges, then sample stations should be placed to bracket individual discharges to better characterize each source. When sampling downstream of the discharge pipe, the investigator should avoid the immediate vicinity of the discharge/influence point

and select a sample point far enough downstream to allow for mixing between the discharge and stream flow.

To decide where an acceptable downstream sampling point should be located, consider the following. For pollutants controlled by acute concerns, enforcement of numeric criteria is at the point of complete mix, or after 15 minutes of travel time, whichever occurs first. For pollutants controlled by chronic concerns, enforcement of numeric criteria is at the point of complete mix, or after 12 hours of travel time, whichever occurs first. These are all projections at design conditions (Q7-10 or harmonic mean flow). The actual point of complete-mix depends on the stream size, its width and depth, its flow on that day, the velocity of the plume, the angle at which it enters the stream, and the roughness of the stream bed.

Field specific conductance measurements may help determine the point of complete mix. If the point of complete mix is unclear or too far downstream for representative sampling, then multiple samples should be collected across the width of the waterbody. For very large rivers it may be necessary to composite water samples collected along a cross channel transect to accurately characterize water quality of the sampled stream segment.

Before collecting data to assess the Potable Water Supply Use (PWS), collectors should consider that while 25 Pa. Code § 93.4 identifies the PWS Use as applying to all surfaces waters, with the exception for which § 96.3(d) states,

> *(d) As an exception to subsection (c), the water quality criteria for total dissolved solids, nitrite-nitrate nitrogen, phenolics, chloride, sulfate and fluoride established for the protection of potable water supply* [PWS] *shall be met at least 99% of the time at the point of all existing or planned surface potable water supply withdrawals unless otherwise specified in this title*

For these parameters, the PWS Use can be evaluated by collecting samples from the facilities raw water spigot or from the source waterbody as close to the intake as possible. Different rules apply regarding sample locations in streams vs. lakes. Sample collections from streams will follow those protocols described in *Chapter 2.5, Stream and River Assessment Survey Design* (Shull and Arnold 2023), and sample collections from lakes will follow protocols described in *Chapter 2.4, Lake Assessment Survey Design* (Arnold 2023).

Quality Assurance

Field blanks and sequential replicates are the most common quality control samples collected. Generally, for every type of sample collected, field blanks (if applicable) and sequential replicates are to be collected every 20th sample or once per project at a minimum. Split replicates are encouraged to be submitted as part of the collector’s routine QC schedule, additional split replicates can also be sample design and protocol dependent. Field blanks are to be collected stream side or lake side. Equipment blanks are recommended on any new sampling equipment and at the start of each field season or on a case by case basis. Equipment blanks are to be collected in a controlled environment

like the office, lab, or warehouse. When collecting blank QC samples, Ultra Purified water is to be used. Ultra Purified water can be obtained from the BOL, Central Office (CO), or another supplier. When implementing QC sampling, the same sampling procedures that are or would be used to collect an environmental sample will be used to collect the QC sample. Both sample blanks and sample duplicates are assigned unique, sequential sample numbers. The collector needs to carefully annotate which sample is a duplicate or blank. Duplicates and blanks must be documented appropriately in SIS under the 'Comments/Quality Assurance' tab. Additional details regarding DEP's Quality Assurance Procedures are found in Appendix E.

Sample Holding Times

Water samples need to be shipped or delivered to BOL as soon as possible. The collector should understand that certain laboratory analyses have "holding times" during which tests must be conducted for result validity. Nitrate concentrations, for example, must be measured within 48 hours of sample collection. If a sample surpasses holding time requirements, the results will not be reported unless a "Request to Analyze Voidable Samples" form (see the BOL website) is submitted to the BOL. It is not advisable to collect and ship samples on Fridays, as the laboratory does not operate on weekends; samples shipped on Friday will not be received and logged until Monday morning. Doing so will guarantee that holding times of 48 hours or less will not be met. Collectors essentially need to plan their water sampling from Monday through Thursday, dropping off samples collected those days by 1600 hours, and verify shipped samples will reach DEP BOL by early Friday morning at the latest. Samples collected for Carbonaceous Biological Oxygen Demand (CBOD) and Biological Oxygen Demand (BOD) analyses have a 48-hour holding time. Holding time begins at the time of sample(s) collection. Initial DO is not performed on CBOD/BOD samples until Wednesday due to the five-day incubation period; consequently, if samples must be collected on Mondays, collect only after 1300 hours to ensure the test is within holding time.

Shipping Samples

All DEP district and regional offices are designated pick-up locations for water samples; the samples must be dropped off by 1600 hours for pick up at 1630 hours. Other locations exist, such as at some Pennsylvania Department of Transportation facilities and some private businesses, but these drop-off locations may require call-ahead notice to the current courier, as they may not be visited daily. Furthermore, some drop-off locations may require a drop-off specific key to open the drop-off entrance lock, and access should be arranged with the point of contact prior to sampling. Drop-off locations are available on the BOL website.

The collector should vertically insert bottles into a cooler, right-side up. The samples should be cooled with cubed or crushed ice. A sufficient amount of ice should be added to the cooler to ensure samples remain at ≤6°C during overnight shipping. Laboratory personnel will note whether samples were shipped properly. Improperly shipped samples may be subject to a data release request. Dry ice will freeze water samples and should never be used for storage or shipping of water chemistry samples. The "Sample Submission Sheet" should be filled out, inserted into a zip-top bag, and attached to the inside of the cooler lid. Courier shipping labels should be printed out during ordering so they can be

attached to the top of the cooler lid during sample drop-off. Shipping labels are secured to the cooler lip with two pieces of packing tape on the left and right side; taping all sides of the label makes removal difficult for lab technicians.

Other Sampling Considerations

For storm water surveys, a minimum of one sample is collected during low or dry weather flow to determine background conditions and from 3 to 5 high flow (storm) events in conjunction with stream flow measurements to characterize pollutant loadings. For storm events, it is important to make collections during the first flush and/or while the hydrograph is rising. Analyses should be performed for metals (Fe, Al, Cu, Pb, Zn, Cd, Cr, Hg), oils and grease, pathogens, and for total and dissolved nutrients. Analysis is not limited to the above, and parameters of special concern (e.g., fertilizers, pesticides, and other organic chemicals) may be added as necessary.

If deemed necessary by the investigator, nutrient sampling will occur during the growing season at least once a month from May through October. Sampling should occur during both dry and wet weather to adequately characterize loadings. Wet weather samples should be collected during the rising hydrograph. In addition, stream discharge will be measured at least once. Water quality analyses should be conducted for total and dissolved nutrients using SAC 047.

For abandoned mine discharges or acid mine discharges, samples should be collected from the point(s) of discharge, if possible. In addition, flow from the discharge(s) should be measured to determine loading rates for Total Maximum Daily Load (TMDL) development. Flow and channel cross section are measured in the field per standard USGS stream gauging techniques (USGS 2006). Analyses are performed for metals, alkalinity, and acidity using SAC 909.

Streams affected by atmospheric deposition typically experience stressful water quality conditions in late winter/early spring during heavy snowmelt and/or storm events. Prolonged intense precipitation events during summer and fall can also result in depressed water quality. Sampling should target these high streamflow episodes to document worst-case conditions that are prone to depressed pH. Sampling should also target seasonal base flow (preferably summer or fall), when pH can rebound to circumneutral values. This sampling structure will allow a characterization of best-case and worst-case water quality conditions at a site, and the difference, which serves as a measure of a stream’s ability to buffer acidic inputs. Analyses are performed for metals, alkalinity, acidity, organic carbon, and carbon dioxide using SAC 087 (Shank 2023). Field pH readings should be taken with accurately calibrated field meters. the pH sensors should have an optimal response life (0 to -20 millivolts in 7 pH buffer).

For PWS assessments, eight samples should be collected during the spring, summer, and fall months in order to capture varying conditions, but there is no seasonal requirement. Sampling during or immediately after significant rain fall is discouraged. Analyses are performed for total nitrites, total and dissolved metals, chloride, fluoride, sulfate, color, and dissolved solids using SAC 166 for streams. Some Human Health (HH) parameters are included in SAC 166 to account for influences often

associated with acid mine drainage, brownfield contamination, or other pollution sources. Additional microbiological and HH parameters can be added on a site-specific basis. For PWS assessments of lakes or reservoirs, three samples should be collected throughout the spring, summer, and fall months. SAC 166 can also be used for collecting PWS data from lakes.

Should excursion(s) of any PWS criteria be apparent early in the collections, sampling should continue until the desired or recommended number of samples are collected.

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4.3 CONTINUOUS PHYSICOCHEMICAL DATA COLLECTION PROTOCOL

Chapter 4 Chemical Data Collection Protocols

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INTRODUCTION

DEP uses continuous instream monitors to assess the quality of Pennsylvania's streams. Data collected using continuous instream monitoring will be used in conjunction with other data for evaluation of water quality standards (WQS) supporting the protected use. Most instream monitoring configurations include at least four parameters: water temperature, specific conductance, pH, and dissolved oxygen. Monitors can also be configured to measure additional properties, such as turbidity, water stage, and fluorescence. Sensor data are valuable for a variety of purposes including but not limited to, characterizing baseline physicochemical stream and lake conditions, describing seasonal and diel fluctuations, and documenting potential exceedances of water quality criteria. These data are also used in conjunction with flow measurements and chemical analyses of grab samples to estimate chemical loads. In lakes, continuous monitors can be deployed to capture water quality at specific depths or deployed at multiple intervals to characterize the lake's vertical water quality profile. Sensors that are used to measure water quality field parameters require careful field observation, cleaning, and calibration procedures. The resulting data requires systematic actions for the computation and publication of final records.

This protocol provides guidelines for site and monitor selection, sensor inspection and calibration procedures, field maintenance, data evaluation and correction, and reporting procedures. Many of these criteria and procedures were developed from and mirror the USGS *Guidelines and Standard Procedures for Continuous Water Quality Monitors: Station Operation, Record Computation, and Data Reporting* (Wagner et al. 2006).

Water quality parameters are dynamic, necessitating frequent and repeated measurements to adequately characterize variations in quality. When the time interval between repeated measurements is adequately small, the resulting water quality record can be considered continuous. A device that measures water quality in this way is called a continuous water quality monitor. These monitors have sensors and recording systems to measure physicochemical water quality field parameters at discrete time intervals and at discrete locations. Operation of a water quality monitor provides a record of water quality that can be processed and reported. The water quality data provides a record of changes in water quality that also serve as the basis for computation of constituent loads at a given site. Data from the sensors are also used to estimate other constituents if a significant correlation (typically through regression analyses) can be established.

Water temperature, conductivity, dissolved oxygen (DO), pH, and turbidity are commonly recorded using continuous monitors. Water temperature and conductivity are true physical properties of waterbodies, whereas DO and pH are measured concentrations. Turbidity is an expression of the optical properties of water. Additional sensors are available to measure other field parameters, such as oxidation-reduction potential, water stage, ammonia, nitrate, chloride, and fluorescence. Some monitors also include algorithms to report calculated parameters, such as specific conductance, total dissolved solids, and percentage of DO saturation. Calculated parameters can be useful; however, consideration for the potential error in the algorithms should be taken into account. Sensor technology

broadens the variety of measurable chemical constituents and reduces the limits of detection. As a result, continual progress is being made to improve applications and refine quality-control procedures.

The vast majority of continuous monitoring of water quality field parameters takes place in Pennsylvania's lotic surface waters which may vary significantly in size, clarity, chemical composition, and biological production. Procedures for continuous monitoring in pristine, headwater streams differ from those in large, impacted rivers. Continuous monitoring in impacted environments can be challenging because of rapid biofouling from microscopic and macroscopic organisms, corrosion of electronic components from salts, and wide ranges in values of field parameters associated with changing weather.

This protocol provides basic guidelines and procedures for use by DEP personnel in site/monitor selection, field maintenance and calibration of continuous water quality monitors, record computation, review, and data reporting. This protocol details the primary technique used by DEP for deploying and servicing continuous instream monitors. Additionally, DEP has record keeping and QA/QC requirements specific to this protocol which are detailed in Appendix E.

This protocol highlights basic guidelines that may need to be modified to meet local environmental conditions. Data management procedures provided in this protocol are designed to offer basic guidelines and may need to be manipulated to suit other purposes and different software. In-depth knowledge of equipment operation and familiarity with the watershed are imperative in the data evaluation process. Examples of the application of scientific judgment in the evaluation of data records are discussed and are, by necessity, site specific.

MONITOR DEPLOYMENT

Major considerations in the design of a continuous instream monitoring station include site selection, monitor selection, monitor configuration, and sensor selection. Sensor and site selection are guided by the purpose of monitoring and the data objectives. The main objective in the placement of the sensors is the selection of a stable, secure location that is representative of the aquatic environment.

Site Selection

The main factors to consider in selecting a water quality monitoring site are the purpose of monitoring and the data quality objectives. All other factors used in the site selection process must be balanced against these two key factors. Defining the purpose of monitoring includes making decisions about the field parameters to be measured, the period and duration of monitoring, and the frequency of data collection. More site-specific considerations in monitor placement include site-design requirements, monitor-installation type, physical constraints of the site, and servicing requirements.

Once the purposes of monitoring and data-quality objectives are defined, balancing the numerous considerations for placement of a continuous water quality monitoring system still can be difficult. Obtaining measurements representative of the waterbody usually is an important data quality

objective. The optimum site consideration for achieving this objective is placing the sonde in a location that best represents the waterbody being measured. Thus, an optimal site is one that permits sensors to be located at a point that best represents the section or depth of interest for the aquatic environment being monitored.

Cross-section variability and upstream influences are major factors for site selection. Cross-section surveys of field parameters must be made to determine the most representative location for monitor placement. A site must not be selected without first determining that the data-quality objective for cross-section variability will be met. Sufficient measurements must be made at the cross-section to determine the degree of mixing at the prospective site under different flow conditions and to verify that cross-section variability at the site is not greater than conditions necessary to meet data-quality objectives. Cross-section measurements must continue to be made after equipment installation to ensure that the measurements are representative of the stream during all seasons and hydrographic flow conditions (Wagner et al. 2006). Additional information on cross-section surveys can be found in *Chapter 4.1, In-Situ Field Meter, Transect, and Profile Data Collection Protocol* (Hoger and Arnold 2023).

Some aquatic environments may present unique challenges for optimal site location. In lakes, confounding conditions created by stratification, wave action and fluctuating water levels dictate depth at which water quality is represented. Lateral mixing in large rivers often is not complete for tens of miles downstream from a tributary or outfall. Therefore, a location near the streambank may be more representative of local runoff or affected by point-source discharges upstream, whereas a location in the channel center may be more representative of areas farther upstream in the drainage basin. Turbulent streamflow may aid in mixing, but turbulence can create problems in monitoring field parameters, such as DO or turbidity. The ideal location for a CIM site is often one that is best for measuring surface-water discharge. Both purposes require a representative site that approaches uniform conditions across the entire width of the stream.

Instream Site Selection

The measurement point in the vertical dimension also needs to be appropriate for the primary purpose of the monitoring installation. Flow condition should be considered during this evaluation. For a medium to small stream with alternating pools and riffles, the best flow and mixing occurs in the riffle portion of the stream; however, if flooding changes the locations of shoals upstream of the monitoring site, the measurement point may no longer represent the overall water quality characteristics of the waterbody. Streams subject to substantial bed movement can result in the sensors being lost or located out of water following a major streamflow event, or at a point no longer representative of the flow. A site may be ideal for monitoring high flow but not satisfactory during low flows.

Assessment of a site also is dependent on fouling potential, ease of access, and susceptibility to vandalism. The configuration and placement of water quality monitoring sensors in cold regions require additional considerations in order to obtain data during periods of ice formation. Overall, a

monitoring site should be safe and accessible, meet minimum depth requirements of the equipment, avoid vandalism, and be characteristic of the system. It may be necessary to reconnoiter the site under several flow conditions before a determination is made. The site should continue to be evaluated throughout the deployment, and if necessary, the monitor moved to a more appropriate location.

Lake Site Selection

In a lake, monitoring sites can be found near the shoreline or in open water. If site selection is near the shoreline, consideration needs to be given to inputs in the vicinity of the proposed site. Aerial maps should be referenced to determine if any tributary influences will impact a shore CIM site. In addition, direct discharges should be considered during the final placement of the sensors. Field reconnaissance should be completed prior to monitor placement. For shoreline deployments, wave action should also be considered. Placement of sensors should be placed far enough from the shore where wave action does not impact the monitoring site.

Open water site selection should, in general, be placed either mid-lake or where water is the deepest. Both would ensure the representativeness of water quality being measured. If interested in inflow water quality and outflow water quality site locations of monitors should be placed in open water near the inlet and near the outfall of the lake. Once site location is determined, profile measurements should be taken to help guide final placement of the monitor. Methods to collect profile data is found in *Chapter 4.1, In-Situ Field Meter, Transect, and Profile Data Collection Protocol* (Hoger and Arnold 2023).

Assessment of a site selection and monitor location also is dependent on fouling potential, ease of access, and susceptibility to vandalism. Overall, a monitoring site should be safe and accessible, avoid vandalism, and be characteristic of the system. It may be necessary to reconnoiter the site under several conditions before a determination is made. The site should continue to be evaluated throughout the deployment, and if necessary, the monitor moved to a more appropriate location.

Monitor Selection

According to Wagner et al. (2006) the selection of a water quality monitor involves four major interrelated elements: (1) the purpose of the data collection, (2) the type of installation, (3) the type of sensor deployed at the installation, and (4) the specific sensors needed to satisfy the accuracy and precision requirements of the data-quality objectives. The DEP Water Quality Division uses a wide variety of instream monitors; however, these monitors are all designed to work with DEP’s preferred monitor configuration which eliminates the need to consider the type of installation (element number 2 above).

Sensors are available as individual instruments or as a single combined instrument that has several different sensors in various combinations. For clarity, in this protocol, a sensor is the fixed or detachable part of the instrument that measures a particular field parameter. A group of sensors configured together commonly is referred to as a sonde. A sonde typically has a single recording unit

or electronic data logger to record the output of multiple sensors. The term monitor refers to the combination of sensor(s) and the recording unit or data logger (Wagner et al. 2006). The most widely used water quality sensors in monitoring installations are water temperature, conductivity, DO, pH, turbidity, and water stage. These sensors are the focus of this protocol.

Monitor Configuration

The monitor configuration preferred by DEP is an internal-logging, multi-parameter, recording monitor that is entirely immersed and requires no external power. Power is supplied by conventional batteries located internally, and sensor data are stored within the sonde on nonvolatile recording devices. DEP uses monitors from multiple companies but prefers models that can provide live readings to facilitate fouling and calibration checks outlined below, and that allow calibration of the sensors to minimize corrections of recorded data. Models that do not have these features can be used but performing the necessary checks will be more challenging.

The standard deployment procedure for streams and rivers consists of the monitor placed inside a well vented schedule 80 Poly Vinyl Chloride (PVC) shroud attached to two form stakes with carabiners and a steel cable (Figure 1). The form stakes are driven into the sediment paralleled to stream flow. In this configuration, the stakes act as a primary and secondary anchor for the monitor. Smaller monitors are also configured in this style, however shrouds are smaller and may only have one form stake anchor based on stream flow characteristics (Figure 2). In both small and large monitor configurations, equipment is covered by large rocks for protection and concealment. Figure 3 illustrates the standard instream configuration for DEP. The standard deployment method for lakes consists of a buoy and anchored lined system.

The primary advantages of the internal-logging configuration are that AC power or large batteries and shelters are not needed. As a result, the upfront cost associated with emplacement is greatly reduced and mobility of the monitors is increased. Additionally, mid-channel deployment is much easier. Some disadvantages to this configuration include the lack of telemetry and solar panels which increases the need for regular maintenance and data retrieval visits. Additional monitor configurations are discussed in Wagner et al. (2006).

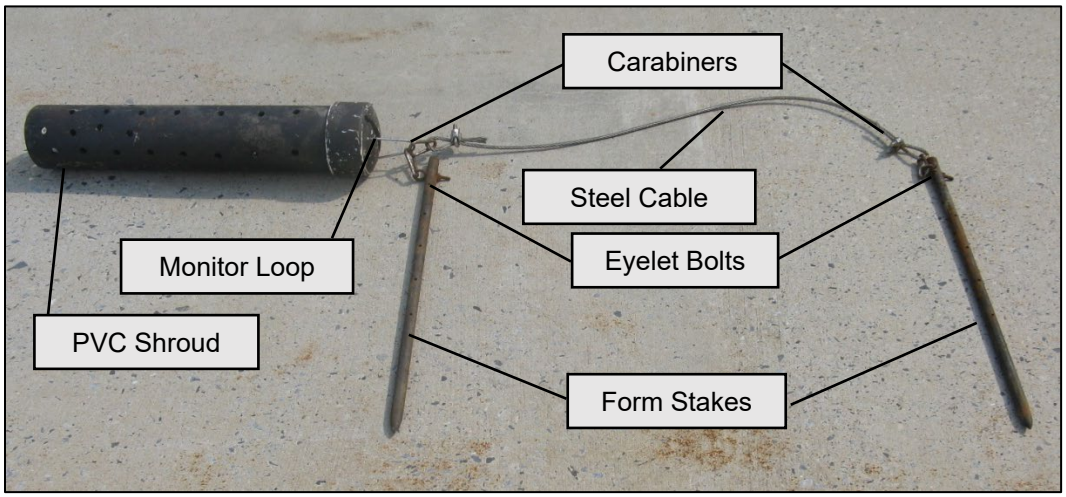

Figure 1. Standard deployment configuration for large, multi-parameter monitors uses two rebar/form stakes used as sediment anchors. Cables and carabiners are attached to stakes using 1/4 in. eyelet bolts. Once attached, the bolt threads are stripped to avoid backing out.

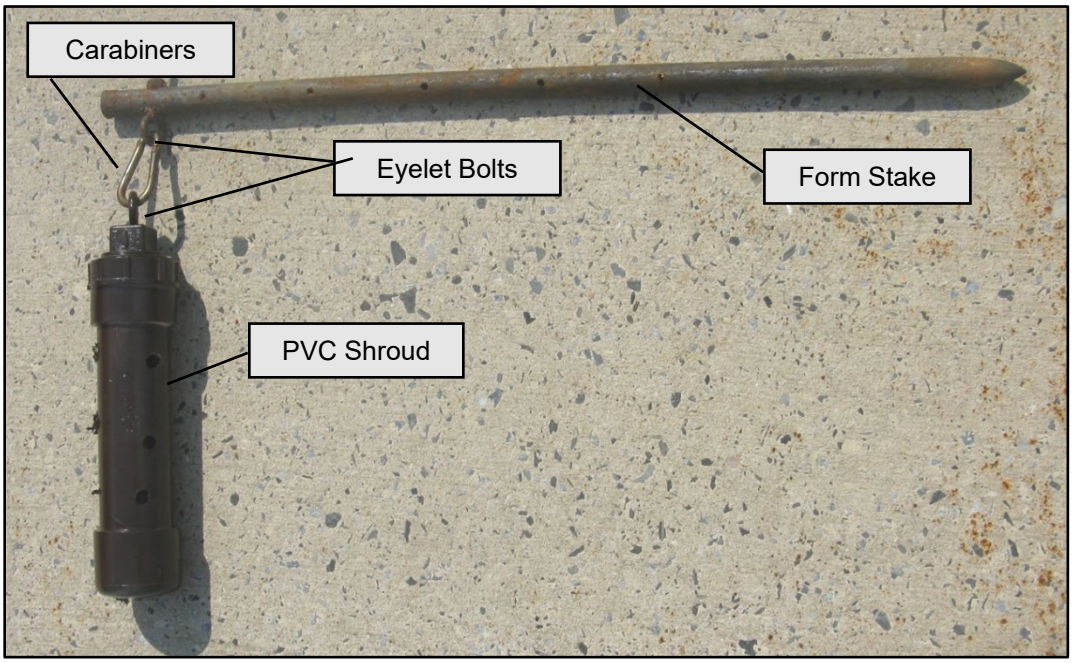

Figure 2. Deployment configuration for smaller monitors typically only includes one form stake.

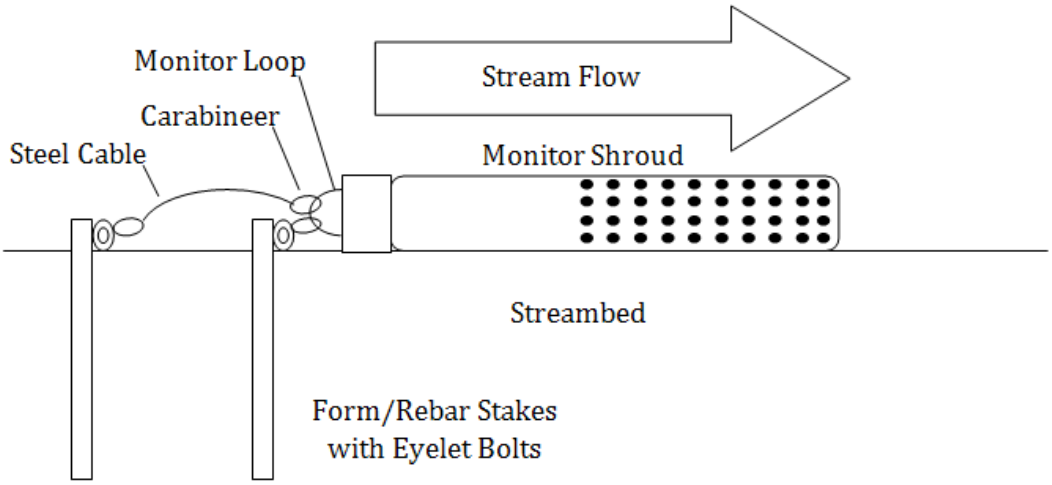

Figure 3. The standard instream configuration for monitors operated by DEP. Large, but manageable, rocks (not shown in figure) are placed around the monitor for stability, concealment, and protection.

Adjusting for Sediment Fouling

In streams characterized by high sediment deposition, monitors can quickly become buried if deployed on the stream bottom. Even when monitors are equipped with wipers, sediment

accumulation can quickly lead to erratic readings that usually render data unusable. In these environments, DEP suggests trying the following adjustments to the standard deployment:

- *Prop the monitor up slightly off the bottom with a small rock.* With mild deposition, this procedure is often sufficient.
- *Angle the monitoring into the flow of the stream.* Support this angle with the rocks covering the sonde. This angle can increase flow across the probes and help flush sediment.
- *Shorten the PVC shroud.* When the sonde guard and probes protrude from the PVC, sediment accumulation is often reduced. Employing this strategy does leave the probes less protected.

When severe sediment fouling conditions are present, it is necessary to deploy the sonde in a way that keeps it off the stream bottom. One effective strategy is to anchor a section of PVC to rocks or trees on the bank and slide the sonde down the PVC into the water (Figure 4). A long bolt through the PVC is used to create a stop for the sonde at the correct height, suspending it in the water column. Cross-section surveys are crucial when employing this strategy to ensure that the water quality near the bank is representative of the stream. Another option to keep the sonde off the bottom of the stream is to suspend the sonde using a bridge or bridge pier (Figure 5). With either strategy, the deployment should be made on the back side of the tree or structure to protect the equipment.

Figure 4. PVC deployment used to minimize sediment fouling at Swamp Creek, near Pottstown, PA.

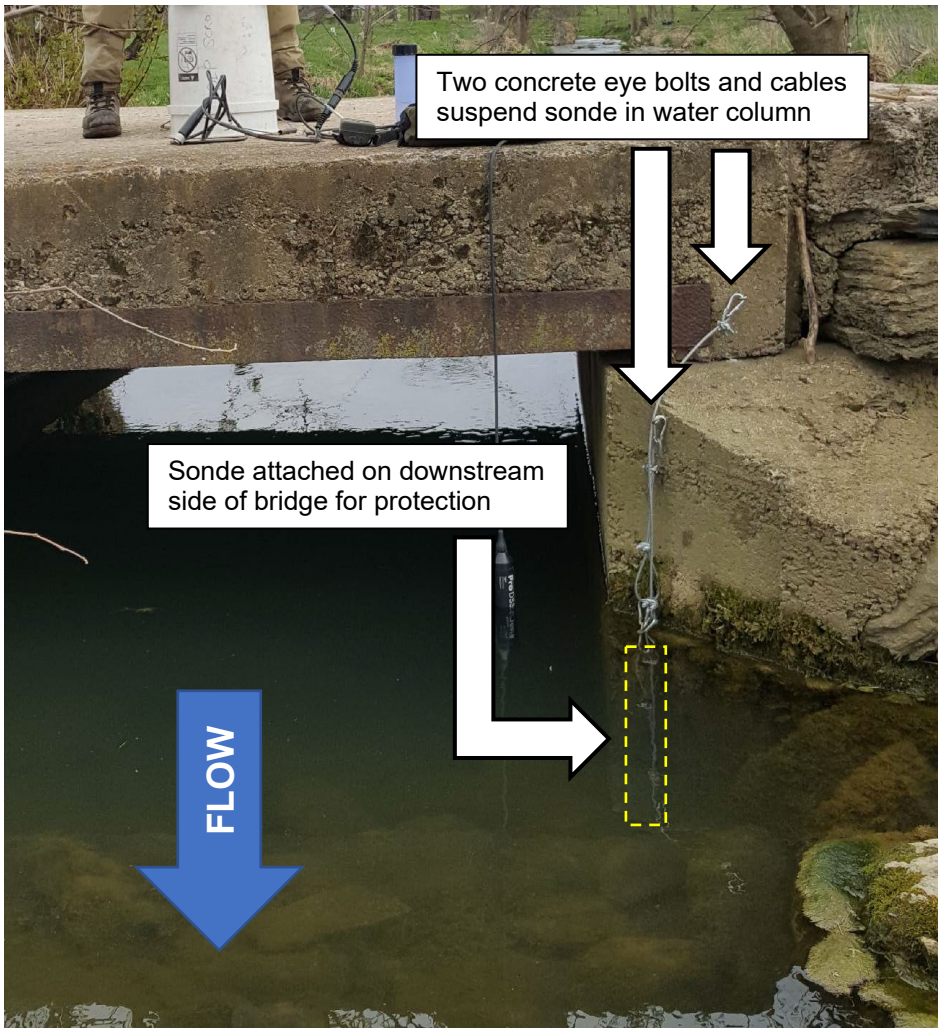

Figure 5. Bridge deployment used at Groff Creek, Lancaster County, PA, to minimize sediment fouling.

Large River Deployment

Accessing sondes at various flows in large rivers can be difficult using the standard deployment procedure. Accessible sections in the spring are often dewatered by a couple feet in late summer. To facilitate regular maintenance throughout the year, even during periods of sustained high-flows, a 10ft section of PVC is used to slide the sonde into place. Rock outcrops or old bridge piers are used to anchor the PVC mid-channel. Large boulders are then stacked on the PVC to protect the deployment (Figure 6). Cables are used to secure the deployment to rock, trees, or form stakes and to feed the sonde down the PVC. If well-protected, these deployments can even survive the scouring of ice sheets in winter.

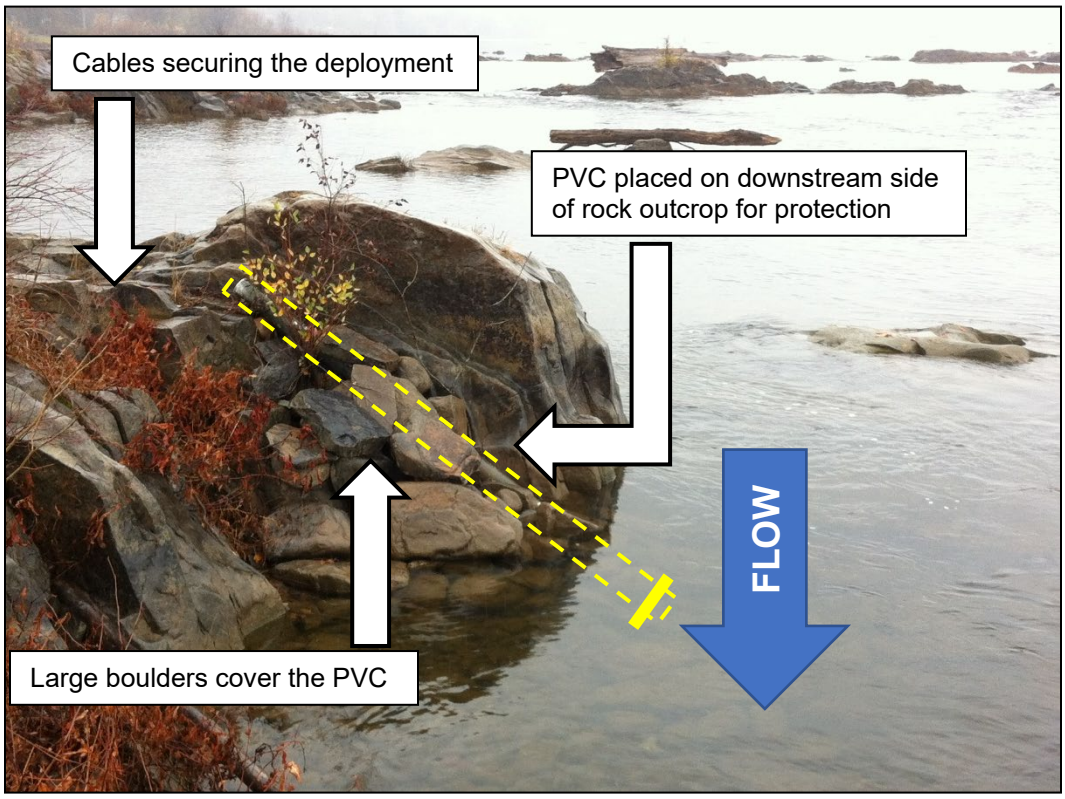

Figure 6. Large river deployment on Susquehanna River at Rockville, PA, employed to facilitate access at various flows.

SENSOR SELECTION

Types of Sensors

Sensors are available for continuous measurement of many field parameters and chemical constituents, but six of the most commonly used sensors are water temperature, specific conductance, DO, pH, turbidity, and water stage. Common concepts and calibration procedures are described in this protocol, but manufacturers' instructions and recommendations should be followed closely. Equipment should be used that has well-documented calibration and maintenance information and acceptable accuracy ratings as described below.

Water Temperature

Water temperature affects density, solubility of constituents (such as oxygen in water), pH, specific conductance, the rate of chemical reactions, and biological activity in water. Continuous water quality sensors usually measure temperature with a thermistor, which is a semiconductor having resistance that changes with temperature. Thermistors are reliable, accurate, and durable temperature sensors that require little maintenance and are relatively inexpensive. The preferred water temperature scale for most scientific work is the Celsius scale. Modern thermistors can measure temperature to ± 0.1°C, but accuracy should be verified with the manufacturer (Wilde 2004).

Specific Conductance

Electrical conductivity is a measure of the capacity of water to conduct an electrical current and is a function of the types and quantities of dissolved substances in water. As concentrations of dissolved ions increase, conductivity also increases. Specific conductance is the temperature specific calculation of conductivity expressed in units of microsiemens per centimeter (Radtke et al. 2005). DEP's Water Quality Division measures and reports specific conductance in microsiemens per centimeter (µS/cm) at 25 °C. Specific conductance measurements can be a good surrogate for total dissolved solids and total ion concentrations, but there is no universal linear relation between total dissolved solids (TDS) and specific conductance. A relation between specific conductance and constituent concentration must be determined for each site (Radtke et al. 2005). In many circumstances, the amplitude and variability of specific conductance is not sufficient to predict other constituent concentrations (such as TDS) within the stream. Therefore, it is important to note that TDS measurements from monitors are calculated from conductivity and are not always accurate or reliable without further evaluation of the ion constituents.

Monitoring systems used by DEP usually contain automatic temperature compensation circuits to compensate specific conductance to 25°C. This can be verified by checking the manufacturer's instruction manual. Most modern sensors are designed to measure specific conductance in the range of 0–2,000 µS/cm or higher. Specific conductance sensors are reliable, accurate, and durable but are susceptible to fouling from aquatic organisms and sediment. Typical accuracy of specific conductance probes in freshwater is ± 1 µS/cm or 0.5% of reading, whichever is greater.

pH

The pH of a solution is a measure of the effective hydrogen-ion concentration. Solutions having a pH below 7 are described as acidic, and solutions with a pH greater than 7 are described as basic or alkaline. Dissolved gases, such as carbon dioxide, hydrogen sulfide, and ammonia, affect pH. Degasification (for example, loss of carbon dioxide) or precipitation of a solid phase (for example, calcium carbonate) and other chemical, physical, and biological reactions may cause the pH of a water sample to change appreciably soon after sample collection (Ritz and Collins 2008).

The electrometric pH-measurement procedure, using a hydrogen-ion electrode, commonly is used in continuous water quality pH sensors. A correctly calibrated pH sensor can accurately measure pH to ± 0.2 pH units; however, the sensor can be scratched, broken, or fouled easily. If flow rates are high, the accuracy of the pH measurement can be affected by streaming-potential effects (Ritz and Collins 2008).

These types of probes typically have a life-span of 1-2 years as the reference solution is depleted (some manufacturers offer refillable reference solution receptacles to extend the life of the probe). This life-span is affected by the ionic concentration of the solution and water in which a probe is stored and deployed. Lower ionic concentrations more quickly deplete the reference solution. The pH mV readings provide insight to the current condition of the probe. DEP's Water Quality Division

recommends recording these values at each calibration. The pH mV values should be within the following criteria:

- Buffer 7: readings should be -50 to 50 mV, 0 mV is ideal
- Buffer 4: readings should be +165 to +180 mV from the buffer 7 reading
- Buffer 10: readings should be -165 to -180 mV from the buffer 7 reading.

As pH probes age and reach the thresholds listed above, they can become slower to respond and the data becomes increasingly erratic (Figure 7).

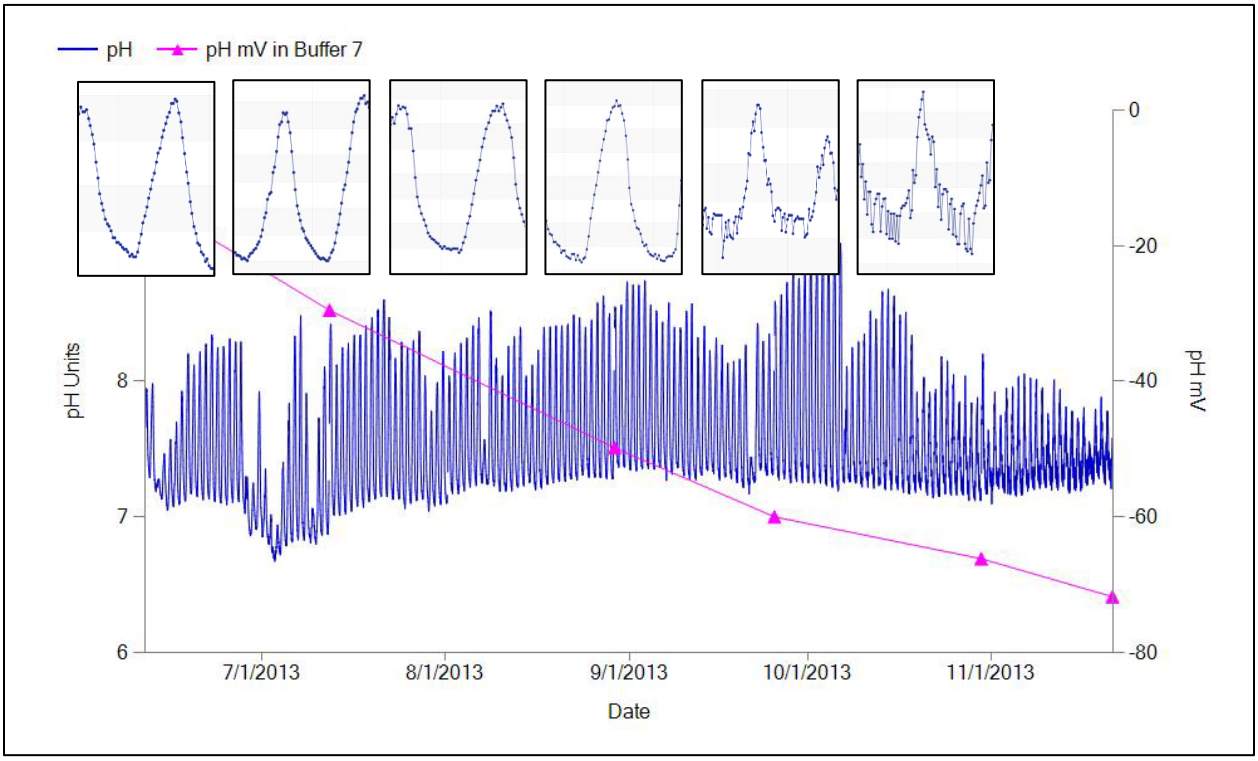

Figure 7. These pH data demonstrate the effects of an aging pH probe, measured by the mV readings departing from 0 mV in buffer 7. The inset boxes are close-ups of the pH at each of the six mV readings during calibration checks. As the mV readings fall outside the optimal range, the pH readings become increasingly erratic.

Dissolved Oxygen

Dissolved oxygen of streams is produced by diffusion from atmospheric oxygen and photosynthetic productivity and drives chemical reactions within and above the substrate and is critical for the survival of aquatic organisms. Expected DO concentrations vary based on temperature and other factors but are generally from 6 to 14 milligrams per liter (mg/L). Dissolved oxygen is also reported as percent saturation, which will decrease with increasing water temperature, and increase with increasing atmospheric pressure. Another impact to DO solubility is salinity. Dissolved oxygen measurements in waters that have specific conductance values >2,000 µS/cm should be corrected for salinity. Most modern sensors automatically compensate for the effects of salinity or have manual

compensation techniques, but this should be verified by checking the manufacturer's instruction manual (Rounds et al. 2013).

There are two different sensor types for measuring DO concentrations and saturations. The first is the luminescent or optical sensor that has a light-emitting diode (LED) to illuminate a specially designed oxygen-sensitive substrate that, when excited, emits a luminescent light with a lifetime that is directly proportional to the ambient oxygen concentration. Although these sensors are more expensive, advantages include: faster response time, few known interferences, no dependence on flow, and increased stability. DEP's Water Quality Division strongly recommends using the optical DO sensor for continuous instream monitoring. Typical accuracy of an optical DO sensor is ± 0.1 mg/L or 1% of the reading, whichever is greater (within the range 0-20 mg/L).

Another DO sensor is the temperature-compensated polarographic membrane-type sensor. Although polarographic membrane-type sensors generally provide accurate results, they are sensitive to temperature and water velocity and are prone to fouling from algal growth and sedimentation. The measuring process consumes DO; therefore, water flow past the sensor is critical. If the water velocity at the point of measurement is less than 1 foot per second (ft/s), an automatic or manual stirring mechanism is required. Chemical alteration of the DO electrodes can be caused by a strong oxidizing or reducing chemical agent, such as a chemical spill, by metal-rich drainage water, or by organic-rich waters. Typical accuracy of a membrane-type DO sensor is ± 0.2 mg/L or 2% of the reading, whichever is greater (within the range 0-20 mg/L).

DEP's Water Quality Division collects and processes DO concentration time series and calculates a DO saturation time series using the DO concentration, temperature, and specific conductance time series. Calculating the DO saturation time series saves significant time by eliminating the grading, correcting, and approving of an additional time series. The time series is generated by creating a calculated-derived series in Aquarius (instructions contained below).

Turbidity

Turbidity is defined as an expression of the optical properties of a sample that cause light to be scattered and absorbed rather than transmitted through a sample. Most turbidity sensors emit light from an LED into the water and measure the light that scatters or is absorbed by the suspended particles in the water. The sensor response is related to the wavelength of the incident light and the size, shape, and composition of the particulate matter in the water. The effect of temperature on turbidity sensors is minimal, and the software for modern sensors provides temperature compensation. Sensors that are regularly maintained and calibrated are somewhat error free. Turbidity sensors should be calibrated directly rather than by comparison with another meter (Anderson 2005). Turbidity can be reported in several different units depending on what type of sensor is being used. One common unit of measure is nephelometric turbidity units (NTU); however, DEP uses sensors that report data in formazin nephelometric units (FNU), because these sensors use near infra-red light (780-900nm) with a detection angle of 90°. For the purposes of measuring a calibration solution, turbidity units are equivalent; however, instruments may not produce equivalent

results for environmental samples, which is why different turbidity units are required. Turbidity sensors typically have a range of 0–1,000 FNU and an accuracy of ± 0.3 FNU or 2%, whichever is greater. Some sensors can report values reliably up to about 4,000 FNU.

Water Stage

Most stream monitors employ pressure transducers to measure water stage. The physical force measured is made up of various factors such as atmospheric pressure, temperature fluctuations, and water pressure. Pressure transducers convert physical force into an electronic signal to be calculated into water stage. Most modern stage sensors will automatically compensate for fluctuations in temperature.

Water stage sensors come in two common forms, non-vented and vented. Non-vented (absolute) sensors measure both atmospheric pressure and water stage simultaneously. Resulting data from non-vented sensors require corrections provided by either a separate barometric pressure monitor or by using barometric data from a near-by airport/weather station. Vented (gage) sensors employ a vent tube that is open to the air. As a result, these sensors will automatically compensate for the changes in atmospheric pressure as long as the vent tube remains unobstructed and dry.
Due to the monitor deployment procedure, DEP generally uses non-vented pressure sensors to record water stage. A nearby barometric pressure monitor is typically used to correct for atmospheric pressure changes. If separate barometric monitors are used, they should be set to the same sampling interval as the stream monitor to facilitate the data management process. Both stream monitor and barometric monitor must be calibrated at the respective monitoring site before logging begins.

When considering the use of a non-vented water stage sensor and a separate barometric monitor it is important to account for distance (between stream and barometric monitor) and elevation. DEP's Water Quality Division generally recommends that the distance between stream monitor and barometric monitor is not greater than 30 miles. This reduces the potential for barometric correction errors due to localized weather patterns. As a general rule for Pennsylvania, barometric pressure changes with elevation at a rate of 1 in-Hg for every 1000 ft. If the elevation difference from stream monitor to barometric monitor is minimal, then no elevation compensation is needed. Correcting for barometric pressure using another instrument adds to the total potential error within the data set. Unless the accuracy of each instrument can be documented, and verified depth measurements are taken at each maintenance visit, the data must be dealt with in a qualitative manner.

USE OF FIELD METERS

The three major uses for a field meter during servicing of a continuous water quality monitor are (1) as a general check of reasonableness of monitor readings, (2) as an independent check of environmental changes during the service interval, and (3) to make cross-section surveys or vertical profiles in order to verify the representativeness of the location of the sonde in the aquatic environment. Data collected by the field meter should not be used to calibrate the instream monitor. However, some instream monitors cannot be calibrated, in which case the calibrated field meter must

be used in the calibration correction of monitor records. In addition, DEP may use a calibrated field meter measurement for undocumented data error corrections (a potential result from the DEP deployment procedure, discussed in detail later). Independent field measurements must be made before, during, and after servicing the monitor in order to document environmental changes during the service. Side-by-side measurements between the field meter and the monitor are made by placing the calibrated field meter as close to the monitor sensors as possible. The field meter should refresh data on the display at 10 second intervals, or more frequently, to ensure the most accurate data are recorded during measurements.

Before site visits, all field meters should be checked for operation and accuracy. Minimum calibration frequency should be once per day in the field. Recalibration will be necessary when a sensor is replaced, and during dramatic changes in elevation or barometric pressure. Calibrations must be recorded in instrument log sheets. The Field Meter Calibration Form is provided in Appendix B.1. DEP recommends that all sensors are calibrated in accordance with manufacturer's specifications. Additional tips and guidance on field meter use are provided in *Chapter 4.1, In-Situ Field Meter, Transect, and Profile Data Collection Protocol* (Hoger and Arnold 2023).

MONITOR OPERATION AND MAINTENANCE

The objective during continuous instream monitoring is to obtain the most accurate and complete record possible. The common operational categories include maintenance frequency, field visits, troubleshooting, and comprehensive record keeping.

This instream monitoring protocol details one operating procedure designed for well-mixed, stable, and relativity slow changing systems. Slowly changing is defined as changes in field measurements during maintenance that are less than the calibration criteria (see Sensor Field Calibration). The wadeable streams of Pennsylvania generally fall into this category.

Field Notes and Instrument Logs

Logs and field notes are essential for accurate and efficient record processing. Operation and maintenance records are maintained in an electronic spreadsheet similar to the USGS standard field form for water quality monitors in Wagner et al. (2006). A copy of the spreadsheet is available upon request. The deployment and maintenance visit field forms from the spreadsheet are provided in Appendices B.5 and B.6, respectively. Field-note requirements for instream monitors include:

1. Station name
2. Date and time of measurements
3. Name(s) of data collector(s)
4. Serial number of field meters and monitor
5. Lot numbers and expiration of dates of standard solutions
6. Location description and picture of monitor in the stream
7. Name of file downloaded from the monitor

8. Monitor values, field meter values, and corresponding time for cleaning checks (for fouling), calibration checks, calibrations/recalibrations, and final readings
9. Battery voltage of monitor at departure and if the batteries were replaced, name of new file, and start time of logging
10. Notes on sensor/monitor changes or replacements, and other comments that facilitate processing of the record
11. Cross-section survey data (locations of points, measured values, and corresponding times), and monitor values before and after the cross-section survey (Hoger and Arnold 202)
12. Measured flow or gage-height data.

Logging Interval and Settings

Most monitors can be set for a wide range of recording intervals. The interval used should account for the expected rate of change in the water quality parameters being measured. In most streams in Pennsylvania, a 30-minute interval adequately captures changes and therefore is the most common interval used by DEP. “Flashy” streams, that are high gradient or have a significant amount of impervious cover in the watershed, change more rapidly and may necessitate a 15-minute interval. When recording turbidity, many prefer to have the additional data points of a 15-minute interval so that the curves of storm events are better represented. A five-minute interval has even been used for a deployment directly downstream of a point-source discharge to capture its short, concentrated discharges. When deploying the monitor independently, however, battery life can be an important consideration and may necessitate a longer interval or a unit with greater battery capacity.

To avoid confusion when comparing continuous data and discrete samples, DEP does not recognize daylight saving time when working with its continuous data. All sondes are set to record in EST (UTC-05:00) and all associated measurements, such as discretes, should be recorded in the same manner to avoid confusion.

Setting the monitor to log is different with each unit, so follow the manufacturer’s instructions. When setting the monitor to log, make note of the interval, name of the file, battery life, and start time. If possible, DEP recommends delaying the start time by at least one hour to allow the monitor plenty of time to stabilize. It is also recommended that the battery voltage parameter is set to record throughout the deployment to aid in determination of battery life. Finally, employing a consistent naming convention (e.g., Newport_1, Newport_2, etc.) can aid in the organization and uploading of files later.

Maintenance Frequency

DEP Water Quality Division monitors are typically placed at a station for approximately one year and revisited 10-12 times during the deployment. However, maintenance frequency will generally depend on fouling rate of the sensors, which varies by sensor type, environmental conditions, and season. The performance of water temperature, specific conductance, and water stage sensors tends to be less affected by fouling than DO, pH, and turbidity sensors. Wiper mechanisms on turbidity and optical DO sensors can substantially decrease fouling in certain aquatic environments. Monitoring sites with nutrient-enriched waters and moderate to high temperatures may require more frequent maintenance. In cases of severe environmental fouling or in remote locations, the use of an observer

(e.g., landowner, volunteer, or local watershed group) to provide more frequent maintenance to the water quality monitor should be considered. Monitoring disruptions as a result of equipment malfunction, sedimentation, electrical disruption, debris, ice, or vandalism also may require additional site visits.

Field Visits

Field visits are undoubtedly the most important step in certifying that quality data are being recorded. The purpose of field visits is to verify that a sensor is working accurately, upload recorded data, confirm calibrations, and provide a reference point for subsequent data management. Quality assurance is accomplished by recording field fouling observations before and after cleaning sensor readings in the environment, calibration checks, and final readings. It is important to conduct field checks at, or close to, the monitor's deployment location to record checks that best represent stream conditions recorded by the monitor. To prevent complications from freezing, caution should be exercised when performing maintenance at temperatures below 0˚C, and maintenance should be avoided at temperatures below -7°C or 20˚F.

The standard protocol for servicing instream monitors is described below:

1. Obtain a discrete measurement from a clean, calibrated field meter at the sonde location.
2. Remove sonde from the monitoring location being careful to minimize disturbance.
3. Connect monitor to field instrument (i.e., computer or handheld device)
    a. If monitor is to be submerged during read-out, ensure the cable is designed to operate under water.
    b. Stop unattened monitoring.
    c. Upload data.
    d. Do a quick review of data to detect any data abnormalities or defects in probes.
    e. Record any significant fouling observed during monitor removal.
4. Conduct before-cleaning, initial monitor inspection.
    a. Record time, readings, and monitor conditions.
    b. With an independent field meter, record instream readings and time near the monitor.
5. Clean sensors (see Sensor Field Cleaning)
6. Return sonde to the stream and conduct after-cleaning monitor inspection.
    a. Record monitor readings and time.
    b. Using an independent field meter, record instream readings near the monitor
7. Remove sonde, and check calibration (see Sensor Field Calibration)
    a. Record calibration-check values.
    b. Recalibrate if necessary.
8. Conduct final readings.
    a. Record monitor readings and time.
    b. Using an independent field meter, observe and record readings near the monitor.
9. Restart unattended sampling with appropriate logging interval, start time, and file name.
    a. DEP recommends a delay start time of 1-2 hours to allow equipment to acclimate following disturbance of the site.

   b. Check monitor's battery levels, change if necessary.
10. Return sonde to monitoring location and inspect anchoring equipment and shroud for deterioration and damage.

The initial monitor readings during a field visit may not accurately represent the recording conditions because of disturbance while retrieving and connecting the monitor. Care should be taken to minimize disturbance and placement of the monitor during retrieval, and the field meter should be upstream of any disturbed area. If observed, significant fouling should be noted on the field sheet. Monitors and field meters should be given ample time to stabilize before readings are recorded. During extreme conditions (extreme cold/heat, early morning, etc.) monitors and field meters may require more time to stabilize.

Comparison of the initial monitor and field meter readings provides a sense of the reasonableness of the recorded data and an indication of any potential errors. The disturbance of a stand-alone monitor during retrieval can prevent the capture of a true fouling error, so care should be taken to minimize this disturbance as much as possible. The difference between the last recorded value on the deployed monitor and the initial reading after retrieval helps establish any effects of disturbance during the monitor retrieval and can be used to calculate what is then referred to as undocumented fouling (described in further detail in Data Correction). A monitor that is connected to an external recording device can typically be accessed for initial side-by-side readings without disturbance—eliminating the potential for undocumented fouling error.

After initial sensor readings, the monitor's sensors are inspected for signs of fouling. These observations are recorded in the field notes before cleaning, and then individual sensors are cleaned according to the manufacturer's specifications. The monitor is then returned to the water. Cleaned sensor readings, field meter readings, and times are recorded in the field notes. If the conditions are steady state, the field meter readings should not change substantially during the time that the monitoring sensors are cleaned. The observed difference between the initial sensor reading and the cleaned-sensor reading is a result of fouling (chemical precipitates, stains, siltation, or biological growths).

After cleaned-sensor readings are recorded, the monitor is removed from the water, calibration is checked in calibration standard solutions, and the readings are recorded. Differences between the cleaned-sensor readings in calibration standard solutions and the expected reading in these solutions are the result of calibration error (drift). The sonde is recalibrated, if necessary, and replaced in the aquatic environment for a final reading.

The set of final readings may not necessarily accurately represent the start of the new record period due to site disturbance. Care should be taken to minimize disturbance and placement of the monitor and field meters should be upstream of any disturbed area. Monitors and field meters should be given ample time to stabilize before readings are recorded. Final sensor readings should be used in

conjunction with the first data record recorded to determine any affect disturbance may have had on final sensor readings, field meter readings, and data collected as part of the new record period.

Sensor Field Cleaning

During the cleaning process, care should be taken to ensure that the electrical connections are kept clean and dry. Water on the connector pins can cause erratic readings. For this reason, a container of compressed air is useful. Procedures for cleaning specific sensors, as described below, are general guidelines and should not replace manufacturer's instructions. Most commercial thermistors can be cleaned with a soft-bristle brush and rinsed with deionized water (Wilde 2004).

Rinse specific conductance sensors thoroughly with de-ionized water before and after making a measurement. Oily residue or other chemical residues (salts) can be removed by using a detergent solution. Specific conductance sensors can soak in detergent solution for many hours without damage. Carbon and stainless-steel sensors can be cleaned with a soft brush, but platinum-coated sensors should never be cleaned with a brush (Radtke et al. 2005). Platinum-coated sensors may be cleaned with a cotton swab.

The pH electrode must be kept clean in order to produce accurate pH values. The body of the electrode should be thoroughly rinsed with de-ionized water before and after use. In general, this is the only routine cleaning needed for pH electrodes; however, in cases of extreme fouling or contamination, the manufacturer's cleaning instructions must be followed (Ritz and Collins 2008).

Optical DO sensors are cleaned with a soft bristle brush and rinsed with deionized water. If the optical DO sensor is equipped with a wiper, ensure the motor is operating properly and parking in the correct position. Also, ensure that the wiping mechanism (pad or brush) is in good condition and clean. The black membrane is sometimes scratched off by coarse debris caught in the wiper. The membrane should be replaced if over half of the surface has been worn off.

Routine cleaning of polarographic DO sensors involves using a soft-bristle brush to remove silt from the outside of the sensor, wiping the membrane with a damp, lint-free cotton swab (available at local electronics stores), and rinsing with de-ionized water. The sensor usually is covered with a permeable membrane and filled with a potassium chloride solution. The membrane is fouled easily and typically will need to be replaced every 2 to 4 weeks. When the membrane is replaced, the potassium chloride solution must be rinsed out of the sensor with de-ionized water followed by several rinses with potassium chloride solution before the sensor is refilled. The membrane must be replaced with care so that the surface of the membrane is not damaged or contaminated with grease, and no bubbles are trapped beneath the membrane. The surface of the membrane should be smooth, and the membrane should be secured tightly with the retaining ring. The sensor must be stored in water for a minimum of 2 to 4 hours, preferably longer, to relax the membrane before installation and calibration. Because of the time required to relax the membrane, replacement of a membrane during a field visit would require having a pre-relaxed membrane on hand to allow for immediate calibration, otherwise it would be necessary to revisit the site after replacing the membrane waiting the required amount of

time before calibration. The retaining ring must be replaced annually or more frequently to prevent loss of electrolytes. Replacing the retaining ring when membranes are changed ensures a tight seal.

The gold cathode of the DO sensor also can be fouled with silver, over an extended period of time, and a special abrasive tool usually is required to recondition the sensor. A fouled anode, usually indicated by the white silver electrode turning gray or black, can prevent successful calibration. As with the cathode, the sensor anode usually can be reconditioned following the manufacturer's instructions. Following reconditioning, the sensor cup must be rinsed, refilled with fresh potassium chloride solution, and a new membrane installed (Rounds et al. 2013).

Turbidity sensors are extremely susceptible to fouling; thus, frequent maintenance trips may be necessary to prevent fouling of the turbidity sensor in a benthic environment high in fine sediment, algae accumulation, or other biological or chemical debris. In environments that cause severe algal fouling, however, algae can accumulate on the wiper pad preventing complete removal of debris from the optical lens, resulting in erratic turbidity data. If the turbidity sensor is not equipped with a mechanical cleaning device that removes solids accumulation or a shutter that prevents accumulation on the lens before readings are recorded, reliable data collection is very difficult. Sensors first should be inspected for damage, ensuring that the optical surfaces of the probe are in good condition. The wiper pad or other cleaning device should be inspected for wear and cleaned or replaced if necessary. Before placing the turbidity sensor in standards, the optic lens should be carefully cleaned with alcohol by using a soft cloth to prevent scratching (or as recommended by the manufacturer), rinsed three times with turbidity-free water, and carefully dried. If the readings are unusually high or erratic during the sensor inspection, entrained air bubbles may be present on the optic lens and must be removed (Anderson 2005).

Sensor Field Calibration

Calibration should only be performed with standards of known quality. All calibrations and calibration checks should be completed with at least two standard solutions. At a minimum, these standard solutions should bracket the entire range of recorded values. An additional standard may be necessary that is proximate to expected environmental conditions to ensure linearity of sensor response. Frequently used standards are described for each parameter below.

Field calibration is performed if the cleaned-sensor readings obtained during the calibration check differ by more than the calibration criteria (Table 1). Spare monitoring sondes or sensors are used to replace water quality monitors that fail calibration after troubleshooting steps have been applied (see Troubleshooting Procedures). All calibration equipment and supplies must be kept clean, stored in protective cases during transportation, and protected from extreme temperatures.

The following example is helpful for understanding the calibration criteria given in Table 1. The calibration criterion for specific conductance is 5 µS/cm or 3%, whichever is greater. Therefore, if a sonde is in 1000 µS/cm standard and reported a reading of 1025 µS/cm the reading would still be within the calibration criteria because 3% of 1000 µS/cm (30 µS/cm) is greater than 5 µS/cm. For a

calibration check in 100 µS/cm standard the criteria would be 5 µS/cm, because 5 µS/cm is greater than 3% of 100 µS/cm. Sensors with readings outside of criteria will be recalibrated during the field visits. Best professional judgment should be used with values that read close to calibration criteria. Data management is often facilitated by eliminating unnecessary calibration events.

Table 1. Calibration criteria for measured parameters.

Field Parameter	Calibration Criteria
Temperature	± 0.2°C
Specific Conductance	± 5 µS/cm or 3%, whichever is greater
pH	± 0.2 units
Dissolved Oxygen	± 0.3 mg/L
Turbidity	± 0.5 FNU or ± 5%, whichever is greater

Using Standard Solutions

Expiration dates and lot numbers for the standard solutions must be recorded and sufficient time provided for the sensor to stabilize in the solution. After the readings in the calibration standard solutions are checked and recorded (without making any adjustments), the monitor is recalibrated, if necessary, using the appropriate calibration standard solutions and following the manufacturer's calibration procedures. When using standard solutions, the process for both field checks and calibration are the same. The sensor, thermistor, and measuring container must be rinsed three times with a standard solution. Gentle tapping will ensure that no air bubbles are trapped in or on the sensor. After the series of rinses, fresh standard solution is poured into the calibration cup ensuring both the thermistor and the sensor are submerged; the sensor values, calibration standard values, and temperature are recorded in the field log sheet. A temperature correction may be necessary if the monitor does not have automatic temperature correction. Standard solution that has been used is discarded. If an error arises during calibration, see manufacturer's guidelines or reference Troubleshooting Procedures below. If these steps fail, the sonde or monitoring sensor must be replaced, and the backup instrument calibrated.

Water Temperature Sensors

Manufacturers generally make no provisions for field calibration of water temperature sensors, but sensors can be periodically NIST certified to ensure accuracy. The water temperature sensor and the calibrated field thermistor are placed adjacent to each other, preferably in flowing water. Sufficient time for temperature equilibration must elapse before a reading is made. The two water temperature sensors must be read and recorded simultaneously. If the monitoring water temperature sensor fails to agree within ± 0.2 °C, troubleshooting steps must be taken; if troubleshooting fails, the sensor must be replaced, as calibration is not possible.

Specific Conductance Sensors

Specific conductance sensors should be checked with at least two calibration standard solutions of known quality before any adjustments are made. Most frequently, the two checks are 100 µS/cm and 1000 µS/cm, however, if stream conditions are likely to be greater than 1000 µS/cm, a 5000 µS/cm

standard is also used to ensure the measured values are within the limits of the standards used. In addition, the zero response of the dry sensor in air should be checked and recorded to ensure linearity of sensor response at low values. If the sensor-cleaning process fails to bring a specific conductance sensor within the calibration criteria (Table 1), the sensor must be recalibrated. Calibration of a specific conductance sensor is a single point calibration. The high standard should be used for the calibration (often 1000 µS/cm but 5000 µS/cm could be necessary in some streams) and a lower standard, nearer the ambient conditions (often 100 µS/cm), is used as an intermediate check of linearity after calibration.

pH Sensors

DEP's Water Quality Division uses three buffers (4, 7, and 10) to first check and then, if necessary, recalibrate pH sensors; this ensures all likely field conditions are bracketed. It is also necessary during checks and calibration to account for deviations from the listed pH value of the buffers at different temperatures. The listed value of the buffer is the pH at a specific temperature (typically 25° C). The manufacturer of the buffer solution should offer a table of expected values at other temperatures. While these tables are very similar across major manufacturers, it is important that tables specific to the manufacturer are used to account for any differences. The tables are often provided at five-degree increments; however, particularly with the 10 buffer, the five-degree interval results in significant jumps in the adjusted pH values. To fill these gaps, regression lines can be fitted to the provided data points. An example table of temperature adjusted pH values and the steps to create the more specific table values are provided in Appendix B.7.

Expiration dates of the buffer solutions are checked and recorded. The pH mV readings in each buffer should also be recorded to track the performance of the sensor (see Sensor Selection for more information). Spare pH sensors or entire backup monitors are carried in case replacement of the sensor is required.

Dissolved Oxygen Sensors

Dissolved oxygen in water is related to water temperature, atmospheric pressure, and salinity. Calibration of optical DO sensors should be checked at 100% saturation and with a fresh zero-DO solution before any adjustments are made. The manufacturer's calibration procedures must be followed closely to achieve a calibrated accuracy of ± 0.1 mg/L concentration of DO (Rounds et al. 2013). Theoretical charts based on water temperature and barometric pressure should be used to confirm calibration of monitors. A general 100% saturation chart for fresh water is provided in Appendix B.8. Customized charts can also be made from the following USGS website: http://water.usgs.gov/software/DOTABLES/.

Most optical DO sensors can be calibrated with only a one-point calibration, usually at 100-percent saturation, although some sondes have the capability of a two-point calibration, at zero-percent and 100-percent saturation. For the sondes that are calibrated only at 100-percent saturation, the DO sensor response is still checked in a zero-DO sodium sulfite/cobalt chloride solution. A fresh zero-DO standard solution should be prepared before each monitor visit by dissolving 0.5 gram of sodium

sulfite and 6 – 10 crystals of cobalt chloride in 500 mL of deionized water. The maintainer should observe the response of the sensor in the solution and remove the sensor at 0.3 mg/L because letting the sensor go completely to zero may cause damage.

Optical DO sensors are calibrated by the manufacturer, and some manuals indicate that calibration may not be required for up to a year. When calibrated, the user should follow the manufacturer's guidance. Regardless of the manufacturer's claims, the user must verify the correct operation of the sensor in the local measurement environment. The standard protocol for servicing should be used for optical DO sensors to quantify the effects of fouling and calibration drift (Rounds et al. 2013). Recalibration should not be necessary if calibration checks show the sensor to be in agreement with the calibration criteria (Table 1) and theoretical chart values. Spare membranes and probes should be available in case replacement is necessary.

Calibration of polarographic sensors in the field presents a problem because replacement of the Teflon™ membrane may be required frequently, and the replaced membrane must be allowed to "relax" in water for 2−4 hours before calibration. One solution to this problem is to carry into the field clean and serviced spare DO sensors, stored in water (or moist, saturated air). The replacement DO sensors then can be calibrated in the field, thus avoiding an interruption in the record and a return site visit (Rounds et al. 2013).

Turbidity Sensors

Optimal calibrations and checks are made with three standard solutions that cover the expected range of values, although many sensors only allow for a two-point calibration. Calibration of the turbidity sensor is made by using standard turbidity solutions (DEP typically uses 0, 124, and 1010 FNU) and by following the manufacturer's calibration instructions. When units only allow a two-point calibration, the zero and high value solutions (e.g., 1010 FNU) should be used to calibrate, and the middle value (e.g., 124 FNU) solution should be used as a check of linearity after calibration. Deionized or ultrapure (filtered and deionized) water may be used as a zero turbidity standard if its turbidity is measured by a laboratory. Laboratory measurements are necessary because deionized or ultrapure turbidity can measure as high as 1.0 FNU. The measured turbidity from the laboratory can be set as an offset calibration in the monitor, or the data can be offset corrected later in data management. Checking or calibrating the turbidity sensor should occur in a stable environment with minimal movement, wind, or exposure to sunlight. Sufficient space between the sensor and the bottom of the calibration cup is also critical during checks and calibration. Calibration in zero standard is best performed with the sensor guard in place to simulate conditions when deployed. Bubbles in the solution can interfere with readings so care should be taken to minimize bubbles when pouring. Running the wiper before each reading or calibration in each solution will help remove any remaining bubbles.

When using polymer-based turbidity standards, it is important to understand that different sensors often read the solution differently. Even different model sensors from the same manufacturer will likely not read a given turbidity standard the same. Therefore, only polymer-based solutions with

documentation for the specific sensor should be used. In most cases this means that genuine manufacturer solution is necessary, as standards from other manufacturers lack the necessary documentation – making proper calibration impossible. Currently, the only alternative is Formazin turbidity standard. Formazin turbidity standard has the benefit of being read the same by all sensors, but it is considered moderately toxic, contains a known carcinogen, and is more difficult to work with. Therefore, DEP recommends using polymer-based turbidity standard—with the proper, sensor-specific documentation—whenever possible.

Water Stage Sensors

Water stage calibration procedures vary slightly with manufacturer, so DEP recommends following manufacture's recommendations. When calibrating water stage for deployment, it is important to calibrate at the monitoring location. Most non-vented sensors will calibrate to a depth of zero in the air. This accounts for the elevation and atmospheric pressure at the monitoring site. Since there is no change in elevation during deployment and most pressure transducers automatically compensate for water temperature, the only corrections needed are changes in barometric pressure (see Calculated Derived Series).

In locations where an immobile reference cannot be found, a staff plate or form/rebar stake may be employed (Figure 8). A staff plate or stake that is driven into the stream bed provides a point of reference for consistent manual stage measurements which can be compared to monitor recordings at the time of a field visit. Staff plate or stake measurements may serve as verified discrete data for subsequent data management purposes.

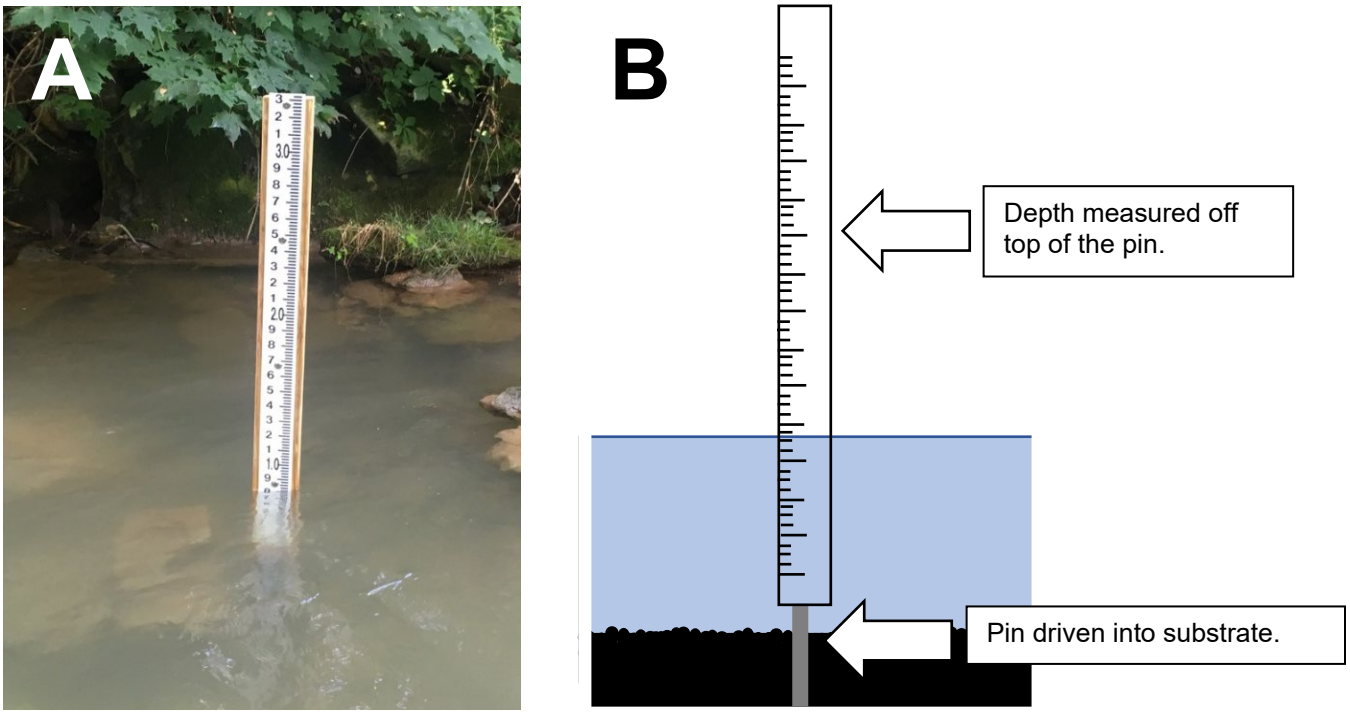

Figure 8. Permanent staff plate deployed at Donegal Creek, near Marietta, PA (A) and depiction of pin used as measuring point (B).

Troubleshooting Procedures

Throughout a deployment a wide variety of issues can often arise. If a problem is encountered with a specific parameter, first determine if the problem is with the sensor or the entire monitor, then make the corrections to ensure correct operation. Spare sensors and monitors should be carried so that corrections can be made during the service visit. Troubleshooting in the field prevents extra trips, saves time, and greatly reduces record loss. A service trip should result in a clean, calibrated, and fully functional monitor. Common problems and potential solutions are listed in Table 2.

Table 2. Troubleshooting procedures for common issues encountered in the field.

Symptom	Possible Issue	Likely Solution
Water Temperature		
Thermistor is not reading accurately	Dirty sensor	Clean sensor
Erratic readings	Poor or wet connection	Dry and tighten connection
Slow to stabilize	Dirty sensor	Clean sensor
Reading off scale	Failure in electronics	Replace sensor or monitor
Specific Conductance		
Will not calibrate	Standard solution old or contaminated	Use fresh standard solution
	Electrodes dirty	Clean with brush
	Air trapped around sensor	Tap gently to expel air
	Weak batteries	Replace batteries
Erratic readings	Poor or wet connection	Dry and tighten connection
Requires frequent calibration	Broken cables	Replace cables Replace monitor
pH		
Will not calibrate	Standard solution old or contaminated	Use fresh standard solution
	Faulty Sensor	Replace sensor
Erratic readings	Poor or wet connection	Dry and tighten connection
	Defective sensor	Replace sensor
Slow response time	Dirty sensor bulb	Clean sensor
	Water is cold or of low ionic strength	Be patient
Dissolved Oxygen		
Will not calibrate	Membrane damaged	Replace membrane
	Electrolyte diluted	Replace membrane and electrolyte
Erratic readings	Poor or wet connection	Dry and tighten connection
	Fouled sensor	Check for obstructions or replace
Slow to stabilize	Gold cathode tarnished	Buff with eraser or recondition sensor
	Fouled membrane	Replace membrane and recondition
	Silver anode corroded	Replace sensor and soak fouled sensor in 3% ammonia for 24 hours
Will not zero	Zero DO solution contains oxygen	Add additional sodium
	Zero DO solution is old	Mix fresh solution
Turbidity		
Unusually high or erratic readings	Entrained air bubbles on the sensor	Gently tap or run wipe cycle
	Damaged Sensor	Replace sensor
	Dirty sensor	Clean, using soft brush
	Poor or wet connection	Dry and tighten connection

DATA MANAGEMENT

The data management process verifies data, corrects for errors, and sets a grade based on overall quality of the record. Accurate and concise field notes and logs are indispensable in processing collected data. The steps in processing data records are: initial data evaluation, data import, data corrections, grading, and final approval. Data Management should begin when the record is uploaded in the field to ensure the equipment is operating properly. Data management is not complete until the monitor is pulled from the stream, data are graded and approved, and a final Continuous Instream Monitoring Report (CIMR) is written, reviewed, and approved by DEP staff. DEP uses Aquarius software from Aquatic Informatics for all data management. The current version of Aquarius that DEP uses is version: 23.2.146.0 (Note: screenshots provided throughout are from Aquarius version: 3.10). This protocol will cover basic operation of the Aquarius software for data management purposes. Two interfaces in Aquarius software aid in data management, Whiteboard and Springboard. This protocol focuses on data management within the Aquarius Springboard application, a more user-friendly environment than Aquarius Whiteboard. Additional training and support can be found at: http://www.aquaticinformatics.com/support.

Initial Data Evaluation

The initial data evaluation is conducted both in the field and back in the office to verify the accuracy of data transfer to the database, to identify erroneous data, and to confirm the monitor is operating as expected. Once data are uploaded from the monitor, they should be quickly reviewed to identify any inconsistencies. Ideally the manufacturer's software will allow the data to be displayed visually. This will allow for a quick determination of the reasonableness of the data including expected range, identification of erratic readings, and/or presence of expected behavior like diel swings or response to changes in flow. Various formats are available to store raw field data. Data formatting will typically depend on the make of the monitor used.

Data Import

Creating New Stations

Data are organized in a cascading folder structure. Stations are organized by internal (to DEP) or external data, basin, HUC, stream name, and finally a specific location. Stations are considered unique if they could represent different water quality conditions. The two main considerations are cross-sectional variability and distance upstream or downstream. If cross-sectional surveys demonstrate variations in water quality, DEP may deploy monitors in multiple locations horizontally across the stream; in Aquarius, each are considered unique stations. Deployments >100 m apart upstream or downstream are considered unique stations, however, deployments separated by shorter distances may be considered unique if conditions warrant. Most deployments by DEP are in relatively shallow, lotic systems; therefore, vertical variations within the water column are often not an issue. If introduced, this variable should also be considered in creating a new station.

Before data can be uploaded to the database server, a station with the appropriate time series needs to be created. To create a new station first navigate to the correct stream in the folder structure. If the stream or HUC folders are missing, they first need to be created. To create the location either right-click on the stream folder and select “New Location” or highlight the stream folder and click the Location Manager button on the toolbar. Under the General tab, the following fields need to be populated:

- **Identifier:** This is a unique number consisting of the ComID from the NHDFlowline the station is on, followed by a unique identifier. If the ComID of the NHDFlowline was 57465467 the Identifier of the first site on that segment would be 57465467-001. If another station is located on that segment at the same time or in the future, its Identifier would be 57465467-002, etc.
- **Name:** The name should include both the stream name and a short description of the location such as a road crossing (e.g., Little Beaver Creek at Little Beaver Rd). Including the stream name allows for better search results when using Aquarius. The short location description should be descriptive enough that anyone can understand its general location.
- **Type:** Water Quality Site
- **Description:** Additional information can be added here such as a more thorough description of the location, whether the location is part of a specific study, etc.
- **Latitude:** Decimal degree format
- **Longitude:** Decimal degree format
- **Elevation:** Required in Aquarius when using latitude/longitude
- **Time Zone:** DEP does not recognize daylight saving time when working with its continuous data; therefore, this should be set to EST (UTC-05:00) for all internal data.

Under the Data Sets tab, create a “Time Series – Basic” for each continuous parameter to be stored in the database. Field Visit (discrete) Time Series do not need to be created in advance; they will be auto-generated when data are input. For each basic time series created, the following fields need to be populated (description and comment fields are optional):

- **Identifier:** This field is auto-populated using the location identifier created in the general tab, the label name created next, and the parameter selected.
- **Label:** Short description of the site (often just the stream name or the stream name and crossing or nearby town). No spaces, capitalize first letter of each word.
- **Parameter:** Use the “…” button to search for a parameter if not in the recently used parameters in the drop-down box. For temperature, use “Water Temperature” not “Temperature”. There are many options for turbidity, “Water Turbidity” is the standard parameter used by DEP. Depth should be entered under “Stage”.
- **Units:** Select the appropriate units.
- **Time Zone:** EST (UTC-05:00).
- **Gap Tolerance:** If time between readings is greater than the value set in this box, Aquarius implies a gap and the points are not connected. Set this value to one minute greater than the recording interval (e.g., if interval is 30 minutes Gap Tolerance should be 31).

Uploading Data

Data can be brought into Aquarius from multiple formats. The most common file type is a delimited text file such as ".csv"; however, Aquarius will recognize some manufacturer-specific files (e.g., YSI's ".dat" file). To upload data, navigate to the site location in Springboard and then select the "Append to Logger" icon . This tool allows for the manual uploading of data and the setup of hot folders, an automated upload process. In the Append to Logger toolbox, first select the file for upload. The second step is to tell Aquarius how to interpret the file. If a configuration file has already been created, select it and click the "Load File" button. (Note that configuration files can be used for different sites as long as the formatting of the files is identical [i.e., headers, parameters, parameter order, etc.]) If a configuration file has not been created, it will be necessary to use the configuration toolbox by clicking "Config Settings…".

Config File Setup

1. Select file type: will most likely be "Text File (CSV, etc)". Click Next.
2. Make sure the proper delimiter is selected and establish the row to start import and number of headers. The number of headers used is not important but it is helpful to include enough to see what the parameter is for the next step. If done correctly, data will be broken into appropriate columns, header rows will be gray, and data rows will be white (Figure 9). Click Next.

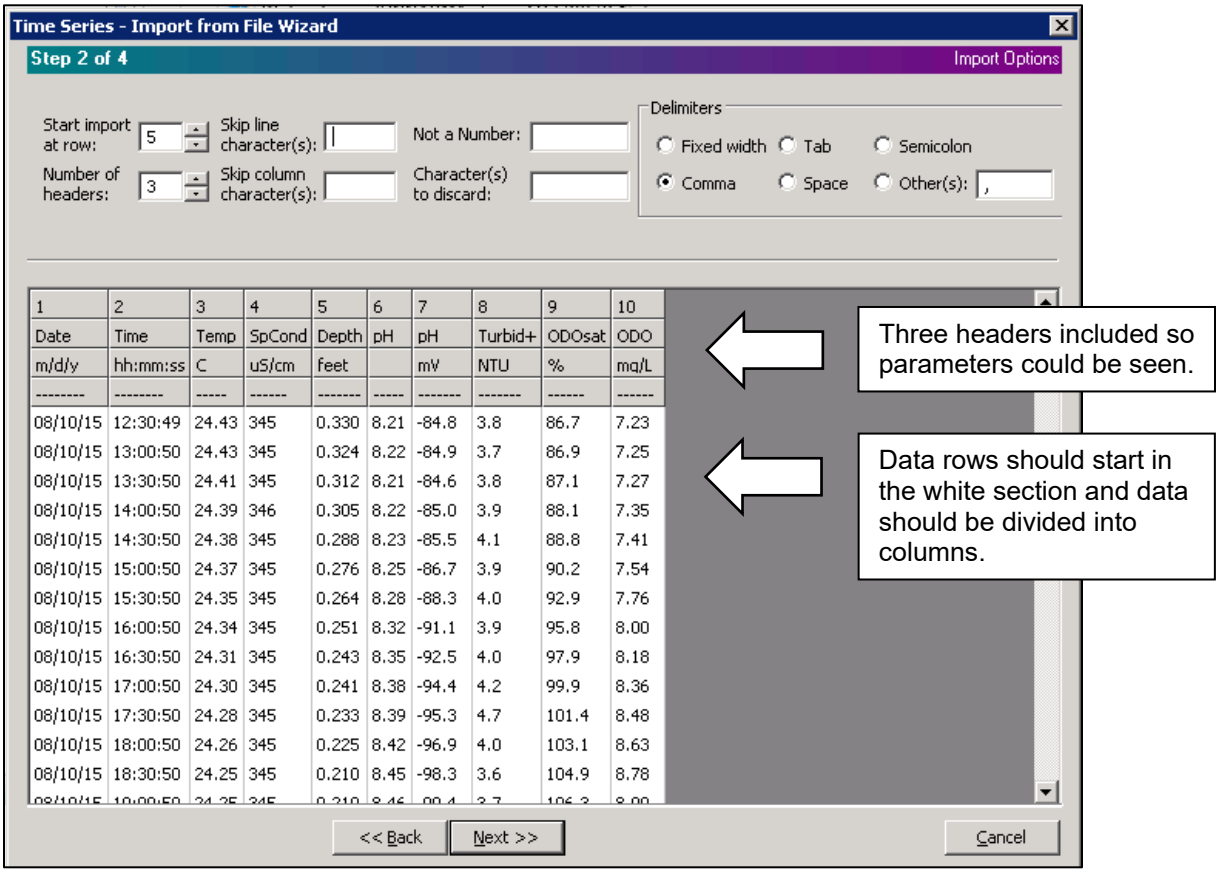

Figure 9. Step 2 of the Config File setup.

3. Use blue arrows to navigate through columns and establish settings for each parameter (Figure 10). Click Next.
    a. Date/Time column(s) requires format and time zone to be selected.
    b. Data columns require parameter, units, gap tolerance, and label to be specified.
    c. If any columns are not wanted (e.g., pH mV, battery voltage), select the “Do not import column (skip)” radio button for that column.

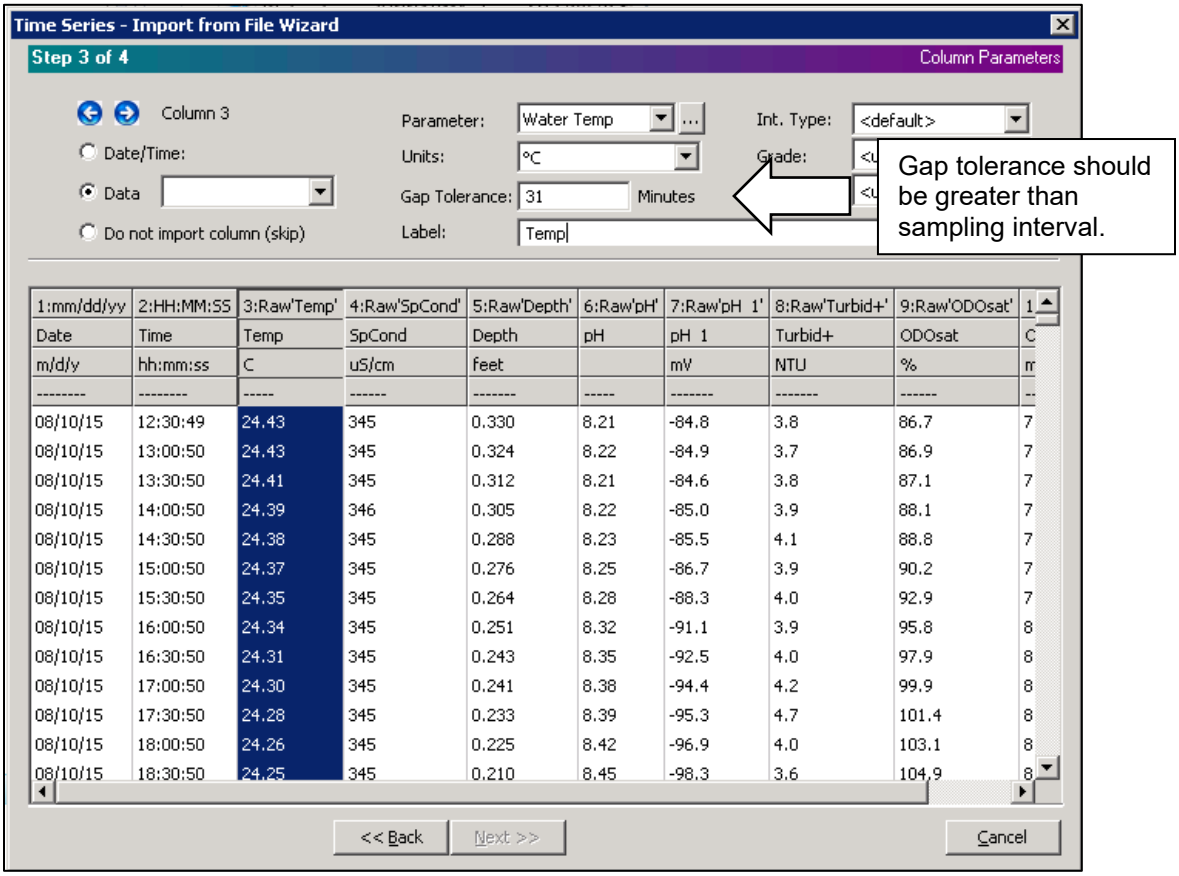

Figure 10. Step 3 of the of Config File setup showing the proper settings for a data column.

4. “Apply Gap Processing Tolerance”, “Join Signals from Multiple Files”, and “Save Configuration” should all be selected. Click Finish.

Once the Config File is loaded, the parameters will be listed in Step 2 of the Append Toolbox (Figure 11). Use the dropdown box for each parameter and designate to which Data Set the data should be added. When all parameters have been designated, Click Append to append that file or first create a hot folder using the “Create Hot Folder…” button.

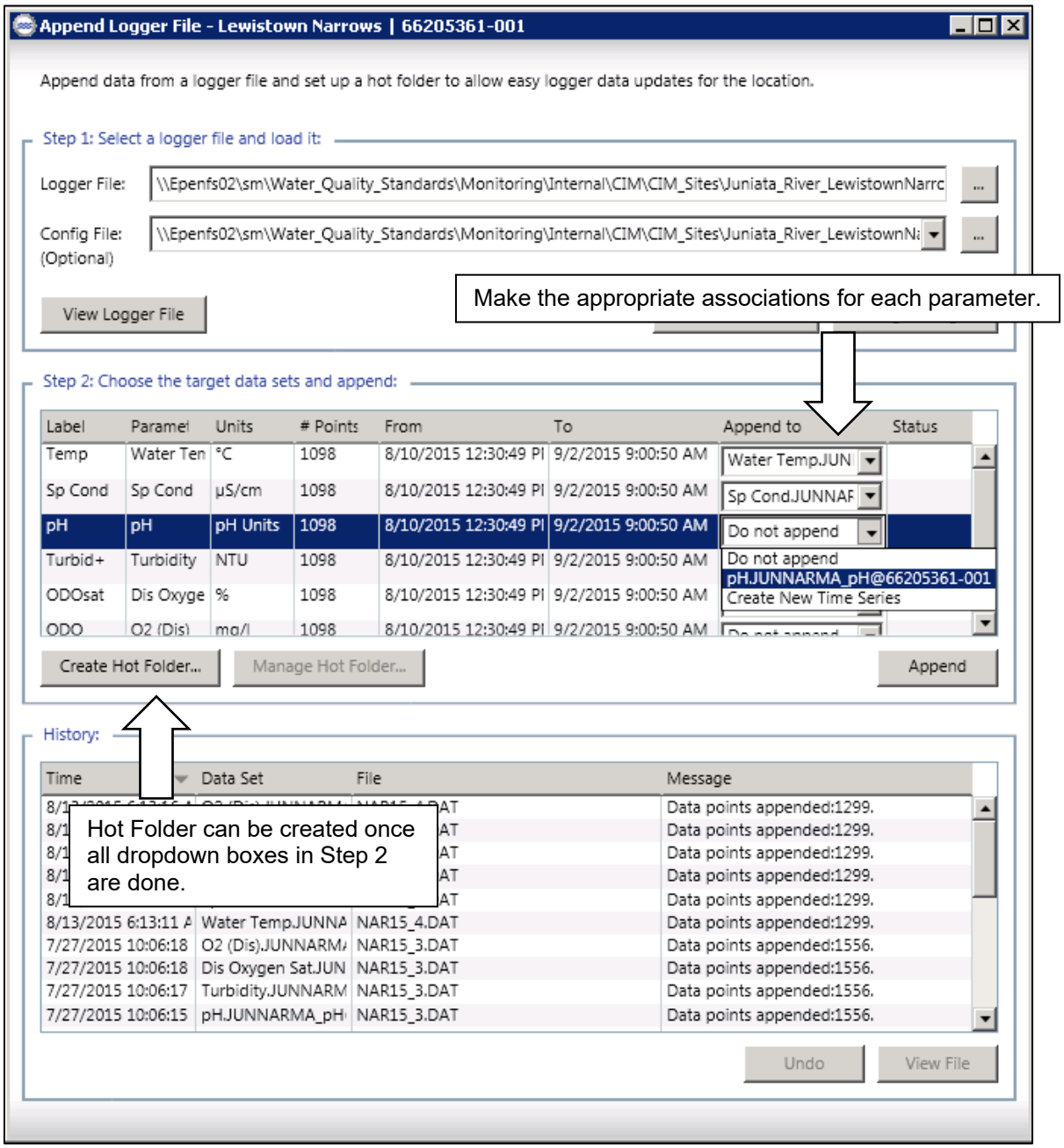

Figure 11. Appending data in the Append Logger File toolbox.

Hot folders expedite the process of uploading the data into Aquarius. Once established, data upload is as simple as dragging and dropping the files. In the Create Hot Folder toolbox only the Description and the Filename Filter need to change (Figure 12). The description should reflect the type of file or where the data came from. The filename filter allows only specific files that follow a certain naming scheme to be recognized by the hot folder. An asterisk is used as a replacement for the part of the name that is variable. Portions of the name and file type (e.g., .csv, .txt) can be specified. By utilizing these features correctly, multiple parsers within the hot folder can be created. For example, to establish features as seen below for the data sonde, and additional parsers for other data for the same location such as atmospheric pressure or secondary piece of equipment. When the Description and Filename Filter boxes are complete, Click OK.

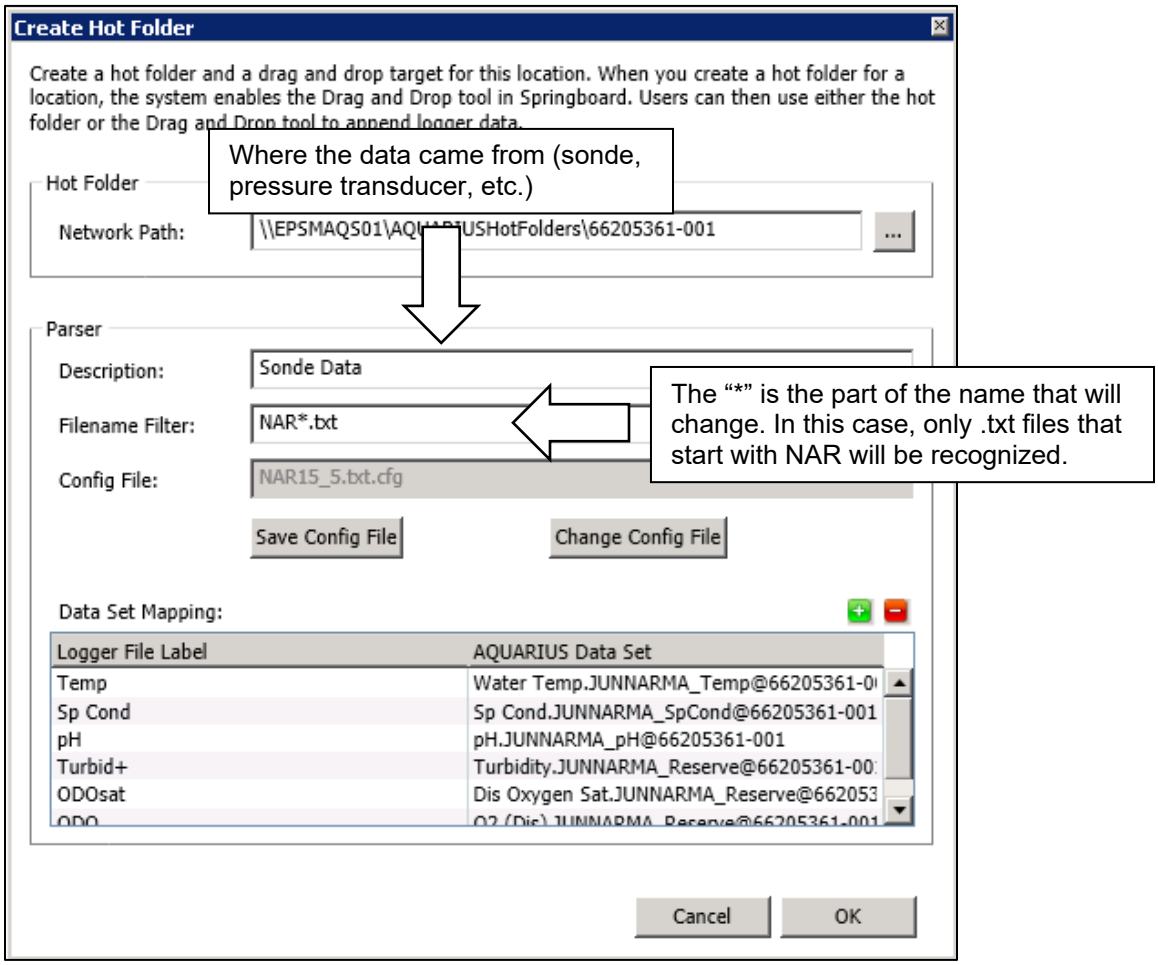

Figure 12. Establishing the settings for a hot folder in the Create Hot Folder toolbox.

Once the hot folder is created, utilize it in one of two ways. The first is to navigate to the folder, and drag and drop files into it. Alternatively, once a hot folder is created the Drag and Drop icon is available on the Springboard toolbar. *Note: the icon will only appear after browser is refreshed.*

If dropping files directly into the folder, within a few seconds they should move to a Processing folder and then a Succeeded folder. The first time a file is dropped, these folders will be created automatically. If using the DRAG and DROP icon within Springboard, a new tab will open in the browser with a drag and drop box. Drop the files into the box and the progress is tracked below.

To add additional file type parsers within the hot folder, go through the same process in the Append toolbox as above. Once the parameter has been designated for a specific parameter, an Add to Hot Folder button will appear. This process will be identical to the first time around, just specify a description and a filename filter. To change any of these specifications later, go through the same process in the Append toolbox and select the Manage Hot Folder button that will appear.

Important Notes Regarding Hot Folders

Consistency of the naming of files and the layout of the content within the files themselves is imperative; therefore:

- Give files a consistent name for each site (e.g., Newport1, Newport2, etc.)
- Do not edit the content of files before uploading. While technically this is not an issue, the edited files are more likely to be inconsistent than the original, equipment-generated file. Therefore, do not edit or clean up headers, move columns, etc.
- Do not edit the parameters during deployment. This means do not add or delete parameters or in the case of Eureka sondes, do not change the order of display. This would in turn change the layout of the data file, so the Config File will need updated.
- If the recording sonde is changed, it may be necessary to update the Config File unless the data files are formatted in the same way**.**
- The Config File that is associated with the hot folder is copied and stored elsewhere once the association is made. This means that after making any edits to a Config File in the Append window, the file must be reassociated to the hot folder.

Entering Discrete Data

Discrete data are stored as Field Visits in Aquarius. With the site selected, click the Field Visit icon . Select New from the toolbar, and choose Field Visit. Enter the date and time of the discrete reading. Make sure the new Field Visit is highlighted in the left column, select New from the toolbar again, and choose Measurement Activity. Click the green circle with a white plus sign to add a parameter. In the Parameter column, select a recent parameter from the drop-down menu by clicking the magnifying glass to find a parameter. Make sure to choose the exact parameter as established for the continuous data sets. Enter the reading in the Value column and confirm the units in the next column are correct. Continue adding parameters until all discrete data are entered (Figure 13). After entering the last value, be sure to click outside the box before clicking Save.

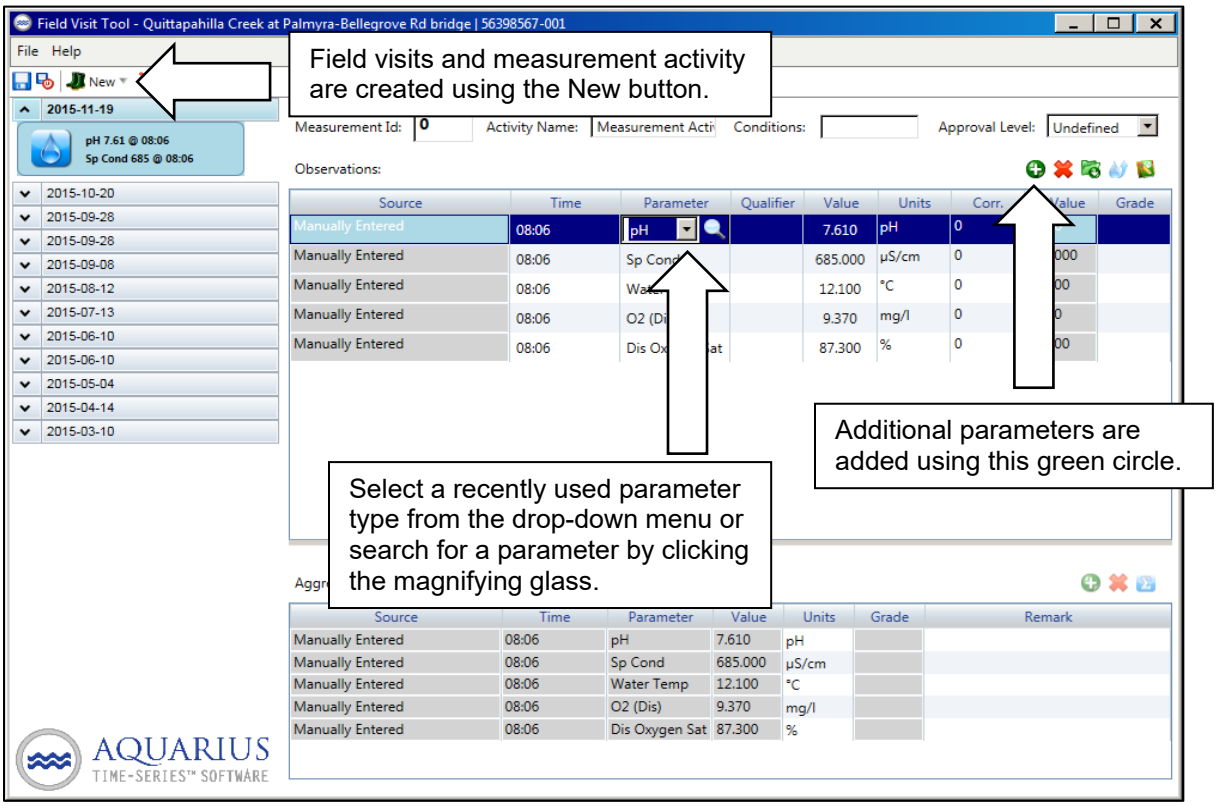

Figure 13. Entering discrete data into the Field Visit toolbox.

Removing Data

If data were erroneously uploaded and need to be removed from the database (e.g., data uploaded under the wrong parameter, data uploaded from the wrong site, etc.), this action is performed using the Append tool. This should not be done using the Data Correction tool described below. The Data Correction tool uses a layers-based system of correction and therefore if the delete function from data correction is used, the data still exist in the database, just behind a deletion layer. This is especially problematic if there are other data to append in its place.

To remove data, select the site in Springboard and click Append To Logger. Under History at the bottom of the Append window, there is a list of the individual uploads by parameter. One or more can be selected and removed from the database by selecting the Undo button. Removing data cannot be undone. It is also important to note that the append history only stores the data appended in the past 12 months. Most errors will be caught within this window but if not, an API script will need to be run to remove the data. The script and instructions can be accessed on Aquatic Informatics support website.

Calculated-Derived Series

Calculated-derived series are generated through formulas that reference one or more other time series (Figure 14). Like basic times series, calculated-derived series are created under location manager. In addition to completing the details as described previously for creating a basic time series, there is a Calculation section. Time series from any station may be selected as inputs. Each input is assigned a name to be referenced in the formula. The formula must be validated before the

series can be saved. These series will update automatically when any changes are made to the underlying time series are made including corrections, deletions, and uploading of additional data. In addition, the calculated-derived series themselves can be edited or corrected like a basic time series. Calculated-derived series adopt their Grade and Approval status from the input time series. The lowest Grade and Approval status from the underlying time series is automatically applied to the calculated-derived series. If there is a gap any of the underlying time series, a gap will appear in the calculated-derived series.

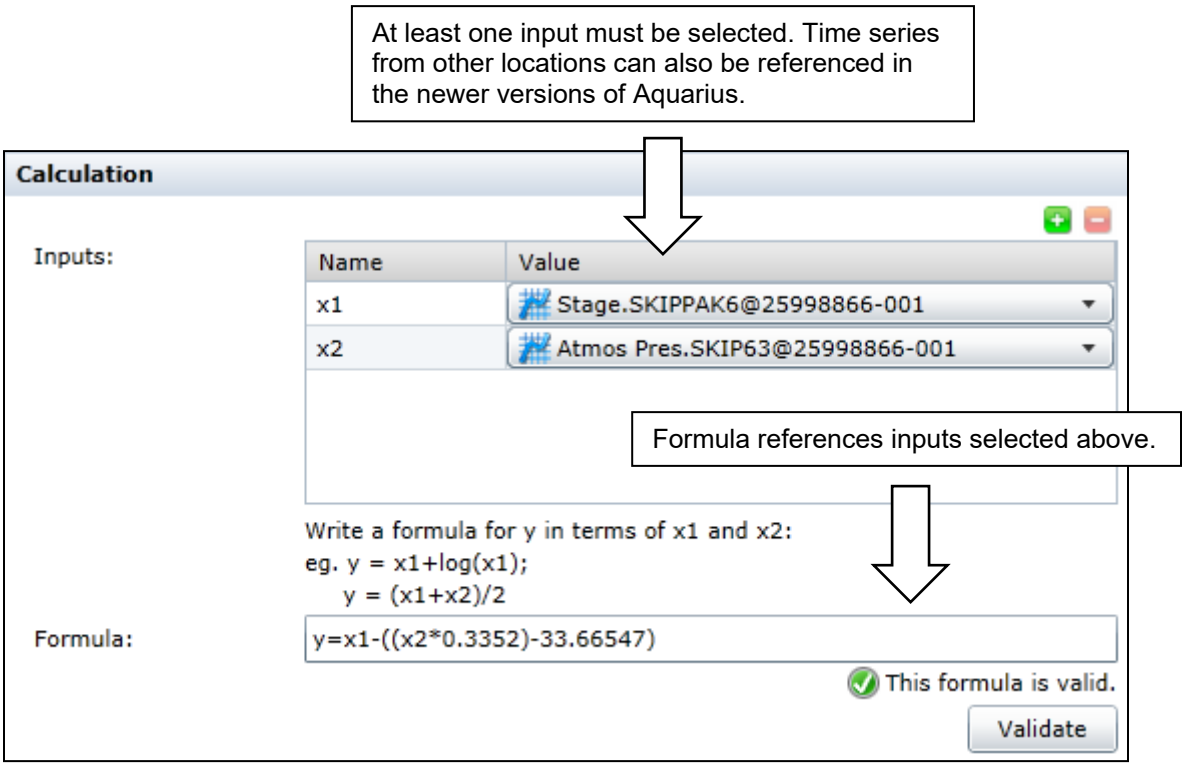

Figure 14. An example of a calculated-derived series in Aquarius that corrects the raw stage for changes in atmospheric pressure for Skippack Creek at Mainland Rd, PA.

One of the most common uses of calculated-derived series used by DEP is for correcting non-vented stage data for changes in atmospheric pressure. Non-vented sondes record changes in atmospheric pressure as changes in stage. These changes in atmospheric pressure are often large enough to mask small flow events (Figure 15). Changes in atmospheric pressure from a logger deployed at the site or a nearby weather station (e.g., airport) can be converted to water column pressure (Table 3) and subtracted from stage data.

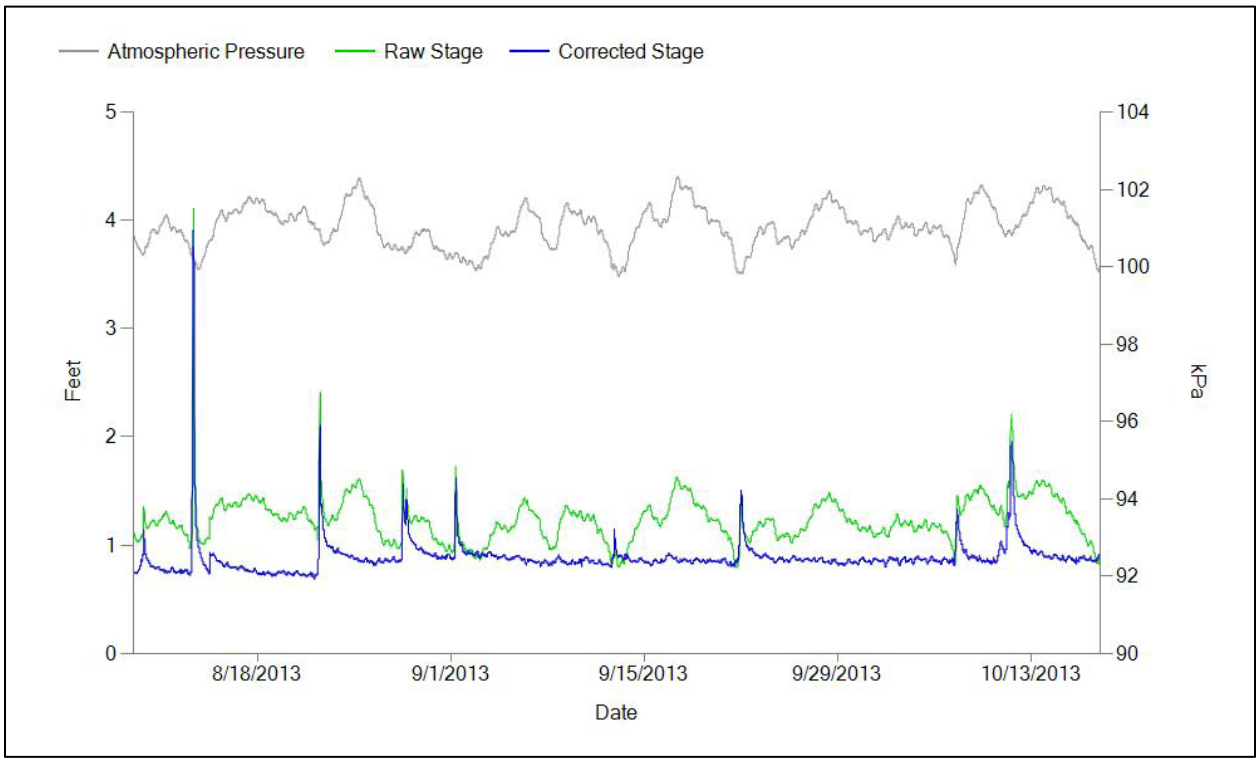

Figure 15. Elimination of changes in atmospheric pressure from non-vented stage data from Skippack Creek at Mainland Rd, PA. Large flow events (e.g., 8/13/13) are apparent in the raw data, but many small flow events are concealed by changes in atmospheric pressure.

Table 3. Common barometric units with water column conversion in feet.

Barometric Unit	Water Column Conversion (ft)
1 psi	2.3108
1 atm	33.959
1 kPa	0.3352
1 mmHg	0.04469
1 inHg	1.1330
1 mb (hPa)	0.03352

Non-vented stage can be corrected for changes in atmospheric pressure using equation (1) in an Aquarius calculated-derived series.

(1) $y=x1-((x2-a)*b)$

Where

x1 = stage (feet) time series

x2 = atmospheric pressure time series

a = first reading from the atmospheric pressure data file (only subtract the changes in atmospheric pressure)

b = conversion factor from Table 3 for atmospheric pressure series x2

Other DEP applications of the calculated-derived series include conversion of conductivity data to specific conductance and the generation of continuous ammonia criteria using the acute and chronic formulas from Chapter 93 water quality criteria, and calculating DO saturation from DO concentration, temperature, and specific conductance. Conductivity can be converted to specific conductance at 25°C using equation (2), assuming a temperature coefficient of 0.0191. This is the standard coefficient used by YSI water quality instruments.

(2) y=x1/(1+(0.0191*(x2-25)))

Where

x1 = conductivity time series
x2 = temperature time series (°C)

Formulas for ammonia criteria from PA §93.7(a), Table 3 have been converted into equations for acute (3) and chronic (4) criteria time series. These time series are generated to establish continuous ammonia criteria thresholds and identify critical time periods. This analysis saves time and money by focusing collection of ammonia grab samples during these critical periods.

(3)
```
if (x2<10) {
y=0.12*(((1+pow(10,(9.73-x1)))/(1+pow(10,((0.09+(2730/(x2+
273.2)))-x1))))/(1+pow(10,(1.03*(7.32-x1)))))*((pow(10,((0.09+
(2730/(x2+273.2)))-x1)))+1);
}
else {
y=0.12*(1/(1+pow(10,(1.03*(7.32-x1)))))*((pow(10,((0.09+(2730/
(x2+273.2)))-x1)))+1);
}
```

(4)
```
if (x2<10) {
if (x1<7.7) {y=0.025*(((1+pow(10,(9.73-x1)))/(1+pow(10,((0.09+
(2730/(x2+273.2)))-x1))))/(pow(10,(0.74*(7.7-x1)))))*((pow(10,
((0.09+(2730/(x2+273.2)))-x1)))+1);
}
else {
y=0.025*(((1+pow(10,(9.73-x1)))/(1+pow(10,((0.09+(2730/(x2+
273.2)))-x1))))/1)*((pow(10,((0.09+(2730/(x2+273.2)))-x1)))+1);
}
}
else {
if (x1<7.7) {
y=0.025*(1/(pow(10,(0.74*(7.7-x1)))))*((pow(10,((0.09+(2730/(x2+
273.2)))-x1)))+1);
```

```
}
else {
y=0.025*((pow(10,((0.09+(2730/(x2+273.2)))-x1)))+1);
}
}
```

Where

x1 = pH time series
x2 = temperature time series (°C)

Dissolved oxygen saturation can be calculated using DO concentration, temperature, and specific conductance converted to salinity (5). In freshwater systems, specific conductance has a very small influence on the DO saturation calculation. Despite this, specific conductance is still included to account for any times (e.g., winter road salt run-off) or locations (e.g., concentrated discharges, estuary influenced) where high salinity is possible. Because specific conductance usually has a small influence, it may be appropriate to substitute the specific conductance time series variable with a constant equal to the median of the specific conductance time series when gaps are present in the specific conductance time series; this will allow DO saturation to be calculated across gaps in the specific conductance time series. The inclusion of salinity to the equation and the nested conversion of specific conductance to salinity makes the equation very long but this has not affected the performance of the calculated-derived series.

(5)
```
y=x1/(0.01*exp((-862194900000*pow((1/(x2+273.15)),4)+
                12438000000*pow((1/(x2+273.15)),3)-
                6423080*pow((1/(x2+273.15)),2)+
                157570.1*(1/(x2+273.15))-139.344)-
                (0.008+(-0.1692)*(pow(((x3*(1+(0.0191*(x2-
25)))/1000)/42.914)/((1+((10.1325*(0.000207+(-
0.0000000637)*10.1325+0.000000000003989*pow(10.1325,2)))/(1+0.03426*x2+
0.0004464*pow(x2,2)+(0.4215+(-0.003107)*x2)*((x3*(1+(0.0191*(x2-
25)))/1000)/42.914))))*(0.6766097+0.0200564*x2+0.0001104259*pow(x2,2)+(-
0.00000069698)*pow(x2,3)+0.0000000010031*pow(x2,4))),0.5))+25.3851*((x3*(
1+(0.0191*(x2-25)))/1000)/42.914)/((1+((10.1325*(0.000207+(-
0.0000000637)*10.1325+0.000000000003989*pow(10.1325,2)))/(1+0.03426*x2+
0.0004464*pow(x2,2)+(0.4215+(-0.003107)*x2)*((x3*(1+(0.0191*(x2-
25)))/1000)/42.914))))*(0.6766097+0.0200564*x2+0.0001104259*pow(x2,2)+(-
0.00000069698)*pow(x2,3)+0.0000000010031*pow(x2,4)))+14.0941*(pow(((x3*(
1+(0.0191*(x2-25)))/1000)/42.914)/((1+((10.1325*(0.000207+(-
0.0000000637)*10.1325+0.000000000003989*pow(10.1325,2)))/(1+0.03426*x2+
0.0004464*pow(x2,2)+(0.4215+(-0.003107)*x2)*((x3*(1+(0.0191*(x2-
25)))/1000)/42.914))))*(0.6766097+0.0200564*x2+0.0001104259*pow(x2,2)+(-
0.00000069698)*pow(x2,3)+0.0000000010031*pow(x2,4))),1.5))+(-
```

```
7.0261)*(pow(((x3*(1+(0.0191*(x2-
25)))/1000)/42.914)/((1+((10.1325*(0.000207+(-
0.0000000637)*10.1325+0.000000000003989*pow(10.1325,2)))/(1+0.03426*x2+
0.0004464*pow(x2,2)+(0.4215+(-0.003107)*x2)*((x3*(1+(0.0191*(x2-
25)))/1000)/42.914))))*(0.6766097+0.0200564*x2+0.0001104259*pow(x2,2)+(-
0.00000069698)*pow(x2,3)+0.0000000010031*pow(x2,4))),2))+2.7081*(pow(((x3
*(1+(0.0191*(x2-25)))/1000)/42.914)/((1+((10.1325*(0.000207+(-
0.0000000637)*10.1325+0.000000000003989*pow(10.1325,2)))/(1+0.03426*x2+
0.0004464*pow(x2,2)+(0.4215+(-0.003107)*x2)*((x3*(1+(0.0191*(x2-
25)))/1000)/42.914))))*(0.6766097+0.0200564*x2+0.0001104259*pow(x2,2)+(-
0.00000069698)*pow(x2,3)+0.0000000010031*pow(x2,4))),2.5))+((x2-
15)/(1+0.0162*(x2-15)))*(0.0005+(-0.0056)*(pow(((x3*(1+(0.0191*(x2-
25)))/1000)/42.914)/((1+((10.1325*(0.000207+(-
0.0000000637)*10.1325+0.000000000003989*pow(10.1325,2)))/(1+0.03426*x2+
0.0004464*pow(x2,2)+(0.4215+(-0.003107)*x2)*((x3*(1+(0.0191*(x2-
25)))/1000)/42.914))))*(0.6766097+0.0200564*x2+0.0001104259*pow(x2,2)+(-
0.00000069698)*pow(x2,3)+0.0000000010031*pow(x2,4))),0.5))+(-
0.0066)*((x3*(1+(0.0191*(x2-25)))/1000)/42.914)/((1+((10.1325*(0.000207+(-
0.0000000637)*10.1325+0.000000000003989*pow(10.1325,2)))/(1+0.03426*x2+
0.0004464*pow(x2,2)+(0.4215+(-0.003107)*x2)*((x3*(1+(0.0191*(x2-
25)))/1000)/42.914))))*(0.6766097+0.0200564*x2+0.0001104259*pow(x2,2)+(-
0.00000069698)*pow(x2,3)+0.0000000010031*pow(x2,4)))+(-
0.0375)*(pow(((x3*(1+(0.0191*(x2-
25)))/1000)/42.914)/((1+((10.1325*(0.000207+(-
0.0000000637)*10.1325+0.000000000003989*pow(10.1325,2)))/(1+0.03426*x2+
0.0004464*pow(x2,2)+(0.4215+(-0.003107)*x2)*((x3*(1+(0.0191*(x2-
25)))/1000)/42.914))))*(0.6766097+0.0200564*x2+0.0001104259*pow(x2,2)+(-
0.00000069698)*pow(x2,3)+0.0000000010031*pow(x2,4))),1.5)) +
0.0636*(pow(((x3*(1+(0.0191*(x2-
25)))/1000)/42.914)/((1+((10.1325*(0.000207+(-
0.0000000637)*10.1325+0.000000000003989*pow(10.1325,2)))/(1+0.03426*x2+
0.0004464*pow(x2,2)+(0.4215+(-0.003107)*x2)*((x3*(1+(0.0191*(x2-
25)))/1000)/42.914))))*(0.6766097+0.0200564*x2+0.0001104259*pow(x2,2)+(-
0.00000069698)*pow(x2,3)+0.0000000010031*pow(x2,4))),2))+(-
0.0144)*(pow(((x3*(1+(0.0191*(x2-
25)))/1000)/42.914)/((1+((10.1325*(0.000207+(-
0.0000000637)*10.1325+0.000000000003989*pow(10.1325,2)))/(1+0.03426*x2+
0.0004464*pow(x2,2)+(0.4215+(-0.003107)*x2)*((x3*(1+(0.0191*(x2-
25)))/1000)/42.914))))*(0.6766097+0.0200564*x2+0.0001104259*pow(x2,2)+(-
0.00000069698)*pow(x2,3)+0.0000000010031*pow(x2,4))),2.5))))*(2140.7*pow((
1/(x2+273.15)),2)-10.754*(1/(x2+273.15))+0.017674)))
```

Where

x1 = DO concentration time series (mg/L)
x2 = temperature time series (°C)
x3 = specific conductance time series (µS/cm)

DATA EVALUATION

Systematic adoption of a standardized data-evaluation process, including rating criteria and maximum allowable limits are vital in finalizing instream monitoring records. Corrections are completed and then data are graded by the individual responsible for the data. If the measured error deviates by more than the maximum allowable limits, the data are deleted and not included in reports or analyses. Final approval of the data is completed by a separate individual before it can be finalized in a report.

Data Correction

During the deployment period, the sensors can experience calibration drift or be influenced by various types of fouling (biological growth, accumulation of sediment or other debris, etc.). The checks performed during the field visits allow these influences on the data to be corrected in post-processing. These checks, however, should not be blindly applied as corrections without review. If for instance, a correction would move the continuous data away from both a discrete field measurement and the subsequent continuous data set, the field check directing that correction is likely erroneous and the correction should not be applied. Final correction of data is determined by best professional judgment, guided by the field checks.

Typically, a data correction period begins and ends on servicing dates. If fouling, calibration, or undocumented errors are small (within the data correction criteria, Table 4), corrections are not necessary. If an error is larger than the criterion, a correction should be applied. The decision to apply data corrections when total errors are smaller than the correction criterion or to correct for other factors is left to best professional judgment. Corrections to be applied are calculated using the DEP digital CIM Field Form spreadsheet. The spreadsheet contains correction worksheets for each field parameter. These worksheets use the field checks performed at the various site visits to calculate the corrections and errors for each period. These calculations are then used in Aquarius to apply corrections. If access to the digital field form is not available, manual error calculations are provided below.

Table 4. Correction criteria for measured field parameters.

Field Parameter	Data Correction Criteria
Temperature	± 0.2°C
Specific Conductance	± 5 µS/cm or 3%, whichever is greater
pH	± 0.2 units
Dissolved Oxygen	± 0.3 mg/L
Turbidity	± 0.5 FNU or ± 5%, whichever is greater

Manual Error Calculations

Quantifying error is performed in accordance with Wagner et al. (2006). Numerically, total error (E_t), as defined by USGS, is equal to the sum of the absolute values of fouling error (E_f) and calibration drift error (E_c):

(5) $E_t = |E_f| + |E_c|$

When highly reliable discrete data are available, it may be used to bracket the time series of data for which error is being determined, thereby allowing the computation of an undocumented error and/or discrete error. This error is equal to the difference between data corrected for fouling and calibration drift and the discrete data. If discrete data are in line with the subsequent data set, an undocumented drift correction may be warranted and the error is referred to as an undocumented error (E_u). If an undocumented drift correction is not warranted, the difference between the corrected data and the discrete data is referred to as a discrete error (E_d). If present, these errors should be included in the total error calculation, modifying equation (5) to:

(6) $E_t = |E_f| + |E_c| + |E_u| + |E_d|$

Total error, as shown in equation (6) may then be applied in data grading.

One important distinction when calculating errors is the difference between a percent correction and percent error. A percent correction is the calculated percentage that is used to correct the continuous data. Percent error is the percentage difference that the raw continuous data are from the correct value. The difference in the calculations is the denominator. Correction calculations are made in relation to the original, raw data and therefore the raw value is used in the denominator. Error calculations are made in relation to the “true” value and therefore the denominator is the “true” value (e.g., reading in standard or buffer, reading after cleaning, etc.). Percent correction should only be used for correcting data and should not be used as a measure of error. Percent error should be used to quantify error but should not be used to correct data. When the unit error is small, the difference between these two percentages will be minimal; however, as the error increases, the difference between these calculations will quickly become significant.

Fouling error (E_f), percent fouling correction ($\%C_f$), and percent fouling error ($\%E_f$) may be determined using equations (7), (8), and (9), as shown below.

(7) $E_f = (Sonde_{ac} – Sonde_{bc}) – (FM_{ac} – FM_{bc})$

(8) $\%C_f = 100 * (E_f / Sonde_{bc})$

(9) $\%E_f = 100 * (E_f / Sonde_{ac})$

Where

$Sonde_{ac}$ = sonde measurement after cleaning
$Sonde_{bc}$ = sonde measurement before cleaning
FM_{ac} = field meter measurement after cleaning
FM_{bc} = field meter measurement before cleaning

Calibration drift error (E_c), percent calibration drift correction ($\%C_c$), and percent calibration drift error ($\%E_c$) may be determined using equations (10), (11) and (12), as shown below.

(10) $E_c = V_{std} - V_{sonde}$

(11) $\%C_c = 100 * [(V_{std} - V_{sonde}) / V_{sonde}]$

(12) $\%E_c = 100 * [(V_{std} - V_{sonde}) / V_{std}]$

Where
V_{std} = value of the standard or buffer
V_{sonde} = value measured by the sonde

Types of Data Corrections

The parameter type and the data obtained during the checks determine the type of correction applied. Multipoint corrections that bracket the range of recorded values are the most accurate corrections and should be used whenever possible. Fouling checks only have a single point of reference (the stream conditions at the time) and therefore, multipoint corrections cannot be used. Calibration checks for specific conductance, pH, and turbidity should all consist of multiple standards that bracket the range of values so those checks should be used to apply a multipoint correction. If a multipoint correction is not possible, a drift (unit based) or percent correction should be applied. Guidelines for which correction to use are summarized in Table 5.

Table 5. Types of corrections to use based on parameter and type of error being corrected. Undocumented errors should use the same correction type as the fouling correction listed.

Parameter	Correction Type	
	Fouling	**Calibration**
Temperature	Drift*	N/A
Specific Conductance	Percent	Multi-point
pH	Drift	Multi-point
Dissolved Oxygen	Percent	Percent
Turbidity	Drift**	Multi-point

*Percent correction may be appropriate if range is large or values near zero
**Percent correction may be appropriate if range is low

In general, percent corrections are preferred over drift corrections because they adjust to the scale of the measurements like the sensors (a 10 µS/cm difference in specific conductance at 1000 µS/cm is

not equivalent to a 10 µS/cm difference at 100 µS/cm). Drift corrections are appropriate for temperature because the range is typically small. If the range is large or is close to zero, a percent correction may be more appropriate. Drift corrections are also used for pH because it is a log-based scale.

Because of the potential for a very large range of values, turbidity can present some difficulty in selecting between drift and percent corrections. Because turbidity is often low during base flow in Pennsylvania streams, a drift correction is usually most appropriate. Small errors can represent large percent differences when values are low. Using a percent correction in these circumstances could lead to a dramatic, inappropriate adjustment to peaks during the period. Likewise, if a maintenance visit occurred during one of these peak events, a drift correction could lead to large, inappropriate shifts in the baseline values. Best professional judgment will need to be used in these circumstances.

All of the above correction types apply the correction in an incremental, linear fashion where no correction is applied at the beginning of the period and the correction increases evenly to the end of the period where the full correction is applied. A less frequently used correction is an offset correction, which applies the same correction to all points in the period. One common use for an offset correction is for stage. When a sonde is returned to the stream, the depth is not always exactly the same as the previous deployment. This change in stage is consistent for the duration of the deployment so an offset correction is used to adjust the data to the previous deployment.

Applying Corrections in Aquarius

Corrections can be applied using alternative procedures; however, using software like Aquarius has multiple advantages. First, is the tracking of all changes to data. All events (corrections, deletions, approvals, etc.) are time stamped and logged with the user ID who made the change. This provides transparency and the ability to implement quality assurance checks. The second benefit is that all changes are input as layers over the raw data. These layers provide great flexibility as they can be turned on/off or adjusted at a later time. It also maintains both raw and corrected datasets without requiring a complete second set of data to be stored. A third benefit is the easy application of complex corrections like multipoint corrections. Multipoint corrections apply the correction not only incrementally and linearly over the period but with respect to the raw value's position relative to the multiple checks entered in the system.

To apply corrections in Aquarius, select a parameter and click Data Correction . Multiple parameters can be selected by holding Ctrl. Select the parameter to edit first; all other parameters will be considered surrogates and will appear as reference(s) only. Including discrete measurements and discharge or stage data as surrogates is suggested to aid in the correction process. Once in the Data Correction window, additional parameters can be added by clicking Select Data Sets under File. In this way, parameters can be selected from other sites as well. The layout of the Data Correction window can be customized but the default layout is described in Figure 16.

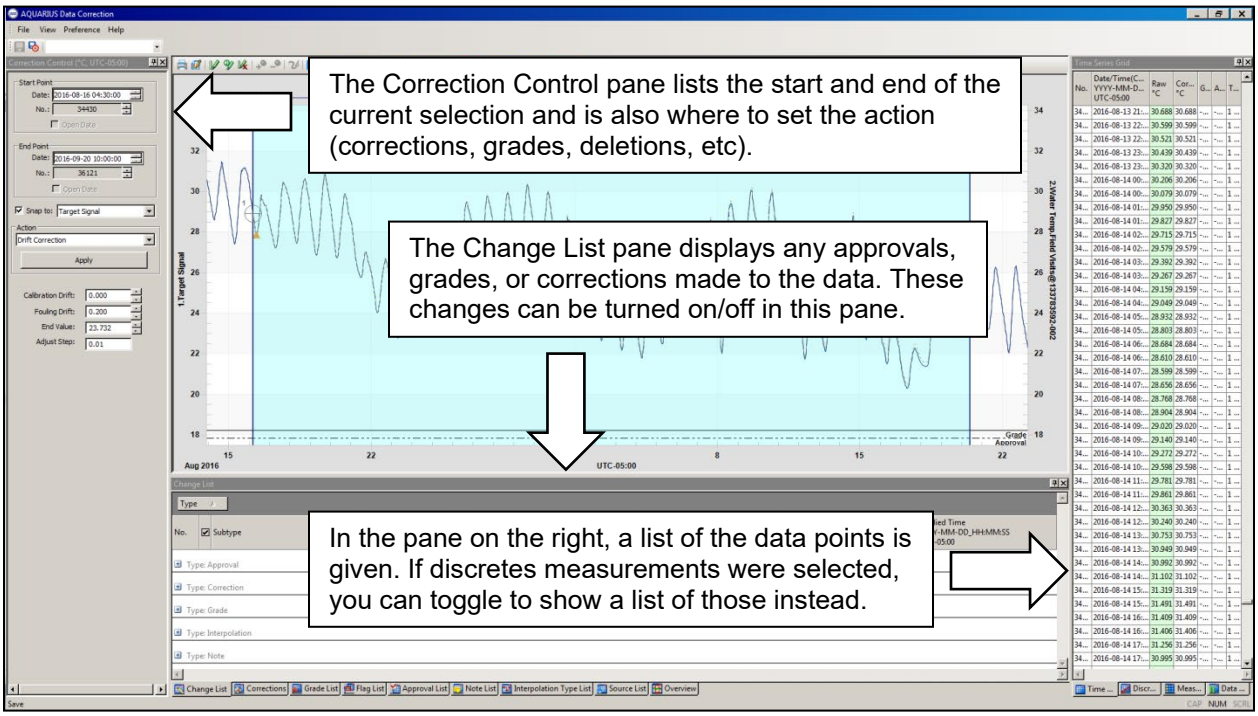

Figure 16. Default layout of the Data Correction window.

Selecting a Period

The first step in applying a correction is determining the period that the correction should apply to. As stated previously, under most circumstances, the correction period is from one service date to the next. By default, the measured error is assumed to have accumulated linearly over that time. An example of an exception to this assumption would be when a significant flow event occurs between service dates resulting in the fouling of a sensor. This flow event upsets the assumption of linear accumulation of fouling error over the entire period. A more appropriate application of the correction may be to limit the fouling correction to the period from the flow event to the next service date.

If there is an extended gap between data series the selection should include that gap, up until the site visit, for the application of a correction. This sometimes happens when the batteries fail prior to the maintenance visit. In these cases, the fouling and calibration checks were made at the time of the site visit not when the batteries failed, so corrections associated with the checks are made by selecting the period up to the maintenance visit. This may require unchecking the “Snap to” box above the Action drop-down menu, allowing the selection to float between data points. This type of selection may also be necessary if a deletion creates a gap at the end of a period.

To select a period to be corrected (or graded, approved, deleted, etc.) select the Mark Region tool. Drag and release over the area to select (Figure 17). Zoom into the beginning and end of the period to make adjustments to the selection; the beginning/end lines can be dragged into place.

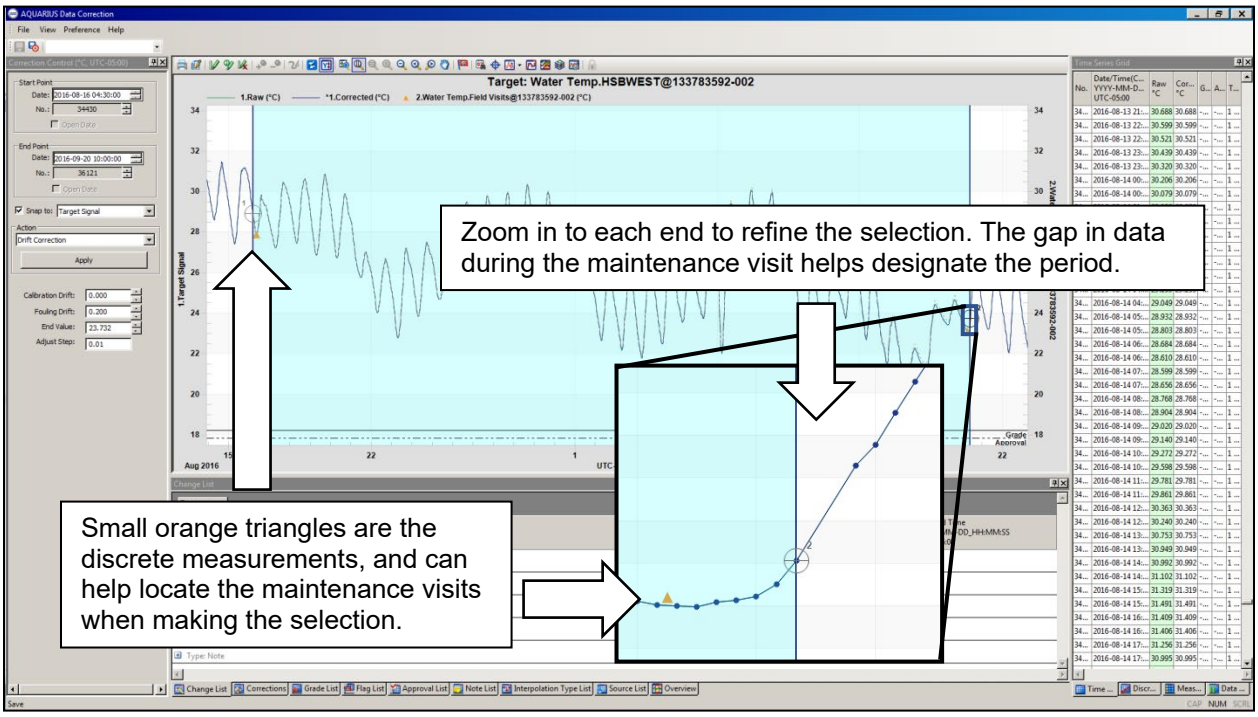

Figure 17. Use the Mark Region tool to select the data to be corrected. Selected region will be highlighted in blue.

Deleting Data

The first editing of data is often the elimination of obviously erroneous data points or sections of data. Often these data are identified visually. These data may be single outliers or a section of erratic readings. The decision to delete data is often made using best professional judgment, guided by knowledge of the location, equipment tendencies, and water quality in general. Examples of factors involved in the decision making process include interruption of diel swings or other expected patterns, very rapid changes (in particular, recoveries normally happen more gradually then the initial response), inconsistencies not aligning with changes in flow, and known equipment tendencies such as the false spikes with turbidity sensors.

Multiple actions can be used to delete data in Aquarius. The most direct way is to use the Delete Region action. All points selected will be deleted including the points at the beginning and end of the selection. To delete a single point with this action, turn off the Snap To option in the Correction Control pane before making the selection. An alternate procedure is to set a deletion threshold value using the Threshold Correction/Flagging action. Using this action, a max or min can be set, or a rate of change threshold can be established. These actions are applied only for the period selected. A more sophisticated threshold can be established using the Adjustable Trim Correction action. This action allows for up to eight pivot points to be established for a variable, upper or lower threshold over the selected region (Figure 18). One important note is that deletion thresholds, whether hardline or variable, will adjust with data if they are moved with subsequent corrections. In other words, the data AND the thresholds are adjusted with any corrections applied after thresholds have been set.

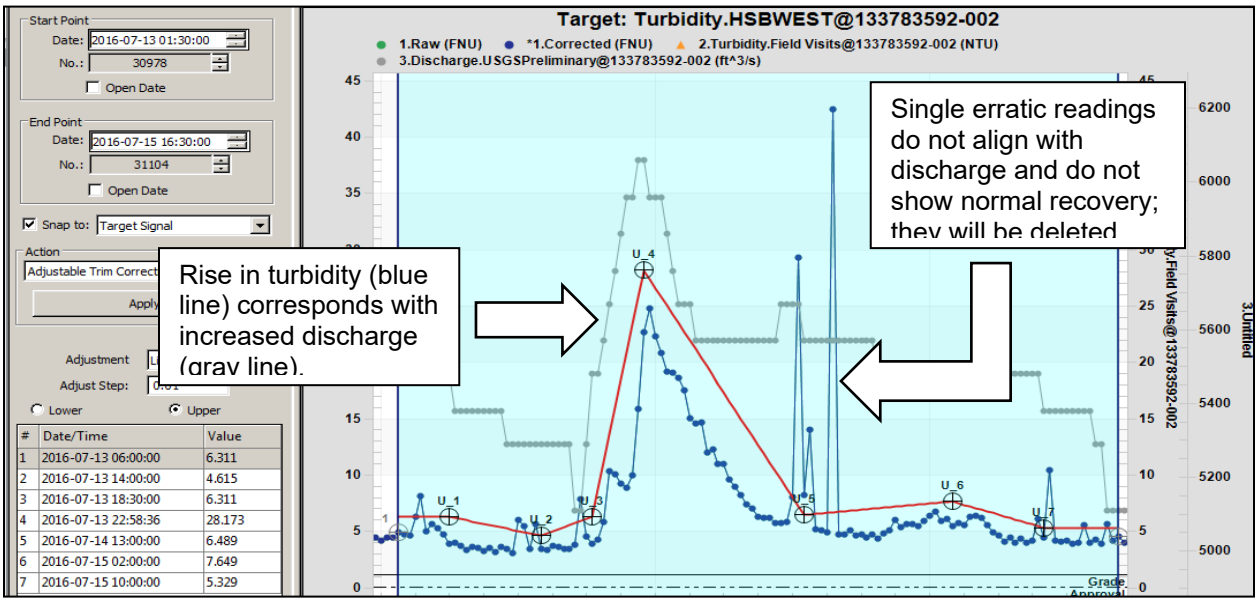

Figure 18. The Adjustable Trim Correction action (red line) allows for a variable threshold to be established. This adjustable threshold can be used to contour data while establishing an upper or lower threshold. This example demonstrates the correction being used to establish an upper threshold to delete erroneous spikes in turbidity data while preserving a rise in turbidity (blue line) from a rain event.

Drift Corrections

Drift corrections are unit-based corrections that apply no change at the start of the interval and the full correction at the end of the interval. Data between the beginning and end points are linearly interpolated. In Aquarius, calibration or fouling drift corrections can be entered in the same correction. Figure 19 shows an example of a 0.2 unit drift correction for pH fouling drift.

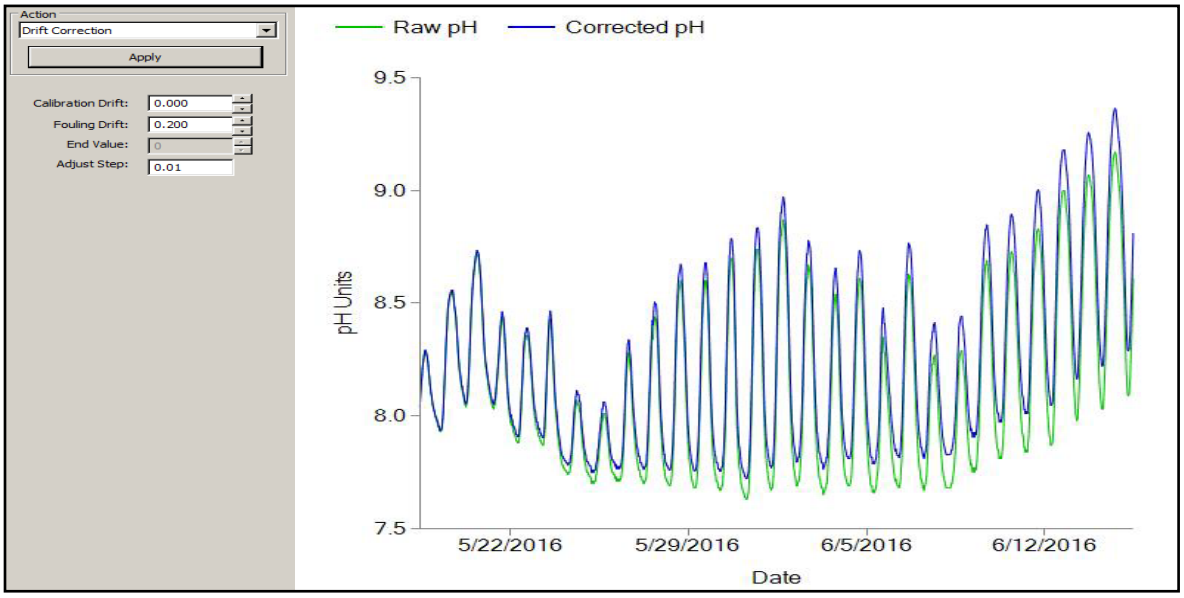

Figure 19. Continuous pH data from the Susquehanna River at Rockville, PA with a drift correction of 0.2 units applied.

Percent Corrections

Percent corrections function similarly to drift corrections but, instead of a unit-based correction, the correction is a percentage of the raw value. This has most importance when the range of values is high. Figure 20 shows specific conductance from Goose Creek at Mosteller Park, PA. This watershed has high impervious cover and the stream is very “flashy”. Baseline specific conductance is over 800 µS/cm but during rain events the specific conductance drops dramatically. If a drift correction was applied, corrections during periods following rain events would be almost four times greater relative to their raw value; therefore, a percent correction should be used instead.

One difference in the application of the correction in Aquarius is that there is not an option to enter fouling and calibration corrections in separate boxes for the same correction. The corrections must be done separately or their sum can be entered and the individual values given in the notes section of the correction. Additionally, Aquarius offers to correct the start, end, or both ends of the selected region.

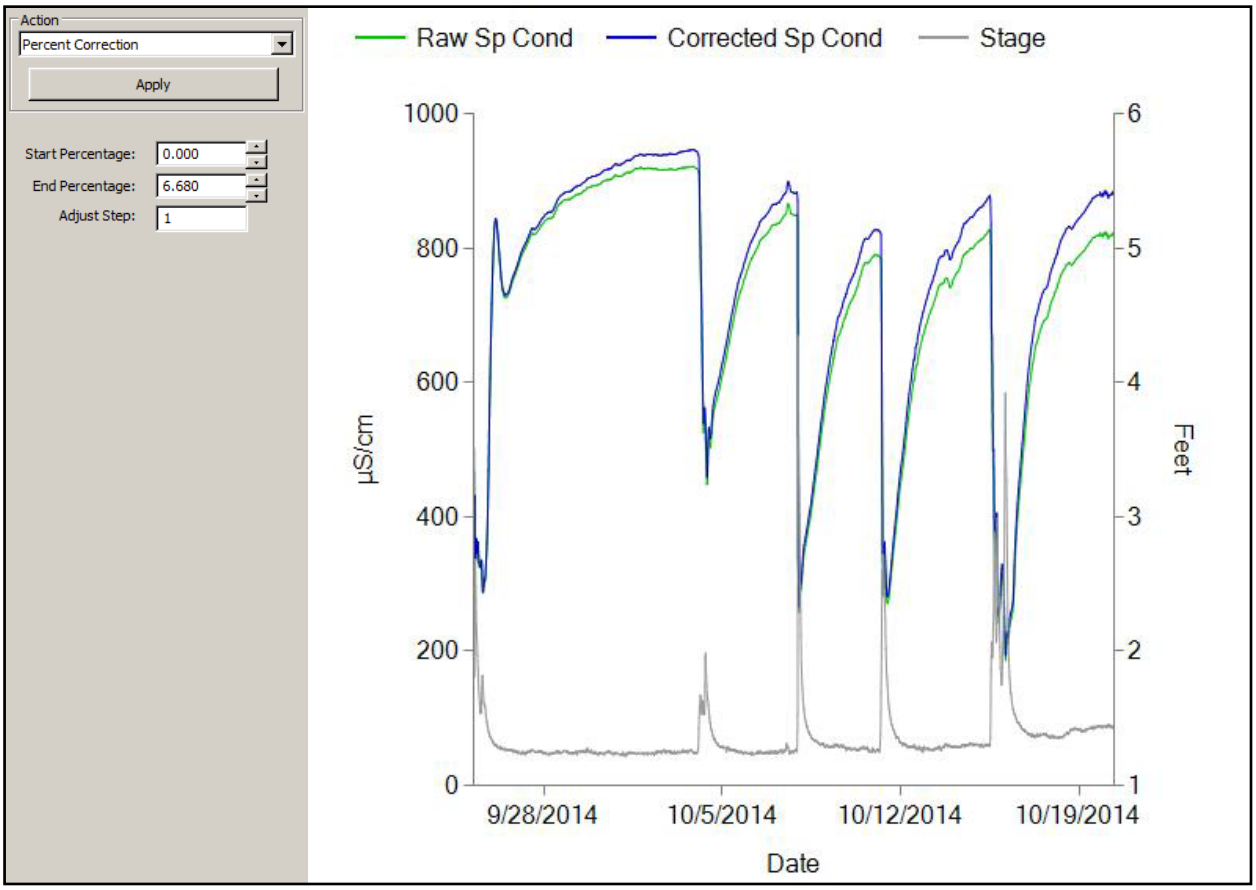

Figure 20. Continuous specific conductance data from Goose Creek at Mosteller Park, PA with a percent correction of 6.68%. Larger raw values are corrected more than the smaller raw values following rain events.

Multipoint Corrections

While drift and percent corrections apply the change incrementally and linearly over the span of the selected region, neither correction can account for potential non-linearity in a sensor's response. Multipoint corrections can address this issue by using checks in multiple standards to correct the data. The action in Aquarius for multipoint corrections is the USGS Multipoint Correction.

The multipoint correction in Figure 21 demonstrates the power of this correction; not only is the correction to the pH dataset incrementally applied over the selected region, but the correction changes relative to the raw value. The strong diel swings exhibit larger corrections as the values approach the pH 10 buffer correction and smaller corrections as the values approach the pH 7 buffer correction. The Correction V Diagram shows the correction applied across the range of values. In this example, the pH 4 buffer correction entered has no influence on the data because all values are between 7 and 10 pH. In order to catalog a record of corrections in Aquarius, the correction is still entered. Additionally, if a subsequent correction were to move any data below 7 pH, the pH 4 buffer correction would become applicable. Like the percent correction, both the start and end of the period can be adjusted.

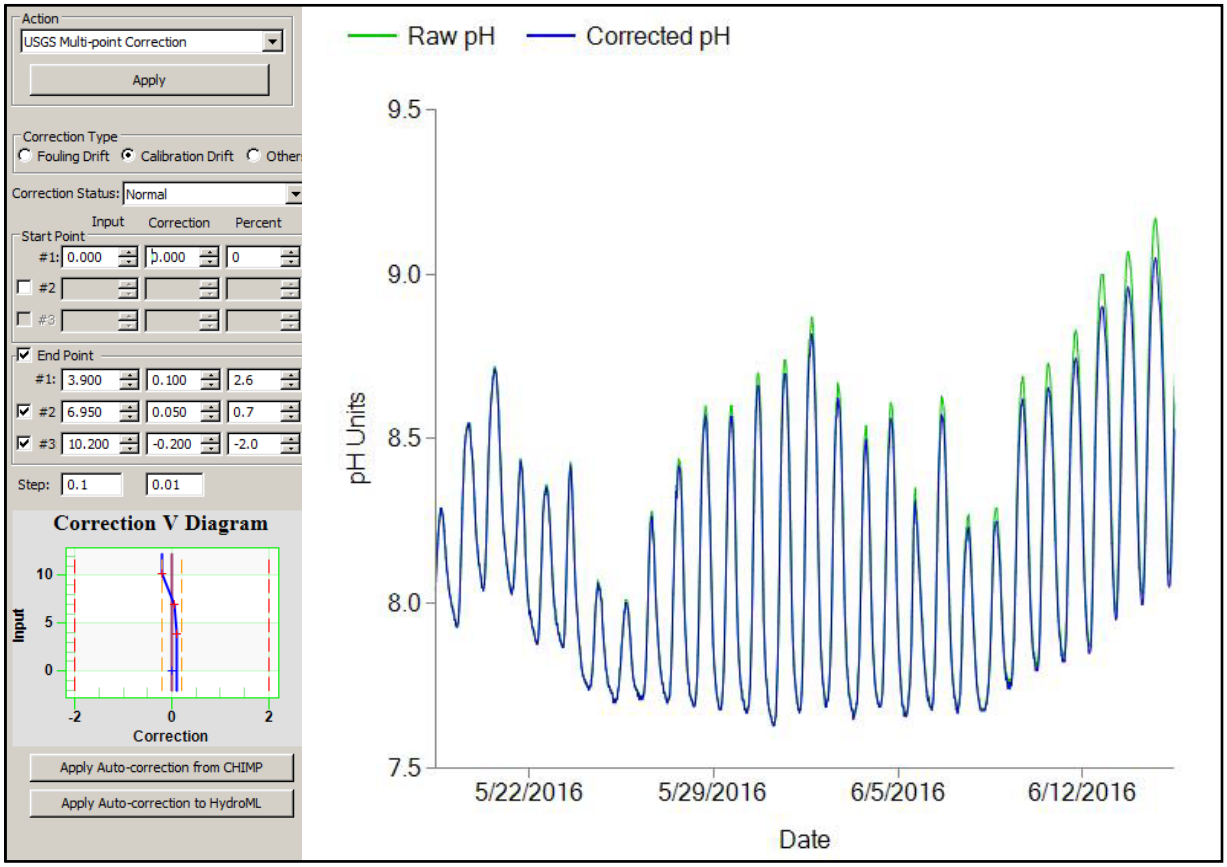

Figure 21. Continuous pH data from the Susquehanna River at Rockville, PA with a USGS multipoint correction applied. Buffers 4, 7, and 10 were used to check pH calibration. Values in buffer are entered under Input (3.90, 6.95, 10.20, respectively). The second column is the correction necessary (0.10, 0.05, -0.20, respectively).

Offset Corrections

Offset corrections are a unit-based correction; however, unlike drift corrections, the value of adjustment is applied equally to the marked region. Offset corrections can be used on data that has not been calibrated or was incorrectly calibrated; however, percent or multipoint corrections can also be used in these circumstances, and often are the more appropriate procedure. A common use of an offset correction is to correct stage data when the equipment was returned to the stream at a different depth. This change in depth is constant for the duration of the next period and is best corrected by an offset correction (Figure 22).

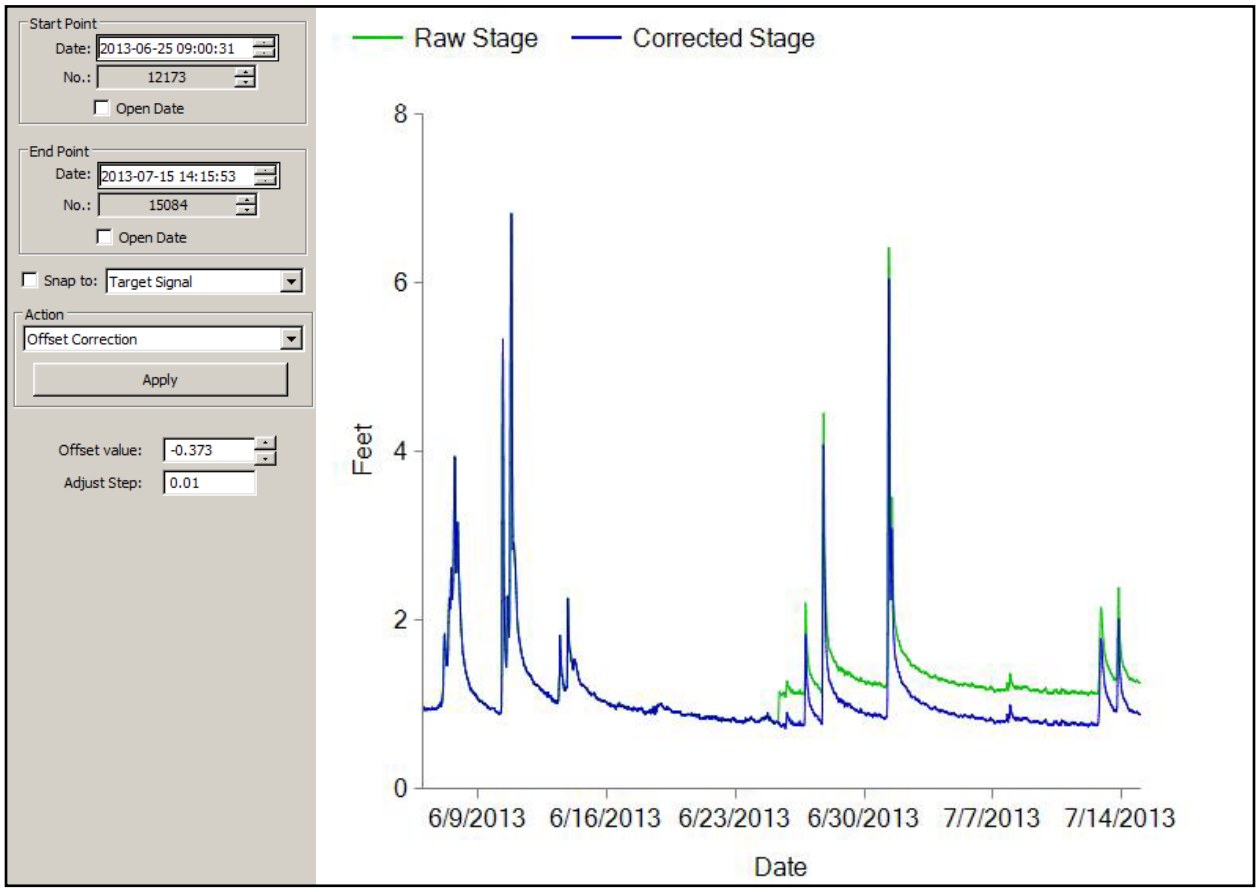

Figure 22. Stage data from Skippack Creek at Mainland Rd, PA with an offset correction of -0.373 feet applied from 6/25/13 to 7/15/13. Data were previously corrected for changes in atmospheric pressure. Only the offset correction is shown here.

Undocumented Corrections

Undocumented error in data is typically the result of fouling error that was not captured through the fouling checks during maintenance. This can happen when the sonde needs to be disturbed to be connected to laptop or handheld unit. Undocumented error is realized by comparing recorded data to verified discrete data or adjoining data periods after fouling and calibration corrections are applied. Verified discrete data must be from either a calibrated field meter or from a laboratory grab sample. Due to the nature of DEP Bureau of Laboratory reported values for pH, and specific conductance,

these parameters should generally be considered less reliable than those obtained in the field from a properly maintained and calibrated field meter.

Another potential source of undocumented error is a faulty thermistor. Because specific conductance, pH, and DO data are dependent on temperature, these records will need to be closely reviewed—and perhaps deleted—if temperature data are found to contain significant error. Wagner et al. (2006) states that for each 1°C change in temperature, specific conductance, and DO concentrations vary 3 percent. For Yellow Spring Instrument (YSI) equipment, a 1°C change in temperature affects conductivity, pH, and DO by 4.0%, 0.5%, and 2.0%, respectively, while a 5°C change in temperature results in changes of 22.0%, 2.0%, and 9.0%, respectively (YSI Tech Support, personal communication, January 18, 2017). The equations establishing these relationships are often proprietary and therefore the exact influence of errors in temperature data cannot be derived. In addition, without these equations, specific conductance, pH, and DO cannot be corrected for errors in temperature data.

Undocumented corrections are corrections that move the continuous data based on a verified discrete sample and/or an adjoining dataset (Figure 23). The type of correction used depends on the parameter and should be the same as that used for fouling corrections. For example, if based on the parameter type, the type of fouling correction is a percent correction, an undocumented correction would also be applied as a percent correction. It’s important to note that undocumented corrections should be made with care; the reviewer should ensure that the discrete datum agrees with the continuous data after redeployment before continuous data are corrected to the discrete

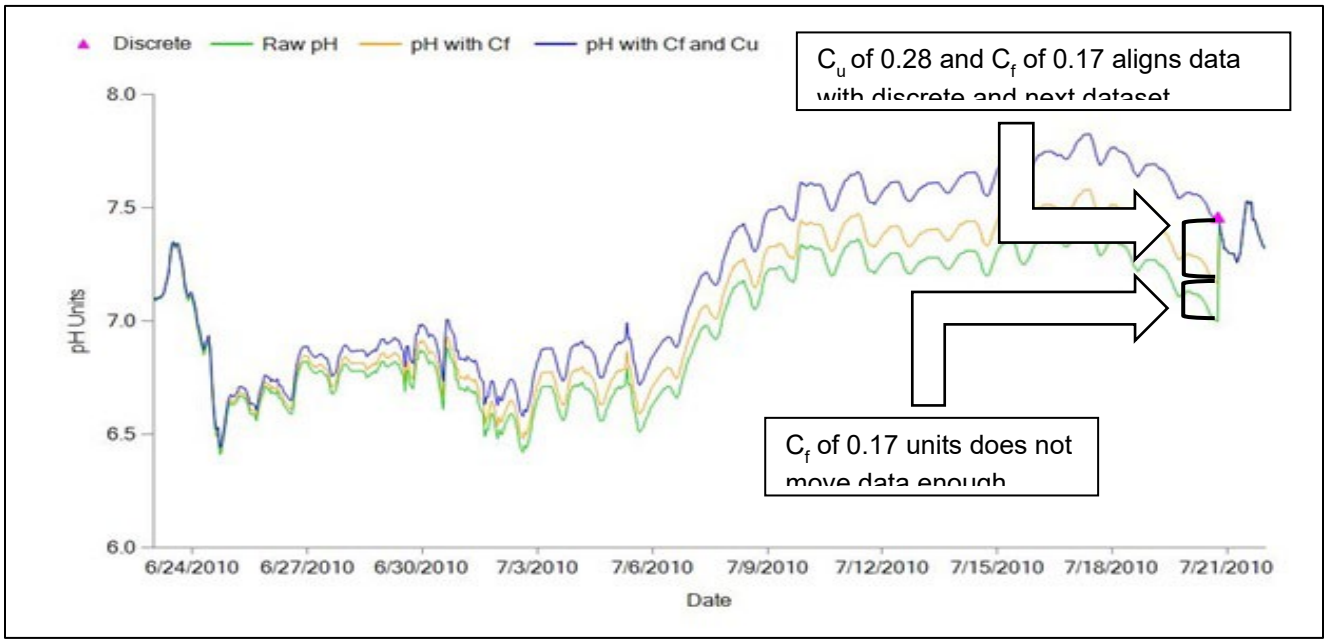

Figure 23. Data correction at Little Kettle Creek, PA. There was no calibration error during this period and fouling error was 0.17 pH units. Correcting for just fouling error (orange line) does not account for the full difference between the continuous data and either the discrete sample point or the beginning of the next series of continuous data. Since the continuous data after redeployment and the discrete datum are in agreement, the data were corrected an additional 0.28 pH units to match—an undocumented correction. Both documented fouling and undocumented corrections were input in Aquarius as drift corrections.

Grading

Grades are determined based on total error (E_t) as defined in equation (2) and are applied using the Data Correction window in Aquarius. Division of Water Quality uses a standardized procedure of grading to remain consistent throughout the data evaluation process (Figure 24). Data are generally classified as either usable or unusable. Data that fall outside the maximum allowable limits (Table 6) are graded Unusable and deleted in Aquarius. The maximum allowable limits are established at approximately 6–10 times the calibration criteria for all standard continuous-monitoring data-collection activities. These limits and rating criteria are taken from the USGS and will be considered minimum standards for DEP. The criteria in Table 7 are used to establish ratings for each period of *usable* data. The Field Form spreadsheet will help determine cutoff points between ratings. This process is described in the Correction and Grading Example.

Some data are not classified as either usable or unusable because they lack the required validations. These data are graded as Unverified and are generally not reported. One exception to not reporting unverified data is stage data collected by a non-vented transducer sensor with no barometric pressure data. These data cannot be accurately verified or corrected, yet stage data provide an important reference point for rain events and help explain what may be occurring in a watershed. This unverified data may be presented in a final report as qualitative data only.

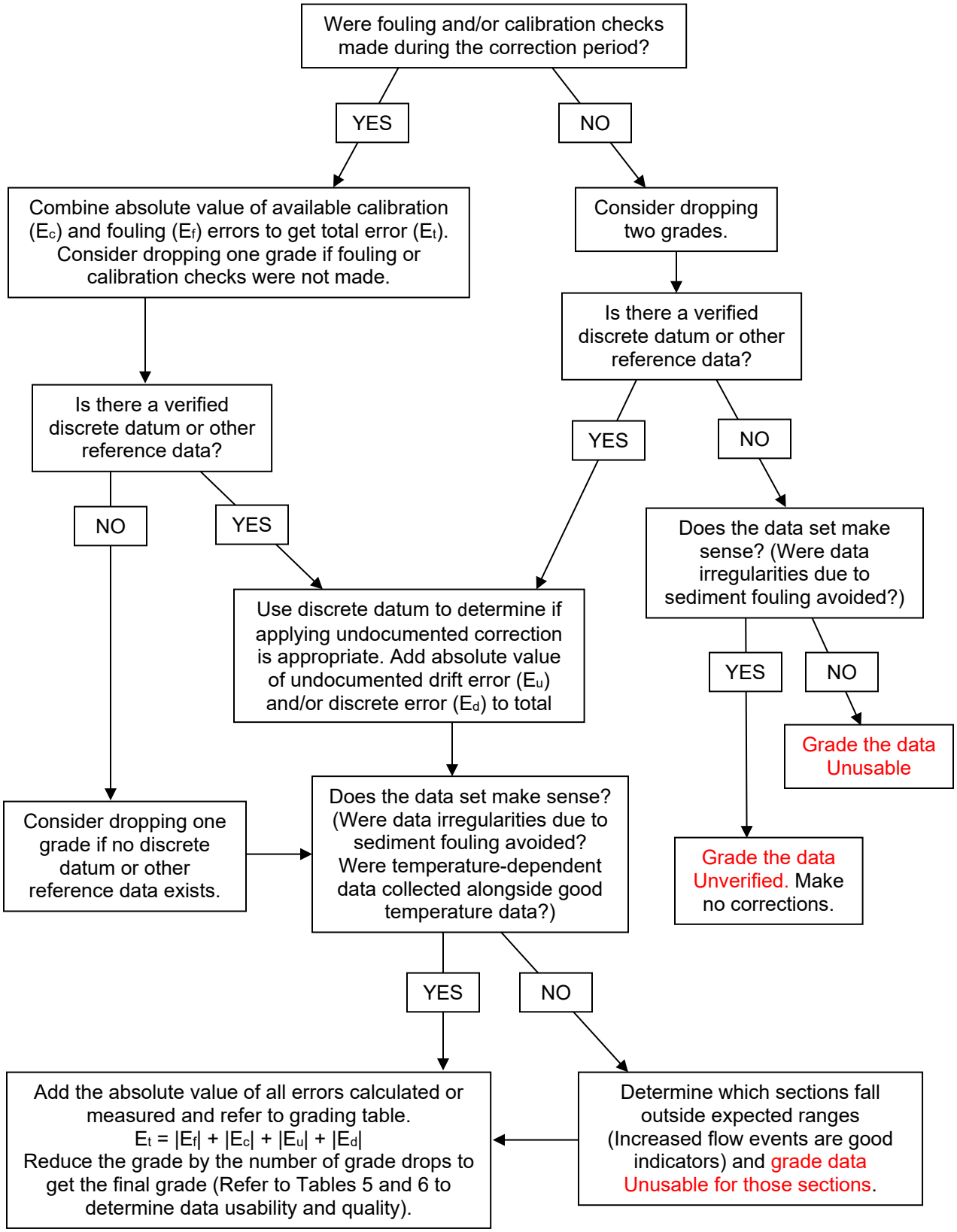

Figure 24. Water Quality Division standardized grading flow chart

Table 6. Maximum Allowable Limits for Reporting Data

Field Parameter	Maximum Allowable Limits
Temperature	± 2.0°C
Specific	± 30%
pH	± 2.0 units
Dissolved Oxygen	± 2.0 mg/L or ± 20%, whichever is greater
Turbidity	± 3.0 FNU or ± 30%, whichever is greater

Table 7. Rating criteria used to qualify usable data.

Field Parameter	Rating (Based on total error [E_t] calculation)			
	Excellent	Good	Fair	Poor
Water Temperature	≤ ± 0.2°C	> ± 0.2 - 0.5°C	> ± 0.5 - 0.8°C	> ± 0.8°C
Specific Conductance	≤ ± 3%	> ± 3 - 10%	> ± 10 - 15%	> ± 15%
pH	≤ ± 0.2 units	> ± 0.2 - 0.5 units	> ± 0.5 - 0.8	> ± 0.8 units
Dissolved Oxygen	≤ ± 0.3 mg/L or ≤ ± 5%, whichever is greater	> ± 0.3 - 0.5 mg/L or > ± 5 - 10%, whichever is greater	> ± 0.5 - 0.8 mg/L or > ± 10 - 15%, whichever is greater	> ± 0.8 mg/L or > ± 15%, whichever is greater
Turbidity	≤ ± 0.5 FNU or ≤ ± 5%, whichever is greater	> ± 0.5 - 1.0 FNU or > ± 5 - 10%, whichever is greater	> ± 1.0 - 1.5 FNU or > ± 10 - 15%, whichever is greater	> ± 1.5 FNU or > ± 15%, whichever is greater

When a multipoint correction is used, the measured change in the data should be used to determine the error for grading purposes. This is the preferred procedure over the alternatives of using the error at the closest standard or averaging the errors in the various standards. Percent error of a multipoint correction (%E_m) can be calculated by substituting raw value (V_r) and corrected value (V_c) into equation (12) for V_{sonde} and V_{std}, respectively:

$$(13) \quad \%E_m = 100 * [(V_c - V_r) / V_c]$$

Using the last data point in the period usually provides the maximum error applied, however, if there is a significant change in the data near the end of the period the maximum error may be at the preceding peak or valley in the data.

When both percent and unit-based thresholds are present in Table 7 (i.e., DO and turbidity), the procedure to be used is determined in the same way as the calibration criteria described earlier—whichever procedure would result in a better grade is the procedure used. This is because when values are low, even small changes can result in very high percentage differences that would lead to unusable data. Similarly, when values are high, small percent differences represent large unit differences that would lead to unusable data.

Selecting whether to use percent or unit-based rating criteria can be problematic with turbidity data when multiple discretes were taken at drastically different values. Unit-based ratings are usually used for turbidity data from Pennsylvania streams because turbidity at base flow is typically low. Discrete readings, however, are often targeted during peak flow events to facilitate the building of rating curves. These turbidity discretes at peak flow may have low percent error but high unit-based error. Using exclusively percent or unit-based rating criteria in this situation will often result in data being rated as unusable. In order to properly rate the data under these circumstances, it is necessary to convert the percent error for the discretes taken at peak flows to a unit-based error that can be summed with the rest of the equation. The turbidity rating scale has a 1:10 ratio of FNU to percent units and therefore the conversion can be made by dividing the percent error by 10 to get the unit-based rating equivalent. Alternatively, a unit-based error can be multiplied by 10 to get a percent rating equivalent.

After the rating is determined based on E_t, the grader must determine if any “grade drops” are warranted. A grade is decreased if there is added uncertainty to the data. The most common reason for decreasing a grade is a missing verification (fouling check, calibration check, or discrete measurement during the period). Because the verification is missing, the grade is dropped by one rating. If two verifications are missing, the grade should be dropped by two ratings. If all verifications are missing, the data should be graded unverified. Grade drop determinations are outlined in Figure 24.

To aid the approval process, the person applying the grade should include a short note in the comment section in Aquarius. The comment section is provided after clicking Apply. The note, at a minimum, should include the errors calculated and note the reason for any grade drops.

Correction and Grading Example

An example of the entire correction and grading process is outlined in Figures 25 and 26 for specific conductance data from the Susquehanna River at Rockville for the period from 6/9/15 to 7/9/15. The basis for these corrections is taken from the Specific Conductance tab of the DEP Field Form. These parameter-specific tabs calculate the errors and anticipated corrections to be applied using the data entered during the maintenance visit. The section of the Specific Conductance tab related to the period being corrected is displayed in the upper portion of Figure 25. Many of the cells in this spreadsheet are populated straight from the individual field visit tabs. The cells highlighted in purple are the cells to be used to enter corrections in Aquarius or used in the grading process. Only the cells highlighted in yellow should be edited.

The period was first reviewed visually for outliers or abnormalities such as sections of erratic readings that needed to be deleted. While these data were slightly more erratic point-to-point than average, no points needed to be deleted. The erratic behavior of the sensor was likely due to fouling as the subsequent dataset, after cleaning the sensor, looked much smoother.

Next, the information from the calibration checks was used to apply a USGS Multi-point Correction. Because the meter was not calibrated during the maintenance visit on 6/9/15, the correction included adjustments to both the beginning and end of the period. Under Start Point, the inputs were 0, 103, and 1019 with corrections of 0, -3, and -19, respectively. End Point inputs were 0, 103, and 1020 with corrections of 0, -3, and -20, respectively. The Start Point inputs and corrections are the End Point inputs and corrections from the previous section of the spreadsheet (not depicted in Figure 25). Once the correction is applied (Figure 26), the error can be calculated. The raw value was 234.0 µS/cm and the corrected value was 228.6 µS/cm; therefore, using equation (13) the calibration error for the period is -2.36%:

$\%E_m = 100 * [(228.6 – 234.0) / 228.6] = -2.36\%$.

It is recommended that calibration corrections are applied first for parameters that require multi-point corrections. This isolates the multi-point correction, allowing the above calculation to be made. If the multi-point correction is made after other corrections, those other corrections will need to be temporarily turned off in Aquarius in order to make the calibration error calculation.

A Percent Correction was then applied to the period to correct for fouling drift. The Start Percentage was 0% because the sonde was cleaned at the 6/9/15 maintenance visit, and therefore it is assumed that no fouling is present. The End Percentage was -0.88% which was taken from the Fouling Correction box in the spreadsheet (Figure 25).

If the either correction did not make sense (i.e., data were moved away from the discrete and/or adjoining datasets), the beginning, end, or both parts of the correction(s) could have been removed. In this case, the calibration and fouling corrections made sense because both moved the data closer to the discrete and adjoining datasets; therefore, both corrections remained applied.

After applying calibration and fouling corrections, the discrete was still 2.0% different than the corrected data (Figure 26). Because the discrete lined up with the subsequent dataset, an undocumented correction was deemed appropriate. A Percent Correction with a Start Percentage of 0.0% and an End Percentage of 2.0% was applied to offset the undocumented error. It is important to note that this determination was made after applying the calibration correction to the next period. Because the sensor was calibrated during the 7/9/15 visit, the beginning of the next period also needed to be corrected.

After all corrections were applied, total error (E_t) was calculated using equation (6):

$E_t = |E_f| + |E_c| + |E_u| + |E_d| = |-0.88\%| + |-2.36\%| + |-2.0\%| + |0.0\%| = 5.24\%$

Fouling error (E_f) was taken from the Fouling Error box of the spreadsheet. This number happened to be the same as the fouling correction applied because the error was small in this case. If the error was larger, these numbers would have been different from each other; therefore, care should be taken to use the correct value. Calibration error (E_c) was $\%E_m$, from the calculation above. Undocumented drift error (E_u) was from the final correction applied. Because there was only one discrete and an undocumented drift correction was applied to meet the discrete, discrete error (E_d) was zero for the period.

The grade is “Good” for specific conductance, when $E_t = 5.24\%$ (Table 7). Additionally, E_t can be entered into the spreadsheet (“Total Error (%)” box, Figure 25) to generate a schedule of prorated grades for the period. For simplicity, these prorated grades are typically not applied. Instead, the lowest grade is applied for the entire period. One exception is when E_t results in unusable data. In this circumstance, the division between poor and unusable is used to isolate only the data that are unusable.

Date	Time arrived	Time left	Fouling Correction: Site Monitor Dirty	Site Monitor Clean	Field Probe Before	Field Probe After	Calibration Check: Standard 1 Standard	Standard 1 Reading	Standard 2 Standard	Standard 2 Reading	Standard 3 Standard	Standard 3 Reading	Standard 4 Standard	Standard 4 Reading	Fouling Correction	Fouling Error (GRADING)	USGS Multi-point Correction Inputs: Reads	Correction	Calibration Error (GRADING)	Remarks
7/9/15	800	1130	228	226	223	223	0	0	100	103	1000	1020	5000		-0.88%	-0.88%	0	0.00	0.00%	
																	103	-3.00	-3.00%	
																	1020	-20.00	-2.00%	

Date & Time	Number of days	Total Error (%) (measured or applied)	Error per day	Dates rated Excellent	Dates rated Good	Dates rated Fair	Dates rated Poor	Dates to be deleted
7/9/15 11:30	30.0	5.24%	0.1745%	6/10/15 through 6/25/15	6/26/15 through 7/9/15			

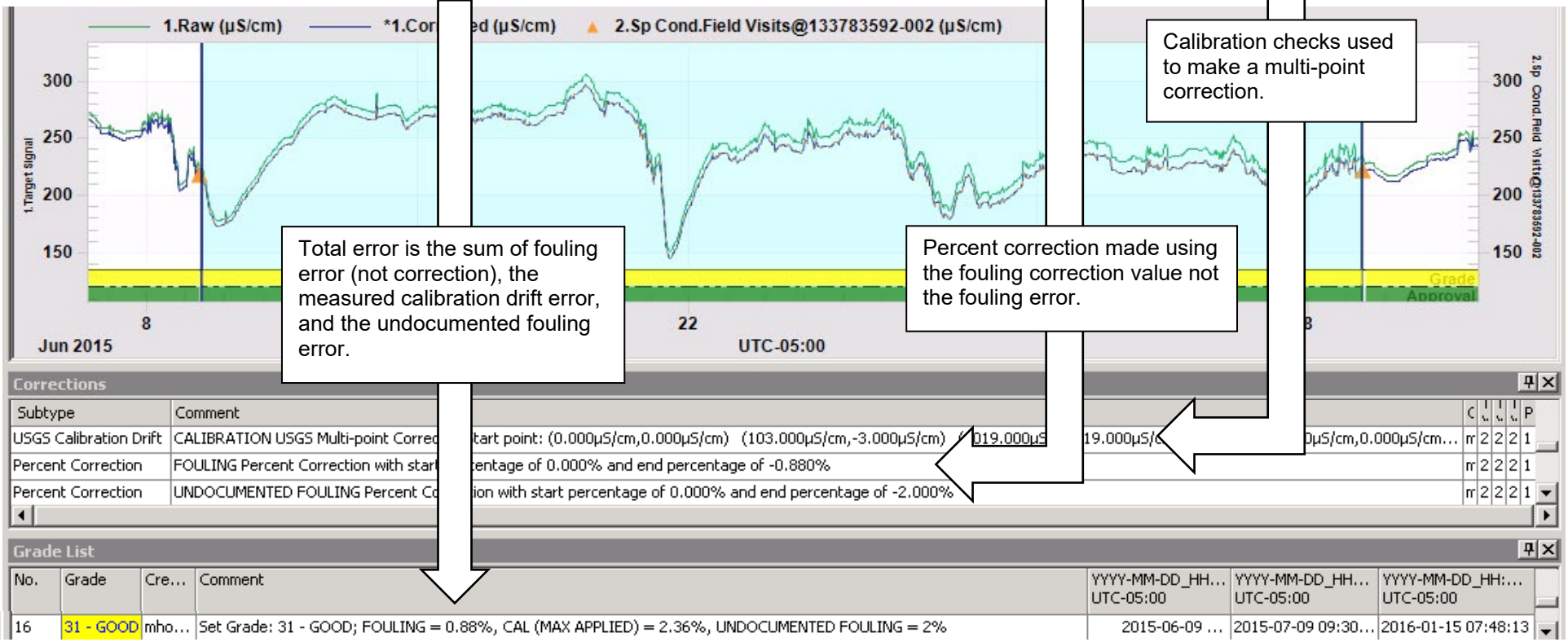

Figure 25. Corrections and grade applied to data from the Susquehanna River at Rockville for the period from 6/9/15 to 7/9/15. The upper section of the figure is taken from the Specific Conductance tab of the Field Form spreadsheet.

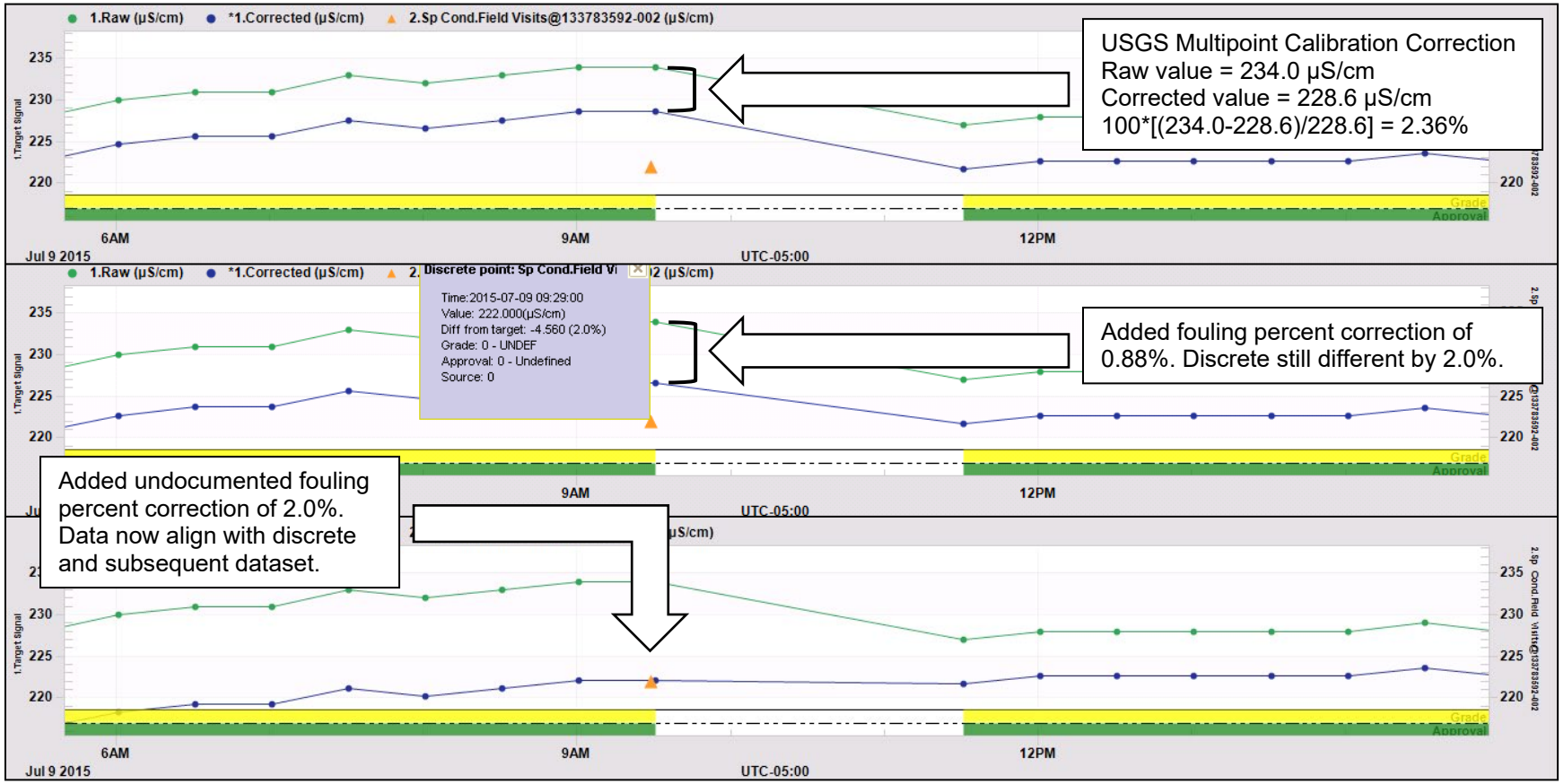

Figure 26. Series of corrections applied in Aquarius to data from the Susquehanna River at Rockville for the period from 6/9/15 to 7/9/15. The errors associated with these corrections are summed to determine the grade.

Final Approval

Final approval is the last step in the data management process that confirms the data by validating the corrections, grading, and decision-making processes of the individual originally responsible for the data. Final approval is typically conducted by individuals with the most experience with continuous instream monitoring. In Aquarius, approvals are applied using the Set Approval action in the data corrections tool box. Division of Water Quality uses two types of approvals, “Approved” and “In Review”. If a reviewer disagrees with, or has a question about a correction or grade, the section is marked “In Review” and the section is discussed with a third party. The third party helps come to a conclusion on the data management, at which time the data are then approved. Once data are approved (Figure 27), it may be placed into the report and used in analyses.

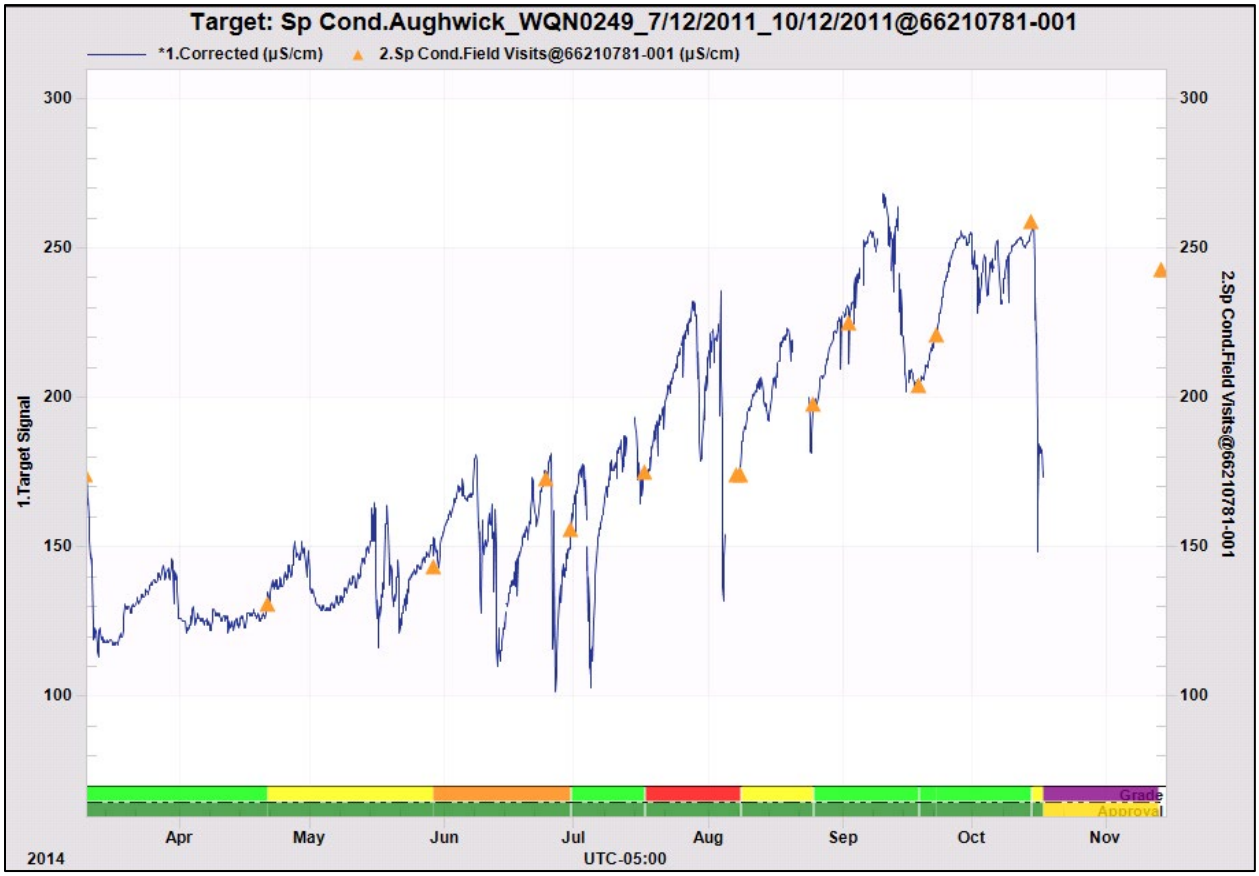

Figure 27. Specific conductance data at Aughwick Creek from 2014 displaying the grades and approval status within the Aquarius data correction tool box. The upper and lower rows of colored bars at the bottom represent grades and approvals, respectively. Because the last period was graded unusable (purple), the data were deleted. All sections were approved (green) except the last section. Since the final reviewer did not agree with the correction and/or grade, that period of data was marked “In Review” (yellow) until the discrepancy was resolved with a third party.

REPORTING

DEP's Water Quality Division compiles, interprets, and reports instream monitor data in Continuous Instream Monitoring Reports (CIMR). Continuous instream monitors produce large amounts of data that, without the proper software, can be difficult to manage, interpret, and report. Division of Water Quality staff uses both numeric and visual procedures to report on time series data. Reporting conventions for time series data often include the maximum, minimum, and median or mean for a measured value as well as graphical representation of the entire dataset. Data are made available to the public online after final review and approval are complete. Standard reporting units and resolutions are detailed in Table 8.

Table 8. Field parameter reporting conventions.

Field Parameter	Reporting Units	Reporting Resolution
Temperature	°C	0.01 °C
Specific Conductance	µS/cm	0.1 µS/cm
pH	Standard pH units	0.01 standard pH unit
Dissolved Oxygen Concentration	mg/L	0.01 mg/L
Dissolved Oxygen Saturation	%	0.1 %
Turbidity	FNU	0.1 FNU

Aquarius software is used for graphical representation of time series data. When possible, these graphs will incorporate at least one measured parameter with either a measured depth or a calculated discharge (Figure 28). Depth or discharge will often contextualize events of interest (i.e., sudden changes) within the dataset.

A great deal of significance is placed on the relationship of the five water quality properties and discharge variations, but other event-related changes are equally vital and can be factored into the relation only through historical measurements, field experience, and on-site observation. Some examples include but are not limited to changes in ambient temperature, periods of sustained cloud cover, chemical spills, increased photosynthetic production, increased wind conditions, sewage overflows, road construction, and ice formation. Understanding these relations is an essential element of accurate instream record management (Wagner 2006).

A CIMR not only includes time series data, it also contains any other associated data collected during the survey period. These data include, but are not limited to, chemical grab samples, macroinvertebrates, fish, and habitat assessments. When combined, these data provide a powerful assessment tool and excellent information on the aquatic environment.

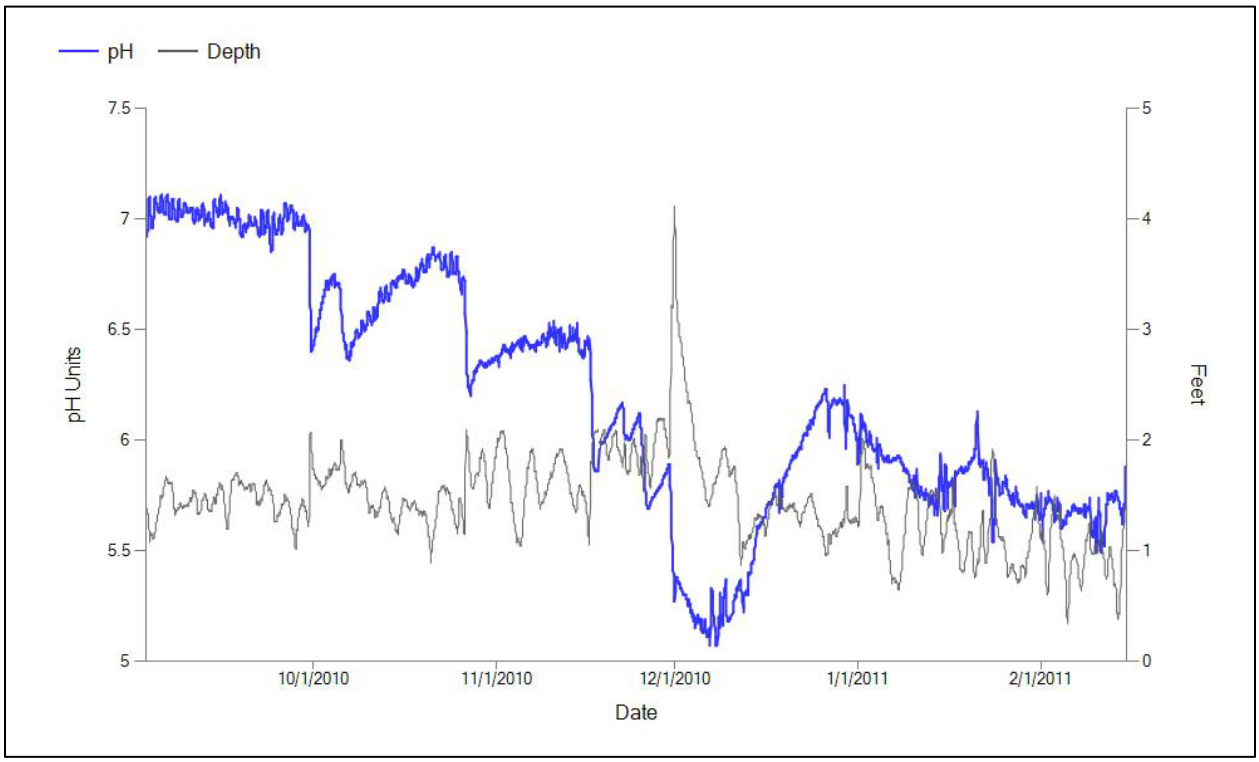

Figure 28. Depth and pH data for the East Branch Clarion River near the mouth of Gum Boot Run from September, 2010 to February, 2011. A critical depression in pH can be seen around the beginning of December, 2010. The depth time series helps identify and explain that the problem is most likely due to atmospheric deposition through precipitation.

ARCHIVING RECORDS

Once a report is written and reviewed it will be archived into digital stream files within DEP and made public online. Monitoring Section personnel within DEP are responsible for the collection, analysis, manipulation, and storage of instream monitoring data, and must ensure that the specified requirements of archiving electronic data are fulfilled.

In addition to electronic data, original water-quality monitoring data on paper may include field notes, field measurements, calibration notes, and service requests. Field notes and measurements should be included in the appropriate electronic Field Form for the site that is then archived in the network CIM folder (field measurements are also stored in Aquarius). Physical calibration logs are scanned and archived in the CIM folder and all documentation of equipment servicing is logged in the sonde management database. It is the responsibility of Monitoring Section to ensure that project files are organized, complete, and entered into the instream monitor archive. The archive should be well documented and maintained by appointed personnel in the Monitoring Section of DEP.

SUMMARY

This protocol offers guidelines for DEP personnel and others in site and monitor selection considerations, field inspection and calibration procedures, data evaluation and correction, and data reporting processes. Evolving sensor technology increases the variety of measurable chemical constituents, allows lower detection limits, and provides improved stability and accuracy. Protocol guidelines and quality assurance procedures will continue to be refined as equipment and software progress.

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4.4 SEDIMENT CHEMISTRY DATA COLLECTION PROTOCOL

Chapter 4 Chemical Data Collection Protocols

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INTRODUCTION

DEP strives to better understand environmental issues and systems through various forms of data collection. Water, macroinvertebrates, and fish are routinely collected to help determine a waterbody's health and analyze pollution sources and causes. Streambed sediment is another parameter that can explain a lot about a system.

Bed sediments can accumulate substances that may not be detectable in a single discrete water sample. They have the potential for accumulating a variety of trace elements and toxins. Additionally, they could re-enter the water column as suspended sediment, introducing those substances into the system and causing further problems downstream. It has been shown that bed and suspended sediments accumulate more trace metals than are normally present in the water column (Horowitz 1985).

Monitoring streambed and lakebed sediment parameters can assist in point and non-point source investigations, spill and complaint issues, and routine monitoring. Testing sediment could show if contaminants are present, such as high levels of metals, radionuclides, or organic compounds, and assist in making assessment decisions on the status of water quality standards (supporting or impaired) of flowing waterbodies.

This document provides guidelines for the standardized collection of streambed and lakebed sediment samples. The procedures described here are adapted from scientific, peer-reviewed procedures, and were developed, field tested, and implemented by DEP's technical experts. This protocol does not attempt to describe the entire spectrum of sediment sample collection techniques, and review of other documentation is encouraged depending on the specific sampling situation.

Because sampling situations vary largely, no single sediment sampling procedure can be universally recommended. This document describes sediment sampling procedures appropriate for typical DEP investigations and may require modification as situations dictate. Variations to this protocol will be dependent upon site conditions, equipment limitations, or limitations imposed by the procedure. Investigators should document modifications and report the final procedures and equipment employed.

Investigators should be aware of, and work to prevent, the potential for sample contamination at all phases of the sample collection process by observing proper sample collection, handling, and preservation procedures described here. The most common sources of error (also known as "interference") are cross-contamination and improper sample collection and preservation. Parameter-specific equipment requirements, sample collection, labeling, and storage will be covered in subsequent sections below.

SAMPLING CONSIDERATIONS

Determining sample design is one of the most important parts of a field collection plan. It is necessary to determine whether a statistical or judgment-based approach is desired. This depends substantially on the project questions – for example, whether or not background samples are being collected, or a spill or discharge effect is being investigated. See *Chapter 2, Sampling Design and Planning* (Lookenbill et al. 2023) for suggested sampling plans.

During or after completing a sampling plan, the collector should perform field reconnaissance of the site(s). Ease of site access, stream flow, and sediment deposition locations should be reviewed. One should ideally avoid sampling sediment immediately after a significant precipitation event where runoff from the surrounding land could occur (Shelton and Capel 1994). Manmade structures, such as bridges, should be noted and sampling immediately downstream of them should be avoided. Runoff from structures can easily impact surrounding sediment downstream. Point and non-point sources of pollution should be noted. Field reconnaissance is also an opportunity to determine necessary equipment for sediment collection, such as dredges versus scoops. Additionally, it can assist in determining locations where it is most likely for sediment to deposit and where concentrations of contaminants could be highest. Locations where sediment can accumulate, and therefore accumulate contaminants, include areas with aquatic vegetation, where velocity decreases, and inside bends in the stream (Oak Ridge Associated Universities (ORAU) Training 2012). Sediment can also accumulate near man-made structures, but again, care must be taken to ensure sediment in those locations is of interest to the project and not a result of surrounding land runoff if that is not the target.

Sampling Supplies and Equipment

DEP programs can and do employ a variety of program-specific sediment sampling techniques that require a multitude of supplies (sampling bottles, bags, etc.), and can include specialized equipment (En Core™ soil corer, Ponar dredge, etc.). This section describes equipment and supplies required to employ the most commonly used sampling techniques and does not include all streambed sediment sampling techniques that could be employed. Additional techniques may be added as they become applicable and as standard procedures are solidified. Much of the equipment used to sample soils can be used to sample sediments as long as sediment is not lost in the water column on the way to the surface; sampling equipment will be determined by water depth, velocity, and other physical parameters. Recommended sampling equipment and other gear are listed in the below check list:

Equipment:
☐ spatulas/trowels/scoops (plastic = metals collecting; stainless steel = organics collecting)
☐ dredges (e.g., Eckman, Ponar)
☐ corers with vials (e.g., En Core™)

Sample containers:
☐ 500 mL plastic sample bottles – trace elements
☐ 500 mL amber glass sample bottles – organics
☐ 40 mL amber glass vials – organics moisture determination
☐ plastic bags, 4 Mil thickness - radiological samples

Sample processing:
☐ compositing bowl(s) (plastic = metals collecting; stainless steel = organics collecting)
☐ sieve(s) - < 63 micron
☐ cloth mesh (disposable sieve)

Other
☐ brushes
☐ Munsell color chart

Equipment cleaning:
☐ wash bottle/deionized water
☐ detergent (0.2% phosphate-free)
☐ acetone (to rinse organics equipment)
☐ methanol (to rinse organics equipment)
☐ hexane (to rinse organics equipment)
☐ 5% HCl (to rinse trace elements equipment)
☐ NoCount® (radioactive decontaminant)

Forms
☐ sediment field forms
☐ laboratory sediment chem. sheets

Shipping
☐ courier shipping forms
☐ tape & dispenser

Shipping (cont.)
☐ ice
☐ coolers

Misc.
☐ hip boots
☐ waders
☐ nitrile gloves
☐ markers (black Sharpies), pens, & pencils
☐ GPS/maps
☐ Ziploc® bags (for bottles and/or sample submission sheets)
☐ field meter (e.g., YSI), calibration solutions, spare batteries
☐ calculator
☐ insect repellent
☐ screwdriver/tools
☐ batteries (D-cell, other:_____)
☐ other:
_____

The numbers and types of sample bottles field staff need for one sediment sample depend on the specific SAC or suite. Sample bottle and preservative requirements can be obtained by contacting DEP's Bureau of Laboratories (BOL) directly.

The choice to use scoops/spatulas, corers, or dredges depends heavily on-site conditions and the specific project requirements. Hand-held scoops and spatulas are convenient for shallow, slow-moving, wadeable streams. They are easy to use and clean. Unfortunately, with this procedure, it can

be easy to lose fine sediment, which is desirable for most tests. Additionally, it can be more difficult to gather in deeper water, and if contamination is a concern, it increases the likelihood of skin contact with material. Deeper and faster-moving water may require the use of a hand-held or mechanical dredge, such as an Eckman or Ponar. The use of a boat may be required or, alternatively, dredges can often be lowered from bridges. However, the project aim should be considered in choosing where to lower a dredge from; runoff from the bridge may or may not be desired. These are convenient ways to gather bed sediment when the bottom cannot be manually reached, but a downside is that it is easy to gather too much deeper sediment, and there is far less control over where and what is actually collected. If historical sediment information is desired, corers are an option. These range from large, cumbersome varieties to small, handheld corers, such as those used for volatile organic compounds (e.g., En Core™). In all cases, excessive ornamentation or wooden parts are discouraged to permit easy and thorough decontamination (USEPA 2012). Collectors are encouraged to seek guidance on the variety of dredges and corers available from the literature at the end of this document.

Some chemical analyses require laboratory technicians to calibrate specialized laboratory equipment, prepare specialized reagents, or otherwise perform pre-analytical preparation before samples can be analyzed. If a collector is going to submit several samples involving specialized preparation, such as allowing radionuclide samples to ingrow, he or she should contact the appropriate technician at the laboratory to ensure enough time is allocated for the pre-test procedures. Additionally, large quantities of samples should always be pre-authorized with the laboratory to avoid surpassing holding times.

DATA COLLECTION

General Considerations

Collectors need to first ensure they have formed an adequate sampling plan that will be representative of the system under investigation. Care must be utilized during collection to reduce contamination from outside sources and maximize the integrity of the sample. The most common causes of sample interference during collection include poor sample-handling and preservation techniques, input from atmospheric sources, and contaminated equipment or reagents. Each sampling site needs to be selected and sampled in a manner that minimizes bias caused by the collection process and that best represents the intended environmental conditions at the time of sampling.

As stated above, the lab should be contacted prior to large or unique sampling events. Previous weather conditions and discharge may be analyzed to ensure samples are being collected with the characteristics required to answer the problem in question. Large precipitation events resulting in high discharge and runoff are not the best time to sample to judge maximum sediment contamination from a point source discharge. In addition, rapid water velocities and increased turbidity make sediment collection with traditional trowels difficult. Discharge data for continuous monitors throughout the state can be found on the United States Geological Survey (USGS) website. Equipment should be

gathered, and a checklist made prior to departure. Once at the site, before handling sample containers, the collector should ensure his or her hands are clean and not contaminated from sources such as food, coins, fuels, mud, insect repellent, sunscreen, sweat, or nicotine. Gloves should be worn for all sediment sampling due to the high possibility of sample contamination. Unlike water samples, sediment samples are handled quite a bit more and there are many more opportunities to introduce contaminants.

For most sediment sampling, the surficial 1 to 6 cm of sediment with grain sizes < 0.06 mm (silts and clays) are desired. It is best to avoid sands (0.06 – 2 mm) and larger grain sizes. Fine grained particles tend to accumulate more trace elements than coarser particles. This is due to a number of factors, including higher available surface area on finer particles (Horowitz 1985). For some organics analyses, grain size < 2.0 mm (sand, silt, clay) is acceptable (Shelton and Capel 1994). In general, the way to distinguish between sand, silt, and clay is displayed in Table 1.

Table 1. Sediment grain sizes. *Adapted from:* Ohio EPA. 2001. Sediment sampling guide and methodologies. 2nd edition.

Soil Type	Grain Size	Description
Sand	0.06 – 2.0 mm	gritty, non-plastic, loose particulates
Silt	0.004 – 0.06 mm	smooth, talc-like, non-plastic, loose particulates
Clay	< 0.004 mm	dense, moldable like putty, cohesive

If a site contains large amounts of sandy material, sieving may be necessary if the aim is to determine the highest concentrations present. In general, it is a good idea to make sure the sample contains > 30% fine material, i.e., silts and clays (Ohio Environmental Protection Agency 2001).

All equipment is cleaned prior to being used. It is advisable to first wash and soak it for 30 minutes in a 0.2% phosphate-free detergent (Shelton and Capel 1994). After soaking, rinse the equipment thoroughly in deionized water, air dry (if possible), and store in individual, sealable containers or bags. If sampling for organic contaminants, clean the stainless steel or Teflon™ equipment with soap and tap water, rinse with deionized water, dry, rinse with acetone, and, lastly, rinse with hexane (as recommended by DEP BOL staff). Wrap organics equipment in aluminum foil. It is highly advised to have several sets of equipment, particularly if planning to sample reference (background) and impacted sites in one trip. It is difficult to fully clean equipment in the field. If doing so, save the rinsate and dispose of it properly. It is also advisable to occasionally collect a rinsate blank, which is obtained by running blank water through or over the equipment used to collect sediment.

Sampling Procedures

First, calibrate any field sampling equipment, such as field meters according to *Chapter 4.1, In-Situ Field Meter, Transect, and Vertical Profile Data Collection Protocol* (Hoger and Arnold 2023). If dealing with a possibly impacted site and reference location(s) have been chosen, sample reference

locations first if combining impacted and reference sites into one trip. This will help lessen the possibility of contamination between sites. It is important to note that safety is always first – avoid wading into high flow areas or locations where slippage could occur, wear appropriate wading gear, and have the proper length and type of gloves on if dealing with impacted sites or chemicals.

If sampling is taking place due to a point-source pollution incident, it is advised to sample downstream of the discharge, at the discharge, and upstream of the discharge, at minimum. In order to collect a sample representative of a particular location, plan to collect at between 5 to 10 depositional zones within a study site and composite those into one sample (Shelton and Capel 1994). For example, if collecting at a point of acid mine discharge (AMD), composite sediment at between 5 to 10 depositional zones downstream of the discharge, composite sediment at between 5 to 10 depositional zones at the discharge, and composite sediment at between 5 to 10 depositional zones upstream of the discharge. Compositing several “zones” of sediment will allow for a more representative description of the sample area and will not target one spot. Additionally, due to lack of sediment buildup at some locations, compositing several spots of sediment buildup facilitates obtaining only the first 1 to 6 cm of sediment. Of course, specific project needs may deviate from this, so plan and document the study accordingly. Additionally, composite sampling is *not* appropriate for the collection of volatile organics samples because compounds could be lost.

Sampling stations located upstream of the discharge pipe should be in non-impacted areas to serve as controls. If there are multiple discharges, then sample stations should be placed to bracket individual discharges in order to better characterize each source. For sampling downstream of the discharge pipe, if the investigator is interested in determining the downstream point where the discharge ceases to be an effect in the sediment, the investigator should avoid the immediate vicinity of the discharge/influence point and select a sample point far enough downstream to allow for mixing between the discharge and stream flow. Sampling can occur at any noted deposition points between in order to characterize effects of the discharge. Depending on stream size, flow, velocity of the plume, and angle at which it enters the stream, solids may deposit at a variety of locations.

As a summary, composite-sampling 5 to 10 depositional zones in a single location (upstream of a discharge, for example) is recommended in most cases. In addition, the sampling reach is discretionary and based upon site-specific characteristics and questions being answered. Regardless of the technique implemented, similar sampling procedures should be used if sampling several locations in one single study, to allow for comparability of data.

At each site, always collect water samples first to avoid stirring up sediment that could contaminate the water sample. Take any water field measurements before sediment sampling. Additionally, always work in a downstream to upstream fashion at a site. Lastly, always document the appearance, odor, sheen, and feel of the sediment on the ‘Sediment Field Data Collection Sheet’ (see Appendix B.4).

Wadeable Protocol

Gather the equipment (scoop, appropriate sampling container [see parameter-specific instructions, below], gloves) and enter stream at the most downstream of the sampling locations. If interested in surficial, recent deposition, sample only the top 1 – 6 cm of sediment; if interested in historical sediment accumulation, sample > 6 cm. Facing upstream, rinse the sampling container several times with native water, emptying it behind you (downstream) to avoid stirring up any additional sediment. In addition, rinse all sampling equipment in native water as well, including scoops, sieves, and bowls. Enter water column at the first sediment depositional area, using care to gather upstream of the current standing position and not stepping where collection will occur, and gather a scoopful of sediment. Slowly raise the scoop out of the water to avoid losing fine material and place sediment into sampling container. Move to the next depositional zone and repeat, working from downstream to upstream. If too much water is collected, carefully decant as it is collected, trying to allow it to settle first as much as possible to avoid loss of fine materials. Additionally, avoid collecting vegetation or other debris. The lab needs to pulverize samples before analysis, so the finer sediment and the less debris collected, the better.

Once enough sediment is collected, exit the stream and empty the sample into an appropriate compositing container. Remove any large pebbles, sticks, vegetation, or other debris. Allow the sediment to settle and carefully decant the water off, or, alternatively, sieve the sample to ensure only fine grained materials are collected. Generally, if one sample is sieved in a project, all samples in that project should be sieved for consistency. In any case, fully note on the field sheet the texture, color, and odor of the sediment. Homogenize the sample using a spatula or trowel. One recommendation to fully homogenize is to “quarter” the sample into four parts, mix those parts, and then mix the entire sample together (USEPA 2012). Place sample back into the sampling container and record all details about the site and collection procedures on the field sheet. Add fixative to the sample, if required. If refrigeration is necessary, place the sample in a cooler with ice in order to cool the sample to 4°C. Do not use dry ice because the sample may freeze. Record GPS coordinates of the location and take photographs, if necessary.

Corers may be necessary if the interest is in collecting historical sediment or volatile organic compounds that cannot be exposed to air. Coring allows stratification of the sediment and the possibility of testing individual layers. A corer is pushed into the sediment and the core is captured in the sample tube (ORAU Training 2012). They can be particularly useful in fast-moving streams where grab samplers would be difficult to use. They come in a variety of shapes and sizes for different needs. Many include a valve or catcher that will prevent loss of the core as it is being brought to the surface. The DEP BOL requires the use of an En Core™ corer (or similar) or a corer using pre-preserved (methanol) vials for volatile organic compound soil and sediment samples. If it is not possible to use these types of corers, a note must be made on the “Sample Submission Sheet” or a data release form will be sent from DEP BOL. USEPA has a detailed description on how to collect volatile organic compounds in USEPA Test Method 5035A (2002) - Appendix A. Refer to the DEP BOL for more information regarding volatile organic compound collecting.

To collect sediment with a corer, either push or hammer it into the sediment (ORAU Training 2012). Be sure to push it straight down and pull it straight up without turning it, and do not fill the tube completely. Keep the sampler vertical when pulled out of the sediment to avoid mixing. Cap the bottom of the sample tube as soon as possible, then the top. If there is some water left in the tube, carefully decant it off and stuff a cloth into the end – this reduces mixing. Augers, often used for soil, can be used for collecting sediment and are turned and pushed into sediment; however, sample loss could be an issue. Refer to the references in the "Literature Cited" section of this document for further information on using a variety of corers, augers, and dredges to collect streambed sediment.

Nonwadeable Protocol

If the water flow is too deep and/or fast moving to manually collect sediment, consider using a dredge. These can either be lowered from a boat or a structure such as a bridge. Depending on size, they can be manually lowered or lowered using a winch. If boating, turn off the engine before using the dredge to eliminate impact on the sample. If an anchor is used, make sure dredging is not collecting the sediment that the anchor disturbed.

Once at the desired location, set the dredge according to user instructions and carefully lower it to the stream or lake bottom. Trip the dredge, and slowly bring it to the surface. Avoid lowering and raising it too fast, which risks disturbing the sediment before it reaches the bottom or losing sediment on the way up. Once at the surface, examine the sample and determine if the dredge was properly tripped; if it was not, discard the sediment and re-sample. Dispense the sample in an appropriate compositing container, collect the rest of the depositional zones, and follow the instructions above for decanting/sieving and compositing the sample. See the references in the "Literature Cited" section of this document for further information on using a variety of dredges to collect streambed sediment.

Parameter-Specific Considerations: Radionuclides

Stainless steel, high-density polypropylene (HDPP), or polyethylene (HDPE) sampling equipment is recommended for sampling for radionuclides (USEPA 2012). Sampling containers should also be made of the same plastics and have a polytetrafluoroethylene (PTFE) or Teflon™ lined lid (USEPA 2012). Verify that the lid will not absorb any water. Stainless steel or plastic compositing containers are recommended. If testing for gamma-emitting radioisotopes, bags are recommended due to large sample volume. DEP BOL recommends collecting one (1) kg of wet sediment in order to verify having enough sample to analyze ½ kg of dry sediment in the lab. Two 500-mL bottles are also a sufficient amount of sample for analysis. Double or triple-bagging samples is highly recommended. Properly close the bags and use a non-absorbing tape to seal. Gamma-emitting radioisotope sediment samples do not require refrigeration or preservatives. The sample holding time is 72 hours if iodine analysis is needed, otherwise it is 6 months. For other tests and details, contact the DEP BOL.

Parameter-Specific Considerations: Metals

High-density polypropylene (HDPP) or polyethylene (HDPE) sampling equipment is recommended; avoid metal sampling equipment whenever possible. There is always the chance of metals in the equipment influencing sample results. Plastic compositing containers are recommended. Most

standard plastic water-sampling bottles can be used to store sediment for metals testing. A typical sediment metals sample at DEP BOL requires a 500-mL plastic or glass container. No preservatives are required and refrigeration to 4°C is necessary. Holding time is 6 months. Contact the DEP BOL for specific parameter details.

Parameter-Specific Considerations: Volatile & Semi-Volatile Organic Compounds

As stated above, DEP BOL requires samples for volatile organics to be collected with corers. There are two coring procedures available: The first is the En Core™ - for each analysis, 2 x 5g En Core™ vials are needed, plus one (1) 40mL amber glass vial, to be used for moisture determination. There is also a 25g size available, but this is not recommended and will also require a data release form. A second procedure uses a small, disposable corer that contains pre-preserved (5mL of methanol), pre-weighed vials. Weight is recorded on the outside of the vial. The core is collected, the sample is ejected into the vial, and the vial is quickly capped. The vial is not to be packed full and care should be used to avoid losing methanol. The threads of the vial should be cleaned before capping so methanol does not leak out. Do not attach additional labels to the vial since this will change the weight of the vial. Two pre-preserved vials and one (1) 40mL amber glass vial are required for analysis. Only “high concentration” vials are required.

If unable to use these types of samplers, specifically state the reasons why on the “Sample Submission Sheet” or a “Client Request for Data Release” form will be sent. In the instance of not using a core sampler, volatile organics samples need to be packed tightly in 2 x 40mL amber bottles (unpreserved). A recommended procedure to avoid air contact is to take a scoop of sediment, remove the top layer in the scoop, and immediately fill the vial with sediment from the scoop. Samples have a holding time of 48 hours using the En Core™ and must be received by the lab within 24 hours after collection. Samples collected and preserved with methanol have a holding time of 14 days. See USEPA Test Method 5035A (2002) - Appendix A for more details.

If testing for semi-volatile organic compounds, fill a 500mL amber glass sampling jar. Volatile and semi-volatile organics sediment samples need to be refrigerated to 4°C and do not require field preservation, unless collecting volatile organics in the pre-preserved vials. When possible, *always* schedule with the lab before collecting samples for organic analyses.

Labeling

While the ***minimum requirement*** is the collector number and sequential sample number, collectors are encouraged to add date and time collected, general test(s) description (total metals, etc.), and preservation indication. This will help prevent confusing what bottles are from which tests and to help ensure the sample is properly preserved and stored. Labeling should be done so that at least 1” of space is left at the top of the bottle to allow BOL to apply lab labels. For details regarding labeling and sample information requirements see *Chapter 4.2, Discrete Water Chemistry Data Collection Protocol* (Shull and Arnold 2023).

Permanent marker will rub off HDPE bottles during collection and transport. Therefore, clear packing tape should be wrapped around the bottle to protect hand-written labels. BOL discourages the use of masking tape. Collectors should keep a log book of all samples they take, and should not re-use sequential numbers in order to avoid confusion. The sample log should annotate the unique collector identification and sample number, date and time, the waterbody name, sample location, Standard Analysis Code (SAC), and any additional analytical tests performed or excluded.

Quality Assurance

Quality assurance sampling follows the same guidelines as described in *Chapter 4.2, Discrete Water Chemistry Data Collection* Protocol (Shull and Arnold 2023) and Appendix E. Additionally, “rinsate blanks” may be necessary at times if a highly-impacted site was sampled. After cleaning equipment, a sample of the cleaning rinsate water is saved and analyzed for parameters of concern (USEPA 2012). This verifies whether or not the equipment was properly cleaned. DEP BOL does not require sediment or soil blanks, although they can be submitted. Normally these are purified sand or water.

Post-Sampling Decontamination

If sampling multiple locations in one trip, try to have multiple sets of equipment, since decontamination in the field can be difficult. If only one set of equipment is available and multiple locations must be sampled, decontamination must occur in the field. Whether completed in the field or back at the office, wash all used equipment first in phosphate-free detergent. Brushes are helpful for removing caked-on soil. Rinse thoroughly. Then, rinse all non-plastic equipment with methanol. Rinse non-metallic equipment, such as those used to sample metals, with a dilute acid, such as 5% HCl (Radtke 2005, Shelton and Capel 1994). It may be necessary to rinse equipment used to sample for radioisotopes with a radioactive decontaminant, such as NoCount®. Afterwards, rinse all equipment thoroughly with deionized water and allow to air dry (if possible). Equipment that is used to sample for organic contaminants should be rinsed with acetone and then hexane, rather than methanol or acid, after the soap and water wash. Discard disposable equipment rather than clean it. Always be sure to store contaminated/dirty equipment separately from unused/clean equipment. Store equipment in individual, sealable containers or plastic bags. Store equipment used to sample organics in aluminum foil.

Sample Submission

Samples need to be shipped or delivered to the lab as soon as possible. Sediment samples being shipped follow the same procedures described in *Chapter 4.2, Discrete Water Chemistry Date Collection Protocol* (Shull and Arnold 2023).The collector should understand that certain laboratory analyses have “holding times” during which tests must be conducted for result validity. Volatile organics, for example, must be received by the laboratory within 24 hours after collection. If a sample surpasses holding time requirements the results will not be reported unless a “Client Request for Data Release” form (see the BOL website) is submitted to the Bureau of Laboratories. *It is not advisable to collect and ship samples on Fridays*, as the laboratory does not operate on weekends; samples shipped on Friday will not be received and logged until Monday morning. Collectors essentially need

to plan their sampling from Monday through Thursday and verify the samples will reach DEP BOL by early Friday morning at the latest. The days before holidays will also need to be considered.
2023

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4.5 PASSIVE WATER CHEMISTRY DATA COLLECTION PROTOCOL

Chapter 4 Chemical Data Collection Protocols

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INTRODUCTION

The Pennsylvania Department of Environmental Protection (DEP) often utilizes unique and innovative techniques to explore environmental issues and determine if environmental problems exist. One technique that has been employed in recent years is the use of passive sampling devices to collect water quality samples of low-level environmental contaminants.

Some contaminants, such as hormones and other endocrine-disrupting compounds (EDCs), can have detrimental effects on aquatic life at extremely low levels (in the ng/L range). In addition, it is very easy to miss a time period where a low-level compound may be detected, which a passive sampler would capture. Therefore, using a sampling device to collect compounds in a large volume of water over a period of time (days) is a great way to collect low-level or sporadically-present compounds.

Although this document refers to the use of passive samplers in surface water, they are also used to sample groundwater and air. There are many types of passive water samplers available today. The samplers employed by the Water Quality Division have been the Semi-Permeable Membrane Device (SPMD) and the Polar Organic Chemical Integrative Sampler (POCIS). SPMDs are used for sampling neutral organic compounds that have a log octanol-water partition coefficient (K_{ow}) that is greater than 3 (Alvarez 2010). Examples of compounds that can be sampled with SPMDs are organochlorine pesticides, polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs), and polycyclic aromatic hydrocarbons (PAHs). A POCIS is used to sample water soluble organic compounds with a log K_{ow} less than 3. Examples of compounds that can be sampled with POCIS are various wastewater indicators, many currently-used pesticides, pharmaceuticals, and hormones.

This document provides guidelines for the standardized collection of passive water samples from flowing waterbody systems. The procedures described here are adapted from scientific, peer-reviewed procedures, and were developed, field tested, and implemented by DEP's technical experts. This protocol does not attempt to describe the entire spectrum of passive sample collection techniques, and review of other documentation is encouraged depending upon the specific sampling situation.

Because sampling situations vary largely, no single passive sampling procedure can be universally recommended. This document describes passive sampling procedures appropriate for typical DEP investigations and may require modifications as situations dictate. Variations to this protocol will be dependent upon site conditions, equipment limitations, or limitations imposed by the procedure. Investigators should document modifications and report the final procedures and equipment employed.

Investigators should be aware of, and work to prevent, the potential for sample contamination at all phases of the sample collection process by observing proper sample collection, handling, and

preservation procedures described here. The most common sources of error (also known as "interference") are cross-contamination and improper sample collection and preservation.

SAMPLING CONSIDERATIONS

Sampling Supplies

This section describes equipment and supplies required to employ POCIS and SPMD sampling techniques and does not include all passive water sampling techniques that could be employed. Additional techniques and equipment may be added as they become applicable and as standard procedures are solidified or altered. Very little equipment is needed to deploy a passive sampler; the sampler, a canister or holder for it, gloves, and a securing pin are all that is necessary. The following supplies are recommended:

- Passive sampler(s), already assembled and in a tin until deployment in the field
- Canister or other holder for passive sampler(s)
- Nitrile Gloves (material cannot be composed of any compounds to be tested)
- Stake/pin for securing in streambed
- Carabineers and eye bolts for securing passive sampler holder to stake/pin
- Sledge hammer

Samplers should be pre-assembled and stored in a tin or other container in a refrigerator (POCIS) or freezer (SPMD or POCIS). New paint can tins are perfect containers because they seal tightly; no air should be allowed to escape or get in. Sampler contamination pre-deployment could be an issue; therefore, assemblage in a sterile environment is necessary.

Factors such as temperature and biofilm buildup can affect the samplers' ability to collect chemicals. Performance reference compounds (PRCs) can help alleviate this problem (Alvarez 2010). These are compounds added to the samplers pre-deployment, and are measured after deployment to see how much chemical is lost. Adjustments can then be made to targeted chemical measurements based on the amount of loss measured in the PRCs. Photo-sensitive PRCs can also be used if the compounds of interest are prone to photo-degradation and the samplers will be exposed to direct sunlight.

Field blanks are also required. Blanks are just regular samplers that will be exposed to air in the field while the passive samplers are being deployed and retrieved from the water. Any air contamination will be picked up and measured by these blanks. Air contamination is common, particularly with SPMDs, depending on the compounds measured. Industrial areas and cities may produce airborne contaminants that are easily picked up by SPMDs. The number of blanks implemented is up to the collector's discretion – DEP normally uses one blank per three sites/deployments, but more blanks are always optimal. The only issue with this procedure is that any contamination that results will be unable to be pinpointed to any one site or sampler.

Analysis Lab(s)

Since many of the compounds that may be analyzed by passive water samplers are emerging contaminants, there are a limited number of labs that may be able to analyze extracts. DEP BOL may be able to perform some analyses. Past analyses that have been performed by BOL on passive sampler extracts include PAHs, pesticides, and PCBs. DEP will also use USGS and private laboratories to analyze additional parameters.

Site Selection and Sample Design

A sample collection plan is recommended before commencing a sampling project. Sampling design procedures are described in *Chapter 2, Sampling Design and Planning* (Lookenbill et al. 2023). Plans should include the location(s) to be sampled, reasons for sampling, number of samples, media of interest (water, sediment, soil, macroinvertebrates, etc.), parameters to sample, QA/QC plans, equipment, preservatives, sample container types, estimated costs, maps, and any other notes or comments pertaining to the project. More information on developing Project Plans can be found in USEPA 2002a, USEPA 2002b, and USEPA 2006. It can be very helpful and more descriptive of a site to take grab water samples, at a minimum, in conjunction with sediment sampling. Macroinvertebrate and fish samples can also help describe overall impacts to a system regardless of the chemicals of interest or the problem being investigated.

Prior to sampling, any historical information, aerial photography, and/or topographic maps of the locations should be gathered and analyzed. Geomorphology and underlying geology around the waterbody may be helpful in determining parameters of interest. Point and non-point source pollution possibilities should be documented. Past water quality and/or biological monitoring results should be gathered. Additionally, it should be noted whether sampling locations are in close proximity to gaging stations or other continuous monitoring stations – this data could prove useful during final data analysis.

Determining sample design is one of the most important parts of a field collection plan. It is necessary to determine whether a statistical or judgment-based approach is desired. This depends substantially on the project questions – for example, whether or not background samples are being collected, or a spill or discharge effect is being investigated. Many sampling design options exist, with many options being statistical in nature. These include systemic sampling, where the distance between sampling locations is kept consistent (Horowitz 1985). This is not appropriate if a known contaminant is present in an area of sediment. Another statistical approach is random sampling, where sample locations are arbitrarily selected so that one area is not more likely to be chosen over another. If the area is homogenous, this is recommended (Horowitz 1985). Stratified random sampling is another option. This involves dividing a target area that may have a contaminant or area of interest into several areas that are homogenous. Random sample sites are then chosen within those areas. This helps remove heterogeneity at a site (Horowitz 1985). A non-statistical design approach is judgmental or targeted sampling – sampling sites are chosen based on professional judgment, *not* scientific theory. This is a good approach to use for sites where a known contaminant is present (i.e., discharge pipe) and can be extremely resource-efficient if there is a lack of funds or equipment.

It can be helpful to anticipate how the data will be used. Consider if it will impact legal or regulatory actions, and how the data will be analyzed and presented. Also consider what criteria may be used during its interpretation. DEP does not have narrative or numeric passive sampler criteria at this time, but does have numeric water quality criteria for many chemicals that may be sampled. One can refer to 25 Pa. Code Chapter 93 for details. One thing to keep in mind is that these chemicals are being sampled over a long period of time, normally at least a month, so the criteria should not be applied as written since they are normally based on grab samples.

Some SACs/suites are available for passive sampler extract analysis from DEP BOL. Contact DEP BOL organic section for more details and to set up a sample submission schedule.

During or after completing a sample collection plan, the collector should perform field reconnaissance of the site(s). It's a good idea to view the site or deploy at a lower flow, particularly if rain or increased flow is expected in the weeks after deployment. If samplers are deployed at high flow, low flow levels should be approximately known. Samplers cannot be exposed to the air during low flow conditions or sample results will be compromised.

DATA COLLECTION

General Considerations

Collectors need to first ensure they have formed an adequate sampling plan that will be representative of the system under investigation. Care must be utilized during collection to reduce contamination from outside sources and maximize the integrity of the sample. The most common causes of sample interference during passive sampler collection include input from atmospheric sources and contaminated equipment. Each sampling site needs to be selected and sampled in a manner that minimizes bias caused by the collection process and that best represents the intended environmental conditions at the time of sampling.

Samplers should be stored in their containers under refrigerated or frozen conditions until deployment. SPMDs must be kept frozen (to -20°C, or the temperature that the laboratory requires) until deployment. POCIS can be stored at ambient temperature for up to two weeks until deployment; any longer than that and they should be frozen (to -20°C, or the temperature that the laboratory requires). Once in the field, nitrile gloves should be put on before sample containers are opened. Blank samplers should be opened and exposed to air. For stream deployments, samplers should be placed in canisters/holders in a timely fashion and moved to the water where stakes have been pre-set. Samplers should be fastened to stakes using carabineers, with careful attention paid to how noticeable the canisters are. Canisters can be supported with rocks but care should be used to avoid covering them entirely so that water flow is still possible. Samplers should not be placed directly in areas of large sediment deposits, if possible, or sediment will clog the filters/membranes. If the samplers are too visible from shore or from bridges, tampering of the equipment could occur. Samplers also cannot be exposed to air during periods of low flow – lowest possible flow during the time period of deployment should be anticipated. Stakes should be very securely hammered into

sediment, or high flows could loosen samplers. For lake deployments a buoy and anchored line system should be used to deploy passive samplers. Samplers will be placed along the anchored buoy system at the desired depth, typically 1 meter below the surface in order to characterize surface water quality. Blanks should be tightly closed after deployment and stored frozen until sampler retrieval. They should be labeled appropriately so they are used at the same site(s) during retrieval.

The number of passive samplers needed for any one sample will depend on the analyses desired (Alvarez 2010). Discuss this with the analysis lab(s) and equipment lab(s).

Date and time of deployment, as well as location site conditions (such as pH, temperature, conductivity, and flow) should be noted. Passive samplers are designed to be deployed for days to months, although 30 days is the average time (Alvarez 2010). Short deployments reduce the advantages of using passive samplers; too long of a deployment could compromise the data. DEP normally deploys passive samplers for around 30 days, or one month. At the time of retrieval, date, time, and field conditions should be noted again. The same blanks used during deployment should be air-exposed and samplers should be removed from the water with the same caution as deployment. If available, new blanks could be used. The passive samplers should be placed immediately into containers that remained sealed during deployment (to avoid contamination). Samplers should then be frozen until received by the lab. Samplers should be extracted as soon as possible or mailed overnight on ice (packs, not loose ice, to avoid water contamination as the ice melts) to a lab for extraction. Analysis should occur in the specified timeframe of holding times of the compounds of interest.

Labeling

Container labeling is not necessary until samplers are retrieved. Be sure to put POCIS in POCIS tins and SPMDs in SPMD tins since solvents may remain in the tins. Once they are retrieved, label the containers with sampling locations, dates, and times. Label blanks accordingly.

Quality Assurance

Because of the expense of deploying passive samplers, DEP does not often do duplicates as it would other samples; however, at least one duplicate per set of around 10 is recommended. Blanks are described above.

Post-Sampling Decontamination of Equipment

After deployment, passive sampler canisters/holders need to be decontaminated. They should be scrubbed with non-ionized soap and water to remove sediments and organic contaminants. If organic compounds were sampled, containers should be rinsed with acetone to remove any water and then rinsed with hexane (Environmental Sampling Technologies SOP E-13). Soaking canisters briefly in a solution of hydrochloric acid (2-4N) with hot water will remove rust and calcium.

Sample Submission

Holding times depend on the compounds analyzed. Discuss with the analysis lab(s) regarding holding times.

Samplers need to be processed as soon as possible after retrieval or shipped as soon as possible to the lab performing the processing. Samplers will need to be shipped priority overnight in coolers with ice packs. No loose ice should be used, as it may melt and get inside the containers, compromising the samples. Processed extracts should be mailed immediately to the labs performing the analyses.

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4.6 SAMPLE INFORMATION SYSTEM (SIS) DATA ENTRY PROTOCOL

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INTRODUCTION

The Sample Information System (SIS) is an Oracle® application that DEP collectors use to store, manage, and retrieve chemical sample data, except for continuous physicochemical data. Continuous physicochemical data are stored and managed using a separate system due to the structure of the data, which is not handled by SIS. This protocol outlines the data requirements and documentation procedures for handling chemical data after it has been collected in the field.

SIS REQUIREMENTS

Sample collectors, at the very least, must have security roles for their program to perform Sample Entry and Querying. Collectors must obtain a SIS login name and password to enter/edit sample information. Collectors will also need to contact a system coordinator or eFACTS coordinator to obtain the correct SIS securities (see SIS Security Request Form, doc # 1300-FM-IT1016 SIS), which will allow them to manage sample information in SIS. SIS securities are broken down into roles. Roles include (1) Querying, (2) Project Entry, (3) Monitoring Point Entry, and (4) Sample Entry. Each role is then applied to one of 52 specific programs or business units such as (1) Watershed Conservation, (2) Water Supply Management, (3) Land Recycling and Waste Management. Program or business unit names periodically change, pending reorganizations and other circumstances.

Samples submitted to the Bureau of Laboratories (BOL) will have the following information populated in SIS: collector identification number, sample sequence number, sample time and date, and sample results. It is the responsibility of the collector to populate, at the minimum, sample medium, sample collection location, field parameters, quality control identification, and general comment information.

SIS can be accessed through the DEP Intradep website by selecting ‘Oracle Applications’. DEP maintains several Oracle applications, so users must select ‘SIS - Samples Information System’. Users will be prompted to enter a unique Commonwealth of Pennsylvania (CWOPA) username and password, in addition to a database identifier. The database identifier is ‘prod’. Samples can be entered into SIS by the sample collector before or after BOL populates sample results. It is important to enter the collector identification number, sample sequence number, and date and time collected correctly. If samples are entered into SIS before BOL populates sample results these attributes will be used to associate sample results. If samples are entered after BOL populates results, the sample collector will need to query to find the sample and populate attributes. Additional information and step-by-step instructions are available through the Sample Information Users Guide (Appendix B.3).

CHAPTER 5 PHYSICAL DATA COLLECTION PROTOCOLS

5.1 STREAM HABITAT DATA COLLECTION PROTOCOL

Chapter 5 Physical Data Collection Protocols

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INTRODUCTION

DEP has adopted a modification of the habitat evaluation procedures outlined in the U.S. Environmental Protection Agency's (USEPA) Rapid Bioassessment Protocols (Barbour et al. 1999). DEP riffle/run stream habitat is evaluated based on 12 parameters rather than the 10 parameters of Barbour et al. (1999) and multihabitat stream habitat is evaluated on nine of the 10 parameters, dropping channel sinuosity of Barbour et al. (1999). The matrix used to assess habitat quality is based on key physical characteristics of the waterbody and surrounding lands. All parameters evaluated represent potential limitations to the quality and quantity of instream habitat available to aquatic biota. These, in turn, affect community structure and composition. The main purpose of the habitat assessment is to account for the limitations that are due to existing stream conditions. This is particularly important in cause/effect and cumulative impact studies where the benthic community at any given station may already be self-limited by background watershed and habitat conditions or impacts from current land uses. In order to minimize the effects of habitat variability, every effort is made to sample similar habitats at all stations. The habitat assessment process involves rating twelve parameters for riffle/run prevalence waterbodies or nine for low gradient waterbodies as excellent, good, fair, or poor, by assigning a numeric value (ranging from 20 - 0), based on the criteria included on the Habitat Assessment Field Data Sheets (Appendix C) and the information provided below.

DATA COLLECTION

The habitat assessment parameters used in the evaluations for riffle/run prevalent waterbodies as well as for low gradient waterbodies are identified and discussed below. The first six parameters evaluate stream conditions within the surveyed reach.

- **Instream Fish Cover** (riffle/run) / **Substrate/Available Cover** (low gradient)
  (riffle/run) – Evaluates the percent makeup of the substrate (boulders, cobble, other rock material) and submerged objects (logs, undercut banks) that provide refuge for a variety of fish including both large bodied pelagic species as well as smaller benthic specialists.
  (low gradient) – Evaluates the relative quantity and variety of natural structures in the stream, such as large rocks, fallen trees, logs and branches, and undercut banks.
- **Epifaunal Substrate** (riffle/run)
  Evaluates riffle quality, i.e., areal extent relative to stream width and dominant substrate materials (cobble, boulders, gravel) that are present.
- **Embeddedness** (riffle/run)
  Evaluates the extent to which rocks (gravel, cobble, and boulders) and snags are covered or sunken into the silt, sand, or mud of the stream bottom. The rating of this parameter may be variable depending on where the observations are taken. To avoid confusion with sediment deposition (another habitat parameter), observations of embeddedness should be taken in the upstream and central portions of riffles and cobble substrate areas.
- **Pool Substrate Characterization** (low gradient)

Evaluates the type and condition of bottom substrates found in pools. Firmer sediment types (e.g., gravel, sand) and rooted aquatic plants support a wider variety of organisms and are scored higher than a pool substrate dominated by mud or bedrock and no plants.

- **Velocity/Depth Regime** (riffle/run)
  Evaluates the presence/absence of four velocity/depth regimes (fast-deep, fast-shallow, slow-deep, and slow-shallow). Generally, shallow is < 0.5m and slow is < 0.3m/sec.
- **Pool Variability** (low gradient)
  Evaluates the overall mixture of pool types found in streams, according to size and depth (large-shallow, large-deep, small-shallow, and small-deep).

The next four parameters evaluate a larger area surrounding the sampled reach. This expanded area is the stream length defined by at least how far upstream the investigator can see from the downstream point of the sample reach, but at least 100 meters upstream of the reach.

- **Channel Alteration** (riffle/run & low gradient)
  Evaluates the extent of channelization or dredging, but can include any other large-scale changes in the shape of the stream channel that would be detrimental to the habitat. Channel alteration is present when artificial embankments, riprap, and other forms of artificial bank stabilization or structures are present; when the stream is very straight for significant distances; when dams and bridges are present; and when other such changes have occurred.
- **Sediment Deposition** (riffle/run & low gradient)
  Estimates the extent of sediment effects in the formation of islands, point bars, and pool deposition. Deposition is typically evident in areas that are obstructed by natural or manmade debris and areas where the stream flow decreases, such as bends.
- **Riffle Frequency** (riffle/run)
  Estimates the frequency of riffle occurrence based on stream width and thus the heterogeneity occurring in a stream. For riffle/run prevalent streams where distinct riffles are uncommon, a run/bend ratio is used as a measure of meandering or sinuosity.
- **Channel Flow Status** (riffle/run & low gradient)
  Estimates the areal extent of exposed substrates due to water level or flow conditions. The flow status will change as the channel enlarges (e.g., aggrading stream beds with actively widening channels) or as flow decreases as a result of dams and other obstructions, diversions for irrigation, or drought. In riffle/run prevalent streams, riffles and cobble substrate are exposed; in low gradient streams, the decrease in water level exposes logs and snags, thereby reducing the areas of good habitat.

The last four parameters apply to riffle/run prevalent, as well as low gradient streams, and evaluate an even greater area. For these parameters, consideration can be given to upstream land use activities.

- **Condition of Banks** (riffle/run & low gradient)
  Evaluates the extent of bank failure, signs of erosion, or the potential for erosion. The stream bank is defined as the area from the water's surface to the bankfull delineation (Figure 1). Steep banks are more likely to collapse and suffer from erosion than are gently sloping banks and are therefore considered to be unstable. Signs of erosion include crumbling, unvegetated banks, exposed tree roots, and exposed soil.
- **Bank Vegetative Protection** (riffle/run & low gradient)
  Estimates the extent of stream bank that is covered by plant growth providing stability through well-developed root systems. The stream bank is defined as the area from the water's surface to the bankfull delineation (Figure 1). This parameter supplies information on the ability of the bank to resist erosion as well as some additional information on the uptake of nutrients by the plants, the control of instream scouring, and stream shading. This parameter is made more effective by defining the native vegetation for the region and stream type (i.e., shrubs, trees, etc.). In some regions, the introduction of exotics has virtually replaced all native vegetation. The value of exotic vegetation to the quality of the habitat structure and contribution to the stream ecosystem must be considered in this parameter. In areas of high grazing pressure from livestock or where residential and urban development activities disrupt the riparian zone, the growth of a natural plant community is impeded and can extend to the bank vegetative protection zone.
- **Grazing or Other Disruptive Pressures** (riffle/run)
  Evaluates disruptions to surrounding land vegetation due to common human activities, such as crop harvesting, lawn care, excavations, fill, construction projects, and other intrusive activities. Grazing and other disruptive pressures are evaluated from the bankfull delineation to through the riparian zone (Figure 1).
- **Riparian Vegetative Zone Width** (riffle/run & low gradient)
  Estimates the width of natural vegetation from the edge of the stream bank out through the riparian zone (Figure 1). Narrow riparian zones occur when roads, parking lots, fields, lawns, bare soil, rocks, or buildings are near the stream bank. Residential developments, urban centers, golf courses, and rangeland are the common causes of anthropogenic degradation of the riparian zone. Conversely, the presence of "old field" (i.e., a previously developed field not currently in use), paths, and walkways in an otherwise undisturbed riparian zone may be judged to be inconsequential to altering the riparian zone and may be given relatively high scores.

After all parameters in the matrix are evaluated, the scores are summed to derive a total habitat score for that station. Please refer to the Assessment Book, *Chapter 2.11, Physical Habitat Assessment Method* (Walters 2017) for accompanying assessment method.

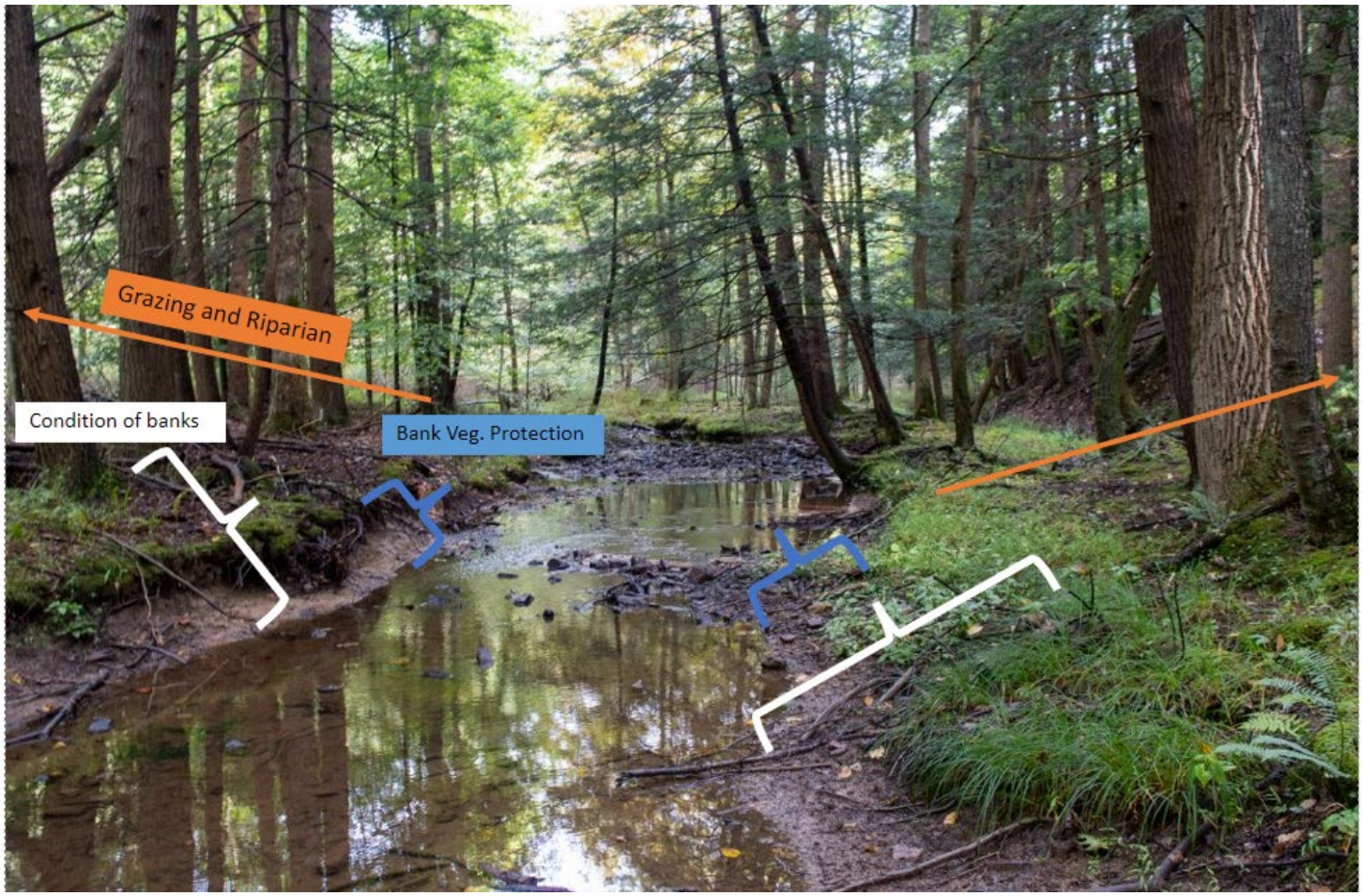

Figure 1. Bank evaluation schematic

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5.2 PEBBLE COUNT DATA COLLECTION PROTOCOL

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INTRODUCTION

This data collection protocol is to be applied to riffle/run dominated, gravel or cobble bed stream segments to determine substrate stability. Flow regime alteration (change in volume and/or timing of discharge) is a major cause of stream instability and habitat alteration. One aspect of concern is the delivery of fine sediments to streams and their effects on aquatic habitat. One approach to monitoring these sediment effects is *A Pebble Count Procedure for Assessing Watershed Cumulative Effects* by Bevenger and King (1995). This procedure utilizes a reference stream approach in evaluating the stability of study or candidate streams. The procedure characterizes particle size distributions of reference and study streams, where reference streams are defined as “natural” or “least disturbed” and study streams as “disturbed” or “impacted”. These particle size distributions can be used for comparative purposes to determine, with statistical reliability, if there has been a shift toward finer size materials in the study stream. This protocol employs a modification of the Wolman (1954) pebble count procedure to a zigzag pattern through a continuum along a longitudinal reach of the stream. This allows for numerous meander bends and associated habitat features to be sampled as an integrated unit.

SAMPLING CONSIDERATIONS

Wadeable reference and study streams should be selected from the same ecoregion, and the streams should be classified according to the Rosgen stream classification system (Rosgen 1994, Rosgen 1996) prior to conducting the field collection. Streams classification can be accomplished in the office using topographic quadrangles and aerial photographs, and the classification should be confirmed when the sample site is visited. This protocol should only be applied to those streams that are classified as B and C types with cobble (B3 or C3) or gravel beds (B4 or C4). If the classification results in stream types G, F, or D, then data collection may not be necessary since, in most cases, these stream types are the result of channel instability. If the instability were a result of natural conditions the stream would not be classified as impaired. Also, if the classification results in stream types A and E, which are ordinarily stable, then data collection is not necessary. In addition, this procedure should not be conducted on “natural” sand or silt/clay bottom streams, as fine particles will be the predominate substrate type, thus resulting in potentially misleading indications of instability.

DATA COLLECTION

Once reference and study streams have been identified, the sample stream reach should include at least two riffle and two pool habitat units if present, or a minimum of 200 meters. The chosen sample reach habitat units should be representative of the streams. Study and reference streams must have a minimum mean width of 3 meters. If mean stream width is greater than 20 meters, then sample reach should be extended 100 meters for each 10-meter increment increase in width. Sampling of reference streams should occur within a few days of the sampling of study streams when possible and should always occur within the same year and season. In order to confirm stream classification, at least two

stream cross-sections (one riffle and one pool) should be measured from bankfull elevation to bankfull elevation within the study reach, prior to conducting the pebble count.

Pebble counts are conducted on the selected reach beginning at the head of a riffle and continuing through 4 habitat units (2 riffle, 2 pools if present), or for a minimum of 200 meters. At least 200 particles are to be sampled from the stream reach. Pebble counts are conducted along a zigzag transect from bank toe to bank toe in the active channel (Figure 1). The angle of the transect from the bank should be maintained as best as possible and can be aided by identifying a location to walk to on the opposite bank. Particles are selected beginning at the start point by placing a finger at the toe of one boot, and without looking, sliding a finger down to the stream bottom until making contact with a particle (Figure 1). Each particle selected is measured along the intermediate axis (Figure 1) and the measurement is recorded on the Pebble Count Data Collection Form (Appendix C.3). Alternatively, each particle measurement may be tallied according to Wentworth size classes (<2 mm, 2-4 mm, >4-8 mm, >8-16 mm, etc.) on the Alternative Pebble Count Field Data Collection Form (Appendix C.4). The investigator then paces off a chosen distance to the next point and samples another particle in the same manner as the first. The distance to the next sample point should be no less than 2.1 meters to avoid correlation between particles sampled.

Data analysis is conducted as described in the Assessment Book, *Chapter 2.11, Physical Habitat Assessment Method* (Walters 2017) for habitat assessment method.

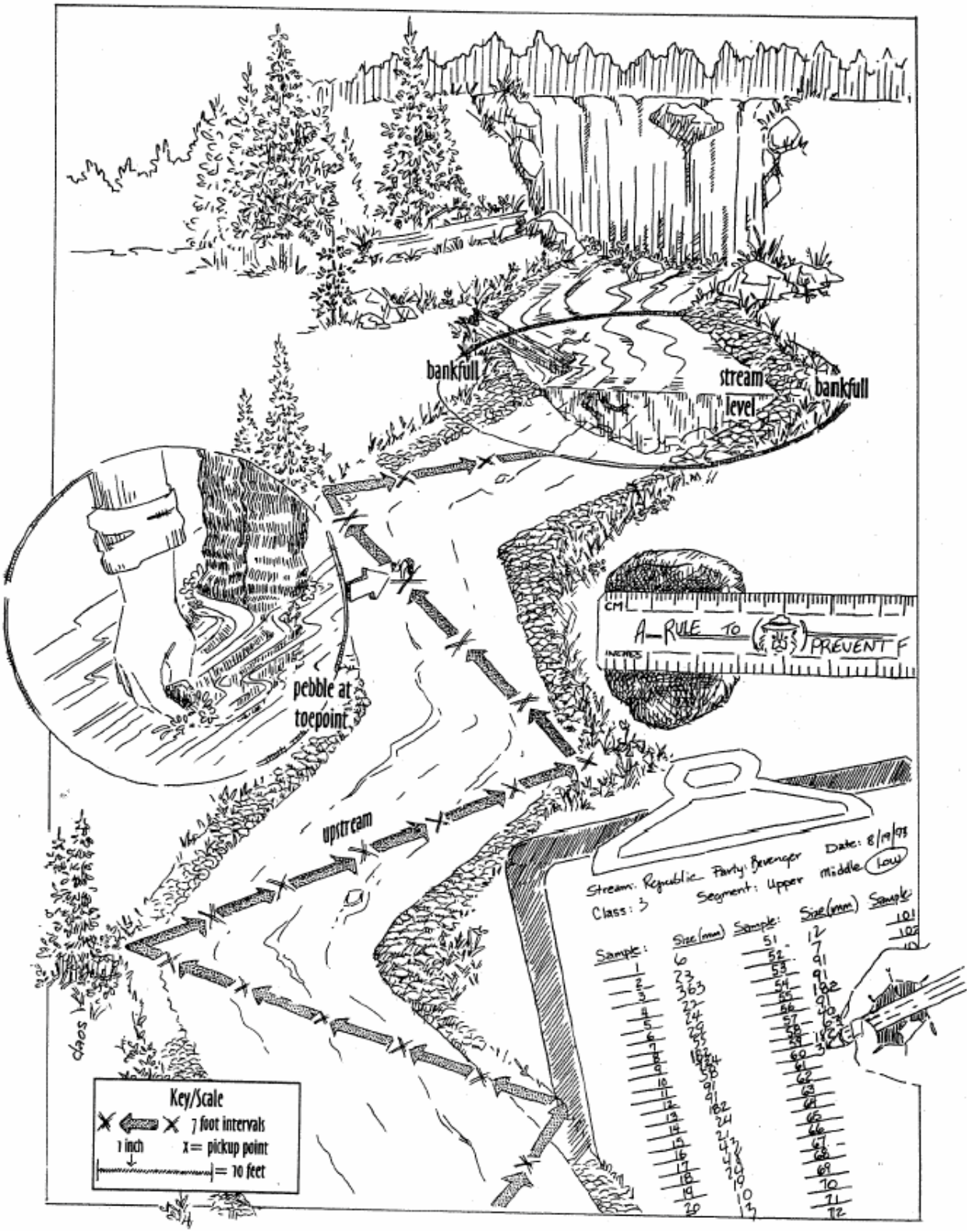

Figure 1. Zig-zag pebble count procedure from Bevenger and King, 1995.

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5.3 WATER FLOW DATA COLLECTION PROTOCOL

Chapter 5 Physical Data Collection Protocols

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INTRODUCTION

Water flow (hereto referred to as discharge) is the volume of water that passes through a conduit over a period of time. For the purposes of this document, the conduit is typically an open stream channel, but it could also include storm drain infrastructure and other water conveyances. Discharge data are collected along with other water quality data in order to characterize yields or quantities of pollutants that are transported by waterbodies. Chemical data are collected and reported as concentrations. Discharge data allows those concentrations to be further characterized as standardized quantities or yields. This is required in characterizing the amount of a pollutant transported by a waterbody that may be affecting water quality.

Historically, DEP has collected discharge data by implementing cross-section measurements using the “Mid-Section” approach described by Buchanan and Somers (1969); where depth and velocity is measured at subdivisions across a transect, discharge is calculated at each subdivision, and the discharge at each of the subdivisions is summed to calculate total discharge. This approach has been utilized in small to medium wadable waterbodies and continues to be a valid discharge data collection protocol.

Over time the utility and consequently the number and frequency of discharge measurements has dramatically increased. Routine discharge is required to regulate water allocation, pollution control programs, generate flood prediction forecasts, and support other important socioeconomic functions. The U.S. Geological Survey (USGS) originally set the standard for collecting discharge data with USGS Techniques of Water Resources Investigations Book 3, Chapter A8 (TWRI 3–A8), “Discharge Measurements at Gaging Stations” by Buchanan and Somers (1969). Since then technology and data collection protocols have evolved, and USGS has continued to set the standard for collecting discharge data. Additional USGS publications include “Measurement and Computation of Streamflow, volumes 1 and 2,” by Rantz et al. (1982); “Discharge measurements using a broad-band acoustic Doppler current profiler,” by Simpson (2002); “Quality-assurance plan for discharge measurements using acoustic Doppler current profiler,” by Oberg et al. (2005); and “Measuring discharge with acoustic Doppler current profilers from a moving boat,” by Mueller and Wagner (2009).

DEP no longer maintains a detailed discharge data collection protocol, but references and implements the latest USGS techniques and standards for collecting discharge data. USGS Techniques and Methods book 3, chap. A8, “Discharge Measurements at Gaging Stations” (Turnipseed and Sauer 2010) is the latest techniques and standards that supersedes or supplements previous USGS publications.

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5.4 SECCHI DEPTH DATA COLLECTION PROTOCOL

Chapter 5 Physical Data Collection Protocols

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INTRODUCTION

Secchi depth is used as a measurement of water clarity. Water clarity not only affects esthetic quality of surface waters but can also be biologically important. Secchi readings vary by season and are affected by three factors, biological (phytoplankton and zooplankton), suspended sediments, and stained water color. Each factor has attributing harmful impacts to a waterbody. General observations of transparency, coloration and tint should be considered when interpreting Secchi disk readings. Waters with an opaque or brown tint are likely impacted by suspended sediment. Waters that have green coloration are likely impacted by algal biomass. Waters tinted or stained to look like tea, commonly as a result of pigmented tannins from organic matter, are generally not cause for concern as tannins are a natural factor contributing to conditions.

Specifically, Secchi depth has a long history as a lake assessment tool. Secchi readings are an important parameter for assessing lake trophic status (see below) and are one of three Trophic State Index (TSI) calculations relied upon to assess lake status. Collecting Secchi depth is one of the simplest lake methods to provide insight into the quality of the lake. DEP employs the use of this *Secchi Depth Data Collection Protocol* for the use of TSI calculations, to perform Esthetics assessments and/or to provide supplemental data for other monitoring objectives. Additionally, Secchi depth will be collected as part of the *Mussel Data Collection Protocol* (Spear, Counahan and Shull 2017).

DATA COLLECTION

Secchi depth is measured using an 8 inch (20 centimeter) black and white quartered disk; this disk is known as a Secchi disk. The Secchi disk is attached to a rope which is marked off in 0.50 meter increments. When collecting Secchi depth measurements, data will be collected off the shady side of the boat and the collector is to remove sunglasses. Removal of sunglasses allow for an unbiased reading. Often, sunglasses are polarized which help reduce glare to enhance vision; this is bias we do not want in results.

To collect a Secchi depth reading, the Secchi disk is lowered into the water until it is no longer visible, then raised until it is just visible again. If the depth does not fall on a 0.50 meter increment use a meter measuring tape to measure to the nearest tenth place. Both values are to be recorded on the Lake Assessment and TSI Field Data Sheet (Appendix A-11). The average of these two depths is the final Secchi depth measurement and will be indicated on the field data sheet.

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5.5 TRASH DATA COLLECTION PROTOCOL

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INTRODUCTION

The presence of trash in the environment is an issue all over the world and some governmental agencies are working to remedy this issue. Oftentimes, trash presents as litter, which is improperly or intentionally disposed trash due to human behavior or mismanagement. Trash can be found in every ecosystem and remediation presents different challenges depending on the ecosystem type. Streams and rivers present unique challenges because sources of trash in these ecosystems can be from direct littering or from upstream influences. A few U.S. states have addressed trash in surface waters through their regulatory programs. For instance, California has developed a standardized method for monitoring and assessing trash in the *California Trash Monitoring Methods and Assessments Playbook* (Moore et al. 2020) and has passed total maximum daily loads for trash. In addition, the Metropolitan Washington Council of Governments has been implementing a detailed sampling methodology for many years (COG 2019). In Pennsylvania, monitoring trash and litter has mainly occurred on land rather than water.

Pennsylvania has taken measures to address land-based trash through evaluation, agency collaboration, and legislation. The *Pennsylvania Litter Research Study* (Burns and McDonnell 2020) concluded that cigarette butts and plastic materials composed most of the litter found along Pennsylvania's roadways. Through public surveys, the study concluded that many people – 54.8% of respondents – agreed that litter ends up in waterways and is a major problem. Furthermore, 18.6% of respondents said they saw the most litter in waterways. DEP and the Pennsylvania Department of Transportation (PennDOT) have also worked together to develop a litter remediation plan called the *Statewide Litter Action Plan* (DEP and PennDOT 2021), which aims to prevent littering from occurring in the first place. In addition, there are numerous laws in place to try to prevent littering and dumping of trash (DEP and PennDOT 2021). Even with these actions in place, Pennsylvania has an observed trash problem in its waterways.

In the case of Pennsylvania streams and rivers, trash is largely a problem in urban and suburban areas. In recent years, listing trash as a stream impairment cause for Recreational Use (RU) activities (mainly esthetics) has been of interest. Trash is an available cause of impairment for Pennsylvania streams. However, there has not been either a quantitative or qualitative way to justify impairing a stream with the trash cause. This document provides a data collection protocol for sampling trash in Pennsylvania's wadable streams and rivers that will support RU assessment decisions for Cause – Esthetics.

SAMPLING DESIGN

This data collection protocol employs a targeted sampling design as outlined in *Chapter 2, Sampling Design and Planning* (Lookenbill et al. 2023) in *Water Quality Monitoring Protocols for Streams and Rivers* (Lookenbill and Arnold 2023). There are many sources and causes of trash deposition to streams and rivers that should be considered. Land characteristics can help determine where to target a trash sampling event. Illegal dump areas are prevalent across Pennsylvania and close

proximity to streams could make them sources of trash (Keep Pennsylvania Beautiful 2011). Areas downstream of combined sewer overflow outfalls could also accumulate litter that comes from the surrounding landscape. Municipal separate storm sewer systems can release trash into waterbodies. Urban areas tend to accumulate more litter than rural and forested areas and subsequently, may have trash accumulation in streams without an obvious source. Bridges, parking lots, and areas with dumpsters may contribute trash to streams. Sometimes riparian buffers in urban areas can keep trash out of the stream and result in accumulating more trash in riparian areas instead of the stream banks or channels. Considering these potential sources and causes will aid in selecting representative streams and stream reaches for sampling.

It is also important to make every attempt to choose a survey reach that is representative of the land-based area. Trash accumulation may change dramatically between stream reaches that are close to each other. Choosing a stream with an accumulation of trash can show the potential amount that could end up in any of the area's streams, either from riparian areas or from downstream transport.

General Considerations

This protocol is only applicable for wadable streams with drainage areas < 500 square miles. Semi-wadeable and non-wadeable procedures have not been developed at this time. This protocol relies upon viewing the stream bottom which is typically not possible across the entire width of semi-wadeable and non-wadeable streams. A reach selection may need to be reevaluated or revisited if visibility of the stream bottom is inadequate. Precipitation and seasonality may also impact the benthic visibility.

Rainfall should be a consideration in deciding when to survey. Trash surveys should not be conducted when turbidity – such as that caused by some storm events – is high enough to impede viewing the stream bottom. However, it may be beneficial to sample after a recent rainfall event as stormwater runoff can deposit trash into streams. If sampling soon after a storm event, the trash survey should occur after most sediment has settled and all other safety considerations are met, as outlined in *Appendix F: Biosecurity and Safety Procedures* (Lookenbill and Arnold 2023). Seasonality is also a necessary consideration when choosing when to sample.

The recommended trash sampling season is from March through October, although this may differ per year and by region. It is generally not recommended to sample during and after leaf fall in the autumn. It is also difficult to sample over the winter when streams are filled with leaf debris and ice. It may be recommended to sample more than once per reach, and over multiple seasons and stream usage scenarios to capture any variation in trash accumulation and to determine frequency of trash returning to the reach. For example, boaters, swimmers, and people fishing may be low during late winter or early spring and high during the summer. This may result in higher trash accumulation during the summer if litter is the dominant source of trash.

Sampling Supplies and Equipment

Several basic supplies are required to sample for trash:

- Measuring tape
- Tally Counter
- Flagging tape or stakes
- Trash grabbers
- Cut-resistant gloves
- Large trash bags
- Buckets (optional, for stream reaches with a low density of trash)
- Backpack
- Field notebook
- Camera

Sample Reach

Delineating a representative 50-meter reach should include both desktop reconnaissance and field-based efforts (Lookenbill et al. 2023). Prior to collecting any field data, finalize the location of the reach after walking along the stream bank and determine the extent of the wetted and bankfull widths (Figure 1). The reach should be clearly marked with flagging tape, stakes, or another form that is easily visible from within the stream. The wetted width is the measured distance across the stream channel that contains water on the day of sampling. The bankfull width, which is often larger than the wetted width, is the measured distance across the stream channel where evidence exists of periodic flow. Evidence of periodic flow may include bank areas without vegetation, undercut banks, areas with vegetation pressed down by recent flows, and/or visible debris lines. This protocol will not include counting trash located outside of the reach's bankfull area. All field data must be collected in a downstream to upstream direction.

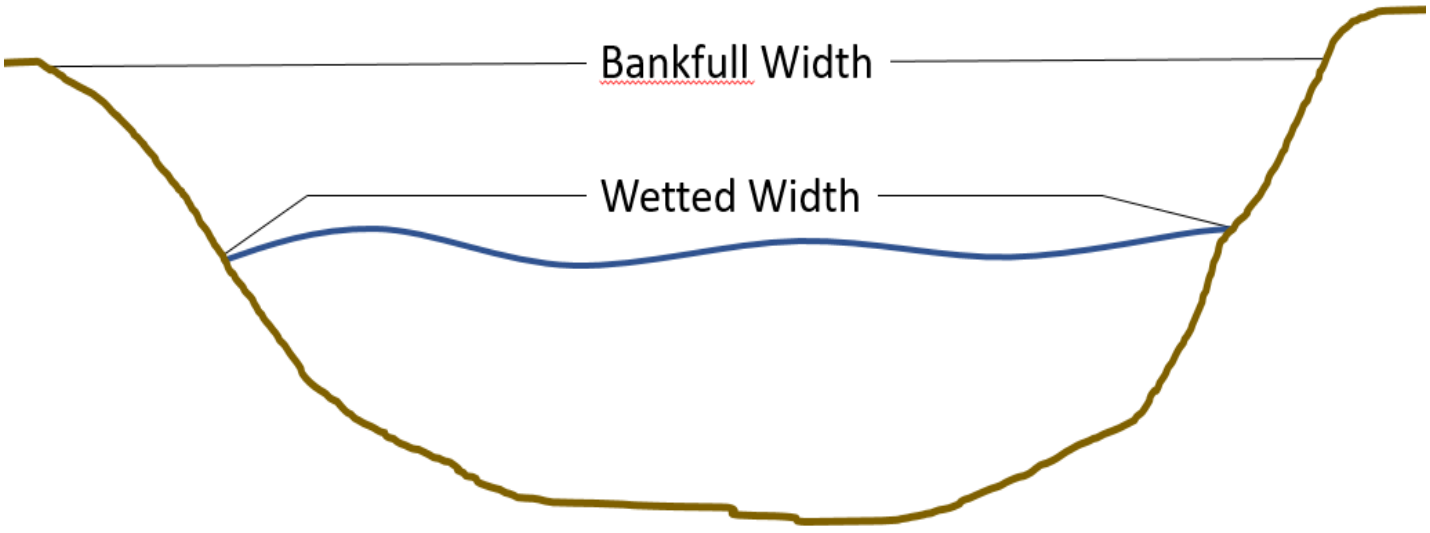

Figure 1. Illustration of wetted and bankfull widths.

DATA COLLECTION

Preliminary Evaluation

After walking along the stream banks and clearly defining the 50-meter reach, measure the most-representative wetted and bankfull widths and record these and all other applicable data on the *Trash Data Collection Preliminary Evaluation Form* (Appendix C.5). It is recommended to record detailed comments and include photo documentation, as they will be helpful in making an assessment determination. Score all parameters included on this form according to the observed conditions within the reach.

If all of the parameters on the form score in the *Optimal* or *Suboptimal* categories, it is optional – but not necessary – to conduct a more comprehensive trash survey. In this case, the scores must be verified by walking the reach from within the stream before the preliminary evaluation may be used to determine a RU assessment decision of supporting. If any of the four parameters on the form score in the *Marginal* or *Poor* categories, a more comprehensive survey of the reach is required.

Comprehensive Trash Survey

A comprehensive trash survey consists of two distinct processes: counting all trash within the reach (the *Counting Procedure*) and "picking" all trash within two subsample areas (the *Picking Procedure*), and all data is recorded on the *Trash Data Collection Comprehensive Form* (Appendix C.6). The counting procedure provides a quantitative measure of trash abundance. The picking procedure provides both quantitative and qualitative details of type of trash (litter, dumping, debris) and trash materials (plastic, metal, glass, etc.) and aims to categorize trash found at the site.

Counting Procedure

The count procedure includes counting all visible trash equal to or larger than half an inch within the targeted reach, it requires one to three people, and is implemented prior to picking. Data collection must begin at the downstream end of the reach (at the farthest downstream marker), end at the upstream extent of the reach (at the farthest upstream marker) and will move in a back-and-forth pattern throughout the reach (Figure 2). Moving downstream to upstream will avoid substrate disturbance that can obstruct views if data were collected by moving from upstream to downstream.

Collectors will begin on either bank and begin counting trash by moving perpendicular to the streamflow until reaching the other bank, counting all visible trash items within an approximate one-meter distance of the perpendicular line (approximately a half meter in the upstream direction and approximately a half meter in the downstream direction) and within in the bankfull area. Count only trash items or pieces equal to or larger than half an inch (roughly half the diameter of a quarter). If a particular trash type occurs in such large quantities to the point where counting each individual item is impractical (e.g., broken pottery, bricks, concrete pieces, broken glass, plastic bag fragments), the collector may consider binning the item as follows: count up to ten items and bin larger quantities as

follows: M (Medium) = 11 – 50 pieces; H (High) = 50 – 100 pieces; VH (Very High) >100 pieces. Use care not to recount items.

Once the collector(s) have reached the other side of the stream, they are to turn and walk upstream approximately one meter, while counting any trash on the adjacent bank within the bankfull extent. Collector(s) continue by turning towards the opposite side of the stream and repeating both the perpendicular movement towards the opposite side of the stream and the counting procedure. Repeat these steps throughout the 50-meter reach. The back-and-forth movement pattern occurs so that the count survey covers the entire sample reach.

It is often useful to have two people side by side counting trash, or to divide the stream in half, and/or to have a third person following behind to count any missed trash and record any field notes. If two people are completing the survey, it can be helpful to have the person who is not conducting the count to stand at the location where the collector (the person who is counting trash) last crossed the stream. This can help avoid missing or double counting trash items. Alternatively, it may be more appropriate to have a person follow along the bank out of the water to tally and take notes. In this case, the collector would walk the stream reach and notify the person standing on the bank each time they see a piece of trash. The person on the bank would be responsible for counting those trash items using a clicker or other method.

After counting all trash within the reach, record the total number trash items in the *Trash Data Collection Comprehensive Form* (Appendix C.6) and move to the *Picking* procedure.

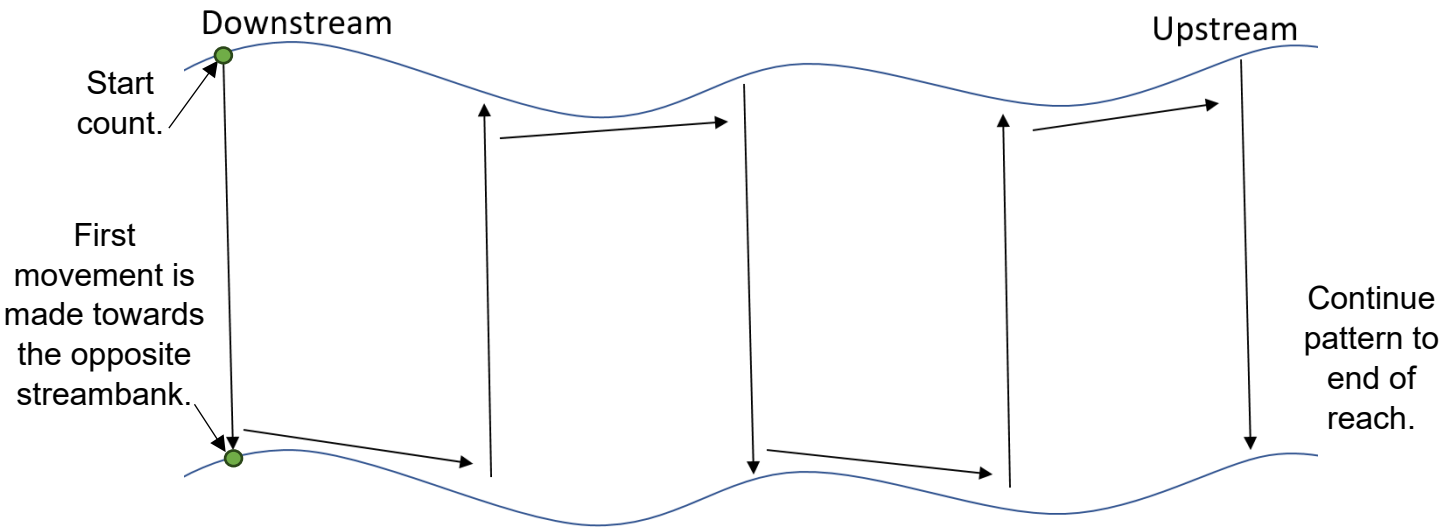

Figure 2. Collection pattern showing the movement from downstream to upstream and crossing the stream perpendicularly. This pattern is repeated throughout the stream reach during the count trash survey (not to scale and not representative of the number of stream crossings or segments).

Picking Procedure

The picking procedure is designed to identify, characterize, and count the relative abundance of the different types of trash within the reach. It is conducted after the count procedure and all data is recorded on the *Trash Data Collection Comprehensive Form* (Appendix C.6). This procedure is to be implemented as described below.

Select two areas within the 50-meter reach that have captured or otherwise prevented some trash from being washed downstream, such as snags or depositional areas. Snags are structures that act as a trash or debris "strainer" (e.g., fallen logs, tree branches, large rocks, Figure 3a) and depositional areas are features where sediment, cobble, etc. have accumulated (e.g., sand bars, riffle areas, Figure 3b) and allow for trash or debris to drop out of the water column. A three-meter length of the stream for each of these two sub-sample areas will be sampled across the entire width of the stream, from bankfull to bankfull. The two sub-sample areas may be adjacent, three-meter lengths of the stream, thereby being a continuous six-meter length across the entire bankfull to bankfull width. In this case, the tally should still include the two distinct subsamples. If two subsamples are not possible due to the lack of trash, include the tally in Subsample 1 and notate the lack of trash for Subsample 2 on the tally sheet.

For each subsample area, the collector(s) will, again, work from downstream to upstream and identify all visible trash that meets the previously mentioned size criteria. Search the subsample areas. Tally and categorize each piece of trash on the *Picking Procedure Tally Sheets* in the appropriate space per Subsample, Category, and Type. Tallying and categorizing may be done while the subsample areas are being searched or, the trash may be collected and removed from the targeted areas and counted afterwards. If the trash is collected during the search, tally and categorize the trash afterwards by spreading it out (preferably on a tarp, trash bag, etc.). This method requires proper disposal of the trash (in a permissible bin) afterwards. Be sure to keep in mind any state or federal laws regarding archeological artifacts. More information on archeological finds in Pennsylvania can be found at *Pennsylvania Archeology: Pennsylvania Historical & Museum Commission*. Trash items may be binned during this *Picking Procedure* the same way as in the *Counting Procedure*.

Record all data in the *Trash Data Collection Comprehensive Form* (Appendix C.6) or in a field notebook while conducting the survey.

Figure 3. Examples of a) a strainer/snag and b) a depositional area.

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APPENDIX A: BIOLOGICAL DATA COLLECTION INFORMATION AND FORMS

A.1 MACROINVERTEBRATE FIELD DATA SHEET

pennsylvania
DEPARTMENT OF ENVIRONMENTAL PROTECTION

COMMONWEALTH OF PENNSYLVANIA
DEPARTMENT OF ENVIRONMENTAL PROTECTION
BUREAU OF CLEAN WATER

FLOWING WATERBODY FIELD DATA FORM

(Information and comments for fields boxed in double lines are required database entries. Other fields are optional for personal use.)

Date-Time-username* **Example 20040212-0312-jsmith**	- - Date Time Username	**Watershed Code (HUC)**	**Stream Code**	**Ch. 93 Use**
Secondary Station ID		Surveyed by:		

*Date as YYYYMMDD, time as military time, and your initials uniquely identify the stream reach.

SWP Watershed	

Survey Type

(1) Antidegradation [Special Protection], (2) Basin Survey, (3) Cause/Effect, (4) Outside Agency, (5) Point of First Use, (6) Probabilistic Survey, (7) Statewide Surface Water Assessment Program, (8) WQN - Macroinvertebrates (9) Other:	

Location

County:		**Municipality:**		**Topo Quad:**	

Location Description:

Landuse

Residential:	%	Commercial:	%	Industrial:	%	Cropland:	%	Pasture:	%
Abd. Mining:	%	Old Fields:	%	Forest:	%	Other:	%		

Landuse Comments:

Canopy cover: open partly shaded mostly shaded fully shaded

Water Quality

	Collector-sequence #	**Field Meter Readings: Temp (^{0}C)**	**DO (mg/L)**	**pH**	**SPC (umhos)**	**Alkalinity mg/l**	**Bottle Notes (N-normal, MNF-metals non-filtered, MF-metals filtered, B-bac't, Others: indicate)**
1.							
2.							
3.							

Water Appearance/Odor Comments: (^see bottom of back for common descriptors)

Findings

Not Impaired:	☐	Impaired biology?	☐	Impaired habitat?	☐	Is impact localized?	☐	Reevaluate designated use?	☐

Decision comments. Describe the rationale for your "Not Impaired" or "Impaired" decision; reach locations for use designation reevaluations; special condition comments; etc.:

IBI Score:		**Total Habitat Score:**	

Macroinvertebrate sampling
Sampling protocol: *(Select One)* – Freestone: ☐ Limestone: ☐ Multihabitat: ☐ Qualitative: ☐ Other (describe): ☐ _____ *(Select One)* – 6D: ☐ 2D: ☐ 10 Jabs: ☐ D-Frame: ☐ Kick Screen: ☐ Other (describe): ☐ _____ *(Select One)* – 200 Subsample: ☐ 300 Subsample: ☐ 200 Subsample (proportional habitat): ☐ Other (describe): ☐ _____
Comments/Abundance Notes:

Habitat Impairment Thresholds	Metric Score
#3 Riff/Run: embeddedness *or* #3 Glide/Pool: substrate character + #6 Sediment Deposition = 24 or less *(20 or less for glide/pool, low gradient streams)*	
#9 Condition of Banks + #10 Bank Vegetation = 24 or less *(20 or less for glide/pool, low gradient streams*	
Total habitat score 140 or less for forested, cold water, high gradient streams *(120 or less for glide/pool, low gradient streams)*	
Habitat Comments:	

Special Condition
Use this block to describe conditions that justify supporting or impairment decision of stations with IBI score <63 and >53.

^Common descriptors: Water Odors - none normal sewage petroleum chemical other; **Water Surface Oils -** none slick sheen globs flecks; **Turbidity -** clear slight turbid opaque; **NPS Pollution** - no evidence some potential obvious; **Sediment Odors -** none normal sewage petroleum chemical anaerobic; **Sediment Oils -** absent slight moderate profuse; **Deposits –** none sludge sawdust paper fiber sand relict shells other. **Are the undersides of stones deeply embedded black?**

A.2 MACROINVERTEBRATE ENUMERATION BENCH SHEET

3800-FM-BPNPSM0403 12/2014

pennsylvania
DEPARTMENT OF ENVIRONMENTAL PROTECTION

COMMONWEALTH OF PENNSYLVANIA
DEPARTMENT OF ENVIRONMENTAL PROTECTION
BUREAU OF POINT AND NON-POINT SOURCE MANAGEMENT

Macroinvertebrate Enumeration

STREAM NAME: _____ STATION #: _____

DATE COLLECTED: _____ COLLECTED BY: _____

Sample Type (Riffle-Run, Multihabitat, Limestone) SORTED BY: INITIAL & DATE

Survey Type (Special Protection, Use status (impairment/supporting), Cause-Effect, Reference, other) IDENTIFIED BY: INITIAL & DATE

QA CHECK BY: INITIAL & DATE

QA RESULTS: Miss / Total x 100

Gear: ☐ **D-Frame** ☐ **Kick Screen** ☐ **Multi-plate** ☐ **Other**_____

	PAN 1	PAN 2
NO. OF GRIDS:		
NO. OF ORGANISMS:		

*Comments:

TAXA	#	QA
1		
2		
3		
4		
5		
6		
7		
8		
9		
10		
11		
12		
13		
14		
15		
16		
17		
18		
19		
20		
21		
22		
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24		
25		

TAXA	#	QA
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27		
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A.3 FISH FIELD DATA COLLECTION SHEET

FIELD DATA SHEET SEMI QUANTITATIVE FISH SURVEY SAMPLING

Site ID: _____ Stream Name: _____ Date: _____

Crew/Agency: _____

_____

Location: _____

Reach Desc: _____

_____

County: _____ Municipality: _____

Start-End Time (Military): _____-_____

Mean Site Width (M): _____ Site Length (M): _____

Latitude (dec. deg.): Longitude (dec. deg.):

Temperature (°C): _____ Sp. Conductance (µs/cm^c): _____ pH (SU): _____	DO (MG/L): _____ Alkalinity (MG/L): _____ Habitat Score: _____

Water Clarity: Turbidity (NTU): _____ Secchi Depth: _____Meters

Or if you forgot your Secchi Disk or Turbidity Meter circle one: **1 2 3** 4 5 6 7 8 9 10

(Turbid ≤ 1 m) (Discolored 1-2 m) (Clear > 2 m)

Gear(s):

☐ Backpack ☐ AC Volts: _____ Button time seconds: _____ # passes: _____

☐ Towboat ☐ DC Watts: _____ Pulse rate (Hz): _____ # netters: _____

☐ Boat ☐ P-DC Amps: _____ Pulse Width (ms): _____ # probes: _____

Make/Model: _____

Comments: _____

% Mesohabitat:

Riffle _____% Run _____% Pool _____%

% **Velocity/Depth Regimes**

Slow-Deep _____% Slow-Shallow _____% Fast-Deep _____% Fast-Shallow _____%

% Habitat: (% fish in habitat (# individuals) / % habitat within reach)

Root Wads __/__% Mud Bank __/__% Macrophyte __/__% Artificial (Docks, Piers, etc.) __/__%

Boulder __/__% Cobble __/__% Gravel __/__% Sand __/__% Silt __/__%

Large Woody Debris __/__% Small Wood Debris __/__%

Comments: _____

% Substrate:

Boulder _____% Cobble _____% Gravel _____% Sand _____% Silt _____%

Detritus _____% Bedrock _____% Artificial or Channelized _____%

Comments: _____

FIELD DATA SHEET SEMI-QUANTITATIVE FISH SURVEY SAMPLING (continued)

Site ID: Stream Name: Date:

Gradient: ☐ Riffle/Run ☐ Glide/Pool	**Wadeability** ☐ Wadeable ☐ nonwadeable (Boatable)

Habitat Parameter	**Riffle/Run – Wadeable & nonwadeable**			
	Optimal	**Suboptimal**	**Marginal**	**Poor**
1. Instream Cover (Fish)	Greater than 50% mix of boulder, cobble, snags, sub-merged logs, undercut banks, or other stable habitat.	30-50% mix of boulder, cobble, or other stable habitat; adequate habitat.	10-30% mix of boulder, cobble, or other stable habitat; habitat avail-ability less than desirable.	Less than 10% mix of boulder, cobble, or other stable habitat; lack of habitat is obvious.
SCORE _____	**20 19 18 17 16**	**15 14 13 12 11**	**10 9 8 7 6**	**5 4 3 2 1**
2. Epifaunal Substrate	Well developed riffle and run, riffle is as wide as stream and length extends two times the width of stream; abundance of cobble.	Riffle is as wide as stream but length is less than two times width; abundance of cobble; boulders and gravel common.	Run area may be lack-ing; riffle not as wide as stream and its length is less than two times the stream width; gravel or large boulders and bed-rock prevalent; some cobble present.	Riffles or run virtually nonexistent; large boulders and bedrock prevalent; cobble lacking.
SCORE _____	**20 19 18 17 16**	**15 14 13 12 11**	**10 9 8 7 6**	**5 4 3 2 1**
3. Embeddedness	Gravel, cobble, and boulder particles are 0-25% surrounded by fine sediment.	Gravel, cobble, and boulder particles are 25-50% surrounded by fine sediment.	Gravel, cobble, and boulder particles are 50-75% surrounded by fine sediment.	Gravel, cobble, and boulder particles are more than 75% surrounded by fine sediment.
SCORE _____	**20 19 18 17 16**	**15 14 13 12 11**	**10 9 8 7 6**	**5 4 3 2 1**
4. Velocity/Depth Regimes	All four velocity/depth regimes present (slow-deep, slow-shallow, fast-deep, fast-shallow).	Only 3 of the 4 regimes present (if fast-shallow is missing, score lower than if missing other regimes).	Only 2 of the 4 habitat regimes present (if fast-shallow or slow-shallow are missing, score lower than if missing other regimes).	Dominated by 1 velocity/depth regime (usually slow-deep).
SCORE _____	**20 19 18 17 16**	**15 14 13 12 11**	**10 9 8 7 6**	**5 4 3 2 1**
5. Channel Alteration	No channelization or dredging present.	Some channelization present, usually in areas of bridge abutments; evidence of past channelization, i.e., dredging, (greater than past 20 yr) may be present, but recent channelization is not present.	New embankments present on both banks; and 40-80% of stream reach channelized and disrupted.	Banks shored gabion or cement; over 80% of the stream reach channelized and disrupted.
SCORE _____	**20 19 18 17 16**	**15 14 13 12 11**	**10 9 8 7 6**	**5 4 3 2 1**
6. Sediment Deposition	Little or no enlargement of islands or point bars and less than 5% of the bottom affected by sediment deposition.	Some new increase in bar formation, mostly from coarse gravel; 5-30% of the bottom affected; slight deposition in pools.	Moderate deposition of new gravel, coarse sand on old and new bars; 30-50% of the bottom affected; sediment deposits at obstruction, constriction, and bends; moderate deposition of pools prevalent.	Heavy deposits of fine material, increased bar development; more than 50% of the bottom changing frequently; pools almost absent due to substantial sediment deposition.
SCORE _____	**20 19 18 17 16**	**15 14 13 12 11**	**10 9 8 7 6**	**5 4 3 2 1**

FIELD DATA SHEET SEMI-QUANTITATIVE FISH SURVEY SAMPLING (continued)

Site ID: Stream Name: Date:

Habitat Parameter	Riffle/Run – Wadeable & nonwadeable (continued)			
	Optimal	**Suboptimal**	**Marginal**	**Poor**
7. Frequency of Riffles	Occurrence of riffles relatively frequent; distance between riffles divided by the width of the stream equals 5 to 7; variety of habitat.	Occurrence of riffles infrequent; distance between riffles divided by the width of the stream equals 7 to 15.	Occasional riffle or bend; bottom contours provide some habitat; distance between riffles divided by the width of the stream is between 15 and 25.	Generally all flat water or shallow riffles; poor habitat; distance between riffles divided by the width of the stream is between ratio >25.
SCORE _____	**20 19 18 17 16**	**15 14 13 12 11**	**10 9 8 7 6**	**5 4 3 2 1**
8. Channel Flow Status	Water reaches base of both lower banks and minimal amount of channel substrate is exposed.	Water fills > 75% of the available channel; or <25% of channel substrate is exposed.	Water fills 25-75% of the available channel and/or riffle substrates are mostly exposed.	Very little water in channel and mostly present as standing pools.
SCORE _____	**20 19 18 17 16**	**15 14 13 12 11**	**10 9 8 7 6**	**5 4 3 2 1**
Riffle/Run – Wadeable Only				
9. Condition of Banks	Banks stable; no evidence of erosion or bank failure.	Moderately stable; infrequent, small areas of erosion mostly healed over.	Moderately unstable; up to 60% of banks in reach have areas of erosion.	Unstable; many eroded areas; "raw" areas frequent along straight sections and bends; on side slopes, 60-100% of bank has erosional scars.
SCORE _____	**20 19 18 17 16**	**15 14 13 12 11**	**10 9 8 7 6**	**5 4 3 2 1**
10. Bank Vegetative Protection	More than 90% of the streambank surface covered by vegetation.	70-90% of the stream-bank surface covered by vegetation.	50-70% of the stream-bank surfaces covered by vegetation.	Less than 50% of the streambank surface covered by vegetation.
SCORE _____	**20 19 18 17 16**	**15 14 13 12 11**	**10 9 8 7 6**	**5 4 3 2 1**
11. Grazing or Other Disruptive Pressure	Vegetative disruption, through grazing or mowing, minimal or not evident; almost all plants allowed to grow naturally.	Disruption evident but not affecting full plant growth potential to any great extent; more than one-half of the potential plant stubble height remaining.	Disruption obvious; patches of bare soil or closely cropped vegetation common; less than one-half of the potential plant stubble height remaining.	Disruption of vegetation is very high; vegetation has been removed to 2 inches or less in average stubble height.
SCORE _____	**20 19 18 17 16**	**15 14 13 12 11**	**10 9 8 7 6**	**5 4 3 2 1**
12. Riparian Vegetative Zone Width	Width of riparian zone >18 meters; human activities (i.e., parking lots, roadbeds, clear-cuts, lawns, or crops) have not impacted zone.	Width of riparian zone 12-18 meters; human activities have impacted zone only minimally.	Width of riparian zone 6-12 meters; human activities have impacted zone a great deal.	Width of riparian zone <6 meters; little or no riparian vegetation due to human activities.
SCORE _____	**20 19 18 17 16**	**15 14 13 12 11**	**10 9 8 7 6**	**5 4 3 2 1**

FIELD DATA SHEET SEMI-QUANTITATIVE FISH SURVEY SAMPLING (continued)

Site ID: Stream Name: Date:

Abv.	Common Name	DELTP*	Description
BODY			
Bg	Black grub (Black spot)	P	Small black cyst approximately one millimeter in diameter, caused by a larval trematode.
Pe	External parasite	P	Ectoparasite attached to body, fins or gills (Leech, Fish louse, Anchor parasite etc.)
De	Deformities	D	Body structure is abnormal, ex. curvature of the spine
Fi	Fungal infection	L	Fungal infections may include a fuzzy (cotton) appearance w/ discolored areas or lesions.
Ma	Melanistic area	L	Darkly pigmented spot(s), not raised from the skin.
Os	Open sore	L	Lesions, (**Note** any mucus or necrotic tissue that may be in or around the sore)
Rr	Raised red sore	L	A bulging or bubbled red sore
Sh	Scales hemorrhagic	L	Evidence of bleeding or hemorrhaging at base of scales
Tu	Tumor	T	Unusual mass or fatty growth
Cw	White cysts	P	Usually a small crème-colored wart or bubble appearance
Cc	Caudal cysts	P	Small cysts specifically found on caudal peduncle, or at the base of anal/dorsal fins
Em	Emaciated	D	Body thin and lacks normal robustness, "starved"
EYES			
Ec	Eye cloudy	L	Eye cloudy or opaque
Ee	Eye exophthalmic	L	Eye bulging out of socket
Eh	Eye hemorrhagic	L	Eye bleeding or has evidence of burst blood vessels
Ed	Eye deteriorated	L	Eye deteriorated, obviously blind, eye missing completely
FINS			
Fe	Fins eroded	E	Erosion of the fin (make note of spawning, as some species will erode fins during this active time)
Fh	Fins hemorrhagic	E	Evidence of bleeding or hemorrhaging of fins
Ff	Fins frayed	E	Fins appear worn out and stressed (make note of spawning)
GILLS			
Ge	Gills eroded	E	Necrotic tissue or eroded to the point that sections of the gill filaments are missing
Gf	Gills frayed	E	Gill lamellae appear worn out and stressed
Gs	Gill spots	P	Small white spots throughout the lamellae
Gc	Gill cysts	P	Cyst attached to gills, usually a small crème-colored wart or bubble appearance
OTHER			
G	Gravid		Female fish full of eggs, releases upon handling
P	Post spawn		Egg sack has already released, abdominal wall stretched but empty, (Only if OBVIOUS)
M	Milting		Male fish releases milt upon handling

Record as- Species- Length(to nearest 5 mm) Anomaly
Example- Smallmouth Bass- 310WcEc, 470, 225Ge
***DELTP- Deformities, Eroded fins/gills, Lesions, Tumors, Parasites**

FIELD DATA SHEET SEMI-QUANTITATIVE FISH SURVEY SAMPLING (continued)

Site ID: Stream Name: Date:

SPECIES (length > 25mm)	# Photo(s)	Field Count	Lab Count	Total Count
1.				
2.				
3.				
4.				
5.				
6.				
7.				
8.				
9.				
10.				
11.				
12.				
13.				
14.				
15.				
16.				
17.				
18.				
19.				
20.				
21.				
22.				
23.				
24.				
25.				
26.				
27.				
28.				
29.				

FIELD DATA SHEET SEMI-QUANTITATIVE FISH SURVEY SAMPLING (continued)

Site ID: Stream Name: Date:

SPECIES (length > 25mm)	# Photo(s)	Field Count	Lab Count	Total Count
30.				
31.				
32.				
33.				
34.				
35.				
36.				
37.				
38.				
39.				
40.				

Comments:

GENERAL INSTRUCTIONS FOR FIELD DATA SHEET SEMI-QUANTITATIVE FISH SURVEY SAMPLING

Station ID: YEARMMDD-TIME-AGENCY, example: (20080601-1200-DEP). TIME = START TIME

Crew/Agency: List each crew member and agency or affiliation.

Location: General description, example: Transect is located 500 meters north of Pine Run and 1000 meters south of Rt. 441 bridge.

Start-End Time: Enter military time, start of shocking to end of shocking.

Mean Site Width: In the first 100 meters, 5 wetted channel widths are measured using a graduated measuring tape or range finder (every 20 meters from the starting point) and averaged.

Site Length: Wadeable = Minimum site length of 100 meters should be surveyed. Increased site length can be used as necessary to cover all habitats (pools, riffles, runs, and cascades).

Average Stream Width (m)	Minimum Site Length
<10m	100m
10 to 40m	10 times the average stream width
>40m	Maximum of 400m

Nonwadeable = Minimum site length of 500 meters should be surveyed. Increased site length can be used as necessary to cover all habitats (pools, riffles, runs, and cascades).

Latitude/Longitude: Decimal Degrees.

Current: Check one.

Species: List species and count all individuals > 25mm.

Column – Photo #: Number fish photographed.

Column – Field Count: The number of the fish identified in the field.

Column – Lab Count: The number of the fish identified in the laboratory.

Comments: Water/weather conditions, problems, etc.

Electrofishing Setup Quick Reference Guide

Standardize
1. Set waveform to Pulsed Direct Current
2. Set duty cycle to 25% (20%-30% based on manufacturer adjustments)
3. Set frequency to 60Hz

Adjust power [wattage (W) = voltage (V) x amperage (I)]
1. Set power adjustment to a minimum
2. Gradually increase power
    a. Refer to manufacturers specifications
    b. A general rule-of-thumb is never make adjustment to the control unit with power applied. Some adjustments with power applied will damage the control unit.
3. Stop adjustments when small bodied fish are adequately immobilized and injury is unlikely

Considerations
1. Low conductivity conditions
    a. Consider using larger anode rings
    b. If larger anode rings are not available or the desired response is still not achieved, the use of alternating current may be needed
    c. High voltage output is generally needed for low conductivity
2. High conductivity conditions
    a. Consider using smaller anode rings
    b. High amperage output is generally needed for high conductivity

(If the expected fish immobilization response is not observed, provide detailed comments and question representativeness)

A.4 FISH TISSUE FIELD DATA COLLECTION SHEET

COMMONWEALTH OF PENNSYLVANIA
DEPARTMENT OF ENVIRONMENTAL PROTECTION
BUREAU OF WATER STANDARDS AND FACILITY REGULATION

GENERAL INSTRUCTIONS FOR FIELD DATA SHEET TISSUE SAMPLING

STATION NUMBER: Enter WQN Station Number, WQF Station Number or Survey Station Number as appropriate. Leave blank if none of these apply.

NOTE: If WQN Number is entered, then Waterbody Name, Location, County and Municipality can be left blank.

DATE, COLLECTOR, AGENCY and COLLECTOR NUMBER: Must be completed.

PROJECT ID: **FISH**

NEW STATIONS: If a WQF station number has not previously been assigned, Quad. Name, Quad. Number, either Lat/Long or inches-N/inches-W and RMI (to nearest 0.1 mile) must be entered (for STORET input).

TISSUE TYPE: Standard samples are:

1. Skin-on, scaled fillets for gamefish, panfish and rough fish.
2. Skinless fillets for catfishes.
3. Skinless one-inch cross-sections for American Eel

SAMPLE: A normal sample is a composite of 10 fillets from 5 fish. For large fish, 5 fillets can be used.

75 PERCENT RULE: The individual fish in each composite should be as close to the same size as possible. A general guideline is that the length of the smallest individual in the composite should be no less than 75 percent of the length of the largest individual.

Fish Health:					
Black grub (Black spot)	**Bg**	Tumor	**Tu**	Fins eroded	**Fe**
External parasite	**Pe**	White cysts	**Cw**	Fins hemorrhagic	**Fh**
Deformities	**De**	Caudal cysts	**Cc**	Fins frayed	**Ff**
Fungal infection	**Fi**	Emaciated	**Em**	Gills eroded	**Ge**
Melanistic area	**Ma**	Eye cloudy	**Ec**	Gills frayed	**Gf**
Open sore	**Os**	Eye exophthalmic	**Ee**	Gill spots	**Gs**
Raised red sore	**Rr**	Eye hemorrhagic	**Eh**	Gill cysts	**Gc**
Scales hemorrhagic	**Sh**	Eye deteriorated	**Ed**		
Life History:					
Gravid	**G**	Post spawn	**Ps**	Milting male	**M**

COMMONWEALTH OF PENNSYLVANIA
DEPARTMENT OF ENVIRONMENTAL PROTECTION
BUREAU OF WATER STANDARDS AND FACILITY REGULATION

FIELD DATA SHEET TISSUE SAMPLING

Station # _____ Waterbody _____ Date & Time _____

Location _____

County _____ Municipality _____

Collector _____ Agency _____ Coll.# _____

COMPLETE FOR NEW STATIONS

Quad. Name _____ Quad.# _____

Lat. (inches N) _____ Long. (inches W) _____ RMI _____

Protocol: ☐ Electrofishing ☐ Rotenone ☐ Seine ☐ Angling

Tissue Type: ☐ Whole Fish ☐ Skin-on Fillet – Scaled ☐ Skinless Fillet ☐ Skin-on Fillet – Not Scaled ☐ Other (specify):

SPECIES	TL (inches)	WT (lbs. oz.)	Fish Health & Life History*
1.			
2.			
3.			
4.			
5.			
6.			
7.			
8.			
9.			
10.			

*Use Fish Health & Life History Codes (if needed)

Comments: (water/weather conditions, man-hours expended, problems, etc.)

_____

_____

_____

_____

_____

A.5 MUSSEL FIELD DATA COLLECTION SHEETS

Date: Site#: Location:

Water Temperature: Air Temperature: page _____ of _____

Dive #	Distance from X site	Distance from Shore	Latitude	Longitude	Diver	Backup Diver	Air In	Air Out	Time In	Time Out	Invasive Mussel Score and Species	Max Depth

Comments:

Bucket
% Boulder:
% Cobble:
%Gravel:
%Sand:
%Silt:

Dive
% Boulder:
% Cobble:
%Gravel:
%Sand:
%Silt:

Dive #	Distance from X site	Distance from Shore	Latitude	Longitude	Diver	Backup Diver	Air In	Air Out	Time In	Time Out	Invasive Mussel Score and Species	Max Depth

Comments:

Bucket
% Boulder:
% Cobble:
%Gravel:
%Sand:
%Silt:

Dive
% Boulder:
% Cobble:
%Gravel:
%Sand:
%Silt:

Dive #	Distance from X site	Distance from Shore	Latitude	Longitude	Diver	Backup Diver	Air In	Air Out	Time In	Time Out	Invasive Mussel Score and Species	Max Depth

Comments:

Bucket
% Boulder:
% Cobble:
%Gravel:
%Sand:
%Silt:

Dive
% Boulder:
% Cobble:
%Gravel:
%Sand:
%Silt:

Date: Location:

Site#	Dive#	Bag#	Genus	Species	Live or Dead	Length (mm)	Width (mm)	Depth (mm)	Weight (g)	Weight W/O Invasive Mussel Attached	Invasive Mussel Length (mm)	Notes

A.6 QBE PERIPHYTON FIELD DATA COLLECTION SHEET

PADEP QBE (Quantitative Benthic Epilithic) Periphyton Field Data Sheet - Page 1

Site / Sample Information

Station ID		**Latitude**	
Chem Coll-Seq			
Chem SAC Code		**Longitude**	
Date			
Time - Arrival		**Days Stable flow**	
Personnel			

Watershed Area (mi^2)			**Time(UTC-5)**	
Inorganic Substrate: % relative			**Temp (°C)**	
Bedrock			**SPC(µS/cm)**	
Boulder		> 10 in.	**pH**	
Cobble		2.5-10 in.	**DO (mg/L)**	
Gravel		0.1 - 2.5 in	**DO (%)**	
Sand		0.06-2 mm	**Turb (NTU)**	
Silt		0.004-0.06		
Clay		<0.004 mm		

Reach Orientation: DWS Facing Orientation taken at Densiometer 1/2 Downstream position (compass degrees CW from magnetic north)	

Densiometer Reading/Orientation	**Canopy Closure Leaf-Off Shaded Dots**	**Canopy Closure Leaf-On Shaded Dots**	**Record at stream widths**
Right Descending Bank			All
1/4 Upstream			> 25 meters
1/4 Downstream			> 25 meters
1/2 Upstream			All
1/2 Downstream			All
3/4 Upstream			> 25 meters
3/4 Downstream			> 25 meters
Left Descending Bank			All
Total / % Closure			

Quantity Rocks Sampled

% Canopy Closure/Density = Divide total by 68 (< 25m) or Divide by 136 (>25m)

Transect Random Rock Pick Calculation (Watershed area ≥ 1,000 sq mi = 27-rock & area < 1,000 sq mi = 9-rock)

Transect 1	**(Upstream)**		Random #'s:	Random #'s:	Random #'s:
RDB	**LDB**	**W / 1/3**			

Transect 2	**(Middle)**		Random #'s:	Random #'s:	Random #'s:
RDB	**LDB**	**W / 1/3**			

Transect 3	**(Downstream)**		Random #'s:	Random #'s:	Random #'s:
RDB	**LDB**	**W / 1/3**			

PADEP QBE - 2 (Quantitative Benthic Epilithic) Periphyton Field Data Sheet - Page 2

Sub-Sample Processing Information:

Total Diluted Algae Slurry Volume (ml): Dilute as needed to achieve min required: 700ml with cyanotoxin test, 400ml without cyanotoxin	

Algal ID Bottles & Notes - 100ml well homoginized slurry in 120ml white plastic sample bottle + 7ml formaldehyde preservative (Collelct min three 100ml replicate bottles).

Phycocyanin Bottle & Notes - 30ml in 120ml white plastic sample bottle. Store/transport on wet ice. Upon arrival at BOL deliver to BOL Bacti refrigerator. BOL will pull 3 sub-samples from this single bottle.

Collector #	Sequence #	

Chlorophyll-a Filter & Notes - 2ml on GFF filter with 1ml MgCO3 fixative - foil wrapped. (Collect 3 replicate filters). Store/transport on dry ice then store at -80°C in BOL Bacti freezer. Indicate if submitting for acidified or non-acidified processing.

Collector #	Sequence #	

AFDM Filter & Notes - 2ml on GFF filter - foil wrapped. (Collect 3 replicate filters). Store/transport on dry ice then store at -80°C in BOL Bacti freezer.

Collector #	Sequence #	

Other Notes

If collecting for cyanotoxin testing: 250ml in whirl-pak bag then place whirl-pak in ziplock bag. Store/transport on dry ice then store at -80°C in Monitoring Section freezer.

A.7 QMH PERIPHYTON FIELD DATA COLLECTION SHEET

PADEP QMH (Qualitative Multi-Habitat) Periphyton Field Data Sheet - Page 1

Site / Sample Information

Station ID		**Latitude**	
Chem Coll - Seq			
Chem SAC Code		**Longitude**	
Date			
Time - Arrival		**Days Stable flow**	
Personnel			

Stream Order	

Time(UTC-5)	
Temp (°C)	
SPC(µS/cm)	
pH	
DO (mg/L)	
DO (%)	
Turb (NTU)	

Total Diluted Algae Slurry Volume (ml): Dilute as needed to achieve min required: 700ml with cyanotoxin test, 400ml without cyanotoxin	

Reach Orientation: DWS Facing Orientation taken at Densiometer 1/2 Downstream position (compass degrees CW from magnetic north)	

Densiometer Reading/Orientation	Canopy Closure Leaf-Off Shaded Dots	Canopy Closure Leaf-On Shaded Dots	Record at stream widths
Right Descending Area			All
1/4 Upstream			> 25 meters
1/4 Downstream			> 25 meters
1/2 Upstream			All
1/2 Downstream			All
3/4 Upstream			> 25 meters
3/4 Downstream			> 25 meters
Left Descending Area			All
Total / % Closure			

% Canopy Closure/Density = Divide total by 68 (< 25m) or Divide by 136 (>25m)

Periphyton Habitats: % relative qualification

Epilithic (Rock) %			**Epidendric (Wood) %**	
Epiphytic (Plant) % [a]			**Episammic (Sand) %**	
Epipelic (Silt) %			**Gravel %**	
Other %			**Other %**	

PADEP QMH (Qualitative Multi-Habitat) Periphyton Field Data Sheet - Page 2

Algal ID Bottles & Notes - 100ml well homoginized slurry in 120ml white plastic sample bottle + 7ml formaldehyde preservative (Collect min three 100ml replicate bottles).

Other Notes: [a] Record -plant taxa from which sampled collected. **If collecting for cyanotoxin testing:** 250ml in whirl-pak bag then place whirl-pak in ziplock bag. Store/transport on dry ice then store at -80°C in Monitoring Section freezer.

A.8 ALGAE IDENTIFICATION AND ENUMERATION LABORATORY SUBMISSION DATA SHEET

Algae Identification and Enumeration Laboratory Submission Data Sheet											
PA DEP GIS Key ID	Date	Sample Type (QMH = Quitative Multi-Habitat, QBE = Quantitative Benthic Epilithic)	Stream Name	Location Description	County	Latitude	Longitude	Surface Area (cm^2)	Initial Sample Volume (ml)	ID Bottle Sub-Sample Volume (ml)	Formaldehyde Added (ml)

A.9 MICROBIOLOGY SAMPLE SUBMISSION SHEET FOR ACIDIFIED CHLOROPHYLL-A, PHYCOCYANIN, AND AFDM

1500-FM-BOL0151 Rev. 10/2015

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DEPARTMENT OF ENVIRONMENTAL PROTECTION
BUREAU OF LABORATORIES
2575 INTERSTATE DRIVE
HARRISBURG, PA 17110-9332

MICROBIOLOGY SAMPLE SUBMISSION SHEET FOR PA DEP

LAB USE ONLY **Date:**

LAB Number: **Received by:**

Temp < 6 C: ☐ Yes or ☐ No

Sample Exceeds Holding time _____
Verify Legal Seal #

Collector ID: **Seq. No.:** **Date Collected: mm/dd/yyyy** **Time collected: Military**

Reason: **Cost Center** **Program:** **SAC or Suite:** **Legal Seal #** Intact Initials

☐ Yes or ☐ No _____

☐ Yes or ☐ No _____

Total # of bottles submitted to Lab: _____ Total # of bottles verified by the Lab (initial): _____

Facility, Source or Case: _____

Address: _____

City, Borough or Township: _____ State: _____ Zip Code: _____

Sample Location or Description: _____

SAMPLE TYPE:

☐ **Drinking Water: [SAC B010 (Presence/Absence) or B017 (count)]**

(√ all that apply) ☐ Public ☐ Non-Public ☐ Regular ☐ Check ☐ Source Water

☐ Reservoir ☐ Well ☐ Spring ☐ Other _____

(√ only one) ☐ Non-reportable ☐ Report to SDWA (Public)

PWSID # **Location ID #**

☐ **Sewage Treatment: [SAC B002]**

(√ all that apply) ☐ Influent ☐ Effluent ☐ Upstream ☐ Downstream ☐ Monitoring Well

☐ **Non-Potable Water:** (√ all that apply)

☐ Total Coliform (B001) ☐ Fecal Coliform (B002) ☐ E. coli (B022) ☐ HPC (B008)

☐ Fecal Strep (B012) ☐ Enterococci (B024) ☐ Plankton (B000) ☐ Chlorophyll (B019)

☐ Biosolids: Multiple Tube Fermentation (SAC B018) and Membrane Filtration (SAC B020)

Collector Name (Print): _____

Relinquished by (Signature): _____

Date: __/__/____ **Phone:** (____) _____ **E-mail:** _____

How Shipped: ☐ Contracted Courier ☐ Hand Delivered to Lab

A.10 BACTERIA MONITORING FIELD AND SITE DATA SHEET

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WATER CONTACT SPORTS RECREATION ASSESSMENT FIELD DATA SHEET

PROJECT :

COLLECTOR : COLLECTOR ID :

DATE : ROUND FOR SAMPLES: OF 5

SITE NAME	*SEQUENCE NO	SAMPLE TIME	WEATHER past 24 hours ?	RAIN past 24 hours ?	WEATHER Present ?	RAIN Present ?	WATER VISIBILITY ?	VELOCITY (Flow), WATER LEVEL DEPTH ?	STORM occurred within # hours ?	Is there evidence a storm occurred ?	Can you SAFELY enter the water ? LAND | WATER	Water SAFE for Recreation ?	TYPES of Recreation ?
											|		
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TOTAL NUMBER OF SAMPLES COLLECTED : ( )

* USE (B) BLANK OR (D) DUPLICATE FOR QA SAMPLES

ATMOSPHERIC CONDITIONS

Weather the past 24 hours? (S) SUNNY (PC) PARTLY CLOUDY (O) OVERCAST (R) RAIN OTHER_____

RAIN the past 24 hours (INCHES)? (N) NONE (0") (LR) LIGHT RAIN (0.15" - 0.25") (HR) HEAVY RAIN (0.5"+)
(TR) TRACE RAIN (0.1" - 0.15") (MR) MODERATE RAIN (0.25" - 0.5") OTHER_____

Weather Present? (S) SUNNY (PC) PARTLY CLOUDY (O) OVERCAST (R) RAIN OTHER_____

RAIN Present (INCHES)? (N) NONE (0") (LR) LIGHT RAIN (0.15" - 0.25") (HR) HEAVY RAIN (0.5"+)
(TR) TRACE RAIN (0.1" - 0.15") (MR) MODERATE RAIN (0.25" - 0.5") OTHER_____

SURFACE WATER CONDITIONS

WATER VISIBILITY? (1) OPTIMAL (Normal/Clear) (3) MARGINAL (Mildly Discolored/Dirty/Stained) (5) POOR (Heavily Discolored/Muddy)
(2) SUB-OPTIMAL (Slightly Discolored/Dirty/Stained) (4) SUB-MARGINAL (Moderately Discolored/Dirty/Stained) OTHER_____

WATER TURBIDITY CAUSE? (S) STORM (C) CONSTRUCTION (B) BOATS/BARGES (A) ANIMALS (LG) LOW GRADIENT (AMD) ACID MINE DRAINAGE
OTHER_____

VELOCITY/FLOW, WATER LEVEL/DEPTH? (1) LESS THAN BANKS (Low Flow/Drought) (3) 1/4 of BANKS FULL (Slightly Elevated Flow) (5) 3/4 of BANKS FULL (Moderately Elevated Flow)
(2) BASE OF BANKS (Base Flow/Normal) (4) 1/2 of BANKS FULL (Mildly Elevated Flow) (6) BANKS FULL (Turbulent Flow/Raging/Storm)
OTHER_____

How many hours since last storm occurred? Number of hours.

Is there evidence a storm occurred? (Y) YES (N) NO If YES, then explain in COMMENTS.
Increased Flow, Water fills (1/4 banks, 1/2 banks, 3/4 banks, both banks), Discolored/Muddy/Turbulent Water, Debris floating in water, Sewage/Storm Discharge.
OTHER_____

Can you safely enter the water from both land and water? (Y) YES (N) NO If NO, then explain in COMMENTS. ANSWERS FOR SAFETY BOTH ON LAND AND IN WATER.
Increased Flow, Mud/Silt Bottom, Vegetation, Wildlife, Woody Debris, Boulders, Trash, Muddy Water, Turbulent Water,
Sewage, AMD, Foul Smell, Sewage/Storm Discharge, Tanic Water, Low Gradient, Beaver Dam, Poison Ivy, Other Vegetation OTHER_____

Is the water safe for RECREATION? (Y) YES (N) NO If NO, then explain in COMMENTS.
Increased Flow, Mud/Silt Bottom, Vegetation, Wildlife, Woody Debris, Boulders, Trash, Muddy Water, Turbulent Water,
Sewage, AMD, Foul Smell, Sewage/Storm Discharge, Tanic Water, Low Gradient, Beaver Dam, Poison Ivy, Other Vegetation OTHER_____

Types of Recreation occurring? (A) ALL (F) FISHING (WD) WADING (B) BOATING/KAYAKING/CANOING/PADDLE BOARDING/JET SKIING
(WC) WATER CONTACT SPORTS (Swimming, Tubing, Skiing, Wake Boarding, Water Play by Children, Skin Diving, Bathing, Surfing) OTHER_____

COMMENTS :

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WATER CONTACT SPORTS RECREATION ASSESSMENT SITE DATA SHEET

Follow up questions after a site completed of sampling.

PROJECT :

COLLECTOR : COLLECTOR ID :

DATE :

SITE NAME	LAND USE ?	SECONDARY LAND USE ?	WATER REGIME ?	SUBSTRATE MATERIAL ?	SITE REGIME ?	SEDIMENT ODOR ?	Is surface water free of scum/debris ?	Is stream bottom free of scum/debris ?	Type(s) of Recreation possible ?	Water Contact Sports recreation possible ?	Do Water Contact Sports recreation activities occur ?	Are Water Contact Sports recreation safe ?

LAND USE? (F) FOREST (A) AGRICULTURE (U) URBAN (W) WETLAND (G) GRASSLAND/SCRUB/SHRUB

SECONDARY LAND USE?
PASTURE, CROPLAND, ANIMAL FARM, NATURAL GRASS/HAY FIELD, MOWED FIELD, WETLAND, ABANDONED MINE DRAINAGE, CEMETARY, HOUSING COMPLEX, BEAVER POND, INDUSTRY/PARKING LOT, DISCHARGE PIPE/CSO/SEWAGE, CEMENT CHANNEL OR WALLS, FISH HATCHERY IMPOUNDMENT, LAKE OTHER_____

WATER REGIME? (RF) RIFFLE (RN) RUN (P) POOL (I) IMPOUNDMENT OTHER_____

SUBSTRATE MATERIAL? (F) FINES/SAND (M) MUD (S) SILT (R) ROCK/BOULDER OTHER_____
(G) GRAVEL

SITE REGIME? (B) BOAT LAUNCH (S) SWIMMING SITE (Known local site) (F) FISHING SITE (Known local site)
(C) CAMPGROUND (M) MARINA (Docks, Sliding Board) OTHER_____

SEDIMENT ODOR? (N) NORMAL (S) SEWAGE/MANURE (O) OIL (M) METALLIC OTHER_____

Is the surface water free of scum/debris? (Y) YES (N) NO If NO, then explain in COMMENTS.
Algae, Algal Bloom, Vegetation, Trash, Floating Debris, Sewage, Animal Feed, Oil. OTHER_____

Is the stream bottom free of scum/debris? (Y) YES (N) NO If NO, then explain in COMMENTS.
Algae, Vegetation, Trash, Submerged Debris, Sewage, Animal Feed, Oil. OTHER_____

What types of recreation are possible?
(A) ALL (F) FISHING (WD) WADING (B) BOATING/KAYAKING/CANOING/PADDLE BOARDING/JET SKIING
(WC) WATER CONTACT SPORTS (Swimming, Tubing, Skiing, Wake Boarding, Water Play by Children, Skin Diving, Bathing, Surfing) OTHER_____

Is water contact sports recreation possible? (N) NEVER (S) SELDOM (F) FREQUENTLY (NA) DO NOT KNOW

Do water contact sports recreation activities occur (N) NEVER (S) SELDOM (F) FREQUENTLY (NA) DO NOT KNOW

Is water contact sports recreation safe? (N) NEVER (S) SELDOM (F) FREQUENTLY (NA) DO NOT KNOW

COMMENTS :

A.11 LAKE ASSESSMENT AND TSI FIELD DATA SHEET

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DEPARTMENT OF ENVIRONMENTAL PROTECTION
BUREAU OF CLEAN WATER

LAKE ASSESSMENT AND TSI FIELD DATA SHEET

Lake Name _____ County _____ TWP or Mun. _____

Station _____ Lat. _____ Long. _____

Date _____/_____/_____Time _____UTC-5 Collector(s) _____

Weather _____

Cloud Cover (%) 0 25 50 75 100 Comments (hazy/foggy) _____

Wind Conditions: none light mod. heavy Direction: _____

Rain Conditions: none drizzle light mod heavy

Surface Turbulence _____ Air Temp. (°C) _____

PROFILE MEASUREMENTS

FIELD METER Make/Model: Serial Number:

DEPTH (meters)	Time (UTC-5)	TEMP (°C)	D.O. (mg/L.)	D.O. (%Sat)	pH	Sp.Cond. (Umhos)	Thermocline (E-M or M-H)†
Surface							

†E-M = Thermocline between Epilimnion and Metalimnion; M-H = Thermocline between Metalimnion and Hypolimnion.

Max. Depth (meters) _____ SECCHI DISK READING (tenths of a meter) _____

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DEPARTMENT OF ENVIRONMENTAL PROTECTION
BUREAU OF CLEAN WATER

LAKE/RESERVOIR FIELD DATA SHEET

SAMPLES COLLECTED

Type	**SAC**	**Location**	**Depth**	**Volume Filtered**	**Time Collected (UTC-5)**	**Collector-Seq#**
CHLOROPHYLL-A		1m below surface	1 m	50 mL		
EPILIMNION WATER		1m below surface	1 m	NA		
HYPOLIMINION WATER				NA		
PLANKTON TOW Net Opening Diameter _____inches		See Instructions below Preservation _____mL Formalin/Lugols		L Towed* ~mL Collected		
Additional Sample(s) _____ _____	SAC(S) _____ _____					

Plankton tow – begin tow 1 meter above lake bottom OR where DO is 2mg/L or more. Perform TWO tows + rinse & store in 500ml bottle. Record depth of tows! *Calculate the volume of lake water filtered during the two tows using this equation ($\pi r^2(d*2))*1000$ by multiplying (depth towed)), where r = radius of the net opening in meters, d = depth of tow in meters.

QA SAMPLES COLLECTED

QA SAMPLE - SAC	**Check if Collected**	**Location (EPILIMNION, HYPOLIMINION, PLANKTON)**	**Time Collected (UTC-5)**	**Collector-Seq#**
Field Blank – Chlorophyll-a				
Field Blank – Chem				
Sequential Replicate – Chlorophyll-a				
Split Replicate – Chlorophyll-a				
Sequential Replicate - Chem				
Split Replicate - Chem				
Sequential Replicate – Plankton				
Split Replicate – Plankton				

COMMENTS _____

_____

_____

_____

_____

_____

_____

_____

SIS Entry Date: _____ Username: _____

A.12 HARMFUL ALGAL BLOOM (HAB) FIELD DATA SHEET

HAB Field Data Collection Sheet

First Name: Last Name: Agency: Phone #: Email:

Waterbody and Location Description:

Location Type:
☐Open Water ☐Beach ☐Boat Launch ☐Shoreline ☐Other/Alternate

Survey Date: Latitude (DD or DMS): Longitude (DD or DMS):

Sample Reason:
☐Routine ☐Bloom (Suspected) ☐Bloom (Response to Report)

Sample Type (Select all that apply):
☐Observation ☐Water Sample

Sample Method (if applicable):
☐Transect(Composite) ☐Scum Grab ☐Surface Grab ☐Depth Grab

If Depth Grab, what is the depth?
Depth (m):_____

Sample Information:
Time of Observation/Water Sample (24-hour Format): _____
Collector ID (if applicable): _____
Sequence # (if applicable): _____
SAC Codes (if applicable): B049 + B041
QA Info (if applicable): ☐Blank ☐Duplicate

Discrete field measurements (if applicable):
Temperature (°C): _____ Specific Conductance (µs/cm): _____ pH (SU): _____
DO (mg/L): _____ DO Saturation (%):_____ Secchi Disk (m): _____
Turbidity (FNU): _____ Chl-a (RFU): _____ PC (RFU): _____

Bloom Description (Select all that apply):
☐No Bloom ☐Bubbling Scum ☐Hairy/silky strands ☐Green Dots/Clumps ☐Green Streaks
☐Spilled Paint Appearance ☐Pea Soup Appearance ☐Other: _____

Bloom Color (Select all that apply):
☐No Bloom ☐Green ☐Blue-green ☐White ☐Other: _____

Bloom Extent:
☐No Bloom ☐Small Localized ☐Large Localized ☐Widespread

Water Uses at Sampling Location (Select all that apply):
☐Primary Regulated (DOH beach) ☐Primary Unregulated (swim at own risk, water contact sports recreation (ie water ski, tubing))
☐Secondary Contact (wading, boating, water sports (ie paddle boarding)) ☐No Known ☐Other (Describe):_____

Photos Available/Submitted: ☐Yes ☐No
Additional Notes: _____

_____

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INSTRUCTIONS: HAB Field Data Collection Sheet

The HAB Field Data Collection Sheet (data sheet) is to be filled out with each observation/sample. The data sheet is universal for both observations and water samples. If an observation is being made WITHOUT a water sample the collector only need to fill in the applicable portions of the data sheet. If an observation is being made AND a water sample is being collected ALL portions of the data sheet are to be completed to include discrete field measurements if equipment is available.

Block 1 INSTRUCTIONS

Block 1 contains contact information, survey date and the description of the waterbody. Portions can either be filled out ahead of time using the PDF Fill Form or at the time of survey. *(NOTE: SURVEY DATE & WATERBODY + LOCATION DESCRIPTION SHOULD BE TRANSFERRED TO THE LAB SAMPLE SUBMISSION SHEET).*

First Name = First Name
Last Name = Last Name
Agency = Agency for whom the data are being collected on behalf of (ie DEP, DCNR, DOH, Army Corps, Private)
Phone # = Best phone number to reach the collector in case there are questions regarding sample submission
Email = Email address in which the collector wants to receive results and other communications
Waterbody and Location Description = Lake or Stream Name with a brief description (ie Lily Lake, west shoreline boat dock)
Location Type = General selection of what the point represents. Selections include beach, boat ramp, shoreline, open water, other/alternative.
Survey Date = Date of visit/sampling event
Latitude and Longitude = Coordinates of the exact location of the observation or water sample. Use either Decimal Degrees (DD) or Degrees Minutes Seconds (DMS), please circle unit of measurement.

Block 2 INSTRUCTIONS

The second block in the field form asks the collector several questions about the sample being collected. This is to be filled out at time of survey and should include the time of the observation or water sample, if applicable record of the sample identification number which is a combination of a Collector-ID and a 3-digit Sequence #. A Collector-ID is to be obtained prior to sample collection if water samples are being collected. A sequence number is randomly selected by the collector and collections should thereafter occur in sequential order. Additional, definitions are provided below to help aid in selection. *(NOTE: TIME, COLLECTOR-ID, & SEQUENCE # SHOULD BE TRANSFERRED TO THE LAB SAMPLE SUBMISSION SHEET)*

Sample Reason (NOTE: THIS INFORMATION SHOULD BE TRANSFERRED TO THE LAB SAMPLE SUBMISSION SHEET)
Routine = A sample that is collected in conjunction with a special project. For example, you are at the lake doing routine water collections and want to take a sample for development purposes. A routine sample will typically not have any “bloom” like characteristics. Waters are usually clear with little to no color that would indicate substantial algae growth.
Bloom (Suspected) = A sample which you collect because you are out monitoring a waterbody and see “bloom” conditions. This would be a sample that was not in a response to requested investigation rather the collector stumbled upon a condition.
Bloom (Response) = A sample that is collected in response to public/managerial notification to an overseeing agency. These samples may or may not have “bloom” characteristics but should be treated as such since there is some level of concern or urgency at the agency level.

Sample Type
Observation = Collection of visual data.
Water Sample = Collection of a water sample to be analyzed for presence of HABs.

Sample Method
Transect (Composite) = A sample in which grab samples are collected at multiple locations HORIZONTALLY, composited into a collection container, combined, and used to obtain the water sample (ie beach composite).
Scum Grab = This is a targeted sample of a concentrated bloom where definitive characteristics describe the occurrence such as a scum, clumps or streaks.
Depth Grab = A sample that is collected at a specific water column depth (ie 5 meters) using Van Dorn, Kemmerer, or Integrated Depth sampler.
Surface Grab = A water sample that is collected from just below the water’s surface and does NOT target a particular concentrated area. For example, there may be no bloom or the bloom is large where most of the water is of one appearance (pea soup or spilled paint).

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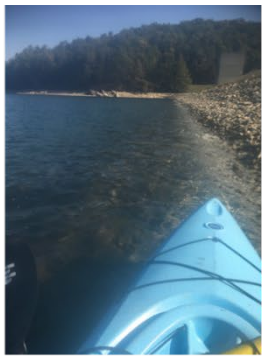
Block 3 INSTRUCTIONS

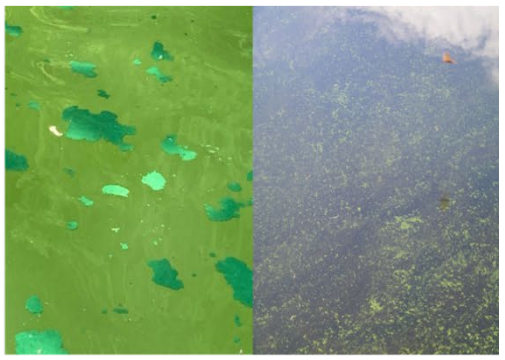
The third block in the field form asks the collector several questions about the observed bloom. If no bloom is observed, please indicate as such. This is to be filled out at the time of survey. Images are provided to aid in interpretation.



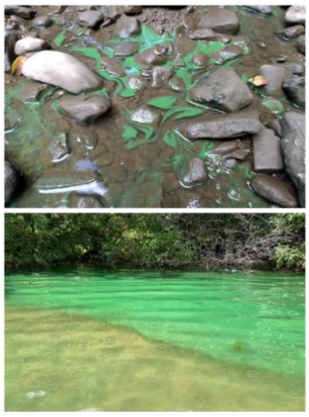
No Bloom

Bubbling Scum

Hairy Silky Streaks

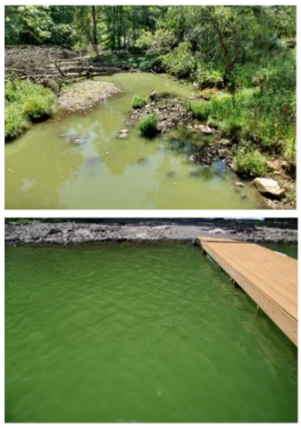


Green Dots/Clumps



Spilled Paint Appearance

Green Streaks



Pea Soup Appearance

Block 4 INSTRUCTIONS

The fourth block in the field form asks the collector to describe the water uses at the surveyed location. There are options for primary contact (regulated), primary contact (unregulated), secondary contact, no known recreation, and an opportunity to describe other activities. Both primary contact options involve activities where full body emersion with a potential of water ingestion exist. The primary contact regulated option should be selected when sampling specifically in a Department of Health (DOH) regulated beach. Primary contact unregulated option should be used when the water is being used for recreation with a great potential for full body emersion. Activities under primary contact unregulated would be water contact sports recreation, such as jet skiing, water tubing and water skiing, or circumstances where swim is not allowed but is being observed (swim at your own risk). Secondary contact activities are those activities where full body emersion is not immanent, and ingestion of water is less likely (ie wading, boating, and other water sports, such as paddle boarding or kayaking). *(NOTE: WATER USE INFORMATION SHOULD BE TRANSFERRED TO THE LAB SAMPLE SUBMISSION SHEET)*

It is requested that photos of the observations in the field be taken, if at all possible, retained for your record, and forwarded to the HAB program coordinator for archiving. This fourth block also gives opportunity for additional notes. Examples of notes include:

- HAB Awareness Signs have been posted.
- Information regarding additional sampling efforts such as the collection of water quality data.
- Observations other than what is requested in the field form. For instance, observations of a pollutant or smells.
- Notes regarding possible environmental effects associated with the bloom (ie fish kill, plant die off, public health, etc.)

A.13 AQUATIC MACROPHYTE FIELD DATA SHEET

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AQUATIC MACROPHYTE FIELD DATA SHEET

(if more than 20 rake tosses required please use an additional sheet)

Lake/Reservoir Name:_____County:_____Twp or Mun.:_____

Date Collected:_____TimeCollected:_____

Collectors:_____

Lowrance File Name(s):_____

Secchi Depth:_____Attenuation Depth (2x Secchi Depth):_____

Plant Density Data Collection

Toss Density Rating Table						
Description	*None*	*Sparse*	*Light*	*Moderate*	*Dense*	*Very Dense*
Range	*0%*	*1 - 20%*	*21 - 40%*	*41 - 60%*	*61 - 80%*	*>81%*
Rating	0	1	2	3	4	5
Bed Types = Floating (F), Emergent (E), Submerged (S)						

Rake #1	Random Distance from Shore (%)	Latitude	Longitude	Bed Type	Depth	Rating

Comments:

_____

_____

_____

_____

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BUREAU OF CLEAN WATER

AQUATIC MACROPHYTE FIELD DATA SHEET
(if more than 20 rake tosses required please use an additional sheet)

Plant Composition/Abundance Data Collection

Abundance Rating Table				
Description	*Present*	*Scarce*	*Common*	*Dominant*
Range	*<2%*	*2 - 20%*	*21-60%*	*>60%*
Abundance Rating	Use the description and range percentages to record a proportional percentage, total abundance rating for each rake will equal 100%			

Rake # / **Species**	Abundance Rating	**Rake #** / **Species**	Abundance Rating	**Rake #** / **Species**	Abundance Rating
Rake # / **Species**	Abundance Rating	**Rake #** / **Species**	Abundance Rating	**Rake #** / **Species**	Abundance Rating

Rake #	Abundance	**Rake #**	Abundance	**Rake #**	Abundance
Species	Rating	**Species**	Rating	**Species**	Rating
Rake #	Abundance	**Rake #**	Abundance	**Rake #**	Abundance
Species	Rating	**Species**	Rating	**Species**	Rating
Rake #	Abundance	**Rake #**	Abundance	**Rake #**	Abundance
Species	Rating	**Species**	Rating	**Species**	Rating

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Rake #	Abundance	**Rake #**	Abundance	**Rake #**	Abundance
Species	Rating	**Species**	Rating	**Species**	Rating
Rake #	Abundance	**Rake #**	Abundance	**Rake #**	Abundance
Species	Rating	**Species**	Rating	**Species**	Rating

Vouchered Specimens

	Vouchered IDs		Vouchered IDs
	(YYYYMMDD-LakeAlias-R#-Collector)		(YYYYMMDD-LakeAlias-R#-Collector)
1		13	
2		14	
3		15	
4		16	
5		17	
6		18	
7		19	
8		20	

9		21	
10		22	
11		23	
12		24	

Comments:

_____
_____
_____
_____
_____
_____
_____

APPENDIX B: CHEMICAL DATA COLLECTION INFORMATION AND FORMS

B.1 FIELD METER CALIBRATION FORM

FIELD METER CALIBRATION FORM	
METER MAKE/MODEL	
METER SERIAL NO.	
DATE	
TIME	
NAME	

SPECIFIC CONDUCTANCE

STD (µS/cm)	TEMP (°C)	READING (µS/cm)	ADJUSTED (µS/cm)	LOCATION
100				
1000				
5000				
Reading in air (should be 0)				

pH

pH BUFFER	TEMP (°C)	TABLE pH	READING (pH units)	ADJUSTED (pH units)	MILLI-VOLTS	LOCATION
7.00						
10.00						
4.00						

TURBIDITY

STD (FNU)	TEMP (°C)	READING (FNU)	ADJUSTED (FNU)	LOCATION
0				
124 / 126				
1000 / 1010				

DISSOLVED OXYGEN

TIME	TEMP (°C)	BARO PRES (mmHg)	TABLE VALUE (mg/L)	READING (mg/L)	READING (%)	ADJUSTED (mg/L)	ADJUSTED (%)	LOCATION

B.2 TRANSECT DATA COLLECTION FORM

TRANSECT DATA COLLECTION FORM

WATERBODY NAME (Juniata River)		**COMMENTS** (Discharge, conditions, etc.)	
TRANSECT NAME (Newport Transect)			
METER MAKE/MODEL (ProDSS, Eureka, etc.)			
METER SERIAL NO.			
DATE			
COLLECTOR(S)			
TIME OF CALIBRATION		**TIME OF AFTER CHECK**	

CALIBRATION

SPECIFIC CONDUCTANCE

STD (µS/cm^c)	TEMP (°C)	READING (µS/cm^c)	ADJUSTED (µS/cm^c)	LOCATION
100				
1000				
5000				
Reading in air (should be 0)				

pH

pH BUFFER	TEMP (°C)	TABLE pH	READING	ADJUSTED	mV	LOCATION
7.00						
10.00						
4.00						

TURBIDITY

STD (FNU)	TEMP (°C)	READING (FNU)	ADJUSTED (FNU)	LOCATION
0				
124 / 126				
1000 / 1010				

DISSOLVED OXYGEN

TIME	TEMP (°C)	READING (mg/L)	READING (%)	LOCATION
BP (mmHg)	TABLE VALUE	ADJUSTED (mg/L)	ADJUSTED (%)	

AFTER CHECK

SPECIFIC CONDUCTANCE

STD (µS/cm)	TEMP (°C)	READING (µS/cm^c)	LOCATION
100			
1000			
5000			
Reading in air (should be 0)			

pH

pH BUFFER	TEMP (°C)	TABLE pH	READING	mV	LOCATION
7.00					
10.00					
4.00					

TURBIDITY

STD (FNU)	TEMP (°C)	READING (FNU)	LOCATION
0			
124 / 126			
1000 / 1010			

DISSOLVED OXYGEN

TIME	TEMP (°C)	READING (mg/L)	READING (%)	LOCATION
BP (mmHg)	TABLE VALUE			

DISCRETE MEASUREMENTS											
POINT	SIS ALIAS ID	COLLECTOR #	SEQUENCE #	TIME	TEMP	SP COND	pH	DO	DO	TURB	COMMENTS
					(°C)	(µS/cm[c])	(SU)	(mg/l)	(%)	FNU	(e.g., YOY habitat, point dry)
1											
2											
3											
4											
5											
6											
7											
8											
9											
10											
11											
12											
13											
14											
15											
16											
17											
18											
19											
20											

COMMENTS

Date entered into SIS: _____ Completed by: _____

Date scanned: _____ Completed by: _____ Scanned file location: □ Archive folder □ CIM folder

B.3 SAMPLE INFORMATION SYSTEM USER'S GUIDE

Sample Information System
User's Guide

Prepared by:	Applications Help Desk
Version:	1.4
Date:	November 19, 2014

Change History

Version	Date	Description
1.0	April 29, 2005	Version for user review
1.1	June 1, 2005	Updated with end user comments (Connie, Gail, John, and Sharon's comments)
1.2	October 19, 2006	Updated based on Web changes
1.3	November 15, 2008	Updated based on screen changes
1.4	November 19, 2014	Updated formatting and to reflect screen changes. Added sections on projects and reports.

PURPOSE

Welcome to the user's guide for the Sample Information System (SIS). This guide provides instruction on how to create and query samples records, create monitoring points, and run reports on sample results. SIS is a repository for chemical analysis results for samples analyzed by the Bureau of Laboratories. SIS also serves as the repository for some self-monitoring samples submitted to the Department of Environmental Protection (DEP). DEP staff may enter information about each sampling event such as the location and field test results. SIS issues a report of the analytical results for each sample analyzed by the Bureau of Laboratories. The system e-mails this report to the sample collector or the assigned group/business unit. The analytical results remain available within SIS and can be queried using the sample details. The application also includes a utility that enables sample information to be exported to a file.

APPLICATIONS SUPPORT HELP DESK

Applications Support Help Desk Line
Number: (717) 705-3768
Hours: Monday to Friday 8:00 am to 4:30 pm
Email: ep-efactshelpdeskteam@pa.gov
Applications Support Help Desk Team

The Applications Support Help Desk Team is composed of members from TreCom Systems Group, Inc. and the DEP. The support team includes help desk specialists, trainers, web masters, on-line help developers, and testing engineers working together to provide complete end-user support for applications within the DEP and applications used by DEP clients.

Applications Support Help Desk Team Services

- Application training
  - Formalized classroom training
  - Small group training

  - One-on-one training
- Participate in meetings to provide application guidance
- Telephone support help desk
- Application web page development and maintenance
- Publish articles identifying solutions to common problems
- Application testing
- Documentation development
- Application on-line help development and maintenance

WHAT IS NHD?

The National Hydrography Dataset (NHD) is a set of digital spatial data created by the USGS and USEPA containing information about naturally occurring and constructed bodies of water, natural and artificial paths through which water flows, and related hydrographic entities. These entities, or features, are combined to form reaches, which provide the framework for linking (or geo-coding) water-related data to the NHD surface water drainage network.

ACCESSING SIS

Security

Security roles assigned to a username determines a user's ability to create, update, or delete information in SIS. To add or modify security roles, contact a system coordinator to submit a security request. The available security roles are:

- Sample Query
- Sample Entry
- Project Entry

Logging on to SIS

A user with security access to SIS can login as follows:

Open the IntraDEP page (http://intradep/).
Click the "Oracle Applications" link.
Click the "SIS – Samples Information System" link and the Logon pop-up window displays.

> 🕮 The first time accessing Oracle applications several security prompts display. Reference the "Special note regarding security warnings" located at the top of the "Oracle Applications" page for instructions on responding to the security prompts.

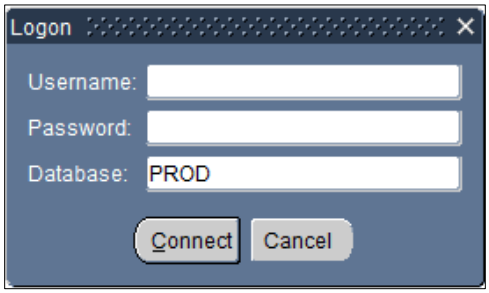


Figure 1: The Logon pop-up window.

Click in the *Username* field and enter a user name.

Click in the *Password* field and enter the password.
Click in the *Database* field and enter “PROD”.
Click the CONNECT button to log on to SIS.

Changing an Oracle Password

Each user has a single password for all Oracle applications. Changing the password used to access SIS also changes the password for *e*FACTS, CTS, etc.

Change an Oracle password as follows:

1. Open the IntraDEP page (http://intradep/).
2. Click the “Oracle Applications” link.
3. Click the CHANGE PASSWORD button and the Logon pop-up window displays.
4. Click in the *Username* field and enter a user name.
5. Click in the *Password* field and enter the current password.
6. Click in the *Database* field and enter PROD.
7. Click the CONNECT button and the “Change Oracle Password” pop-up window displays.

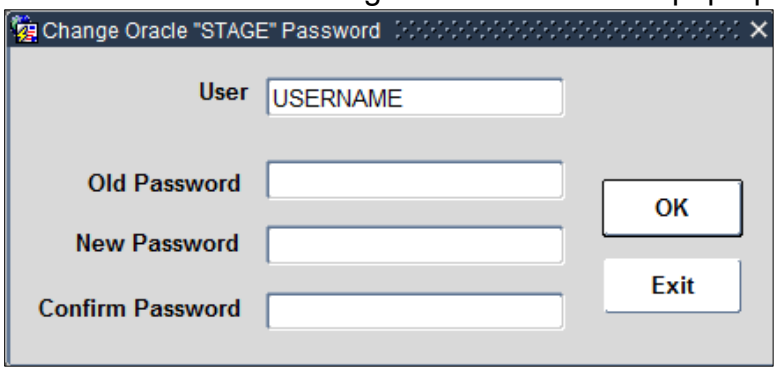


Figure 2: The “Change Oracle Password” pop-up window.

8. Click in the *Old Password* field and enter the current password.
9. Click in the *New Password* field and enter a new password.
10. Click in the *Confirm Password* field and enter the new password a second time.
11. Click the OK button.
12. A message in the hint line at the bottom of the screen will indicate if the password was successfully changed.

SIS OVERVIEW

This section provides an overview of the screen layout, common structure, fields, and buttons within SIS.

Title Bar

The Title Bar is located at the top of each screen in SIS and displays the name of the screen. The Title Bar also includes minimize, maximize, and close window buttons that change the size of the active window.

Menu Bar

The Menu Bar is located below the Title Bar and displays two different layers: the Main Menu Bar and the Screen Menu Bar.

Main Menu Bar

The Main Menu Bar is only available on the SAMPLE INFORMATION SYSTEM Screen that displays after first logging in to SIS. The Main Menu Bar includes menus for each screen in SIS.

Screen Menu Bar

The Screen Menu Bar is available on each screen in SIS and contains menus, commands, and sub-commands to access reports, functions, and other screens in SIS.

The Screen Menu Bar has two levels: menus and commands. The first-level menus are always visible at the top of the screen and do not directly access a screen or report. Click a menu to display a list of commands. Click a command to perform an action such as displaying a screen, saving a record, printing, or displaying a second-level of commands.

Toolbar

Every screen in SIS contains a horizontal toolbar positioned at the top of the window under the Screen Menu Bar. The toolbar displays in two modes: Entry Mode and Query Mode.

Entry Mode

The Entry Mode toolbar displays by default after opening a screen.

Save (F10)

The SAVE button commits to the database any created records or updates and deletions of existing records on the active screen. Information entered on a screen is not added to the database until the modifications are committed to the database by clicking the SAVE button.

Print

The PRINT button sends the visible information displayed on the screen to a printer. Clicking the PRINT button displays a pop-up window to select a printer, paper size, print orientation, and number of copies.

Enter Query (F7)

The ENTER QUERY button displays when the active screen is in Entry Mode. Clicking the ENTER QUERY button changes the screen from the Entry Mode to Query Mode, which is used to search for records in SIS. Clicking the ENTER QUERY button replaces it with three Query Mode buttons: EXECUTE QUERY, CANCEL QUERY, and COUNT HITS. For more information on the three Query Mode buttons, reference the *QUERY MODE* section of the user guide.

Next Record (↓)

The NEXT RECORD button displays, or navigates to, the next record of the current block. Use this button to view additional records when a query is executed and retrieves more than one record.

Previous Record (↑)

The PREVIOUS RECORD button displays, or navigates to, the previous record of the current block.

Create Record (F6)

The CREATE RECORD button clears the current record from the screen and creates a new, blank record.

Delete Record (Shift + F6)

The DELETE RECORD button deletes the current record. Clicking the DELETE RECORD button will delete the current record on which the cursor is residing. The record is not permanently deleted until the SAVE button is clicked.

Duplicate Record

The DUPLICATE RECORD button duplicates the previously displayed record into the current, empty record. Update any fields on the duplicated record and then save the changes. Use this button to enter multiple similar records.

Edit Item (Ctrl + E)

The EDIT ITEM button displays the Editor pop-up window with the contents of the currently selected field. The Editor pop-up window provides the user with a larger space to view or update the contents of a field.

Clear Record (Shift + F4)

The CLEAR RECORD button clears the current record from the screen without deleting it. This button removes the currently displayed record from the buffer of queried records and displays the next record in the queried buffer.

Clear Form

The CLEAR FORM button clears the current screen of all records without updating the records. Clicking the CLEAR FORM button places the screen in a similar state to when it is first opened.

Help

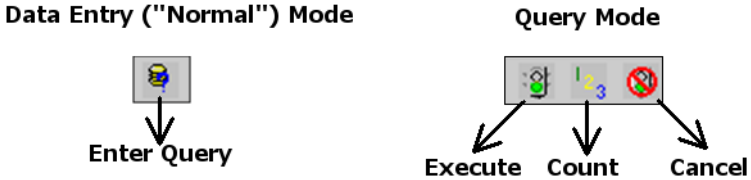
The HELP button provides the technical properties of the field in which the cursor resides.

Exit (CTRL + Q)

The EXIT button closes the current screen.

QUERY MODE

Use Query Mode to display records that match user-specified criteria. Screens open in Entry Mode with the ENTER QUERY button visible. Click the ENTER QUERY button to place the screen into Query Mode, which hides the ENTER QUERY button and displays the EXECUTE QUERY, COUNT HITS and CANCEL QUERY buttons.



Execute Query (F8)

The EXECUTE QUERY button retrieves all records that match the user-defined query criteria.

△ *Caution: If no criteria are specified, a "blind query" is executed which retrieves all records. Running blind queries is not recommended.*

Count Hits (Shift + F2)

The COUNT HITS button displays a number in the hint line indicating how many records match the query criteria. Click this button prior to executing a query to see the number of results and determine whether to specify additional criteria to limit the results.

Cancel Query (Ctrl + Q)

The CANCEL QUERY button exits QUERY MODE and places the screen into the Entry Mode. The CANCEL QUERY button does not stop an executed query that is currently running.

SCREEN STRUCTURE

The screens in SIS use a standard layout with common elements described in this section.

Header and Detail Block

A header block identifies a specific sample displayed on the screen.

A detail block displays information related to the sample identified in the header block.

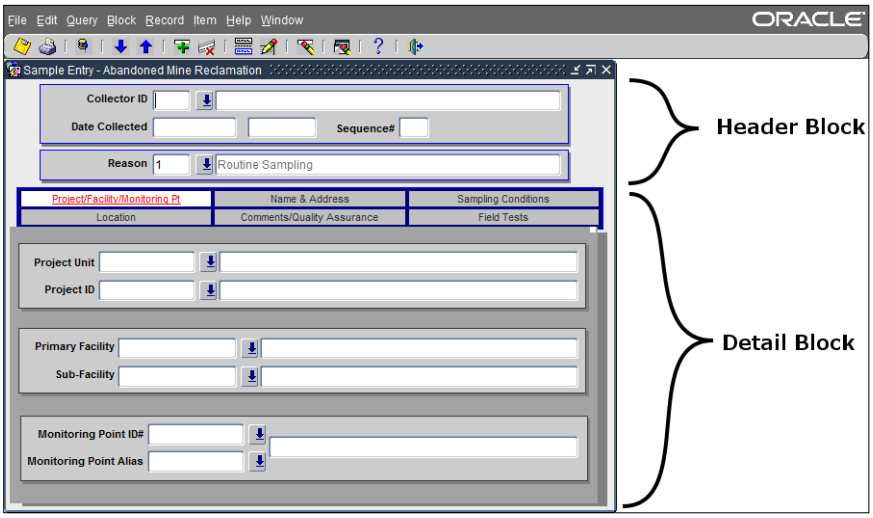


Figure 3: The SAMPLE ENTRY Screen with the header and detail blocks identified.

Tabs

Detail blocks are separated into tabs that display additional information related to the record in the header block. Click on a tab name to activate it and display the related details. The header block continues to display the current sample record and the activated tab displays the related detail record. The label of the currently displayed tab displays underlined and in red font.

List of Values (F9)

A list of values (LOV) button, typically located to the right of a field, displays a list of codes or a calendar for the related field.

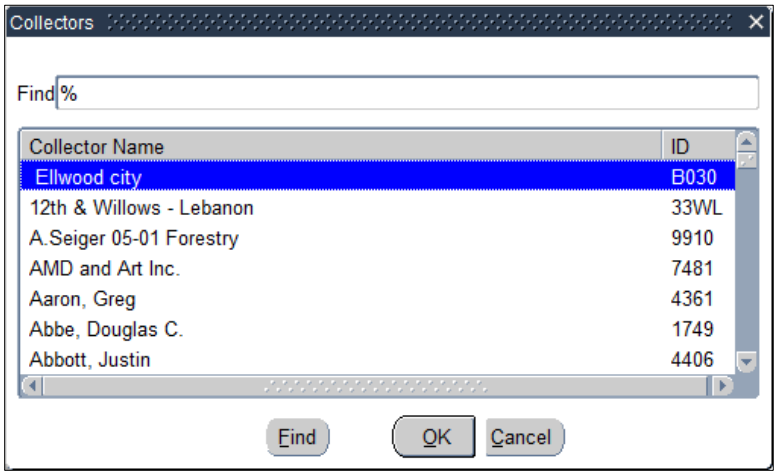


Figure 4: A list of values with the first value selected.

Using the list of values button:

Click the list of values button to the right of the field and a list of values pop-up window displays.

Click on an entry in the list to select it.

> 🕮 The list may contain numerous values. Filter the list by clicking in the Find field, entering a wildcard (%) followed by part of a code or description, and then clicking the FIND button. The list displays only values containing the text entered in the Find field.

Click the OK button to select the highlighted entry or click the CANCEL button to return to the screen without retrieving a value.

Pop-Up Messages

Pop-up messages alert or warn the user about certain actions, information, or functionality throughout the system. Users should read the pop-up message for directions to follow or to confirm an action.

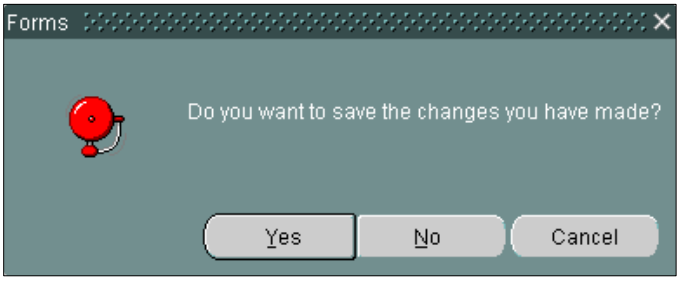


Figure 5: A pop-up message confirming an action.

Hint Line

Every window in SIS, except the SAMPLE INFORMATION SYSTEM Screen, contains a horizontal bar at the bottom of the screen called the hint line. The hint line displays information about the screen, an operation in progress, the result of an operation, or a description of the currently selected field.

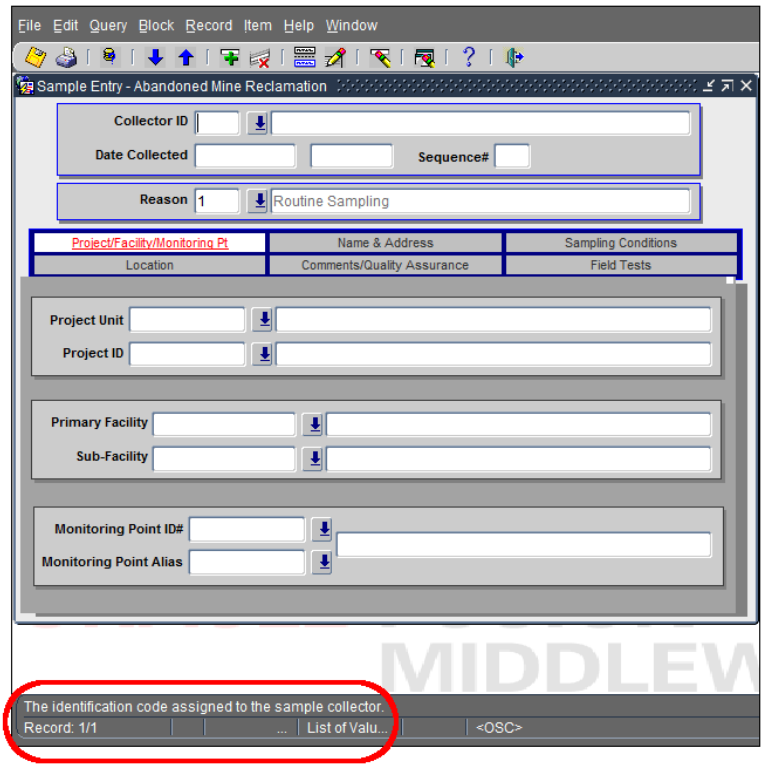


Figure 6: The hint line displaying a description for the *Collector ID* field.

QUERYING FOR SAMPLES

The following sections provide information on how to query an existing sample and the associated details using the SAMPLES Screen. Access the SAMPLES Screen by clicking the Samples Menu and then clicking the Query samples command.

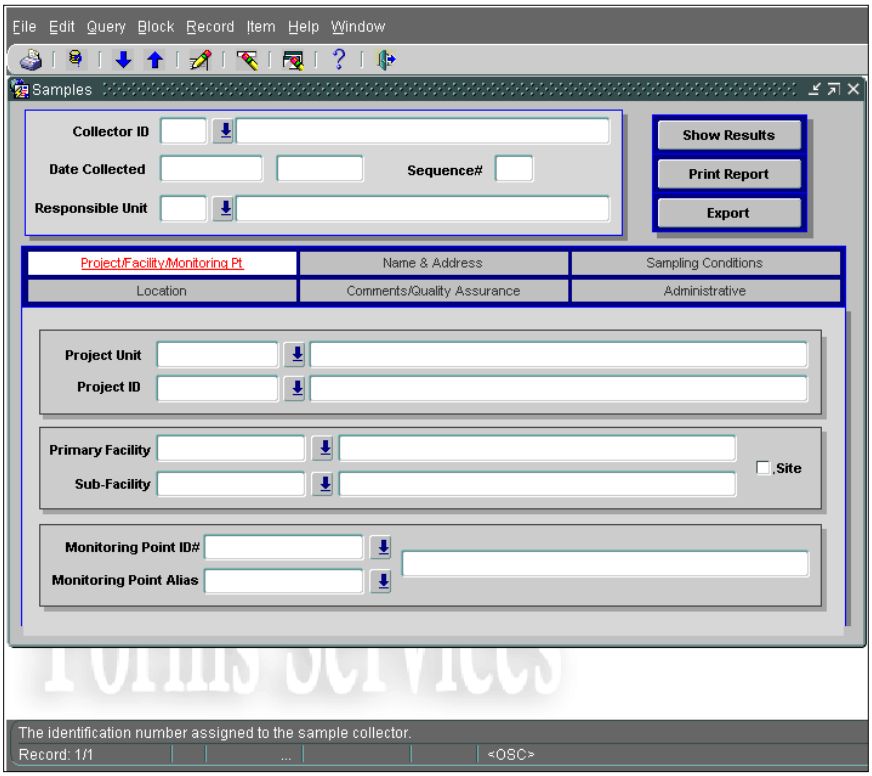


Figure 7: The samples screen.

Querying Using the Collector and Date Collected

This section provides steps for querying an existing sample using the collector, date collector, and/or sequence number.

Access the SAMPLES Screen and then click the ENTER QUERY button on the toolbar or press the [F7] key.
Enter the four-digit collector ID assigned to the employee or monitoring point that collected the sample or select it by clicking the list of values button to the right of the *Collector ID* field.
Press the [TAB] key.
If known, enter the date that the sample was collected. Press the [TAB] key.
If known, enter the time that the sample was collected. Press the [TAB] key.
If known, enter the sequence number assigned to the sample.
Click the EXECUTE QUERY button on the toolbar or press the [F8] key.
If more than one sample is retrieved, use the NEXT RECORD button on the toolbar or press the [↓] key to locate the correct record.

> 🕮 If more than one record was retrieved, “Record: 1/?” displays below the hint line.

Querying using the Query Options Button

The Query Options button allows querying using a range of dates, sequence numbers, and test results.

1. To use the QUERY OPTIONS button, access the SAMPLES Screen and then click the ENTER QUERY button on the toolbar or press the [F7] key.
2. Click the QUERY OPTIONS button and the “Query Options” pop-up window displays.

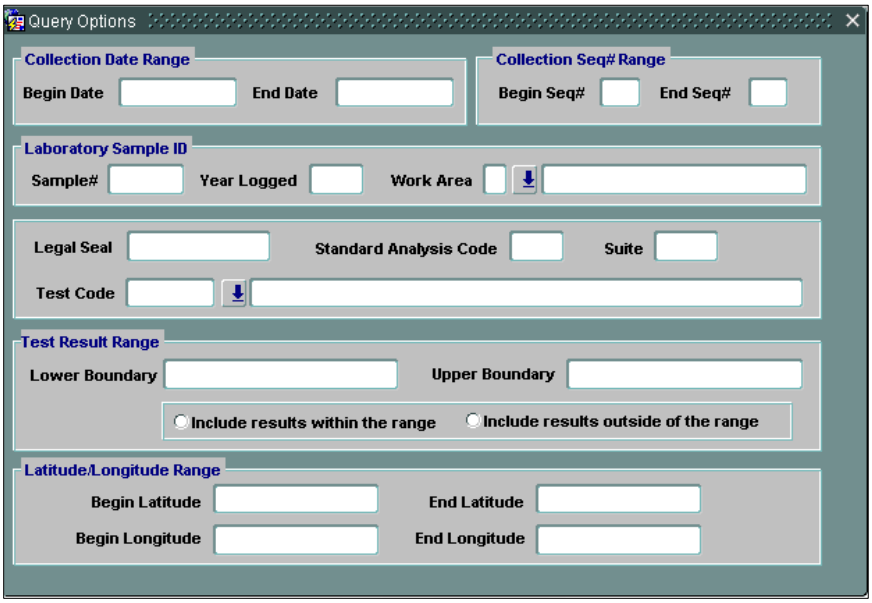


Figure 8: The "Query Options" pop-up window.

3. Enter query criteria in any of the fields on the screen.
4. Click the COUNT HITS button on the toolbar to determine how many records will be retrieved.
5. If the query count is extensive, further qualify the query before executing.
6. Click the EXECUTE QUERY button on the toolbar or press the [F8] key.
7. If more than one sample is retrieved, use the NEXT RECORD button on the toolbar or press the [↓] key to locate the correct record.

> 🕮 If more than one record was retrieved, "Record: 1/?" displays below the hint line.

8. To view the results and details of the sample, reference the *VIEWING SAMPLE DETAILS* section of the user guide.

Querying using Multiple Criteria

This section provides steps for querying a sample using the fields in the header block and the fields under the detail tabs on the SAMPLES Screen.

> 🕮 If the complete query value for a field is not known, use the wildcard (%) at the beginning and/or end of one or two key characters. For example, to match both Lower Paxton and Paxtang municipalities enter: %PAXT%

1. Access the SAMPLES Screen and then click the ENTER QUERY button on the toolbar or press the [F7] key.
2. Click on a tab that contains a desired field for entering query criteria.

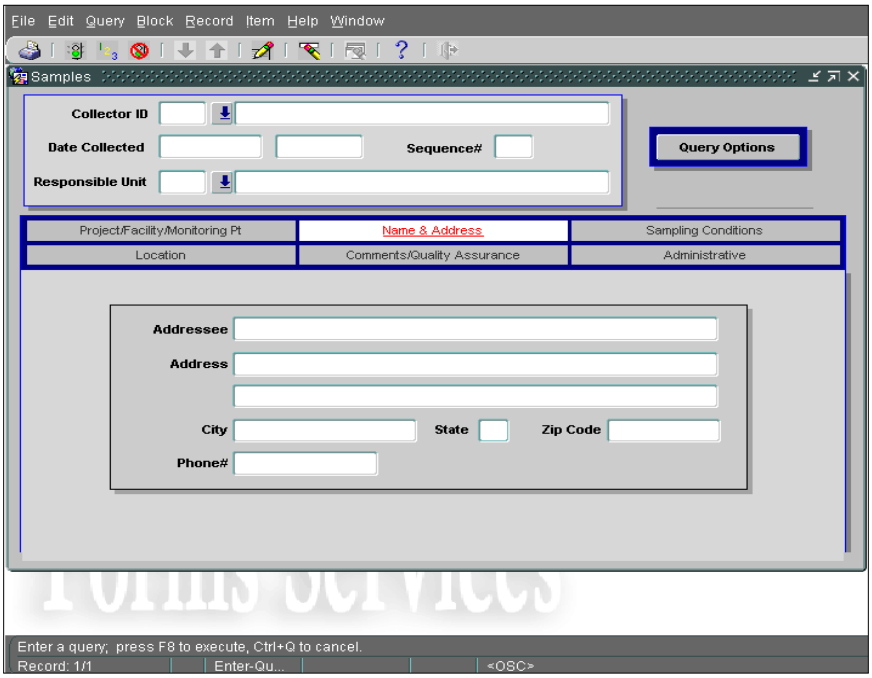


Figure 9: The samples screen with the Name & Address TAB selected.

3. Enter query criteria in any of the queryable fields identified at the end of this section.
4. Click the COUNT HITS button on the toolbar to determine how many records will be retrieved.

> 🕮 If the query count is extensive, further qualify the query before executing.

5. Click the EXECUTE QUERY button on the toolbar or press the [F8] key.
6. If more than one sample is retrieved, use the NEXT RECORD button on the toolbar or press the [↓] key to locate the correct record.

> 🕮 If more than one record was retrieved, "Record: 1/?" displays below the hint line.

7. To view the results and details of the sample, reference the *Viewing the Sample Details* section of the user guide.

Queryable Fields:

Sample Query Header Block

- Collector ID*
- Sequence#
- Date Collected
- Responsible Unit*

Project/Facility/Monitoring Pt TAB

- Project Unit*
- Primary Facility*
- Monitoring Point ID#*
- Project ID*
- Sub-Facility*
- Monitoring Point Alias*

Name/Address TAB

- Addressee
- Address Line 2
- Zip Code
- Address Line 1
- City
- Phone#

Sampling Conditions TAB

- Sampling Reason*
- Medium Type*
- Stream Condition*
- Final Flow
- Determination*
- Units*
- Sampling Protocol*
- Medium*
- Initial Flow
- Units*
- Depth To Water
- Determination*

Location TAB

State*
Municipality
Longitude
Reference Point*
Location Procedure*
Elevation
Altitude Datum*
County
Latitude
Datum
Geometric Type*
Location
Accuracy
Method *

Comments/Quality Assurance TAB

Appearance
Quality Assurance Type*
Confidentiality Reason*
Duplicate of Sample
Voided/Dry Sample

Administrative TAB

Cost Center*
Date Created
Date Last Modified
Entry Complete
Created By
Last Modified By

**A list of values is available.*

VIEWING SAMPLE DETAILS

This section provides steps for how to view the details associated with a sample.

Access the SAMPLES Screen and query a sample record.
Click the SHOW RESULTS button to display the SAMPLE ANALYSES/RESULTS Screen, which includes the lab results of sample testing.

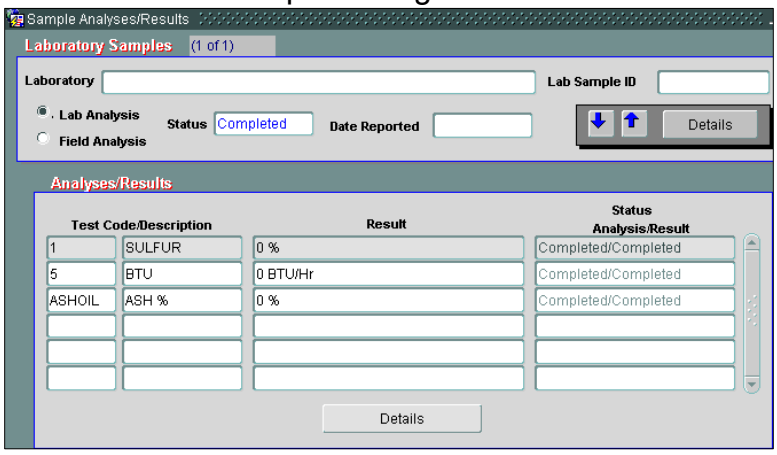


Figure 10: The SAMPLE ANALYSES/RESULTS Screen.

The SAMPLE ANALYSES/RESULTS Screen displays the laboratory that tested the sample and a list of test codes with results. If there are multiple laboratory sample analyses, then click the NEXT RECORD or PREVIOUS RECORD buttons view a different analysis.
To view details about the laboratory identifiers for the sample, click the DETAILS button on the right-hand side of the screen. The "Lab Sample" pop-up window displays.

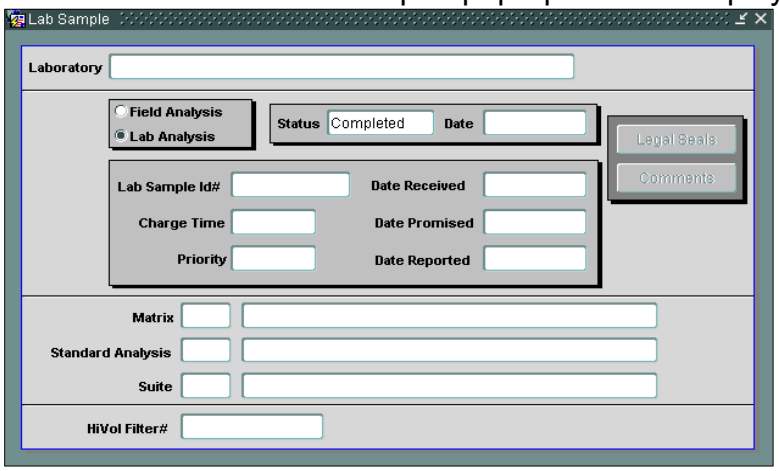


Figure 11: The "Lab Sample" pop-up window.

If available, click the LEGAL SEALS or COMMENTS button to view additional details.
Click the ☒ to exit the pop-up window and return to the SAMPLE ANALYSES/RESULTS Screen.
To view the complete details of a specific test, click on a test code in the Analyses/Results section of the screen to select it and then click the DETAILS button located at the bottom of the screen. The "Analysis/Result Details" pop-up window displays.

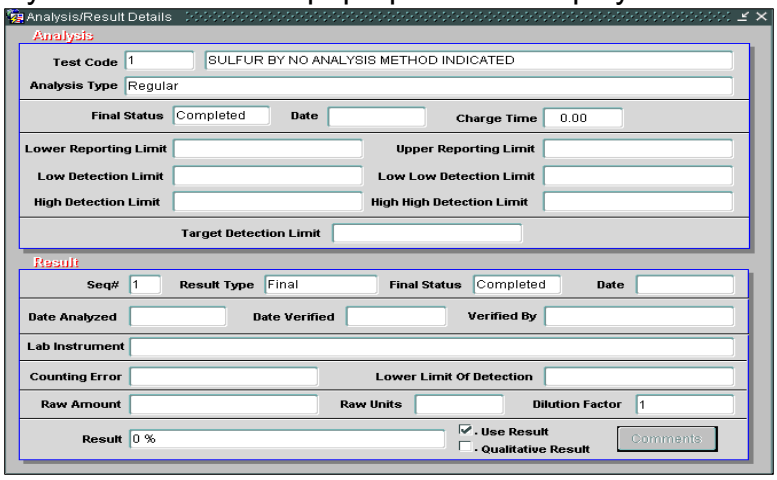


Figure 12: The "Analysis/Result Details" pop-up window.

If available, click the COMMENTS button to view additional comments.
Click the ☒ to exit the pop-up window and return to the SAMPLE ANALYSES/RESULTS Screen.
Click the ☒ again to return to the SAMPLES Screen.
Six tabs containing information about the collection of the sample display in the detail block of the screen.

Figure 13: The six detail tabs on the samples screen.

Click on a tab to display the details:

- Project/Facility/Monitoring Pt TAB
  This tab identifies the specific project for which a sample was taken; the regulated entities (primary and sub-facilities) that may impact the soil, water, or air sample; and/or the monitoring point established for sample collection.
- Name & Address TAB
  This tab displays the name and address at which the sample was collected.
- Sampling Conditions TAB
  This tab displays the conditions under which the sample was collected including the sampling reason, method, medium, and stream condition.
- Location TAB
  This tab displays the location at which the sample was collected including county, municipality, latitude/longitude, and locational metadata.
- Comments/Quality Assurance TAB
  This tab displays comments and quality assurance indicators about the sample.
- Administrative TAB
  This tab displays cost center associated with the sample, who created and last updated the sample, and when the sample was created and last updated.

To generate a printed a report displaying the laboratory that tested the sample, a summary of the analysis methods, and results of the tests, click the PRINT REPORT button on the SAMPLE QUERY Screen.

EXPORTING A SAMPLE

The EXPORT button located on the SAMPLES Screen generates a customizable report using the queried samples. The report is saved as file on the local computer. Contact the Applications Helpdesk to request the "Sample Export" security role required to use this function.

1. Access the SAMPLES Screen and query sample records to export.
2. Click the EXPORT button and the "Export Samples" pop-up window displays.

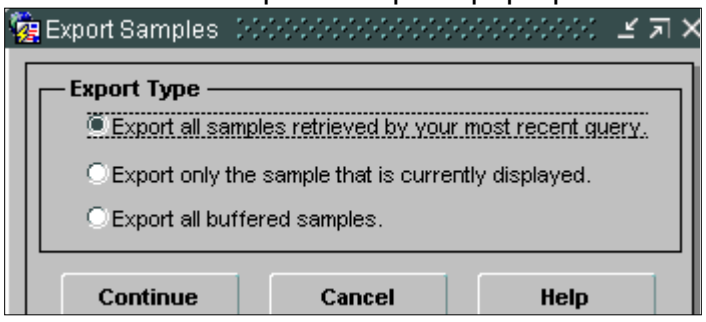


Figure 14: The "Export Samples" pop-up window.

3. Select which samples to include in the report and then click the CONTINUE button. The EXPORT SAMPLES Screen displays.

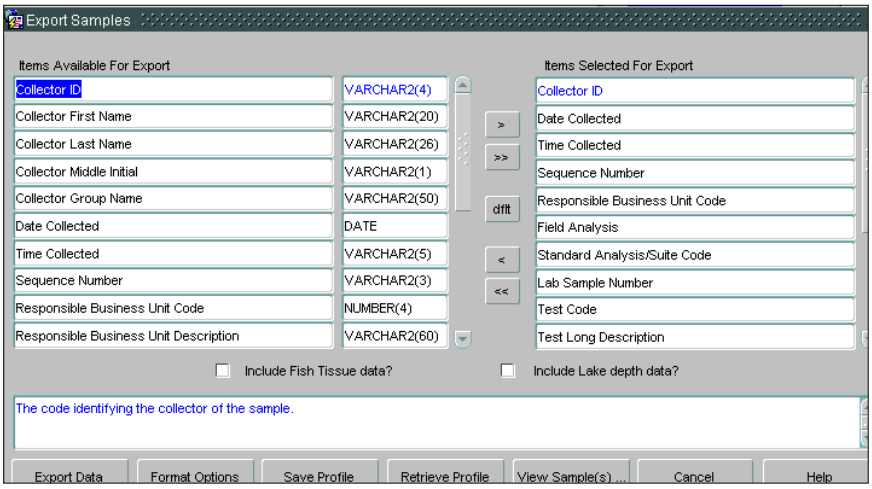


Figure 15: The EXPORT SAMPLES Screen.

4. The column on the left-hand side of the screen lists items available to include in the report. To add an item, click on it and then click the > button.
5. The column on the right-hand side of the screen lists the items selected to include in the report. To remove an item, click on it and then click the < button.

> 🕮 Remove all selected items by clicking the << button or add all available items by clicking the >> button. To return to the selected items that initially display, click the "dflt" button.

6. Click the FORMAT OPTIONS button to specify how to delimit, or separate, the items selected for the report. Use the comma delimited option to generate reports that open in a spreadsheet application like Microsoft Excel or use the fixed width field option to generate reports to open in a word processing application like Microsoft Word.

> 🕮 After selecting items for export and setting a format, click the SAVE PROFILE button to save these settings to a profile on the local computer. Click the RETRIEVE PROFILE button to use the same settings when exporting a different set of samples.

7. Click the VIEW SAMPLE(S) button to verify the list of samples that the report will include.
8. Click the EXPORT DATA button and a save dialog displays.
9. Choose a location for the file and click the SAVE button.

> 🕮 When exporting a comma delimited report, add .csv to the end of the filename to easily open the report in Excel.

10. The amount of time required to create the report varies depending on the number of samples and items selected. An "Export Complete" message displays when the report is finished. Click the OK button.

ACCESSING THE SAMPLE ENTRY SCREEN

Use the SAMPLE ENTRY Screen to create new sample records or update existing sample records. Access the SAMPLE ENTRY Screen by clicking the Samples Menu and then clicking the Sample Entry command.

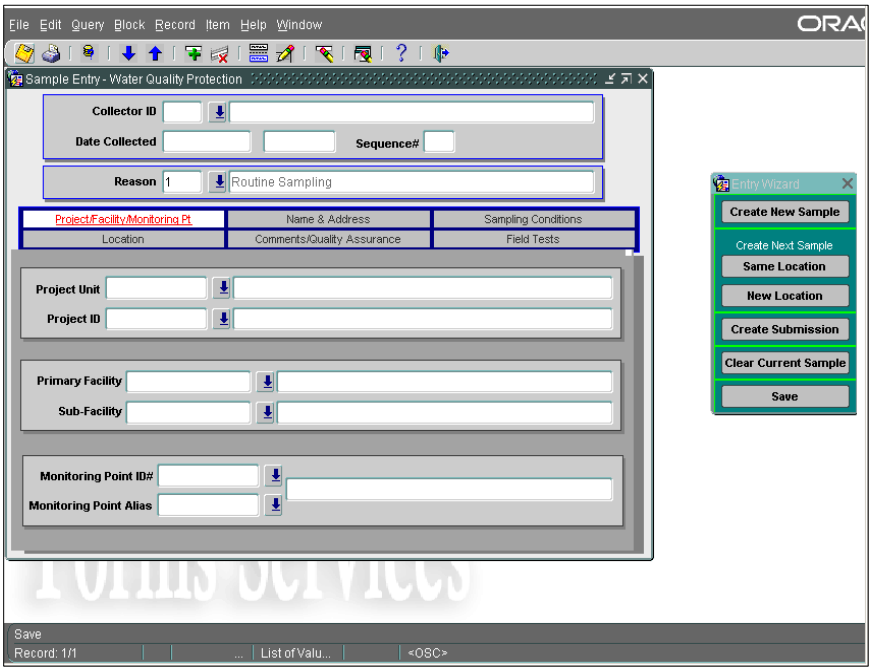


Figure 16: The SAMPLE ENTRY Screen.

> 🕮 The "Business Unit List of Values" pop-up window displays for users authorized for more than one business unit. Select the relevant Business Unit to enter samples for and then click the OK button.

CREATING SAMPLE RECORDS

This section provides steps for creating sample records in SIS using the SAMPLE ENTRY Screen. The Sample Header and Sampling Conditions TAB contain the only required fields. The rest of the fields are optional and should be entered when applicable.

Sample Header

The sample header is required. It identifies the general sample information including collector, date and time collected, sequence number, and sampling reason.

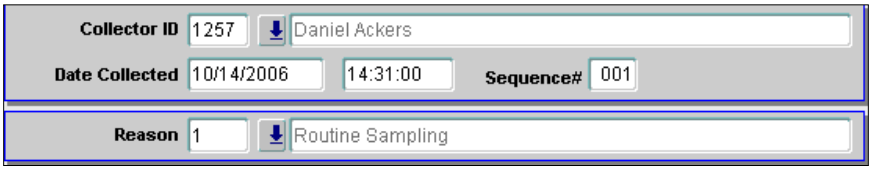


Figure 17: A sample header.

1. Access the SAMPLE ENTRY Screen.
2. Enter the four-digit collector number assigned to the employee, group, or monitoring device that collected the sample in the *Collector ID* field. Press the [TAB] key.
3. Enter the date the sample was collected in the first *Date Collected* field (format MM/DD/YYYY). Press the [TAB] key.
4. Optionally, enter the time the sample was collected in military time (i.e., enter 1:00 pm as 1300, enter 9:00 am as 0900) in the second *Date Collected* field. Press the [TAB] key.
5. Enter the sequence number for the sample in the *Sequence#* field. Press the [TAB] key.
6. Click the list of values button next to the *Reason* field to select a reason for sampling. The field defaults to "Routine Sampling."

Project/Facility/Monitoring Point TAB

This section is used to indicate if the collected sample is related to a project, *e*FACTS facility, or a monitoring point.

Click the Project/Facility/Monitoring Pt TAB.

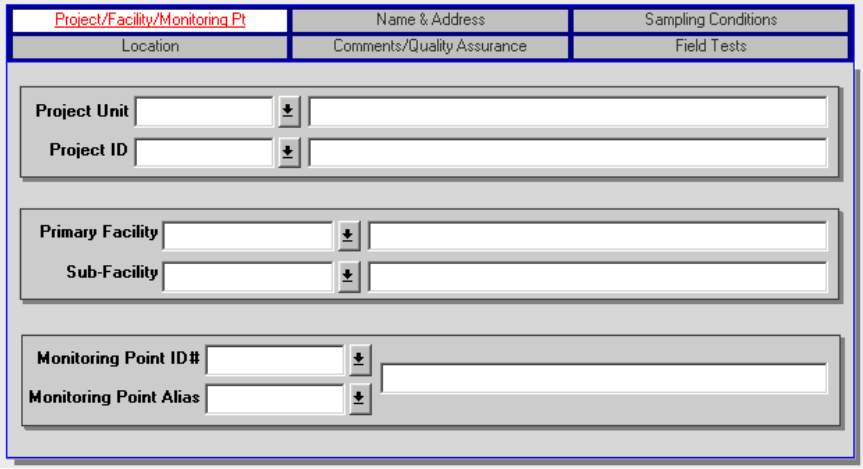


Figure 18: The Project/Facility/Monitoring Pt TAB.

If the sample was collected for an existing project, complete the following steps:

a. Enter the code identifying the project's business unit or select using the list of values button to the right of the *Project Unit* field. Press the [TAB] key.
b. Enter the identification number assigned to the project or select using the list of values button to the right of the *Project ID* field.

If the sample was collected to monitor an existing primary facility and/or sub facility, complete the following steps:

Enter the program-specific identification number in the *Primary Facility* field or select a primary facility from the list of values by entering a facility name or program. Press the [TAB] key.

Optionally, select a sub-facility by clicking the list of values button to the right of the *Sub-Facility* field.

If the sample was collected at a particular monitoring point, identify the monitoring point using one of the following methods:

Enter the Monitoring Point ID assigned to the monitoring point and press the [TAB] key.

Enter the Monitoring Point Alias and press the [TAB] key.

Click the list of values button to the right of either *Monitoring Point* field, enter a latitude and longitude, and click the ACCEPT button to display a list of monitoring points to select from.

> 🕮 Coordinates are entered in the format DD-MM-SS.SSSS (e.g., 41-29-46.3034 or -79-38-09.6071)

If a monitoring point was entered, click the SAVE button on the toolbar or press the [F10] key.

If necessary, proceed to the next tab.

If a monitoring point was not entered, proceed to the Sampling Conditions TAB.

Sampling Conditions TAB

This section is used to enter the conditions under which the sample was collected. The *Medium Type* and *Medium* fields are required. If the sample is linked to a monitoring point on the Project/Facility/Monitoring Pt TAB, the *Medium Type* and *Medium* fields are populated using information from the monitoring point.

1. Click the Sampling Conditions TAB.

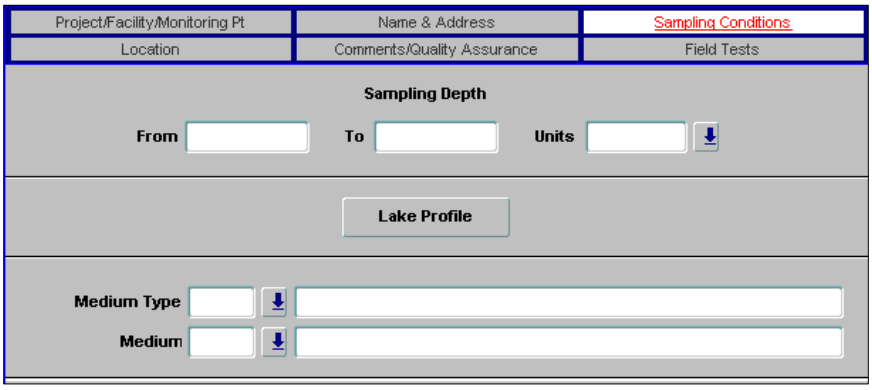


Figure 19: The Sampling Conditions TAB.

2. The *Medium Type* field is required. Enter the code that identifies the type (category) of sample medium (soil, water, air, plants, etc.) or select by using the list of values button to the right of the *Medium Type* field. Press the [TAB] key.
3. The *Medium* field is required. Enter the code that identifies the sample medium or select by using the list of values button to the right of the *Medium* field. Press the [TAB] key.
4. The fields that display vary based on the Business Area selected when first accessing the SAMPLE ENTRY Screen. Enter data in the optional fields as necessary.
5. Click the SAVE button on the toolbar or press the [F10] key.
6. If necessary, proceed to the next TAB.

Location TAB

This section is used to identify the location at which a sample was collected.

> 🕮 The latitude, longitude and datum are required in order to link an NHD record to a sample.

If a sample is linked to a monitoring point on the Project/Facility/Monitoring Pt TAB, the Locational TAB automatically displays the locational information from the monitoring point.

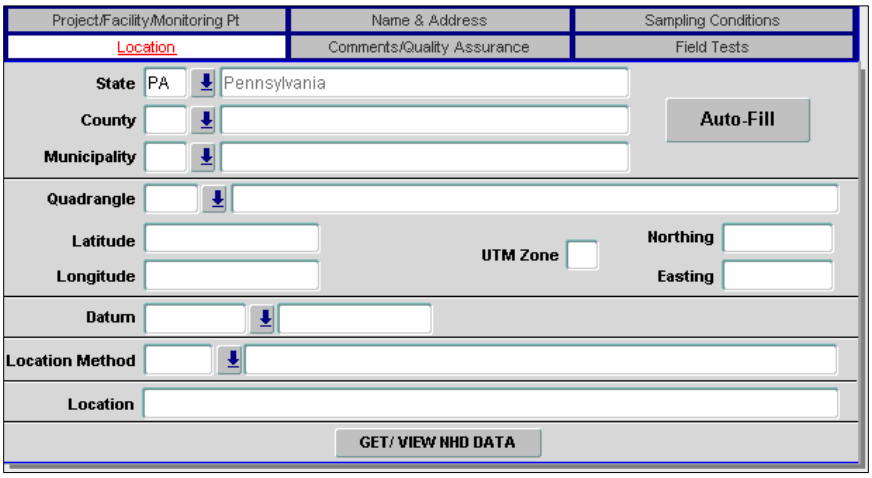


Figure 20: The Location TAB.

1. Click the Location TAB.
2. Click the AUTO-FILL button (The county and municipality displays based on the linked primary facility, sub facility, or monitoring point). If the county and municipality does not display, complete Steps c through e; otherwise, proceed to Step f.
3. The state defaults to “PA”. Update if necessary. Press the [TAB] key.
4. Select the county by using the list of values button.
5. Select the municipality by using list of values button.

6. Select the quadrangle by using the list of values button.
7. (Required to insert NHD) Enter the latitude where the sample was taken in the *Latitude* field. Press the [TAB] key.
8. (Required to insert NHD) Enter the longitude where the sample was taken in the *Longitude* field. Press the [TAB] key.

> 🕮 Coordinates are entered in the format DD-MM-SS.SSSS (e.g., 41-29-46.3034 or -79-38-09.6071)

9. Enter the UTM Zone where the sample was taken in the *UTM* field. Press the [TAB] key.
10. Enter the northing where the sample was taken in the *Northing* field. Press the [TAB] key.
11. Enter the easting where the sample was taken in the *Easting* field. Press the [TAB] key.
12. (Required to insert NHD) Enter the horizontal reference datum used to calculate the point at which the sample was collected or select by using the list of values button to the right of the *Datum* field. Press the [TAB] key.
13. Enter the method used to identify the point at which the sample was collected or select by using the list of values button to the right of the *Location Method* field. Press the [TAB] key.
14. Enter a description of the location at which the sample was collected in the *Location* field.
15. Click the SAVE button on the toolbar or press the [F10] key.
16. Proceed with linking the sample to an NHD record or to the next tab.

Linking the Sample to a NHD Record

This section identifies the steps for linking a sample to a NHD record. Complete the step for entering details on the Location TAB before linking the sample to a NHD record.

> 🕮 If the sample is associated with a monitoring point, the NHD record for the monitoring point will "automatically" display for the sample and cannot be updated. Therefore, this procedure cannot be completed.

Click the Location TAB.
Verify that the latitude, longitude, and datum exist for the sample. If not, insert the latitude, longitude, and datum.

> 🕮 To insert an NHD record for a sample, the latitude, longitude and datum must be entered on the Location TAB.

Click the GET/VIEW NHD DATA button at the bottom of the Location TAB. The NHD pop-up window displays.

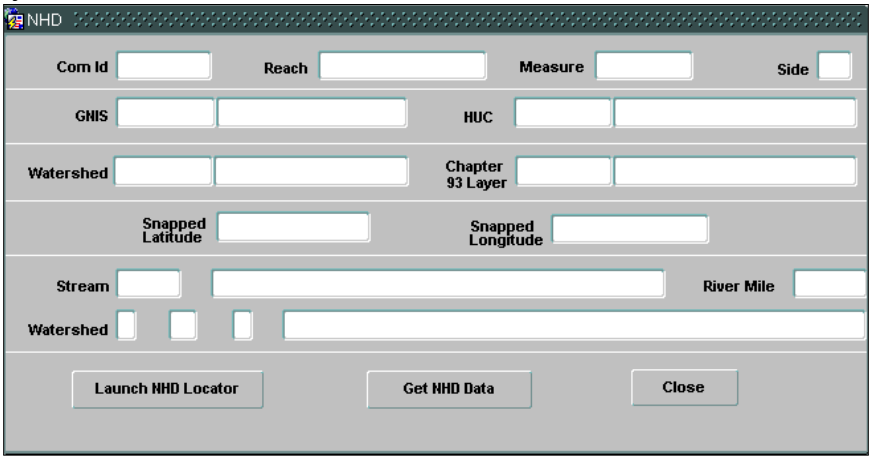


Figure 21: The NHD pop-up window.

Click the LAUNCH NHD LOCATOR button at the bottom of the screen.

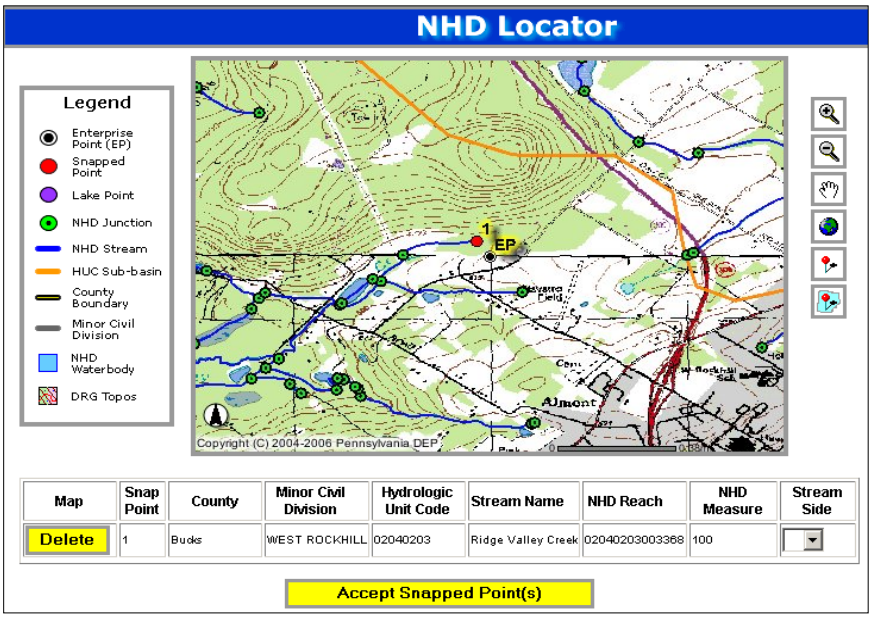


Figure 22: The NHD locator.

Use the NHD Locator Tool to either accept the default snapped point or create a user-defined, new snapped point to accept. Reference the NHD Locator Tool User Guide for the steps.
Click the ACCEPT SNAPPED POINT(S) button and then click the OK button to exit the NHD Locator Tool and return to the SAMPLE ENTRY Screen.
Click the GET NHD DATA button to add the NHD record created via the NHD Locator Tool to the sample.
Click the CLOSE button to return to the Location TAB.

Name and Address TAB

This section is used to enter a name and address associated with the collection location of the sample.

1. Click the Name & Address TAB.

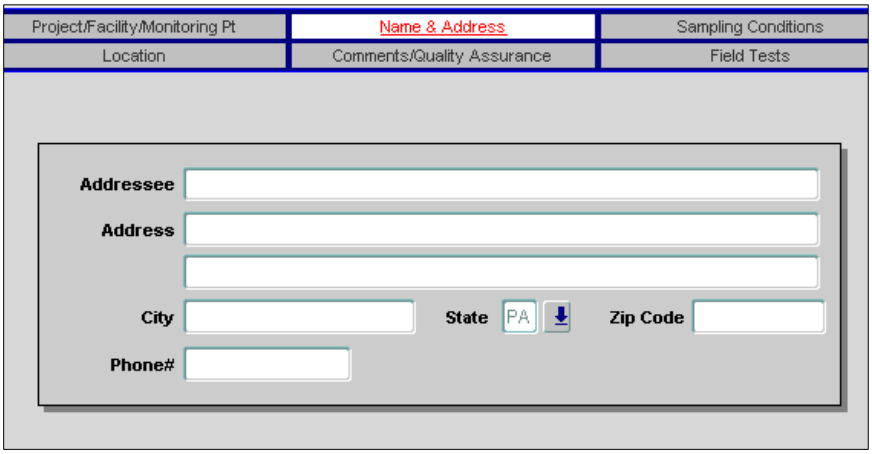


Figure 23: The Name & Address TAB.

2. Enter the name of the location where the sample was collected in the *Addressee* field. Press the [TAB] key.
3. Enter the first line of the address where the sample was collected on the first line in the *Address* field. Press the [TAB] key.

4. If necessary, enter the second line of the address where the sample was collected in the *Address* field. Press the [TAB] key.
5. Enter the city where the sample was collected in the *City* field. Press the [TAB] key. The state defaults to "PA".
6. Enter the zip code where the sample was collected in the *Zip Code* field. Press the [TAB] key.
7. Enter the phone number where the sample was collected in the *Phone#* field.
8. Click the SAVE button on the toolbar or press the [F10] key.
9. If necessary, proceed to the next tab.

Comment/Quality Assurance TAB

This section is used to enter the comments and quality assurance details regarding the sample.

1. Click the Comments/Quality Assurance TAB.

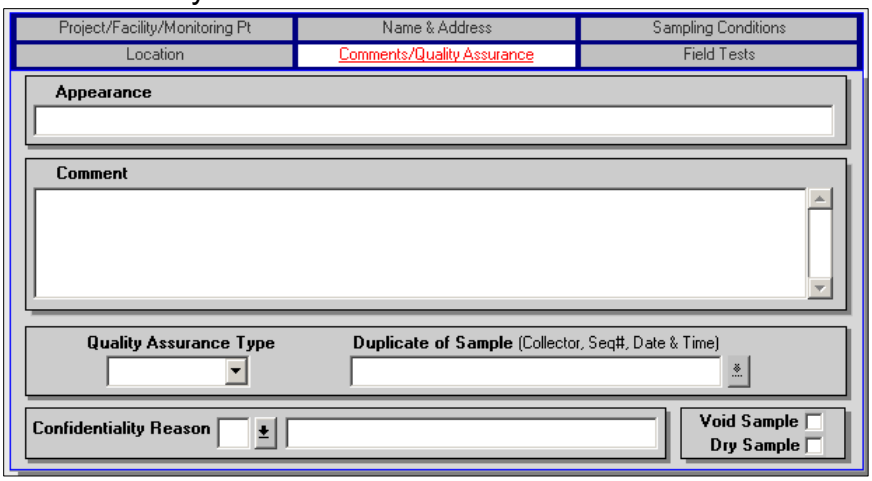


Figure 24: The Comments/Quality Assurance TAB.

2. Enter a description of the sample's appearance. Press the [TAB] key.
3. Enter any additional information regarding the sample. Press the [TAB] key.
4. Enter the quality assurance type (duplicate, blank, or spike).
5. If a duplicate, click the list of values button, enter the ID of the collector for the duplicate sample, the date the duplicate was collected, and then click the ACCEPT button.
6. If necessary, select a confidentiality reason code (e.g., private water supply or legal enforcement action).
7. If the sample is to be voided due to quality assurance reasons, select the "Void Sample" checkbox.
8. If the sample is to be dry, select the "Dry Sample" checkbox.
9. Click the SAVE button on the toolbar or press the [F10] key.
10. If necessary, proceed to the next tab.

Field Tests TAB

This section is used to identify the types of tests conducted in the field on the sample.

Click the Field Tests TAB.

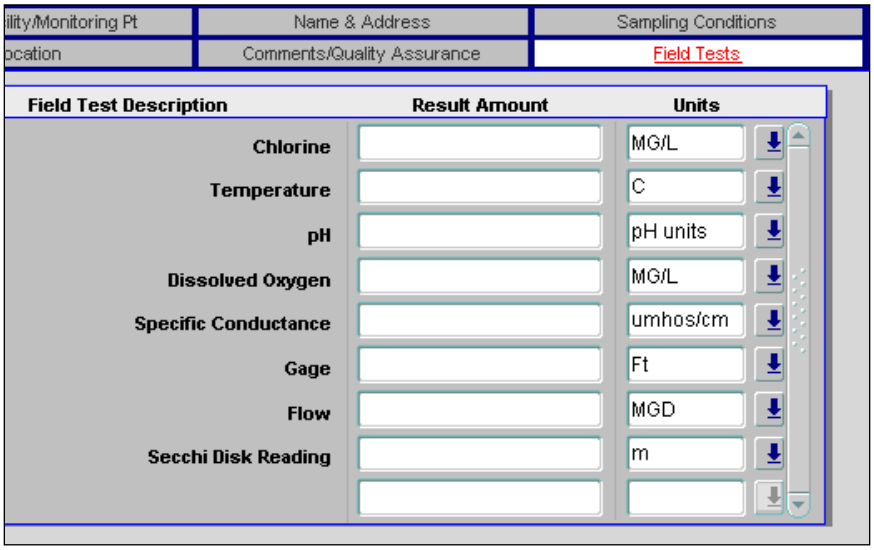


Figure 25: The Field Tests TAB.

Use the scrollbar to locate the field test for the results and click in the *Result Amount* field.

> 🕮 The list of field tests varies based on the Business Unit.

Enter the amount for the test. Press the [TAB] key.
Update the unit of measurement if necessary.
Repeat the previous steps all applicable field tests are entered.
Click the SAVE button on the toolbar or press the [F10] key.

Inserting Additional Samples for the Same Location

This section identifies how to enter an additional sample at the same location as the previously entered sample.

1. While the previous sample is displayed on the screen, click the SAME LOCATION button. A new record matching the header, project, facility, monitoring point, and location details of the previous sample is created.
2. Update the sequence number.
3. Update the sample detail tabs as described in the *CREATING SAMPLE RECORDS* section of the user guide.

Inserting Additional Samples at a New Location

To enter an additional sample at a new location:

1. While the previous sample is displayed on the screen, click the NEW LOCATION button. A new record with header details matching the previous sample will be created.
2. Update the sequence number and reason.
3. Update the sample detail tabs as described in the *CREATING SAMPLE RECORDS* section of the user guide.

Voiding a Sample

1. Access the SAMPLES Screen by clicking the Samples Menu and then clicking the Query sample command.
2. Query the sample to be voided.
3. Click the Comments/Quality Assurance TAB.

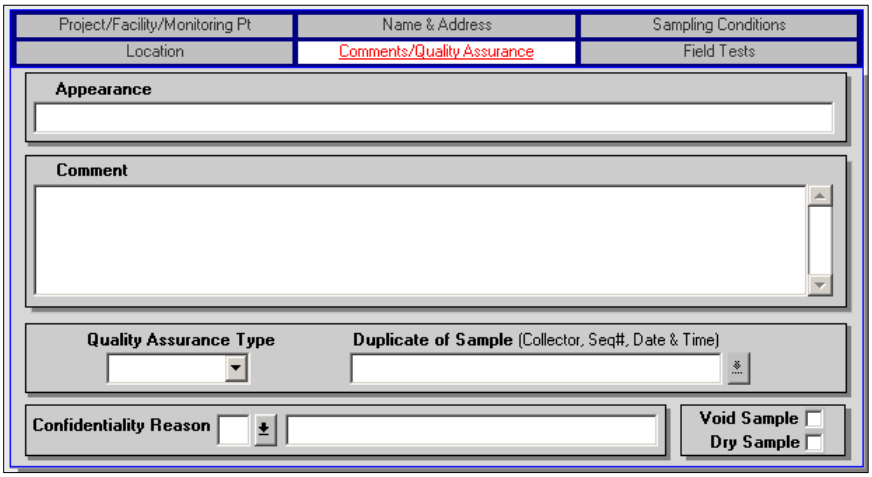


Figure 26: The Comments/Quality Assurance TAB.

4. If applicable, select a quality assurance type to identify a reason for voiding the sample. Press the [TAB] key.

> 🕮 If the quality assurance type is duplicate and only one sample record exists for the collector and date collected, the duplicate sample automatically displays. Otherwise, click the list of values button to identify the duplicate.

5. If applicable, select the Void Sample checkbox.
6. If applicable, select the Dry Sample checkbox.
7. Click the SAVE button on the toolbar or press the [F10] key.

UPDATING SAMPLE RECORDS

Update existing sample records using the SAMPLE ENTRY Screen.

Access the SAMPLE ENTRY Screen by clicking the Samples Menu and then clicking the Sample Entry command.

Click the ENTER QUERY button on the toolbar and query for a sample as described in the *QUERYING FOR SAMPLES* section of the user guide.

Update the fields described in the *CREATING SAMPLE RECORDS* section of the user guide.

When finished click the SAVE button on the toolbar or press the [F10] key.

MONITORING POINTS

This section provides steps for how to create a new monitoring point using the MONITORING POINTS Screen. Access the MONITORING POINTS Screen by clicking the Samples Menu and then clicking the Monitoring points command.

Use monitoring points to indicate a consistent sampling location. Monitoring points can also be linked to an *e*FACTS facility or a SIS project.

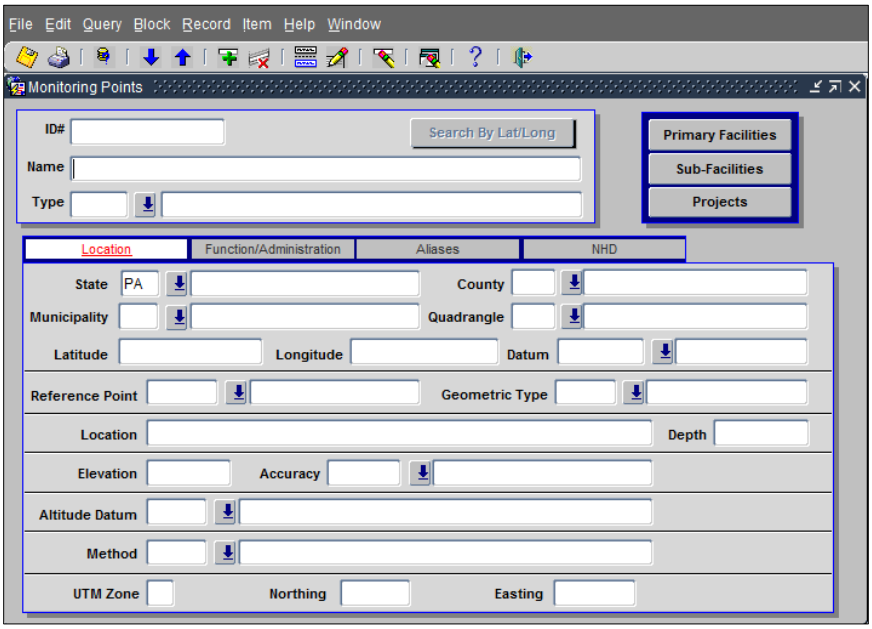


Figure 27: The MONITORING POINTS Screen.

Creating a New Monitoring Point

Step 1: Header Block (required)

Use the header block to identify the monitoring point name and type.

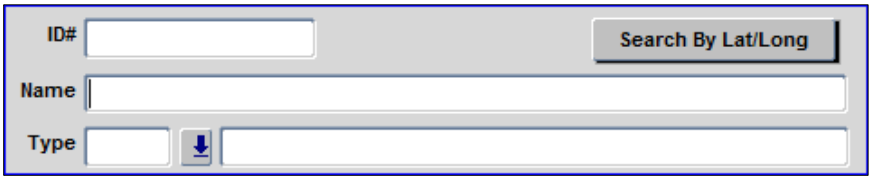


Figure 28: The monitoring point header block.

1. Click the CREATE RECORD button on the toolbar or press the [F6] key.
2. Enter the name of the monitoring point and press the [TAB] key.
3. Enter the code that identifies the type of monitoring point or select one using the list of values button.

Step 2: Location TAB (required)

Use the Location TAB to identify the location of the monitoring point from a general location description to specific coordinates. The following fields are required:

- State
- County
- Latitude
- Longitude

🕮	The Datum field is required if linking an NHD record to the monitoring point.

Click the Location TAB.

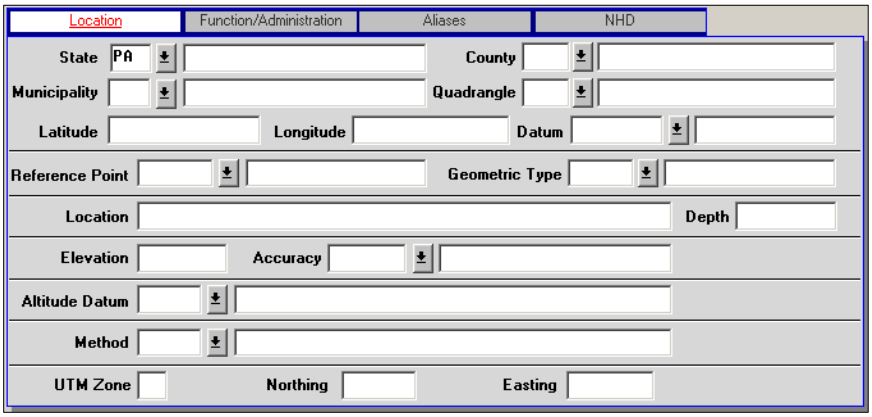


Figure 29: The Location TAB.

The state defaults to "PA". Update if necessary.
Select a code identifying the county in which the sample was collected using the list of values button.
Optionally, select the code identifying the municipality in which the sample was collected by using the list of values button.
Enter the latitude where the sample was taken in Degrees-Minutes-Seconds (e.g., 40-15-42.8394).
Enter the longitude where the sample was taken in Degrees-Minutes-Seconds (e.g., -76-52-46.9194).
Optionally, enter the horizontal reference datum used to calculate the point at which the sample was collected or select by using the list of values button.
Complete any of the optional fields with available information:

- Municipality
- Quadrangle
- Datum
- Reference Point
- Geometric Type
- Location
- Depth
- Elevation
- Accuracy
- Altitude Datum
- UTM Zone
- Northing
- Easting

📖 Click in a field to display a description in the hint line.

Step 3: Function/Administration TAB (required)

Use the Function/Administration TAB to identify the function, medium type, and sample medium for a monitoring point as well as administrative details such as the closed date for a monitoring point. The *Medium Type* and *Sample Medium* fields are required.

1. Click the Function/Administration TAB.

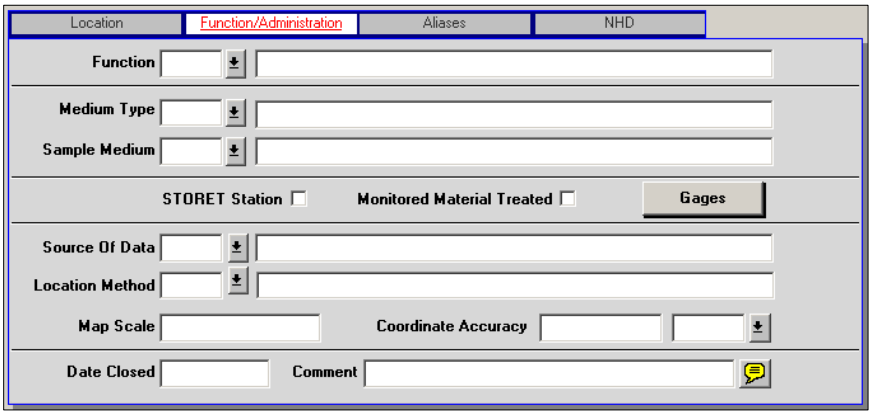


Figure 30: The Function/Administration TAB.

2. Optionally, enter a code identifying the function of the monitoring point or select by using the list of values button.
3. Enter the code identifying the sample medium type monitored by the monitoring point or select by using the list of values button.
4. Enter the code identifying the sample medium monitored by the monitoring point or select by using the list of values button.
5. If the monitoring point is a STORET station, select the STORET Station checkbox.
6. If the monitored material is treated, select the Monitored Material Treated checkbox.
7. If monitoring gages are associated with the monitoring point, complete the following steps:
    a. Click the GAGES button and the "Monitoring Gages" pop-up window displays.

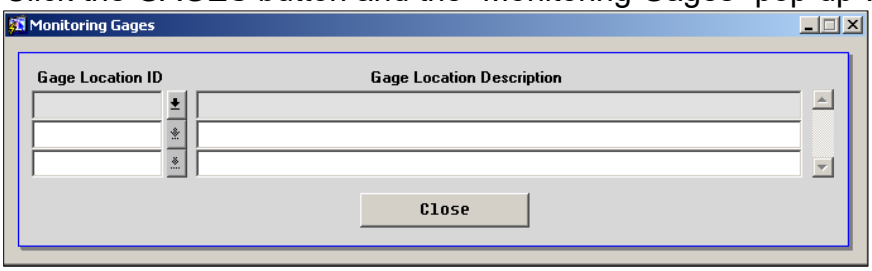


Figure 31: The "Monitoring Gages" pop-up window.

    b. To go to a blank record, click the CREATE RECORD button on the toolbar or press the [F6] key.
    c. Enter the gage location ID or select by using the list of values button.
    d. Repeat Steps ii and iii until all associated gages are selected.
    e. Click the CLOSE button.
8. Enter a code identifying the source of the information regarding the monitoring point (e.g., home owner, DEP, etc.) or select by using the list of values button.
9. Enter a code identifying the method used to identify the point coordinates of the monitoring point or select by using the list of values button.
10. Enter the map scale used to locate the monitoring point.
11. Enter the accuracy measurement of the coordinates and select a code identifying the unit of measure for the accuracy measurement using the list of values.
12. Click the SAVE button on the toolbar or press the [F10] key.

Step 4: Aliases TAB (optional)

Use the Aliases TAB to create identifiers for the monitoring point in addition to the ID generated by SIS.

1. Click the Aliases TAB.

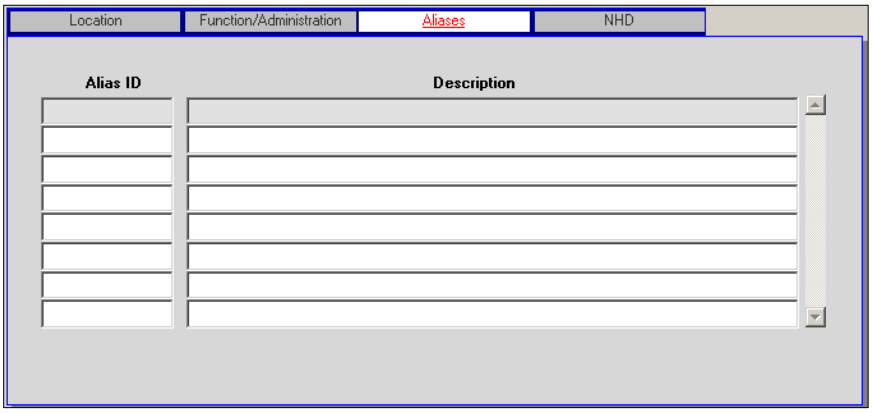


Figure 32: The Aliases TAB.

2. Click the CREATE RECORD button on the toolbar or press the [F6] key to add a new record.
3. Enter the alias ID for the monitoring point. Press the [TAB] key.
4. Enter a description of the alias.
5. Repeat Steps b through d to enter additional aliases.

Linking the Monitoring Point to a NHD Record

Link a monitoring point to a NHD record to monitoring the environmental impact on a water resource.

1. Query an existing monitoring point or create a new one.
2. Click the Location TAB.
3. Verify that the latitude, longitude, and datum are entered.
4. Click the NHD TAB.

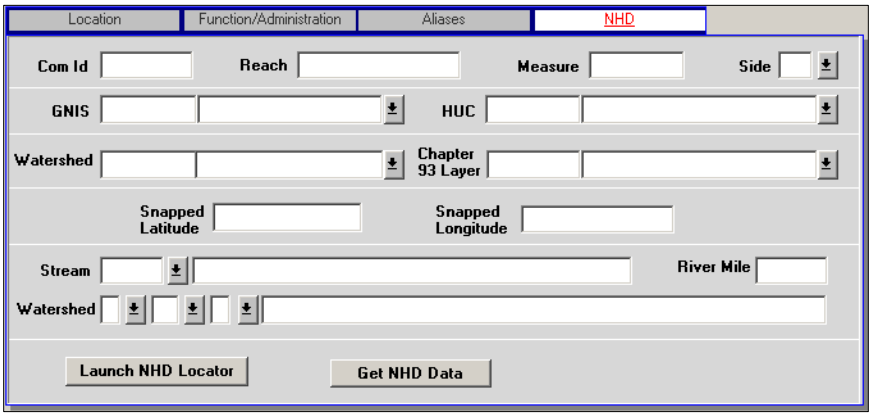


Figure 33: The NHD TAB.

5. Click the LAUNCH NHD LOCATOR button at the bottom of the screen and the NHD Locator Screen displays.

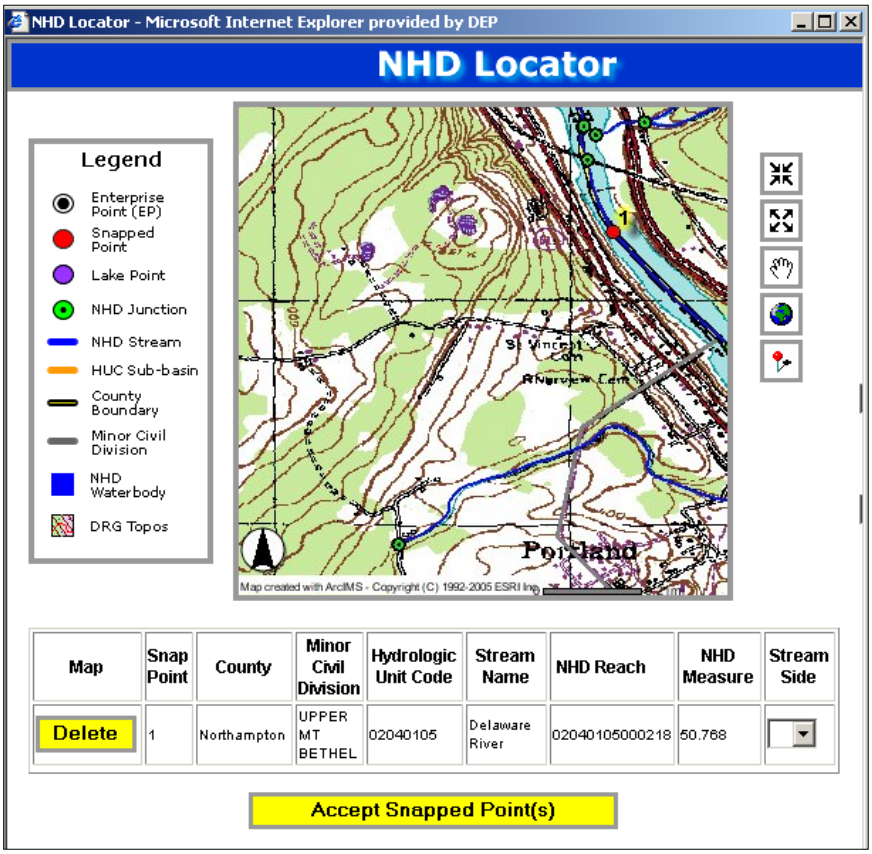


Figure 34: The NHD locator.

6. Use the NHD Locator Tool to either accept the default snapped point or create a user-defined, new snapped point to accept.
7. Click the ACCEPT SNAPPED POINT(S) button and then click the OK button to exit the NHD Locator Tool and return to the SAMPLE ENTRY Screen.
8. Click the GET NHD DATA button to add the NHD record created via the NHD Locator Tool to the sample.
9. Click the SAVE button on the toolbar or press the [F10] key.

Linking Primary Facilities to a Monitoring Point

Link a monitoring point to an eFACTS primary facility to monitor its environmental impact on the soil, water, or air.

△ *Caution: Do NOT link the monitoring point to a primary facility if intending to link to specific sub-facilities of that primary facility. Instead proceed to the section on linking sub-facilities.*

Query an existing monitoring point or create a new one.
Click the PRIMARY FACILITIES button and the “Monitoring Point/Primary Facilities” pop-up window displays.
Click the CREATE RECORD button on the toolbar or press the [F6] key.

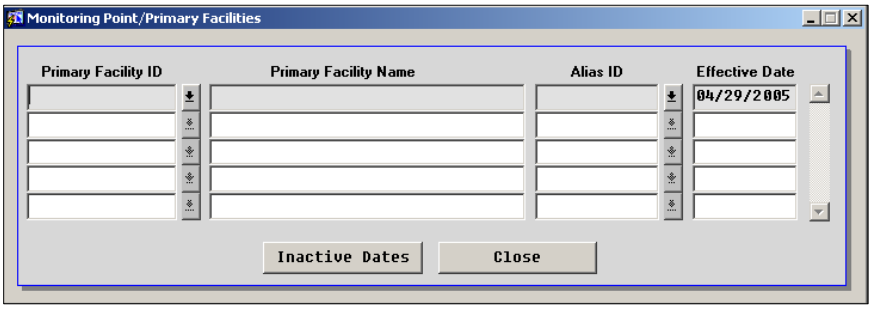


Figure 35: The "Monitoring Point/Primary Facilities" pop-up window.

Select a primary facility by entering the "Other ID" from *e*FACTS (i.e., permit, registration, or license number) or search for a primary facility by name.

To search for a primary facility by name:

Click the list of values button to display the "List Reduction Criteria" pop-up window.
Enter the name of the facility (use the wildcard symbol if necessary)
Optionally, select a program to limit the list of matching primary facilities.
Click the ACCEPT button and the "FIX Primary Facilities" pop-up window displays.
Select the correct facility from the list and click the OK button.
Optionally, select an Alias ID to associate this primary facility with one of the aliases created for the monitoring point
Update the effective date if necessary.
Click the SAVE button on the toolbar or press the [F10] key.
Click the CLOSE button.

Linking Sub-facilities to a Monitoring Point

Link a monitoring point to an *e*FACTS sub-facility to monitor its environmental impact on the soil, water, or air.

1. Query an existing monitoring point or create a new one.
2. Click the SUB-FACILITIES button and the "Monitoring Points/Sub-Facilities" pop-up window displays.
3. Click the CREATE RECORD button on the toolbar or press the [F6] key.

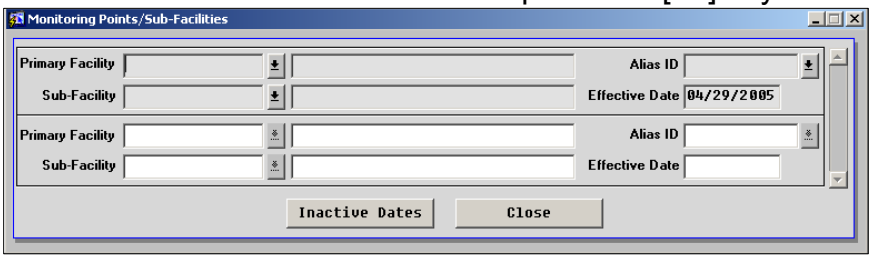


Figure 36: The "Monitoring Point/Sub-Facilities" pop-up window.

4. Select a primary facility by entering the "Other ID" from *e*FACTS (e.g., permit, registration, license) or search for a primary facility by name.
   To search for a primary facility by name:
   a. Click the list of values button to display the "List Reduction Criteria" pop-up window.
   b. Enter the name of the facility (use the wildcard symbol if necessary)
   c. Optionally, select a program to limit the list of matching primary facilities.
   d. Click the ACCEPT button and the "FIX Primary Facilities" pop-up window displays.
   e. Select the correct facility from the list and click the OK button.
5. Optionally, select an Alias ID to associate this primary facility with one of the aliases created for the monitoring point

6. Select a sub-facility using the list of values button.
7. Update the effective date if necessary.
8. Click the SAVE button on the toolbar or press the [F10] key.
9. Click the CLOSE button.

Linking Projects to a Monitoring Point

Link a monitoring point to a project to monitor its environmental impact on the soil, water, or air.

1. Query an existing monitoring point or create a new one.
2. Click the PROJECTS button and the "Monitoring Point/Projects" pop-up window displays.

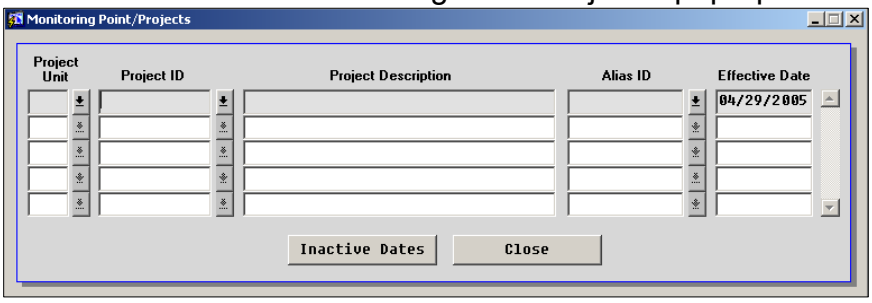


Figure 37: The "Monitoring Point/Projects" pop-up window.

3. Click the CREATE RECORD button on the toolbar or press the [F6] key.
4. Optionally, enter a business unit code in the *Project Unit* field or select one using the list of values.
5. Enter a Project ID or select one using the list of values.
6. Optionally, select an Alias ID to associate this project with one of the aliases created for the monitoring point
7. Update the effective date if necessary.
8. Click the SAVE button on the toolbar or press the [F10] key.

Closing a Monitoring Point

Closing monitoring point disallows linking samples to it.

1. Query an existing monitoring point.
2. Click the Function/Administration TAB.

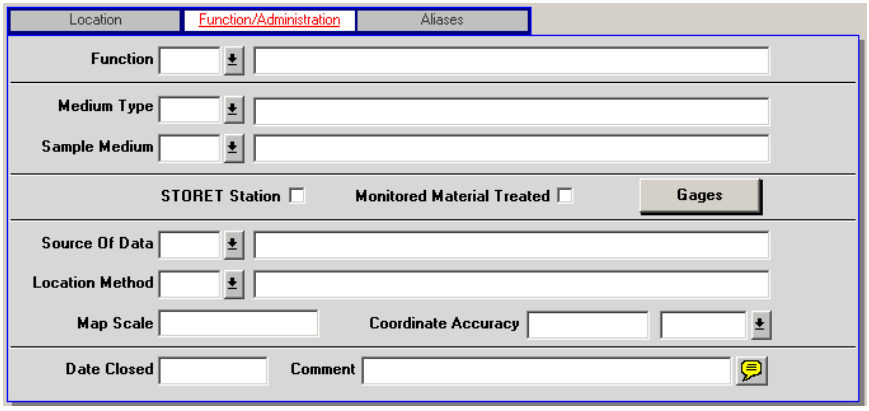


Figure 38: The Function/Administration TAB.

3. Enter a date in the *Date Closed* field. Press the [TAB] key.
4. Enter a comment regarding the closure.
5. Click the SAVE button on the toolbar or press the [F10] key.

PROJECTS

Use projects to relate samples collected for a specific purpose or to group samples or monitoring points together for the purposes of query sample results or running reports. Link a sample to a project using the SAMPLE ENTRY Screen or link a monitoring point to a project using the MONITORING POINTS Screen.

Querying for Existing Projects

1. Click the Samples Menu and then click the Projects command. The PROJECTS Screen displays.
2. Click the ENTER QUERY button on the toolbar or press the [F7] key.
3. Enter exact text criteria or use the wildcard symbol (%) in the *Project ID*, *Description*, and/or *Comments* fields or select a business unit or cost center using the lists of values.

> 🕮 The Comments field is case-sensitive. The Project ID and Description fields are not.

4. Click the COUNT HITS button or press [SHIFT] + [F2] to display the number of matching projects in the hint line.
5. Click the EXECUTE QUERY button or press the [F8] key to display the matching projects.

Inserting a New Project

1. Click the Samples Menu and then click the Projects command. The PROJECTS Screen displays.
2. Enter a unique project ID and press the [TAB] key.
3. Enter a project description and press the [TAB] key.
4. Enter a business unit or select one using the list of values. Press the [TAB] key.
5. Enter a cost center or select one using the list of values.
6. Optionally, enter any comments about the purpose of the project.
7. Click the SAVE button on the toolbar or press the [F10] key.

REPORTS

Requesting Sample Reports

After lab analysis of a sample is complete, a report is e-mailed to the collector, business unit, and report groups linked to the sample. Reports for groups of samples collected during a date range or identified by collector ID and sequence numbers can be e-mailed again or sent to a different address by requesting sample reports.

1. Click the Reports Menu, select Sample reports, and then click the Request sample reports command. The REQUEST SAMPLE REPORTS Screen displays.
2. The screen is automatically placed into query mode. Enter a collector ID, collection date, and/or sequence number to locate a particular set of samples.

> 🕮 To enter a range of collection dates or sequences, click the QUERY OPTIONS button instead of specifying a specific date or sequence.

3. Click the COUNT HITS button on the toolbar to display the number of samples matching the query criteria in the hint line.
4. After verifying the expected or appropriate number of samples will be retrieved, click the EXECUTE QUERY button on the toolbar to display the matching samples.
5. Select the samples to have reports generated by clicking the “Select Sample” checkbox or click the SELECT ALL button.
6. Select either the default report destination or a custom report destination.

> 🕮 Click the SHOW DEFAULT button to see the default report destination. Click the CUSTOMIZE button to enter a custom report destination. The custom report destination defaults to the current user.

7. Click the PRINT REPORTS button and a confirmation dialog displays indicating the selected reports have been e-mailed to the destination specified. Click the OK button.

Generating Sample Reports

The sample reports for samples collected by a specific collector during a date range can be generated and viewed in a PDF file.

Click the Reports Menu, select Sample reports, and then click the Long/Short Sample report command. The LONG/SHORT REPORTS Screen displays.
The short report type is selected by default. Select the desired report type.

> 🕮 The short sample report form displays on a page with a portrait orientation and has a compact layout. The long sample report displays on a page with a landscape orientation and typically uses more pages for each sample than the short report.

Enter a collector ID.
Enter a beginning sample collection date in the *SAMPLE BEGIN DATE* field.
If necessary, change the numbers in the *BEGIN* and *END SEQUENCE NO.* fields
Click the RUN REPORT button. After a period of time that varies depending on the number matching sample reports, a PDF will open with the sample reports.

Missing Sample Header/Results Report

Use the Missing Sample Header/Results report to view a list of sample records entered into SIS that do not have a lab results or to view a list of lab results available in SIS that have not had a sample record entered.

Click the Reports Menu and then click the Missing sample header/results command. The MISSING HEADER/RESULTS REPORTS Screen displays.
Select the Missing Header report to generate a list of sample reports awaiting entry of the sample in SIS or select the Missing Result report to generate a list of samples in SIS awaiting sample reports.
Select a Collector Group, Business Unit, or Collector to run the report for from the lists of values.
Enter a collection date range for either the Missing Header or Missing Results report.
Click the RUN REPORT(S) button.

Monitoring Points Report

The Monitoring Points report lists all of the monitoring points linked to a specified project, primary facility, or sub-facility including the location of the monitoring point, whether it is active, and the date last sampled.

1. Click the Reports Menu and then click the mOnitoring points command. The MONITORING POINT REPORTS Screen displays.
2. Select either a PDF or Excel file for the report destination.
3. Select report type to indicate whether the monitoring points will be identified by project, primary facility, or sub-facility.

If running a report by project:
   a. Optionally, identify a project unit to narrow down the list of projects.
   b. Select a project ID.
   c. Click the RUN REPORT button.

If running a report by primary facility:
   a. Enter the *e*FACTS "Other ID" for a primary facility. The "Other ID" is typically a permit or registration number assigned by the related program.
   b. Alternatively, use the list of values to select a facility by either facility name or associated program.
   c. Click the RUN REPORT button.

If running a report by sub-facility:
   a. First, enter the *e*FACTS "Other ID" for a primary facility. The "Other ID" is typically a permit or registration number assigned by the related program.
   b. Alternatively, use the list of values to select a primary facility by either facility name or associated program.
   c. Select the sub-facility using the list of values.
   d. Click the RUN REPORT button.

4. Optionally, select the checkbox to include the date each monitoring point was last sampled.

> 🕮 Including the date last sampled for each monitoring point lists the most recent sample regardless of which business unit collected the sample. Use the list of values to restrict it to a specific business unit.

Module 8.1A Report

SIS can generate Module 8.1A reports for a specific monitoring point related to a project or *e*FACTS primary facility or for all monitoring points between a pair of latitudes and longitudes.

To run the report for an individual monitoring point related to a project or *e*FACTS primary facility:

1. Click the Reports Menu and then click the module 8.1A command. The MODULE 8.1A REPORTS Screen displays.
2. Select either a PDF or Excel file for the report destination.
3. Select a report type to indicate whether the monitoring points will be identified by project or primary facility.

   If running a report by project:
      a. Optionally, identify a project unit to narrow down the list of projects.
      b. Select a project ID.
      c. Select a monitoring point alias from the MP Alias list of values.

   If running a report by primary facility:

   Enter the *e*FACTS "Other ID" for a primary facility. The "Other ID" is typically a permit or registration number assigned by the related program.

   Alternatively, use the list of values to select a facility by either facility name or associated program.

   Select a monitoring point alias from the MP Alias list of values.
4. Enter a beginning and ending sample collection date range.
5. Optionally, identify to include only samples collected by a specific business unit.
6. Click the RUN REPORT button.

To run the report for all monitoring points between a pair of latitudes and a pair of longitudes:

1. Click the Reports Menu and then click the module 8.1A command. The MODULE 8.1A REPORTS Screen displays.
2. Select either a PDF or Excel file for the report destination.
3. Select the *By Latitude/Longitude* report type.
4. Enter the latitude values in the *low* and *high* fields.
5. Enter the longitude values in the *low* and *high* fields.

> 🕮 Coordinates are entered in the format DD-MM-SS.SSSS (e.g., 41-29-46.3034 or -79-38-09.6071)

6. Enter a beginning and ending sample collection date range.
7. Optionally, identify to include only samples collected by a specific business unit.
8. Click the RUN REPORT button.

B.4 SEDIMENT FIELD DATA COLLECTION SHEET

pennsylvania
DEPARTMENT OF ENVIRONMENTAL PROTECTION

COMMONWEALTH OF PENNSYLVANIA
DEPARTMENT OF ENVIRONMENTAL PROTECTION
BUREAU OF CLEAN WATER

SEDIMENT COLLECTION FIELD DATA FORM

(Information and comments for fields boxed in double lines are required database entries. Other fields are optional for personal use.)

Collector ID:		Sequence Number:		Date:		Time (Military):	
Collector Name:		Stream Name:					

Location

County:		Municipality:		Topo. Quad:	

Location Description (latitude, longitude, etc.):

SACs/Suites:	
QA/QC? (circle one):	Duplicate Blank N/A Other (explain-e.g., split sample)

Landuse

Residential:	%	Commercial:	%	Industrial:	%	Cropland:	%	Pasture:	%
Abd. Mining:	%	Old Fields:	%	Forest:	%	Other:	%		

Landuse Comments:

Canopy Cover: open partly shaded mostly shaded fully shaded

Sediment Sample

Equipment Used (circle one)	scoop/trowel dredge corer other (explain)

Sample Description (DWS discharge in plume X # of meters; at end-of-pipe discharge; reference location; etc.):

Permitted Discharge Name/Permit # (if applicable):			
Number of Deposition Locations:		Depth of Sediment Collection (approx. 1-3 cm, etc.):	
Type, texture, color, & odor of sediment and water:*			

Water

	Collector-Sequence #:	Field Meter Readings: Temp (ºC)	DO (mg/L)	pH	Cond. (umhos)	Alkalinity (mg/L)	Bottle Notes: (N-normal; MNF-metals non-filtered; MF-metals filtered; B-bac't; others: indicate)
1							
2							
3							

Other Notes:

***Common Descriptors**: **Water Odors -** none normal sewage petroleum chemical other; **Water Surface Oils -** none slick sheen globs flecks; **Turbidity -** clear slight turbid opaque; **NPS Pollution** - no evidence some potential obvious; **Sediment Odors -** none normal sewage petroleum chemical anaerobic; **Sediment Oils -** absent slight moderate profuse; **Deposits –** none sludge sawdust paper fiber sand relict shells other _____. **Are the undersides of stones deeply embedded black?**

Soil Types (summarized):
Sand: 0.06-2.0 mm; gritty, non-plastic, loose particulates
Silt: 0.004-0.06 mm; smooth, talc-like, non-plastic, loose particulates
Clay: <0.004 mm; dense, moldable like putty; cohesive
Adapted from: Ohio EPA. 2001. Sediment sampling guide and methodologies. 2nd edition.

Soil Color/Types:
Refer to a Munsell Color Chart

B.5 CIM DEPLOYMENT FORM

STATION NAME	
CALIBRATED/DEPLOYED BY	
METER MAKE/MODEL	
METER SERIAL NO.	
DATE	
TIME	
FILE NAME	
LOGGING START TIME	
BATTERY LIFE (DAYS or VOLTS)	
MEMORY (DAYS)	

******IF THIS IS A SONDE FROM EUREKA MAKE SURE "CablePower_V" (UNITS WITH EXTERNAL BATTERY PACKS) OR "Int_Batt_V" (UNITS WITH INTERNAL BATTERIES) PARAMETER IS ON TO ALLOW FOR BATTERY CHECKS******

If this sonde is equipped with depth, was it calibrated?	
Is a barologger being deployed at this site?	

Comments:

SPECIFIC CONDUCTANCE

(Calibration Criteria: the greater of 5 µS/cm or 3% of measured value)

PROBE SERIAL NO:

STD VALUE (µS/cm)	MANUFAC-TURER	TYPE	LOT NO.	EXP DATE
100				
1000				
5000				

STD VALUE (µS/cm)	TEMP (°C)	READING (µS/cm)	ADJUSTED (µS/cm)	CALIBRATION LOCATION
100				
1000				
5000				
Reading in air (should be 0)				

pH

(Calibration Correction: ± 0.2 pH units)

PROBE SERIAL NO:

pH BUFFER	TEMP ADJUSTED pH	MANUFAC-TURER	LOT NO.	EXP DATE
7.00	7.00			
10.00	10.00			
4.00	4.00			

TEMP ADJUSTED pH	TEMP (°C)	READING (pH units)	ADJUSTED (pH units)	MILLI-VOLTS	CALIBRATION LOCATION
7.00					
10.00					
4.00					

If Eureka sonde, was reference solution changed?	

TURBIDITY

(Calibration Criteria: the greater of ± 0.5 FNU or ± 5% of measured value)

PROBE SERIAL NO:

STD VALUE (FNU)	MANUFAC-TURER	TYPE	LOT NO.	EXP DATE
0				
126				
1000				

STD VALUE (FNU)	TEMP (°C)	READING (FNU)	ADJUSTED (FNU)	CALIBRATION LOCATION
0				
126				
1000				

THE FOLLOWING PARAMETER SHOULD BE CALIBRATED IN THE FIELD

DISSOLVED OXYGEN

(Calibration Criteria: ± 0.3 mg/L)

PROBE SERIAL NO:

TEMP (°C)	BARO PRES (mmHg)	TABLE VALUE (mg/L)	CALIBRATION LOCATION

READING (mg/L)	READING (%)	ADJUSTED (mg/L)	ADJUSTED (%)	READING IN ZERO DO SOLUTION

LOCATION DESCRIPTION

B.6 CIM MAINTENANCE VISIT FIELD FORM

Station Name	
Inspected By	
Date	
Time (UTC-5)	
Monitor Make/Model	
Monitor Serial No.	
Field Meter Make/Model	
Field Meter Serial No.	

DISCRETE MEASUREMENT		Time (UTC-5):	
Temp (°C)		**DO (mg/L)**	
SC (µS/cm)		**DO (%)**	
pH (units)		**Turbidity (FNU)**	
MONITOR FOULING			
FILE UPLOADED			
Comments:			

MONITOR FOULING CHECKS

BEFORE CLEANING		Time (UTC-5):	
		MONITOR	FIELD METER
Temp (°C)			
SC (µS/cm)			
pH (units)			
DO (mg/L)			
DO (%)			
Turbidity (FNU)			
Other:			
If Eureka sonde, was reference solution changed?			

AFTER CLEANING		Time (UTC-5):	
		MONITOR	FIELD METER
Temp (°C)			
SC (µS/cm)			
pH (units)			
DO (mg/L)			
DO (%)			
Turbidity (FNU)			
Other:			

CALIBRATION DRIFT CHECKS

DISSOLVED OXYGEN

(Calibration Criteria: ± 0.3 mg/L)

PROBE SERIAL NO:

Calibration Check					Time (UTC-5):	
TEMP (°C)	BARO PRES (mmHg)	TABLE VALUE (mg/L)	READING (mg/L)	READING (%)	ERROR %	READING IN ZERO DO SOLUTION
					0.0	

Recalibration (if necessary)					Time (UTC-5):	
TEMP (°C)	BARO PRES (mmHg)	TABLE VALUE (mg/L)	READING (mg/L)	READING (%)	ERROR %	READING IN ZERO DO SOLUTION
					0.0	

Comments:

SPECIFIC CONDUCTANCE

(Calibration Criteria: the greater of 5 µS/cm or 3% of measured value)

PROBE SERIAL NO:

STD VALUE (µS/cm)	MANUFAC-TURER	TYPE	LOT NO.	EXP DATE
100		NaCl		
1000		NaCl		
5000		NaCl		

Calibration Check			Time (UTC-5):
STD VALUE (µS/cm)	TEMP (°C)	READING (µS/cm)	ERROR %
100			0.0
1000			0.0
5000			0.0
Reading in air (should be 0)			

Recalibration (if necessary)			Time (UTC-5):
STD VALUE	TEMP (°C)	READING (µS/cm)	ERROR %
100			0.0
1000			0.0
5000			0.0
Reading in air (should be 0)			

Comments:

pH

(Calibration Correction: ± 0.2 pH units)

PROBE SERIAL NO:

pH BUFFER	TEMP ADJUSTED pH	MANUFACTURER	LOT NO.	EXP DATE
7.00	7.00			
10.00	10.00			
4.00	4.00			

Calibration Check Time (UTC-5):

TEMP ADJUSTED pH	TEMP (°C)	READING (pH units)	ERROR	MILLIVOLTS	SLOPE
7.00			0.00		
10.00			0.00		0.0
4.00			0.00		0.0

Recalibration (if necessary) Time (UTC-5):

TEMP ADJUSTED pH	TEMP (°C)	READING (pH units)	ERROR	MILLIVOLTS	SLOPE
7.00			0.00		
10.00			0.00		0.0
4.00			0.00		0.0

Comments:

TURBIDITY

(Calibration Criteria: the greater of ± 0.5 FNU or ± 5% of measured value)

PROBE SERIAL NO:

STD VALUE	MANUFACTURER	LOT NO.	EXP DATE
0			
126			
1000			

Calibration Check Time (UTC-5):

STD VALUE	TEMP (°C)	READING (FNU)	ERROR %
0			N/A
126			0.0
1000			0.0

Recalibration (if necessary) Time (UTC-5):

STD VALUE	TEMP (°C)	READING (FNU)	ERROR %
0			N/A
126			0.0
1000			0.0

Comments:

FINAL READINGS		
Time (UTC-5):		
	MONITOR	FIELD METER
Temp (°C)		
SC (µS/cm)		
pH (units)		
DO (mg/L)		
DO (%)		
Turbidity (FNU)		
Other:		
Comments:		

NEW FILE NAME	
LOGGING START TIME (UTC-5)	
BATTERY LIFE (DAYS or VOLTS)	
MEMORY (DAYS)	
SONDE LOGGING OR PULLED?	

B.7 TEMPERATURE ADJUSTMENTS FOR PH BUFFERS

Table 1. Temperature adjusted pH buffer values from British Drug House (BDH; “Temperature Dependence of pH,” n.d.)

TEMP (°C)	0	5	10	15	20	25	30	35	40	45	50
BDH5022	4.00	4.00	4.00	4.00	4.00	4.00	4.01	4.02	4.03	4.04	4.06
BDH5050	7.12	7.09	7.06	7.04	7.02	7.00	6.99	6.98	6.98	6.97	6.97
BDH5076	10.31	10.23	10.17	10.11	10.05	10.00	9.95	9.91	9.87		9.81

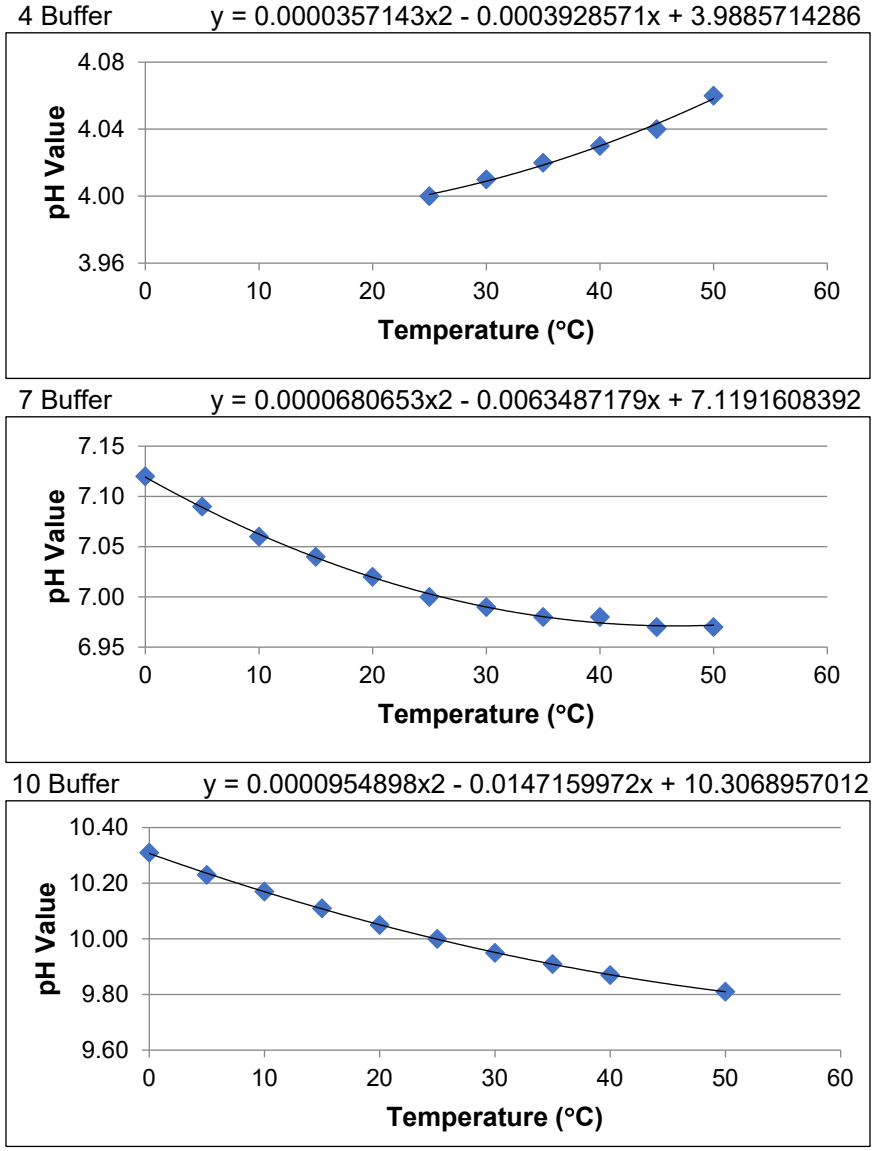


Figure 1. Second-order polynomial regression lines fitted to temperature-corrected pH values for BDH buffers.

TEMPERATURE ADJUSTMENTS FOR pH BUFFERS

(Based on regression equations fitted to values for BDH buffers)

TEMP (°C)	0
pH 4	4.00
pH 7	7.12
pH 10	10.31

TEMP (°C)	1	2	3	4	5	6	7	8	9	10
pH 4	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00
pH 7	7.11	7.11	7.10	7.09	7.09	7.08	7.08	7.07	7.07	7.06
pH 10	10.29	10.28	10.26	10.25	10.24	10.22	10.21	10.20	10.18	10.17

TEMP (°C)	11	12	13	14	15	16	17	18	19	20
pH 4	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00
pH 7	7.06	7.05	7.05	7.04	7.04	7.04	7.03	7.03	7.02	7.02
pH 10	10.16	10.14	10.13	10.12	10.11	10.10	10.08	10.07	10.06	10.05

TEMP (°C)	21	22	23	24	25	26	27	28	29	30
pH 4	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.01	4.01	4.01
pH 7	7.02	7.01	7.01	7.01	7.00	7.00	7.00	6.99	6.99	6.99
pH 10	10.04	10.03	10.02	10.01	10.00	9.99	9.98	9.97	9.96	9.95

TEMP (°C)	31	32	33	34	35	36	37	38	39	40
pH 4	4.01	4.01	4.01	4.02	4.02	4.02	4.02	4.03	4.03	4.03
pH 7	6.99	6.99	6.98	6.98	6.98	6.98	6.98	6.98	6.98	6.97
pH 10	9.94	9.93	9.93	9.92	9.91	9.90	9.89	9.89	9.88	9.87

TEMP (°C)	41	42	43	44	45	46	47	48	49	50
pH 4	4.03	4.04	4.04	4.04	4.04	4.05	4.05	4.05	4.06	4.06
pH 7	6.97	6.97	6.97	6.97	6.97	6.97	6.97	6.97	6.97	6.97
pH 10	9.86	9.86	9.85	9.84	9.84	9.83	9.83	9.82	9.82	9.81

Figure 2. Temperature adjustments for pH buffers.

B.8 DO 100% SATURATION CHART FOR FRESH WATER

Temp °C	Barometric Pressure (mmHg)														
	700	705	710	715	720	725	730	735	740	745	750	755	760	765	770
0.0	13.46	13.56	13.65	13.75	13.85	13.94	14.04	14.14	14.23	14.33	14.43	14.52	14.62	14.72	14.81
0.5	13.27	13.37	13.46	13.56	13.65	13.75	13.84	13.94	14.03	14.13	14.23	14.32	14.42	14.51	14.61
1.0	13.09	13.18	13.28	13.37	13.46	13.56	13.65	13.75	13.84	13.93	14.03	14.12	14.22	14.31	14.40
1.5	12.91	13.00	13.09	13.19	13.28	13.37	13.46	13.56	13.65	13.74	13.84	13.93	14.02	14.11	14.21
2.0	12.73	12.82	12.91	13.01	13.10	13.19	13.28	13.37	13.46	13.56	13.65	13.74	13.83	13.92	14.01
2.5	12.56	12.65	12.74	12.83	12.92	13.01	13.10	13.19	13.28	13.37	13.46	13.55	13.64	13.73	13.82
3.0	12.39	12.48	12.57	12.66	12.75	12.84	12.93	13.02	13.10	13.19	13.28	13.37	13.46	13.55	13.64
3.5	12.23	12.31	12.40	12.49	12.58	12.67	12.75	12.84	12.93	13.02	13.11	13.19	13.28	13.37	13.46
4.0	12.07	12.15	12.24	12.33	12.41	12.50	12.59	12.67	12.76	12.85	12.93	13.02	13.11	13.20	13.28
4.5	11.91	11.99	12.08	12.17	12.25	12.34	12.42	12.51	12.59	12.68	12.77	12.85	12.94	13.02	13.11
5.0	11.75	11.84	11.92	12.01	12.09	12.18	12.26	12.35	12.43	12.52	12.60	12.69	12.77	12.86	12.94
5.5	11.60	11.69	11.77	11.86	11.94	12.02	12.11	12.19	12.27	12.36	12.44	12.52	12.61	12.69	12.78
6.0	11.46	11.54	11.62	11.70	11.79	11.87	11.95	12.04	12.12	12.20	12.28	12.37	12.45	12.53	12.61
6.5	11.31	11.39	11.48	11.56	11.64	11.72	11.80	11.88	11.97	12.05	12.13	12.21	12.29	12.37	12.46
7.0	11.17	11.25	11.33	11.41	11.49	11.58	11.66	11.74	11.82	11.90	11.98	12.06	12.14	12.22	12.30
7.5	11.03	11.11	11.19	11.27	11.35	11.43	11.51	11.59	11.67	11.75	11.83	11.91	11.99	12.07	12.15
8.0	10.90	10.98	11.06	11.14	11.21	11.29	11.37	11.45	11.53	11.61	11.69	11.76	11.84	11.92	12.00
8.5	10.77	10.84	10.92	11.00	11.08	11.16	11.23	11.31	11.39	11.47	11.54	11.62	11.70	11.78	11.86
9.0	10.64	10.71	10.79	10.87	10.94	11.02	11.10	11.18	11.25	11.33	11.41	11.48	11.56	11.64	11.71
9.5	10.51	10.59	10.66	10.74	10.81	10.89	10.97	11.04	11.12	11.19	11.27	11.35	11.42	11.50	11.57
10.0	10.39	10.46	10.54	10.61	10.69	10.76	10.84	10.91	10.99	11.06	11.14	11.21	11.29	11.36	11.44
10.5	10.26	10.34	10.41	10.49	10.56	10.64	10.71	10.78	10.86	10.93	11.01	11.08	11.16	11.23	11.30
11.0	10.15	10.22	10.29	10.37	10.44	10.51	10.59	10.66	10.73	10.81	10.88	10.95	11.03	11.10	11.17
11.5	10.03	10.10	10.17	10.25	10.32	10.39	10.47	10.54	10.61	10.68	10.76	10.83	10.90	10.97	11.05
12.0	9.91	9.99	10.06	10.13	10.20	10.27	10.35	10.42	10.49	10.56	10.63	10.71	10.78	10.85	10.92
12.5	9.80	9.87	9.94	10.02	10.09	10.16	10.23	10.30	10.37	10.44	10.51	10.58	10.66	10.73	10.80
13.0	9.69	9.76	9.83	9.90	9.97	10.04	10.11	10.19	10.26	10.33	10.40	10.47	10.54	10.61	10.68
13.5	9.59	9.65	9.72	9.79	9.86	9.93	10.00	10.07	10.14	10.21	10.28	10.35	10.42	10.49	10.56
14.0	9.48	9.55	9.62	9.69	9.76	9.82	9.89	9.96	10.03	10.10	10.17	10.24	10.31	10.37	10.44
14.5	9.38	9.44	9.51	9.58	9.65	9.72	9.78	9.85	9.92	9.99	10.06	10.13	10.19	10.26	10.33
15.0	9.27	9.34	9.41	9.48	9.54	9.61	9.68	9.75	9.81	9.88	9.95	10.02	10.08	10.15	10.22
15.5	9.18	9.24	9.31	9.38	9.44	9.51	9.58	9.64	9.71	9.78	9.84	9.91	9.98	10.04	10.11
16.0	9.08	9.14	9.21	9.28	9.34	9.41	9.47	9.54	9.61	9.67	9.74	9.80	9.87	9.94	10.00
16.5	8.98	9.05	9.11	9.18	9.24	9.31	9.37	9.44	9.50	9.57	9.64	9.70	9.77	9.83	9.90
17.0	8.89	8.95	9.02	9.08	9.15	9.21	9.28	9.34	9.41	9.47	9.54	9.60	9.66	9.73	9.79

Temp °C	Barometric Pressure (mmHg)														
	700	705	710	715	720	725	730	735	740	745	750	755	760	765	770
17.5	8.80	8.86	8.92	8.99	9.05	9.12	9.18	9.24	9.31	9.37	9.44	9.50	9.57	9.63	9.69
18.0	8.70	8.77	8.83	8.90	8.96	9.02	9.09	9.15	9.21	9.28	9.34	9.40	9.47	9.53	9.59
18.5	8.62	8.68	8.74	8.80	8.87	8.93	8.99	9.06	9.12	9.18	9.24	9.31	9.37	9.43	9.50
19.0	8.53	8.59	8.65	8.72	8.78	8.84	8.90	8.96	9.03	9.09	9.15	9.21	9.28	9.34	9.40
19.5	8.44	8.50	8.57	8.63	8.69	8.75	8.81	8.87	8.94	9.00	9.06	9.12	9.18	9.25	9.31
20.0	8.36	8.42	8.48	8.54	8.60	8.66	8.73	8.79	8.85	8.91	8.97	9.03	9.09	9.15	9.21
20.5	8.28	8.34	8.40	8.46	8.52	8.58	8.64	8.70	8.76	8.82	8.88	8.94	9.00	9.06	9.12
21.0	8.19	8.25	8.31	8.37	8.43	8.49	8.55	8.61	8.67	8.73	8.79	8.85	8.92	8.98	9.04
21.5	8.11	8.17	8.23	8.29	8.35	8.41	8.47	8.53	8.59	8.65	8.71	8.77	8.83	8.89	8.95
22.0	8.04	8.09	8.15	8.21	8.27	8.33	8.39	8.45	8.51	8.57	8.63	8.68	8.74	8.80	8.86
22.5	7.96	8.02	8.08	8.13	8.19	8.25	8.31	8.37	8.43	8.48	8.54	8.60	8.66	8.72	8.78
23.0	7.88	7.94	8.00	8.06	8.11	8.17	8.23	8.29	8.35	8.40	8.46	8.52	8.58	8.64	8.69
23.5	7.81	7.86	7.92	7.98	8.04	8.09	8.15	8.21	8.27	8.33	8.38	8.44	8.50	8.56	8.61
24.0	7.73	7.79	7.85	7.90	7.96	8.02	8.08	8.13	8.19	8.25	8.30	8.36	8.42	8.48	8.53
24.5	7.66	7.72	7.77	7.83	7.89	7.94	8.00	8.06	8.11	8.17	8.23	8.28	8.34	8.40	8.45
25.0	7.59	7.65	7.70	7.76	7.81	7.87	7.93	7.98	8.04	8.10	8.15	8.21	8.26	8.32	8.38
25.5	7.52	7.58	7.63	7.69	7.74	7.80	7.85	7.91	7.97	8.02	8.08	8.13	8.19	8.24	8.30
26.0	7.45	7.51	7.56	7.62	7.67	7.73	7.78	7.84	7.89	7.95	8.00	8.06	8.11	8.17	8.22
26.5	7.38	7.44	7.49	7.55	7.60	7.66	7.71	7.77	7.82	7.88	7.93	7.99	8.04	8.10	8.15
27.0	7.32	7.37	7.43	7.48	7.53	7.59	7.64	7.70	7.75	7.81	7.86	7.91	7.97	8.02	8.08
27.5	7.25	7.30	7.36	7.41	7.47	7.52	7.57	7.63	7.68	7.74	7.79	7.84	7.90	7.95	8.01
28.0	7.19	7.24	7.29	7.35	7.40	7.45	7.51	7.56	7.61	7.67	7.72	7.77	7.83	7.88	7.93
28.5	7.12	7.18	7.23	7.28	7.33	7.39	7.44	7.49	7.55	7.60	7.65	7.71	7.76	7.81	7.87
29.0	7.06	7.11	7.16	7.22	7.27	7.32	7.38	7.43	7.48	7.53	7.59	7.64	7.69	7.74	7.80
29.5	7.00	7.05	7.10	7.15	7.21	7.26	7.31	7.36	7.42	7.47	7.52	7.57	7.62	7.68	7.73
30.0	6.94	6.99	7.04	7.09	7.14	7.20	7.25	7.30	7.35	7.40	7.46	7.51	7.56	7.61	7.66
30.5	6.88	6.93	6.98	7.03	7.08	7.13	7.19	7.24	7.29	7.34	7.39	7.44	7.49	7.55	7.60
31.0	6.82	6.87	6.92	6.97	7.02	7.07	7.12	7.17	7.23	7.28	7.33	7.38	7.43	7.48	7.53
31.5	6.76	6.81	6.86	6.91	6.96	7.01	7.06	7.11	7.16	7.21	7.27	7.32	7.37	7.42	7.47
32.0	6.70	6.75	6.80	6.85	6.90	6.95	7.00	7.05	7.10	7.15	7.20	7.25	7.30	7.36	7.41
32.5	6.64	6.69	6.74	6.79	6.84	6.89	6.94	6.99	7.04	7.09	7.14	7.19	7.24	7.29	7.34
33.0	6.59	6.64	6.69	6.74	6.79	6.84	6.89	6.93	6.98	7.03	7.08	7.13	7.18	7.23	7.28
33.5	6.53	6.58	6.63	6.68	6.73	6.78	6.83	6.88	6.93	6.98	7.02	7.07	7.12	7.17	7.22
34.0	6.48	6.53	6.57	6.62	6.67	6.72	6.77	6.82	6.87	6.92	6.97	7.02	7.06	7.11	7.16

APPENDIX C: PHYSICAL DATA COLLECTION INFORMATION AND FORMS

C.1 RIFFLE/RUN PREVALENCE HABITAT DATA COLLECTION FORM

Physical Habitat Evaluation Form for Riffle/Run Prevalence

Waterbody Name:	GIS Key (YYYYMMDD-hhmm-User):
Location:	
Investigators:	Completed By:

Parameter	Optimal	Suboptimal	Marginal	Poor
†1. Instream Cover[1] *(fish)*	Greater than 50% mix of boulder, cobble, submerged logs, undercut banks, or other stable habitat.	30-50% mix of boulder, cobble, or other stable habitat; adequate habitat.	10-30% mix of boulder, cobble, or other stable habitat; habitat availability less than desirable.	Less than 10% mix of boulder, cobble, or other stable habitat; lack of habitat is obvious.
_____	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1
†2. Epifaunal Substrate[1] *(riffle quality)*	Well-developed riffle and run; riffle is as wide as stream and length extends two times the width of stream; abundance of cobble.	Riffle is as wide as stream but length is less than two times width; abundance of cobble; boulders and gravel common.	Run area may be lacking; riffle not as wide as stream and its length is less than 2 times the stream width; gravel or large boulders and bedrock prevalent; some cobble present.	Riffles or run virtually nonexistent; large boulders and bedrock prevalent; cobble lacking.
_____	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1
†*3. Embeddedness[1] *(evaluate in upstream & central portions of riffles)*	Gravel, cobble, and boulder particles are 0-25% surrounded by fine sediment.	Gravel, cobble, and boulder particles are 25-50% surrounded by fine sediment.	Gravel, cobble, and boulder particles are 50-75% surrounded by fine sediment.	Gravel, cobble, and boulder particles are more than 75% surrounded by fine sediment.
_____	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1
4. Velocity/Depth Regimes[1]	All four velocity/depth regimes present (slow-deep, slow shallow, fast-deep, fast shallow)	Only 3 of the 4 regimes present if fast-shallow is missing, score lower than if missing other regimes.)	Only 2 of the 4 habitat regimes present (if fast-shallow or slow-shallow are missing, score lower than if missing other regimes).	Dominated by 1 velocity/depth regime (usually slow-deep).
_____	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1
5. Channel Alteration[2] *(only include downstream alteration when affecting reach)*	No channelization or dredging present.	Some channelization present, usually in areas of bridge abutments; evidence of past channelization, i.e., dredging (greater than 20 yr.) may be present, but recent channelization is not present.	New embankments present on both banks; and 40 to 80% of stream reach channelized and disrupted.	Banks shored with gabion or cement over 80% of the stream reach channelized and disrupted.
_____	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1
*6. Sediment Deposition[2] *(evaluate in pools & depositional areas)*	Little or no enlargement of islands or point bars and less than 5% of the bottom affected by sediment deposition.	Some new increase in bar information, mostly from coarse gravel; 5-30% of the bottom affected; slight deposition in pools.	Moderate deposition of new gravel coarse sand on old and new bars; 30-50% of the bottom affected; sediment deposits at obstruction, construction and bends, moderate depositions of pools prevalent.	Heavy deposits of fine material increased bar development; more than 50% of the bottom changing frequently; pools almost absent due to substantial sediment deposition.
_____	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1

Note: Wadeable couplet scores only calculated if the Wadeable Riffle-Run Protocol (Chapter 3.1) is used. Semiwadable couplet score only calculated if Semiwadeable Large River Protocol (Chapter 3.4) is used.

*** WADEABLE COUPLET SCORE (EMBEDDEDNESS + SEDIMENT DEPOSITION)** _____

† SEMIWADEABLE TRIPLET SCORE (INSTREAM COVER + EPIFANUAL SUBSTRATE + EMBEDDEDNESS) _____

Parameter	Optimal	Suboptimal	Marginal	Poor
7. Riffle Frequency[2] *(riffle quantity; consider run:bend ratio)*	Occurrence of riffles relatively frequent; distance between riffles divided by the width of the stream equals 5 to 7; variety of habitat.	Occurrence of riffles infrequent; distance between riffles divided by the width of the stream equals 7 to 15.	Occasional riffle or bend; bottom contours provide some habitat; distance between riffles divided by the width of the stream is between 15 to 25.	Generally all flat water or shallow riffles; poor habitat; distance between riffles divided by the width of the stream is >25.
_____	**20 19 18 17 16**	**15 14 13 12 11**	**10 9 8 7 6**	**5 4 3 2 1**
8. Channel Flow Status[2]	Water reaches base of both lower banks and minimal amount of channel substrate is exposed.	Water fills >75% of the available channel; or <25% of channel substrate is exposed.	Water fills 25-75% of the available channel and/or riffle substrates are mostly exposed.	Very little water in channel and mostly present as standing pools.
_____	**20 19 18 17 16**	**15 14 13 12 11**	**10 9 8 7 6**	**5 4 3 2 1**
****9. Condition of Banks**[3] *(edge of water to bankfull delineation)*	Banks stable; no evidence of erosion or bank failure.	Moderately stable; infrequent, small areas of erosion mostly healed over.	Moderately unstable; up to 60% of banks in reach have areas of erosion.	Unstable; many eroded areas; "raw" areas frequent along straight sections and bends; on side slopes, 60-100% of bank has erosional scars.
LDB	**10 9 8**	**7 6**	**5 4 3**	**2 1**
Total _____ **RDB**	**10 9 8**	**7 6**	**5 4 3**	**2 1**
****10. Bank Vegetative Protection**[3] *(edge of water to bankfull delineation)*	More than 90% of the stream bank surfaces covered by vegetation.	70-90% of the stream bank surfaces covered by vegetation.	50-70% of the stream bank surfaces covered by vegetation.	Less than 50% of the stream bank surfaces covered by vegetation.
LDB	**10 9 8**	**7 6**	**5 4 3**	**2 1**
Total _____ **RDB**	**10 9 8**	**7 6**	**5 4 3**	**2 1**
11. Grazing or Other Disruptive Pressure[3] *(bankfull through riparian zone)*	Vegetative disruption through grazing or mowing is minimal or not evident; almost all plants allowed to grow naturally.	Disruption evident but not affecting full plant growth potential to any great extent; more than one-half of the potential plant stubble height remaining.	Disruption obvious; patches of bare soil or closely cropped vegetation common; less than one-half of the potential plant stubble height remaining.	Disruption of stream bank vegetation is very high; vegetation has been removed to 2 inches or less in average stubble height.
LDB	**10 9 8**	**7 6**	**5 4 3**	**2 1**
Total _____ **RDB**	**10 9 8**	**7 6**	**5 4 3**	**2 1**
12. Riparian Vegetative Zone[3] *(bankfull through riparian zone)*	Width or riparian zone >18 meters; human activities (i.e., parking lots, roadbeds, clear-cuts, lawns or crops) have not impacted zone.	Width of riparian zone 12-18 meters; human activities have impacted zone only minimally.	Width of riparian zone 6-12 meters; human activities have impacted zone a great deal.	Width of riparian zone <6 meters; little or no riparian vegetation due to human activities.
LDB	**10 9 8**	**7 6**	**5 4 3**	**2 1**
Total _____ **RDB**	**10 9 8**	**7 6**	**5 4 3**	**2 1**

Note: Wadeable couplet scores only calculated if the Wadeable Riffle-Run Protocol (Chapter 3.1) is used. Semiwadable triplet score only calculated if Semiwadeable Large River Protocol (Chapter 3.4) is used.

**** WADEABLE COUPLET SCORE (CONDITION OF BANKS + BANK VEGETATIVE PROTECTION)** _____

TOTAL HABITAT SCORE _____

[1] Reach scale: Evaluate parameter within the immediate vicinity of biological sampling reach.

[2] Expanded scale: Evaluate parameter within sampling reach and at least 100m UPS of sampled reach, longer if visual extent allows.

[3] Macro scale: Evaluate parameter based on expanded scale; can be extended further to account for characteristics within representative reach.

C.2 LOW GRADIENT HABITAT DATA COLLECTION FORM

Physical Habitat Evaluation Form for Low Gradient (Pool/Glide) Streams

Waterbody Name:	GIS Key (YYYYMMDD-hhmm-User):
Location:	
Investigators:	Completed By:

Parameter	Optimal	Suboptimal	Marginal	Poor
1.Substrate/Available Cover	Greater than 50% of substrate favorable for epifaunal colonization and fish cover; mix of snags, submerged logs, undercut banks, cobble or other stable habitat at stage to allow full colonization potential (i.e., logs/snags that are not new fall and not transient).	30-50% mix of stable habitat; well-suited for full colonization potential; adequate habitat for maintenance of populations; presence of additional substrate in the form of new fall, but not yet prepared for colonization (may rate at high end of scale)	10-30% mix of stable habitat; habitat availability less than desirable; substrate frequently disturbed or removed.	Less than 10% stable habitat; lack of habitat is obvious; substrate unstable or lacking.
	20 19 18 17 16	**15 14 13 12 11**	**10 9 8 7 6**	**5 4 3 2 1**
2. Pool Substrate Characterization	Mixture of substrate materials, with gravel and firm sand prevalent; root mats and submerged vegetation common.	Mixture of soft sand, mud or clay; mud may be dominant; some root mats and submerged vegetation present.	All mud or clay or sand bottom; little or no root mat; no submerged vegetation.	Hard-pan clay or bedrock; no root mat or vegetation.
	20 19 18 17 16	**15 14 13 12 11**	**10 9 8 7 6**	**5 4 3 2 1**
3. Pool Variability	Even mix of large-shallow, large-deep, small-shallow, small-deep pools present.	Majority of pools large-deep; very few shallow.	Shallow pools much more prevalent than deep pools.	Majority of pools small-shallow or pools absent.
	20 19 18 17 16	**15 14 13 12 11**	**10 9 8 7 6**	**5 4 3 2 1**
4. Sediment Deposition	Little or no enlargement of islands or point bars and less than 20% of the bottom affected by sediment deposition	Some new increase in bar formation, mostly from gravel, sand or fine sediment; 20-50% of the bottom affected; slight deposition in pools.	Moderate deposition of new gravel, sand or fine sediment on old and new bars; 50-80% of the bottom affected; sediment deposits at obstructions, constrictions, and bends; moderate deposition of pools prevalent.	Heavy deposits of fine material, increased bar development; more than 80% of the bottom changing frequently; pools almost absent due to substantial sediment deposition.
	20 19 18 17 16	**15 14 13 12 11**	**10 9 8 7 6**	**5 4 3 2 1**
5. Channel Flow Status	Water reaches base of both lower banks and minimal amount of channel substrate is exposed.	Water fills >75% of the available channel; or <25% of channel substrate is exposed.	Water fills 25-75% of the available channel and/or riffle substrates are mostly exposed.	Very little water in channel and mostly present as standing pools.
	20 19 18 17 16	**15 14 13 12 11**	**10 9 8 7 6**	**5 4 3 2 1**

Parameter	Optimal	Suboptimal	Marginal	Poor
6. Channel Alteration	Channelization or dredging absent or minimal; stream with normal pattern.	Some channelization present, usually in areas of bridge abutments; evidence of past channelization, i.e., dredging, (greater than past 20 yr.) may be present, but recent channelization is not present.	Channelization may be extensive; embankments or shoring structures present on both banks; and 40 to 80% of stream reach channelized and disrupted.	Banks shored with gabion or cement; over 80% of the stream reach channelized and disrupted. Instream habitat greatly altered or removed entirely
	20 19 18 17 16	**15 14 13 12 11**	**10 9 8 7 6**	**5 4 3 2 1**
7. Condition of Banks	Banks stable; evidence of erosion or bank failure absent or minimal; little potential for future problems. <5% of bank affected.	Moderately stable; infrequent, small areas of erosion mostly sealed over. 5-30% of bank in reach has areas of erosion.	Moderately unstable; 30-60% of bank in reach has areas of erosion; high erosion potential during floods.	Unstable; many eroded areas; “raw” areas frequent along straight sections and bends; obvious bank sloughing; 60-100% of bank has erosional scars.
	20 19 18 17 16	**15 14 13 12 11**	**10 9 8 7 6**	**5 4 3 2 1**
8. Bank Vegetative Protection	More than 90% of the streambank surfaces and immediate riparian zone covered by native vegetation, including trees, understory shrubs, or nonwoody macrophytes; vegetative disruption through grazing or mowing minimal or not evident; almost all plants allowed to grow naturally.	70-90% of the streambank surfaces covered by native vegetation, but one class of plants is not well-represented; disruption evident but not affecting full plant growth potential to any great extent; more than one-half of the potential plant stubble height remaining.	50-70% of the streambank surfaces covered by vegetation; disruption obvious; patches of bare soil or closely cropped vegetation common; less than one-half of the potential plant stubble height remaining.	Less than 50% of the streambank surfaces covered by vegetation; disruption of streambank vegetation is very high; vegetation has been removed to 5 centimeters or less in stubble height.
_____	**20 19 18 17 16**	**15 14 13 12 11**	**10 9 8 7 6**	**5 4 3 2 1**
9. Riparian Vegetative Zone	Width or riparian zone >18 meters; human activities (i.e., parking lots, roadbeds, clear-cuts, lawns or crops) have not impacted zone.	Width of riparian zone 12-18 meters; human activities have impacted zone only minimally.	Width of riparian zone 6-12 meters; human activities have impacted zone a great deal.	Width of riparian zone <6 meters; little or no riparian vegetation due to human activities.
_____	**20 19 18 17 16**	**15 14 13 12 11**	**10 9 8 7 6**	**5 4 3 2 1**

C.3 PEBBLE COUNT DATA COLLECTION FORM

PEBBLE COUNT FORM

GIS Key: _____ **Survey Crew:** _____

Stream: _____ **County:** _____ **SWP:** _____

Mean Width: _____ **Sample Interval:** _____ **Reach Length:** _____

Station Description: _____

1		35		69		102		135		168	
2		36		70		103		136		169	
3		37		71		104		137		170	
4		38		72		105		138		171	
5		39		73		106		139		172	
6		40		74		107		140		173	
7		41		75		108		141		174	
8		42		76		109		142		175	
9		43		77		110		143		176	
10		44		78		111		144		177	
11		45		79		112		145		178	
12		46		80		113		146		179	
13		47		81		114		147		180	
14		48		82		115		148		181	
15		49		83		116		149		182	
16		50		84		117		150		183	
17		51		85		118		151		184	
18		52		86		119		152		185	
19		53		87		120		153		186	
20		54		88		121		154		187	
21		55		89		122		155		188	
22		56		90		123		156		189	
23		57		91		124		157		190	
24		58		92		125		158		191	
25		59		93		126		159		192	
26		60		94		127		160		193	
27		61		95		128		161		194	
28		62		96		129		162		195	
29		63		97		130		163		196	
30		64		98		131		164		197	
31		65		99		132		165		198	
32		66		100		133		166		199	
33		67		101		134		167		200	
34		68									

Comments: _____

C.4 ALTERNATIVE PEBBLE COUNT DATA COLLECTION FORM

ALTERNATIVE PEBBLE COUNT FIELD FORM

Station GIS Key:	**Station Description:**
Survey Crew:	
Reach Length (meters):	
Sample Interval (meters):	**Mean Steam Width (meters):**

Particle Description	Intermediate Axis of Particle (mm)	Substrate Type	Particle Count Tally	Particle Count Results		
				Total#	Item %	Cumulative %
Silt/Clay	<.062	Silt/Clay				
Very Fine	.062-.125	Sand				
Fine	>.125-.25					
Medium	>.25-.5					
Coarse	>.5-1.					
Very Coarse	>1-2					
Very Fine	>2-4	Gravel				
Fine	>4-6					
Fine	>6-8					
Medium	>8-11					
Medium	>11-16					
Coarse	>16-23					
Coarse	>23-32					
Very Coarse	>32-45					
Very Coarse	>45-64					
Small	>64-90	Cobble				
Small	>90-128					
Large	>128-180					
Large	>180-256					
Small	>256-362	Boulder				
Small	>362-512					
Medium	>512-1024					
Large-Very Large	>1024					
Bedrock		Bedrock				
Sample Size:		**Totals:**				

C.5 TRASH DATA COLLECTION PRELIMINARY EVALUATION FORM

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DEPARTMENT OF ENVIRONMENTAL PROTECTION
BUREAU OF CLEAN WATER

TRASH DATA COLLECTION PRELIMINARY EVALUATION FORM

Waterbody Name:	**GIS Key*:**
Location:	**Latitude, Longitude:**
Secondary ID:	**Avg Stream Wetted Width (m):**
County:	**Avg Stream Bankfull Width (m):**
Surveyors/Collectors:	
* Date as YYYYMMDD–time as military time–Username, which uniquely identifies the stream sample.	

Parameter	Optimal	Suboptimal	Marginal	Poor
1. General Impression *(After walking along the reach, impression of the reach area.*)	Effectively no or very little trash.	Streambed and banks are predominantly free of trash except for a few scattered areas.	Streambed and banks have more than a few areas with trash and about equal, or slightly more, areas that are free from trash.	Trash immediately distracts the eye. Streambed and banks predominantly contain trash.
_____	**12 11 10**	**9 8 7**	**6 5 4**	**3 2 1**
2. Littering (*Litter is trash that has been improperly discarded or inadvertently left lying in a public space.*)	After walking along the reach, little or no litter is evident (*including outside of the bankfull and reach area, but still within sight*).	After walking along the reach, small amounts of litter are evident (*including outside of the bankfull and reach area, but still within sight*).	There is sufficient evidence of litter within the reach, such as *scattered* cans, bottles, food wrappers, and/or plastic bags, etc..	Litter within the reach is immediately noticeable and significant, such as the presence of *many* cans, and/or bottles, food wrappers, and/or plastic bags, etc..
_____	**12 11 10**	**9 8 7**	**6 5 4**	**3 2 1**
3. Dumping (*Presence of illegally or otherwise inappropriately discarded trash in bulk, including one or more bags of trash, yard waste, one or more household items, etc.*)	Little or no evidence of dumping in or around the reach area (*even if outside of the bankfull extent, but still within sight*).	Some evidence of dumping in or around the reach area (*even if outside of the bankfull extent, but still within sight*).	There is sufficient evidence of dumping within the reach.	There is significant evidence of chronic dumping within the reach.
_____	**12 11 10**	**9 8 7**	**6 5 4**	**3 2 1**
4. Downstream Transport (*Trash originated from upstream and was carried to the reach area by the flow. Evidence may include silt marks on the trash, faded colors, or that trash was that was clearly deposited at the high-water mark.*)	Trash (if any) appears to have been deposited at the location and not transported from upstream areas.	Some evidence that trash (*<10 pieces*) appears to have been transported to the location from upstream areas.	Sufficient evidence that trash (*10 to 50 pieces*) was transported to the location from upstream areas.	Significant evidence that trash (*>50 pieces*) was transported to the location from upstream areas. Trash may have accumulated in substantial quantities or may be in various states of degradation based on the ability for it to persist in the environment.
_____	**12 11 10**	**9 8 7**	**6 5 4**	**3 2 1**

TOTAL SCORE: _____

1. Land use within the reach: Agriculture Forest Urban Suburban Rural
   Wetland Grassland/Scrub/Shrub/Pasture

2. Atmospheric conditions:
   a. Weather the past 24 hours? Sunny Partly Cloudy Overcast Rain Other_____

   b. Rain the past 24 hours (inches)? None (*0"*) Trace Rain (*0.1" - 0.15"*) Light Rain (*0.15" - 0.25"*)
   Moderate Rain (*0.25" - 0.5"*) Heavy Rain (≥ *0.5"*)

   c. Weather at present? Sunny Partly Cloudy Overcast Rain Other_____

   d. Rain at present (inches)? None (*0"*) Trace Rain (*0.1" - 0.15"*) Light Rain (*0.15" - 0.25"*)
   Moderate Rain (*0.25" - 0.5"*) Heavy Rain (≥ *0.5"*)

3. Water visibility: Optimal (*Normal/Clear*) Sub-optimal (*Slightly Discolored/Dirty/Stained*)
   Marginal (*Mildly Discolored/Dirty/Stained*) Sub-marginal (*Moderately Discolored/Dirty/Stained*)
   Poor (*Heavily Discolored/Muddy*)

4. Do people use this site for passive recreation (*e.g., sitting, reading, nature viewing, etc.*)?
   Y or N or Unknown

5. Do people use this site for active recreation (*i.e., boating, fishing, other water contact sports*)?
   Y or N or Unknown

6. Do combined sewer outfalls or stormwater outfalls exist in the reach or upstream?
   Y or N or Unknown

7. Are there "strainers" (*e.g., tree branches or other areas that collect trash*) in the stream reach?
   Y or N or Unknown

8. Were pictures of trash taken and, if so, by whom (*photo documentation of trash in streams and/or banks is helpful but not required*)?

9. **Additional Comments** (*May include suspected sources of specific items. For example: if items such as bricks, concrete, etc. are present, do they appear to be remnants of bank stabilization/channelization or a product of illegal dumping? Does some trash appear to have originated from a specific facility nearby, and does it appear that the trash is only from that facility or are there other obvious sources?*):

C.6 TRASH DATA COLLECTION COMPREHENSIVE FORM

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COMMONWEALTH OF PENNSYLVANIA
DEPARTMENT OF ENVIRONMENTAL PROTECTION
BUREAU OF CLEAN WATER

TRASH DATA COLLECTION COMPREHENSIVE FORM

Waterbody Name:	**GIS Key*:**
Location:	**Latitude, Longitude:**
Secondary ID:	**Avg Stream Wetted Width (m):**
County:	**Avg Stream Bankfull Width (m):**
Surveyors/Collectors:	
* Date as YYYYMMDD–time as military time–Username, which uniquely identifies the stream sample.	

TRASH SURVEY		
Counting Procedure Total Count	Total Count of Trash: _____ Binning (Type(s) & Categories):	Comments:
Picking Procedure Subsample (SS) 1 (Downstream) Description	*Circle SS Type:* Strainer/Snag Deposition Binning (Type(s) & Categories):	Comments:
Picking Procedure Subsample (SS) 2 (Upstream) Description	*Circle SS Type:* Strainer/Snag Deposition Binning (Type(s) & Categories):	Comments:

GIS Key or Stream Name:_____

PICKING PROCEDURE TALLY SHEET

CATEGORY	TYPE	TALLY SS 1	SUB-TOTAL SS 1	TALLY SS 2	SUB-TOTAL SS 2
Plastic	Bag – Grocery/takeout				
Plastic	Bag –Trash				
Plastic	Bag – Zip-top				
Plastic	Bag – Other				
Plastic	Bag Pieces				
Plastic	Balloons				
Plastic	Beverage bottles/caps/labels				
Plastic	Beverage containers/cups/lids – plastic/paper				
Plastic	Bottles, Other				
Plastic	Food wrappers//bags				
Plastic	Food containers/lids – plastic/paper				
Plastic	Food/beverage containers/lids – polystyrene				
Plastic	Lighters				
Plastic	Miscellaneous hard plastic/pieces				
Plastic	Miscellaneous soft plastic/pieces				
Plastic	Packaging materials (polystyrene)				
Plastic	Packaging materials (non-polystyrene)				
Plastic	Pens/markers/office supplies				
Plastic	Straws				
Plastic	Tarps/plastic sheeting				
Plastic	Plastic, Other (Describe)				
Tobacco/E-cig	Cigarettes/butts				
Tobacco/E-cig	Cigar Tips				
Tobacco/E-cig	Tobacco packaging				
Tobacco/E-cig	E-cigarette parts/packaging				
Glass	Glass Bottles				
Glass	Glass Pieces				
Glass	Glass, Other (Describe)				
Metal	Aluminum/steel Cans				
Metal	Metal Pipes/rebar				
Metal	Wire (Electrical)				
Metal	Wire (Barb, Chicken, etc.)				

GIS Key or Stream Name:_____

CATEGORY	TYPE	TALLY SS 1	SUB-TOTAL SS 1	TALLY SS 2	SUB-TOTAL SS 2
Metal	Metal, Other (Describe)				
Fabric/Cloth	Natural Fabric				
Fabric/Cloth	Shoes				
Fabric/Cloth	Synthetic Fabric-clothing				
Fabric/Cloth	Synthetic Fabric-other				
Construction	Bricks				
Construction	Concrete/asphalt Pieces				
Construction	Concrete Blocks				
Construction	Lumber				
Construction	Construction, Other (Describe)				
Biodegradable	Yard Waste				
Biodegradable	Paper/Cardboard				
Biodegradable	Biodegradable, Other (Describe)				
Biohazard	Diapers/human waste				
Biohazard	Medical Waste				
Biohazard	Face Masks				
Biohazard	Biohazard, Other (Describe)				
Other	Appliances/electronics				
Other	Automotive				
Other	Batteries				
Other	Ceramic dishes/pieces				
Other	Fishing Gear				
Other	Furniture				
Other	Personal Care Products				
Other	Shopping carts				
Other	Sporting goods				
Other	Tires				
Other	Toys				
Other	Miscellaneous (Describe)				
TOTALS		**TOTAL SS 1:**		**TOTAL SS 2:**	

Items may be binned if abundance if greater than 10 pieces as follows: M (Medium): 11 – 50 pieces H (High): 50 – 100 pieces VH (Very High): >100 pieces

ITEM DESCRIPTIONS

CATEGORY	TYPE	DESCRIPTION (IF NECESSARY)		EXAMPLES (IF NECESSARY)
Plastic	Bag – Grocery/Takeout	Disposable shopping or restaurant bags		Grocery, fast food
Plastic	Bag – Trash			
Plastic	Bag – Zip-top	Disposable storage bags, such as "Ziploc" brand		Sandwich, freezer bags
Plastic	Bag, Other	Plastic bag types that do not fall in above categories		Road salt bags
Plastic	Bag Pieces	Fragments of bags; can be binned into abundance categories		
Plastic	Balloons	Rubberized, plastic, or part-foil balloons		Party store
Plastic	Beverage bottles/caps/labels	Disposable drink bottles and/or caps/labels		Water, soda, juice
Plastic	Beverage container/cups/lids-plastic/paper	Disposable plastic drink cups from restaurants, gas stations, etc.(non-polystyrene)		
Plastic	Bottles, Other	Plastic bottle types that do not fall in above categories		Washing detergent
Plastic	Food wrappers/bags	Disposable foil-lined or non-foil lined food wrappers or bags		Chip bags, candy wrappers
Plastic	Food containers/lids-plastic/paper	Disposable plastic food containers from restaurants, gas stations, etc. (non-polystyrene)		
Plastic	Food/beverage containers/lids-polystyrene	Disposable polystyrene ("Styrofoam") food beverage containers		
Plastic	Lighters	Pocket lighters for cigarettes, etc.		
Plastic	Miscellaneous hard plastic/pieces	Unidentifiable hard plastic pieces/chunks		Broken toys parts, electronic parts
Plastic	Miscellaneous soft plastic/pieces	Unidentifiable soft plastic pieces/chunks		Any soft plastic piece broken/ripped, non-polystyrene
Plastic	Packaging materials (polystyrene)	Any item used to mail/ship packages/envelopes composed of polystyrene.		Styrofoam/polystyrene
Plastic	Packaging materials (non-polystyrene)	Any item used to mail/ship packages/envelopes composed of materials other than polystyrene.	Plastic-lined sleeves, packing tape	
Plastic	Pens/markers/ office supplies	Any office or home office equipment		Pens, pencils, markers, scissors, rulers, tape dispensers, staplers
Plastic	Straws	Disposable plastic straws		
Plastic	Tarps/plastic sheeting	Any large material clearly made of plastic for use as painters' sheeting, tarps, tablecloths, etc.		
Plastic	Plastic, Other (Describe)	Any item clearly made of any type of plastic not falling into remaining categories		
Tobacco/E-Cig	Cigarettes/butts			
Tobacco/E-Cig	Cigar tips			
Tobacco/E-Cig	Tobacco packaging			
Tobacco/E-Cig	E-cigarette parts/packaging			
Glass	Glass bottles	Glass beverage/storage bottles		Soda bottles, beer bottles, Mason jars, spice jars
Glass	Glass pieces	Unidentifiable pieces of glass; can be binned into abundance categories		
Glass	Glass, Other (Describe)	Any item clearly made of any type of glass not falling into remaining categories		
Metal	Aluminum/steel cans	Disposable beverage containers		Soda/beer cans
Metal	Metals Pipes/rebar			

Appendix C Physical Data Collection Information and Forms

CATEGORY	TYPE	DESCRIPTION (IF NECESSARY)	EXAMPLES (IF NECESSARY)
Metal	Wire (Electrical)	Any electrical wire, including those with coverings	
Metal	Wire (Barb, Chicken, etc.)	Any wire other than electrical wires	
Metal	Metal, Other (Describe)	Any item clearly made of any type of metal not falling into remaining categories	
Fabric/Cloth	Natural Fabric	Fabric made from natural materials	Cotton
Fabric/Cloth	Shoes		
Fabric/Cloth	Synthetic Fabric-clothing	Clothing made from synthetic materials	Nylon, polyester
Fabric/Cloth	Synthetic Fabric-other	Any fabric other than clothing made from synthetic materials	Nylon, polyester
Construction	Bricks		
Construction	Concrete/asphalt pieces	Concrete or asphalt chunks	
Construction	Concrete Blocks	Whole or partial concrete block	
Construction	Lumber	Processed wood, generally cut into shapes	Plywood, fencing, boards
Construction	Construction, Other (Describe)	Any item used in construction not falling into remaining categories	
Biodegradable	Yard Waste	Waste from yardwork, generally dumped	Grass clippings, bush cuttings
Biodegradable	Paper/Cardboard	Any item made exclusively from degradable paper materials	Cardboard boxes, tablets, paper towels
Biodegradable	Biodegradable, Other (Describe)	Any biodegradable item not falling into remaining categories	
Biohazard	Diapers/human waste		
Biohazard	Medical Waste	Medical supplies/equipment	Needles, diabetic supplies, medicine/drugs
Biohazard	Face Masks	Disposable face masks, normally thin cloth/paper	
Biohazard	Biohazard, Other (Describe)	Any biohazard item not falling into remaining categories	
Other	Appliances/electronics		
Other	Automotive		
Other	Batteries		Alkaline batteries, lithium batteries, car batteries
Other	Ceramic dishes/pieces	Whole or partial ceramic/pottery; pieces can be binned into abundance categories	
Other	Fishing Gear		Hooks, lines, lures, sinkers
Other	Furniture		
Other	Personal Care Products		Makeup, hair supplies, soaps
Other	Shopping Carts		
Other	Sporting Goods		
Other	Tires		
Other	Toys		
Other	Miscellaneous (Describe)	Any item not falling into remaining categories	

APPENDIX D: PENNSYLVANIA'S SURFACE WATER QUALITY MONITORING NETWORK (WQN) MANUAL

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PENNSYLVANIA'S SURFACE WATER QUALITY MONITORING NETWORK (WQN) MANUAL

2023

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OFFICE OF WATER PROGRAMS

BUREAU OF CLEAN WATER

PENNSYLVANIA'S SURFACE WATER QUALITY MONITORING NETWORK (WQN) MANUAL

2023

Appendix D Pennsylvania's Surface Water Quality Monitoring Network (WQN) Manual

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INTRODUCTION

The Pennsylvania Water Quality Network (WQN) is a statewide, fixed-station water quality sampling system operated by the Pennsylvania Department of Environmental Protection's (DEP) Bureau of Clean Water (BCW). It is designed to track both the quality of Pennsylvania's surface waters and the effectiveness of the water quality management program by accomplishing four basic objectives:

- Monitor temporal water quality trends in major surface streams throughout Pennsylvania.
- Monitor temporal water quality trends in selected reference waters.
- Monitor the trends of nutrient and sediment loads in the major tributaries entering the Chesapeake Bay.
- Monitor temporal water quality trends in selected Pennsylvania lakes.

"Major" streams, for the purposes of this document, are interstate and intrastate waters with drainage areas of roughly 200 square miles or greater. These waters receive both point and non-point source pollutants and are sampled at or near their mouths to measure overall quality before flows enter the next higher order stream or before exiting the state. In this way, trends can be established, and the effectiveness of water quality management programs can be assessed by watershed. For more detailed information on the objectives of the WQN, see Subappendix D.5.

The monitoring of the WQN is conducted collaboratively between DEP, USGS Pennsylvania Water Science Centers, and Susquehanna River Basin Commission (SRBC).

WQN resources can be found on DEP's WQN webpage: www.dep.pa.gov, keywords: Water Quality Network.

STATION TYPES

The WQN consists of 178 active stations grouped into three primary station types: standard, Chesapeake Bay nutrient loading, and reference.

The 119 standard stations are generally sampled every other month for stream discharge measurements and chemical analysis, and every other year for a biological and physical evaluation. Of these, 28 are sampled monthly for chemical analysis and 17 are considered low alkalinity stations which are analyzed for a few additional parameters. See Subappendix D.1 for the list of parameters collected at low alkalinity stations.

The 38 Chesapeake Bay Nutrient Loading (Chesapeake Bay) stations are sampled monthly for stream discharge measurements and chemical analysis, and once a year for biological and physical evaluation. These stations are also part of the Chesapeake Bay Program nontidal monitoring network (CBP NTN) which requires eight additional water chemistry samples to be collected annually during

storm events. More information on the CBP NTN can be found at: CBP_nontidal_water_quality_monitoring.

The 21 reference stations are sampled monthly for stream discharge measurements and chemical analysis and typically once a year (spring or fall) for biological and physical evaluation. For method development purposes, some reference stations are sampled twice a year, once in the spring and once in the fall. Reference stations are selected to represent minimally disturbed waters within Pennsylvania's various bioregions. Bioregions are areas with similar water quality characteristics because of like features such as topography, land use, soils, and potential natural vegetation. Although it may be argued that Pennsylvania has no unaffected waters (by atmospheric deposition), reference stations reasonably describe water quality that could be achieved in the absence of point source discharges and in the presence of minimal or typical non-point source impacts. Thus, these stations can provide, in the absence of site-specific data, a benchmark for use in the water quality management permitting program as well as a measure of changing water quality in areas of the state not directly impacted by development.

WQN lake stations (excludes Lake Erie and Presque Isle Bay which are standard WQN stations) are selected after consideration of size, public access, intensity of use, and availability of existing data. Large lakes with heavy public use and/or historical data are favored for inclusion in this program because changing trends in the water quality of these resources have the potential for serious impacts on water uses. Approximately 90 lakes are included in this network, however, sampling occurs in groups of 15-20 lakes on a rotation. These lake groups are sampled once a year for five consecutive years before initiating a new group. The five-year data sets are then used to assess lake water quality trends. Monitoring at the current group of 12 lakes started in 2021 and will continue through 2025.

Station specific details are included in the Active, Macroinvertebrate, and Discontinued Stations tables found in Subappendices D.2-D.4. A web mapping application is also available to view each station at WQN_web_application.

UPDATES TO THE WQN

This revision of the WQN manual covers changes that occurred from October 2020 through October 2024. All information in this edition is reflective of the WQN's status as of November 1st, 2024. Any changes after this date will be reflected in the next edition.

Fourteen reference stations were monitored for atmospheric deposition between September 2020 and December 2023 using the new Standard Analysis Code (SAC) 122. This SAC included the SAC 015 tests plus analyses for dissolved aluminum (0.1-micron filter) and Total Carbon Dioxide. This additional monitoring has since concluded, and SAC 122 is no longer being used. Reference WQNs 514 and 870 were discontinued in May 2021 to allow for new stations in smaller watersheds. Reference station WQN0293 - Cross Keys Run was discontinued in March 2022 after 17 months

because data analysis showed the station wasn't reference quality. Reference station 287 – Diehls Hollow was changed to a Standard station and will be sampled every other month due to elevated nutrients and sediment indicating non-reference quality. The water chemistry sampling at WQN 201 will now be conducted by the U.S. Geological Survey (USGS) instead of the Susquehanna River Basin Commission (SRBC). In exchange, SRBC took over the water chemistry sampling at WQNs 217 and 224. Water chemistry sampling at WQN 182 also transferred from DEP to USGS. Two new Chesapeake Bay stations, WQN0295 and WQN0298, were created in January 2024 to provide data in small agricultural watersheds. In Oct 2022, 1-4-Dioxane sampling was added to WQN0116 - Perkiomen Creek and WQN0182 - Delaware River at Marcus Hook. Beginning in November 2024, WQN0206 will use SAC 012 instead of SAC 010. All stations using suite 048 (RAD99) for gross alpha and gross beta are now using the updated suite 245 (GABWT). Starting in September 2024, Reference stations are using SAC B052 instead of B021. Numerous laboratory test code changes have occurred throughout the SACs utilized by the WQN to maintain low reporting limits.

The tables and subappendices in this update reflect these changes.

WATER CHEMISTRY SAMPLING

Water chemistry samples are collected at all active WQN stations based on the frequency assigned in the Active Stations table (Subappendix D.2). Reference and Chesapeake Bay stations are sampled once every month. The majority of standard stations are sampled once every other month and lakes are sampled once annually. Sampling for a station, no matter the station type, should be on a fixed scheduled. This allows for the potential of a wide range of sampling conditions.

At least one SAC is assigned to each WQN station depending on the station type. A SAC contains a list of analytes and is used to identify which water chemistry tests should be performed on a sample. A list of the SACs and the station type associated with it can be found in Subappendix D.1. Selected water chemistry tests may be added to any of the WQN stations on a case-by-case basis when the need for additional data is determined. However, the use of additional analyses for extended periods of time is discouraged in favor of creating a new SAC tailored to the needs of the collector. All such changes are coordinated with the DEP Bureau of Laboratories (BOL) through the Bureau of Clean Water's Water Quality Division (WQD).

Chemical Sampling Procedures

Multiple agencies and numerous field staff are part of the WQN sampling effort. It is important that all involved are using the same data collection protocols to ensure reliable and comparable data. Any questions regarding sampling protocols should be directed to the DEP WQD, 717.787.9637.

All WQN collectors must obtain a four-digit collector identification number assigned by BOL. The Collector ID Request Form (ID Request Form) should be filled out and submitted via email to the WQD.

The "DEP Laboratory Submission Sheet" (Submission Sheet) must be filled out and submitted to the BOL. The information required on this form varies between station types. Field staff are responsible for working with the WQD and BOL to ensure the form is filled out correctly.

Surface water samples are collected as isokinetic, depth integrated, single mid-channel or equal-width samples. Samples are normally collected in High-Density Polyethylene (HDPE) or amber glass (for organic compound analysis) sample bottles. A sample volume and field preservation chart are available for reference in Subappendix D.6. Bottles must be labeled with the WQN number, collector ID number, sequence number, date, and time. If space allows, adding the SAC, general test descriptions (total metals, TOC, etc.), filtered, and preservation type can help minimize any questions concerning the sample analyses. Depending on the station, samples may be collected from a bridge, boat, or by wading.

Detailed descriptions on how to collect surface water samples can be found in Chapter 4 of DEP's *Water Quality Monitoring Protocols for Surface Waters* (the Monitoring Book, Lookenbill and Arnold 2023) and in Chapters 4 and 5 of the USGS's National Field Manual for the Collection of Water Quality Data (USGS 2006, Wilde 2004).

Standard Stations

A standard station is typically assigned SAC 010 or 610 and can be located anywhere in Pennsylvania. SACs 016 and 616 are used if the watershed experiences low alkalinity. The 610 and 616 SACs are used for stations in geographic areas that could be underlain by Marcellus shale; these SACs contain a few additional analytes typically associated with Marcellus formation water. Reason Code 01 should be used on the Sample Submission Sheet for all standard stations.

Reference Stations

These stations are assigned SAC 015 which contains the analyte dissolved organic carbon (DOC). The analytical method for DOC requires that a blank sample is associated with every DOC sample. To satisfy this requirement, a blank DOC sample is collected with the first SAC 015 sample in a sampling group. A sampling group can contain the samples collected in the same day, week, project, etc. but should not be more than 20 samples. The collector ID and sequence number for that blank should be recorded on all the Sample Submission Sheets for that sampling group and specify that it is the blank to be associated with these DOC samples. A new blank DOC sample should be collected with each sampling group. Reference stations are additionally sampled for fecal coliforms and *E. coli*; a sterilized 125 mL bacteriological bottle pretreated with sodium thiosulfate (neutralizes the effects of residual chlorine) is used. A separate Sample Submission Sheet should be filled out for this analysis and SAC B052 is used. Reason Code 02 should be used on all reference station Sample Submission Sheets.

Chesapeake Bay Nutrient Loading Stations

This station type is assigned either SAC 012 or 612 and all sites are within the Chesapeake Bay watershed. SAC 612 stations are in geographic areas that could be underlain by Marcellus shale; this

SAC contains a few additional analytes typically associated with Marcellus formation water. Reason Code 01 is used on the Sample Submission Sheet for the monthly, routine sample collected at these Chesapeake Bay stations.

Storm Sampling

Since these stations are also part of the Chesapeake Bay Program's non-tidal network (CBP NTN), in addition to the monthly routine sample, eight additional storm samples are collected throughout the sampling year. A storm event is characterized with a rising discharge (cfs) of at least twice that of the pre-storm average daily discharge. The assigned SAC, field parameters, and a suspended sediment sample is collected. These samples are identified with Reason Code 014 on the datasheet. Ideally, one suspended sediment concentration (SSC) and one SSC with sand and fine particle analyses should be collected per quarter. If a storm event occurs on the scheduled routine sampling day, the sample is identified as "routine, storm-impacted" and should be assigned Reason Code 015 on the datasheet. If feasible, an SSC sample should be collected also. These "routine, storm-impacted" samples are counted as the routine monthly sample and not as one of the eight storm samples. All suspended sediment samples are shipped to the USGS Ohio-Kentucky-Indiana Water Science Center Sediment Laboratory in Louisville, KY for analysis.

Storm sampling for Chesapeake Bay Nutrient Loading stations is the only targeted sampling in the WQN. More detailed information on the sampling requirements for storm samples can be found in the CBP's Non-Tidal Water Quality Monitoring document (CBP 2008).

<u>Lake Stations</u>

Lake stations are assigned SAC 083. Lakes are additionally sampled for chlorophyll-a under SAC B029. Lake Erie and Presque Isle Bay bimonthly samples are collected at mid-depth. For all other lakes, two samples are collected from one site during mid-summer stratification conditions. These sites correspond to the deepest point in each lake with one sample collected a meter below the surface (top sample) and the second sample from one meter from the bottom (bottom sample). A water quality profile, to include temperature, dissolved oxygen, pH, and specific conductance, is recorded at this site through the vertical water column and an aliquot from the top sample is filtered for chlorophyll-a analysis. Chlorophyll-a sampling is conducted according to the Chlorophyll-a protocol found in Chapter 3 of the Monitoring Book (Lookenbill and Arnold 2023). Lake sampling protocols can be found in Chapters 2 through 4 of the Monitoring Book (Lookenbill and Arnold 2023). It is recommended that at least one other site should be collected near the lake inlet. This site will follow the same procedures as the deepest point site.

Other Chemical Sampling

Due to the sampling design of the Water Quality Network, WQN stations are often utilized to gather data for specific projects.

<u>Pesticide Sampling</u>

Pesticide grab samples are currently not being collected.

POCIS and SPMD Sampling

The WQN is being utilized to monitor hormones and other endocrine-disrupting compounds (EDCs). Polar Organic Chemical Integrative Samplers (POCIS) and Semi-Permeable Membrane Devices (SPMDs) are types of passive sampling devices being deployed at several WQN stations to collect water quality samples of low-level environmental contaminants. POCIS and SPMD sampling will follow the procedures outlined in the Passive Water Chemistry Data Collection Protocol in Chapter 4 for the Monitoring Book (Lookenbill and Arnold 2023). Analysis of the samples will be performed at SGS AXYS, NWQL, and the USGS Eastern Ecological Science Center in Kearneyville, WV. Approximately forty estrogenicity grab samples will also be collected and sent to the USGS Eastern Ecological Science Center for analysis.

Per- and Polyfluoroalkyl Substances (PFAS) Sampling

PFAS are anthropogenic substances commonly saturated with fluorine. Sampling for PFAS will occur monthly at several WQN stations. Samples will be depth and width integrated and composited in a HDPE churn splitter. Samples will be submitted to the DEP laboratory or SGS AXYS for analysis.

Wastewater Sampling

Approximately forty wastewater grab samples will be collected between March and September. Samples will be unfiltered and composited into two 1-liter glass amber bottles. Analysis of the wastewater indicators will be performed at the USGS National Water Quality Laboratory (NWQL) in Denver, CO.

BIOLOGICAL SAMPLING

All biological sampling is conducted using the protocols outlined in Chapter 3 of the Monitoring Book (Lookenbill and Arnold 2023). At a minimum, benthic macroinvertebrate data are collected at standard stations every other year between August 1st and September 30th. Standard stations that are wadeable in the non-summer months will be sampled between November 1st and April 30th. Macroinvertebrates are collected annually at Chesapeake Bay stations between November 1st and April 30th. Reference stations either rotate annually between a spring (March 1st to April 30th) and fall (Nov. 1st to Dec. 31st) annual collection or are sampled twice per year (once in spring and once in fall).

Data collection per the wadeable riffle-run protocol is appropriate at most stations, however, WQN stations can be sampled using any of the protocols outlined in Chapter 3 of the Monitoring Book (Lookenbill and Arnold 2023) if appropriate. The appropriate data collection protocols for each WQN is listed in Subappendix D.3.

A physical habitat evaluation is conducted with every macroinvertebrate data collection effort. The procedures in the *Stream Habitat Data Collection Protocol* within Chapter 5 of the Monitoring Book (Lookenbill and Arnold 2023) should be used. The majority of WQN stations will use the riffle/run

habitat parameters, however, a few stations will use the multihabitat parameters if they are determined to be low gradient. Please contact the WQD if clarification is needed. Habitat field data sheets are available in Appendix C in the Monitoring Book (Lookenbill and Arnold 2023).

Qualitative plankton samples are collected annually at Lake Erie stations. Quantitative invertebrate or plankton sampling and quantitative or qualitative fish sampling is optional at any station and may be conducted at the discretion of the collector. Plankton data collection protocols are detailed in Chapter 3 of the Monitoring Book as well as the fish sampling protocols (Lookenbill and Arnold 2023).

Fish tissue is sampled periodically at the rate of approximately 100 stations per year following the protocols outlined in Chapter 3 of the Monitoring Book (Lookenbill and Arnold 2023). Tissue sampling locations are determined annually during the first quarter of the calendar year preceding sample analysis and rotated throughout the WQN to provide periodic but statewide coverage and to maintain surveillance on problem waters. Fish tissue (fillets) is analyzed for appropriate priority pollutants to assess its suitability for human consumption.

FLOW MEASUREMENT

Stream discharge (flow volume) is measured or calculated each time a water chemistry sample is collected. USGS stream gaging facilities and/or extrapolation equations are utilized whenever possible. Where no USGS facilities/equations exist, stream discharge is measured using U.S. Army Corps of Engineers and private gaging facilities or calculated according to procedures outlined by USGS (Turnipseed and Sauer 2010). Lake levels for Lake Erie and Presque Isle Bay stations are measured at the U.S. Coast Guard station at the entrance to Erie Harbor.

Flow Measurement Procedures

The Pennsylvania WQN utilizes several types of stage gages to provide stream discharge. The two main types are the wire-weight (WW) gage and the electric tape (ET) gage. Both are non-recording gages that yield readings indirectly via measurement from a fixed point to the water surface. Other gages that may be used include a staff gage, analog-to-digital recorder (ADR) dial, strip chart, hand tape, crest stage gage, lock chamber or telemark (TMK). Descriptions on the various flow measurement procedures are in Subappendix D.7.

The Water Resources Division of the USGS recommends that an outside gage such as a staff gage or a WW gage be used by DEP personnel. If no outside gage is present or it is unreadable, USGS prefers a reading from the ADR dial or the ET gage, in that order. The base gages for the network stations are primarily ET gages or WW gages. If neither of these types are present, a Staff gage may function as the base gage for that site.

DATA AVAILABITY AND MANAGEMENT

All chemical data analyzed by DEP BOL are entered by BOL staff into DEP's internal Oracle

database. The collector or DEP WQD staff are responsible for entering the field chemistry, station information, comments, and USGS OH-KY-IN Sediment Lab results into the Oracle database. The data are uploaded quarterly by DEP via the Water Quality Exchange (WQX) to the United States Environmental Protection Agency's (EPA) database. These data are made available to the public on the Water Quality Portal (https://www.waterqualitydata.us). Macroinvertebrate samples are processed, and the taxa identified and enumerated by a contractor or by DEP staff. The macroinvertebrate data are entered into the WQD's internal Streams and Lakes Integrated Management System (SLIMS) database. These data are also periodically uploaded to the Water Quality Portal. Analyses of trends at selected WQN stations are published in DEP's Biennial Integrated Water Quality Monitoring and Assessment Report.

QUALITY ASSURANCE

A quality assurance project plan has been developed to describe the procedures used to implement the WQN in both the field and laboratory. Copies of this plan can be obtained from the Bureau of Clean Water's WQD or EPA Region III. One sample "blank" is submitted bimonthly by each field office involved in sample collection and duplicate or "split samples" are collected at the rate of one in every 20 routine WQN samples. Qualified WQD staff routinely audit WQN field staff to ensure sampling consistency between staff and to evaluate the utility of each station. Additional quality assurance for specific data collection protocols can be found throughout the Monitoring Book (Lookenbill and Arnold 2023).

LITERATURE CITED

CPB. 2008. Non-tidal water quality monitoring. Chapter V *in* Non-Tidal Water Quality Field Procedures. Chesapeake Bay Program, Annapolis, Maryland.

Lookenbill, M. J. and E. Arnold (editors). 2023. Water quality monitoring protocols for surface waters. Pennsylvania Department of Environmental Protection, Harrisburg, Pennsylvania.

Turnipseed, D. P. and V. B. Sauer. 2010. Discharge measurements at gaging stations: United States Geologic Survey. Book 3, Chapter A8 *in* National field manual for the collection of water quality data: Techniques and methods. U.S. Department of the Interior, U.S. Geological Survey, Reston, Virginia. (Available online at: https://pubs.usgs.gov/twri/twri3a8/.)

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USGS. 2006. Collection of water samples. Book 9, Chapter A4 (version 2.0) *in* National field manual for the collection of water quality data: Techniques and methods. U.S. Department of the Interior, U.S. Geological Survey, Reston, Virginia. Available online at: https://pubs.er.usgs.gov/publication/twri09A4.

D.1 STANDARD ANALYSIS CODES (SAC)

WQN PARAMETER LISTS

1. ALL STATIONS (standard field analyses)

pH Temperature Dissolved Oxygen Specific Conductance

**** % Dissolved Oxygen and Turbidity should also be reported, if collected, using data collection protocols in Lookenbill and Arnold (2023).

2. STANDARD STATIONS (SAC 010)

Test Code	Test Description	Test Code	Test Description
00410	Alkalinity (Alk)	01105H	Aluminum, Total (Al)
00610A	Ammonia Nitrogen (NH3 N)	99020	Bromide (Br)
00916A	Calcium, Total (Ca)	00940	Chloride (Cl)
01042H	Copper, Total (Cu)	00900	Hardness, Total (Hard)
01045A	Iron, Total (Fe)	01051H	Lead, Total (Pb)
00927A	Magnesium, Total (Mg)	01055A	Manganese, Total (Mn)
01067A	Nickel, Total (Ni)	00620	Nitrate Nitrogen (NO3 N)
00615	Nitrite Nitrogen (NO2 N)	00600A	Nitrogen, Total (N)
00403	pH, Lab (PH)	00665A	Phosphorus, Total (P)
70507A	Phosphorus Orthophosphate, Total P(O)	00937A	Potassium, Total (K)
00929A	Sodium, Total (Na)	00095	Specific Conductivity (SPC)
00945	Sulfate, Total (SO4)	70300U	Total Dissolved Solids @ 180C (TDS)
00530V	Total Suspended Solids (TSS)	01092A	Zinc, Total (Zn)

3. CHESAPEAKE BAY NUTRIENT LOADING STATIONS (SAC 012)

Test Code	Test Description	Test Code	Test Description
00410	Alkalinity (Alk)	01105A	Aluminum, Total (Al)
00608A	Ammonia Nitrogen, Dissolved (NH3 N)	00610A	Ammonia Nitrogen, Total (NH3 N)
99020	Bromide (Br)	00916A	Calcium, Total (Ca)
00940	Chloride (Cl)	01042H	Copper, Total (Cu)
00900	Hardness, Total (Hard)	01045A	Iron, Total (Fe)
01051H	Lead, Total (Pb)	00927A	Magnesium, Total (Mg)
01055A	Manganese, Total (Mn)	01067A	Nickel, Total (Ni)
00631A	Nitrate & Nitrite, Dissolved (NO3 + NO2)	00630A	Nitrate & Nitrite, Total (NO3 + NO2)
00602A	Nitrogen, Dissolved (N)	00600A	Nitrogen, Total (N)
00680	Organic Carbon, Total	00403	pH, Lab (PH)
00671A	Phosphorus Orthophosphate, Dissolved P(O)	70507A	Phosphorus Orthophosphate, Total P(O)
00666A	Phosphorus, Dissolved (P)	00665A	Phosphorus, Total (P)
00937A	Potassium, Total (K)	00929A	Sodium, Total (Na)
00095	Specific Conductivity (SPC)	00945	Sulfate, Total (SO4)
70300U	Total Dissolved Solids @ 180C (TDS)	00530V	Total Suspended Solids (TSS)

01092A	Zinc, Total (Zn)

4. REFERENCE STATIONS (SAC 015 & B052*)

Test Code	Test Description	Test Code	Test Description
70508	Acidity, Total	00410	Alkalinity (Alk)
01106H	Aluminum, Dissolved (Al)	01105H	Aluminum, Total (Al)
00608A	Ammonia Nitrogen, Dissolved (NH3 N)	00610A	Ammonia Nitrogen, Total (NH3 N)
01007H	Barium, Total (Ba)	00314	Carbonaceous Biochemical Oxygen Demand (CBOD5)
01022K	Boron, Total (B)	99020	Bromide (Br)
01025H	Cadmium, Dissolved (Cd)	00915A	Calcium, Dissolved (Ca)
00916A	Calcium, Total (Ca)	00940	Chloride (Cl)
01040H	Copper, Dissolved (Cu)	01042H	Copper, Total (Cu)
MMTECMF	E. coli*	31616	Fecal Coliform* (FC)
00900	Hardness, Total (Hard)	01046A	Iron, Dissolved (Fe)
01045A	Iron, Total (Fe)	01130A	Lithium, Dissolved (Li)
01132A	Lithium, Total (Li)	01049H	Lead, Dissolved (Pb)
01051H	Lead, Total (Pb)	00925A	Magnesium, Dissolved (Mg)
00927A	Magnesium, Total (Mg)	01056H	Manganese, Dissolved (Mn)
01055H	Manganese, Total (Mn)	01065H	Nickel, Dissolved (Ni)
01067H	Nickel, Total (Ni)	00631A	Nitrate + Nitrite N, Dissolved
00630A	Nitrate + Nitrite N, Total	00600A	Nitrogen, Total (N)
00681	Organic Carbon, Dissolved	00680	Organic Carbon, Total
82550	Osmotic Pressure (OP)	00403	pH, Lab (PH)
00666A	Phosphorus, Dissolved (P)	00665A	Phosphorus, Total (P)
00671A	Phosphorus Orthophosphate, Dissolved P	70507A	Phosphorus Orthophosphate, Total P
00937A	Potassium, Total (K)	01147H	Selenium, Total (Se)
00929A	Sodium, Total (Na	00095	Specific Conductivity (SPC)
01082A	Strontium, Total (Sr)	00945	Sulfate, Total (SO4)
70300U	Total Dissolved Solids @ 180C (TDS)	00530V	Total Suspended Solids (TSS)
01090H	Zinc, Dissolved (Zn)	01092H	Zinc, Total (Zn)

5. LOW ALKALINITY STATIONS (SAC 016)

Test Code	Test Description	Test Code	Test Description
70508	Acidity, Total	00410	Alkalinity (Alk)
01106H	Aluminum, Dissolved (Al)	01105H	Aluminum, Total (Al)
00610A	Ammonia Nitrogen, Total (NH3 N)	00314	Carbonaceous Biochemical Oxygen Demand (CBOD5)
99020	Bromide (Br)	00915A	Calcium, Dissolved (Ca)
00916A	Calcium, Total (Ca)	00940	Chloride (Cl)
01040H	Copper, Dissolved (Cu)	01042H	Copper, Total (Cu)
00900	Hardness, Total (Hard)	01046A	Iron, Dissolved (Fe)
01045A	Iron, Total (Fe)	01130A	Lithium, Dissolved (Li)
01132A	Lithium, Total (Li)	01049H	Lead, Dissolved (Pb)

01051H	Lead, Total (Pb)	00925A	Magnesium, Dissolved (Mg)
00927A	Magnesium, Total (Mg)	01056H	Manganese, Dissolved (Mn)
01055H	Manganese, Total (Mn)	01065H	Nickel, Dissolved (Ni)
01067H	Nickel, Total (Ni)	00620	Nitrate Nitrogen (NO3 N)
00615	Nitrite Nitrogen (NO2 N)	00600A	Nitrogen, Total (N)
00403	pH, Lab (PH)	00665A	Phosphorus, Total (P)
70507A	Phosphorus Orthophosphate, Total P(O)	00937A	Potassium, Total (K)
00929A	Sodium, Total (Na)	00095	Specific Conductivity (SPC)
00945	Sulfate, Total (SO4)	70300U	Total Dissolved Solids @ 180C (TDS)
00530V	Total Suspended Solids (TSS)	01090H	Zinc, Dissolved (Zn)
01092H	Zinc, Total (Zn)		

6. ATMOSPHERIC DEPOSITION REFERENCE STATIONS (SAC 122): Includes B021 and all SAC 015 parameters plus:

Test Code	Test Description
00420C	Carbon Dioxide, Total (CO2)

7. STANDARD TDS STATIONS (SAC 610): Includes all SAC 010 parameters plus:

Test Code	Test Description	Test Code	Test Description
01007H	Barium, Total (Ba)	01022K	Boron, Total (B)
01132A	Lithium, Total (Li)	82550	Osmotic Pressure (OP)
01147H	Selenium, Total (Se)	01082A	Strontium, Total (Sr)

8. CHESAPEAKE BAY NUTRIENT LOADING TDS STATIONS (SAC 612): Includes all SAC 012 parameters plus:

Test Code	Test Description	Test Code	Test Description
01007H	Barium, Total (Ba)	01022K	Boron, Total (B)
01130A	Lithium, Dissolved (Li)	01132A	Lithium, Total (Li)
82550	Osmotic Pressure (OP)	01147H	Selenium, Total (Se)
01082A	Strontium, Total (Sr)		

9. LOW ALKALINITY TDS STATIONS (SAC 616): Includes all SAC 016 parameters plus:

Test Code	Test Description	Test Code	Test Description
01007H	Barium, Total (Ba)	01022K	Boron, Total (B)
82550	Osmotic Pressure (OP)	01147H	Selenium, Total (Se)
01082A	Strontium, Total (Sr)		

10. RADIOLOGICAL (Suite 245 - GABWT): Alpha and Beta may be listed on the Sample Submission Sheet as additional parameters, for any WQN station, to supplement the selected analysis code.

11. LAKE STATIONS (SAC 083 & B029*)

Test Code	Test Description
00410	Alkalinity, Total (Alk)
00610A	Ammonia Nitrogen, Total (NH3 N)
01007A	Barium, Total (Ba)
71870	Bromide (Br)
00940	Chloride (Cl)
01042H	Copper, Total (Cu)
01045A	Iron, Total (Fe)
00927A	Magnesium, Total (Mg)
71900I	Mercury, Total
00600A	Nitrogen, Total (N)
00665A	Phosphorus, Total (P)
00929A	Sodium, Total (Na)
01082A	Strontium, Total (Sr)
70300U	Total Dissolved Solids @ 180C
82079	Turbidity, Nephelmetric

Test Code	Test Description
01105H	Aluminum, Total (Al)
01002H	Arsenic, Total (As)
01022K	Boron, Total (B)
00916A	Calcium, Total (Ca)
962*	Chlorophyll a (fluorometric)
00900	Hardness, Total (Hard)
01051H	Lead, Total (Pb)
01055H	Manganese, Total (Mn)
00620A	Nitrate Nitrogen (NO3 N)
00403	pH, Lab (PH)
01147H	Selenium, Total (Se)
00095	Specific Conductivity (SPC)
00945	Sulfate, Total (SO4)
00530V	Total Suspended Solids (TSS)
01092H	Zinc, Total (Zn)

12. FISH TISSUE STATIONS

a. SAC 059

Test Code	Test Description
71946	Barium, Total (Ba)
71939	Chromium, Total (Cr)
71936	Lead, Total (Pb)
71945	Selenium, Total (Se)

Test Code	Test Description
71940	Cadmium, Total (Cd)
71937	Copper, Total (Cu)
71930	Mercury, Total (Hg)
71947	Strontium, Total (Sr)

b. PCBs (SAC PCBF)

CAS #	Test Description	CAS #	Test Description
11104282	Arochlor 1221	11141165	Arochlor 1232
53469219	Arochlor 1242	12672296	Arochlor 1248
11097697	Arochlor 1254	11096825	Arochlor 1260

c. PESTICIDES (SAC PESTF)

CAS #	Test Description	CAS #	Test Description
72548	4,4-DDD	72559	4,4-DDE
50293	4,4-DDT	309002	Aldrin
319846	Alpha-BHC	5103719	Alpha-Chlordane
3734483	Chlordene	5103731	Cis-Nonachlor
60571	Dieldrin	72208	Endrin
5103742	Gamma-Chlordane	58899	Gamma-BHC (Lindane)
76448	Heptachlor	1024573	Heptachlor Epoxide

72435	Methoxychlor	2385855	Mirex
53190	O,P-DDD	3424826	O,P-DDE
789026	O,P-DDT	26880488	Oxychlordane
39765805	Trans-Nonachlor		

D.2 ACTIVE WATER CHEMISTRY STATIONS

DEP WATER QUALITY NETWORK (WQN) – ACTIVE STATIONS REV. 1/2023

WQN	Stream Name	Station Type	Start Date (M/YY)	Samples per Year	SAC	REG	Location	Municipality	County	Lat	Long	USGS Gage	Drain Area (Sq Mi)
001	Genesee Rvr	STN	3/90	6	610	NC	SR1010 (Commercial St Br) - Village of Genesee	Genesee Twp	Potter	41.99426	-77.87011	04221000	86
100	UNT Sixpenny	REF	11/15	12	15	SC	Upstr of Old Railroad Grade Tunnel	Union Twp	Berks	40.24077	-75.77734	Manual	1
101	Delaware Rvr Nr Morrisville	STN	7/62	6	10	SE	SR2026 (Trenton Avenue Br) Nr Morrisville	Morrisville Twp	Bucks	40.21970	-74.77810	01463500	6788
103	Delaware Rvr @ Port Jervis	STN	5/62	6	610	NE	US Rts 6 & 209 Br - Port Jervis	Port Jervis	Orange, NY	41.37190	-74.69700	01434000	3077
104	W Br Delaware Rvr	STN	5/62	6	616	NE	SR191 Br	Buckingham Twp	Wayne	41.95227	-75.29208	01426500	651
105	Brandywine Crk	STN	7/62	6	15	SE	RXR Br 400 Mi dwnstr of SR1 Br - Chadds Ford	Birmingham Twp	Delaware	39.86950	-75.59330	01481000	288
110	Schuylkill Rvr	STN	7/62	12	10	SE	Falls Br	Philadelphia	Philadelphia	40.00840	-75.19750	01474500	1879
111	Schuylkill Rvr	STN	7/62	12	10	SE	SR4038 Hanover St Br	Pottstown	Montgomery	40.24206	-75.65124	01472000	1147
113	Schuylkill Rvr	STN	7/62	6	16	SC	T954 (Water St) Br - Berne	Bern Twp	Berks	40.52220	-75.99800	01470500	357
115	Wissahickon Crk	STN	7/62	6	10	SE	Ridge Ave Br	Philadelphia	Philadelphia	40.01483	-75.20670	01474000	64
116	Perkiomen Crk	STN	7/62	6	10	SE	T325 (Arcola Rd) Br	L Providence Twp	Montgomery	40.15319	-75.45559	01473000	301
117	Tulpehocken Crk	STN	7/62	6	10	SC	Grings Mill Br	Bern Twp	Berks	40.36281	-75.96795	01471000	217
121	Neshaminy Crk	STN	7/62	6	10	SE	SR213 Br Nr Langhorne	Middletown Twp	Bucks	40.17421	-74.95733	01465500	209
123	Lehigh Rvr	STN	7/62	6	16	NE	SR2012 (Glendon Ave) Br - H. Moore Park	Easton	Northampton	40.66669	-75.23834	01454700	1360
126	Lehigh Rvr	STN	5/62	6	16	NE	SR115 Br	Tobyhanna Twp	Monroe	41.13040	-75.62601	01447500	92
130	Jordan Crk	STN	7/62	6	10	NE	Martin Luther King Jr Blvd	Allentown	Lehigh	40.60199	-75.46231	01452000	82
131	Aquashicola Crk	STN	7/62 - 12/87, 10/2018	12	16	NE	SR 248 Br @ mouth	Lower Towamensing	Carbon	40.79333	-75.61250	1450600	78

137	Brodhead Crk	STN	5/62	6	10	NE	SR2028 (LR45061) Br	Smithfield Twp	Monroe	40.99358	-75.13764	01442500	288
138	Brodhead Crk	STN	5/62	6	16	NE	SR191 Br - Analomink	Stroud Twp	Monroe	41.03700	-75.21000	01440400	123
139	Bushkill Crk	STN	5/62	6	16	NE	T523 Br Nr Shoemakers	M Smithfield Twp	Monroe	41.08835	-75.03763	01439500	117
141	Wallenpaupack Crk	STN	5/62	6	16	NE	T353 - East Sterling	Greene Twp	Pike	41.33624	-75.34006	01431600	69
142	Tobyhanna Crk	STN	1/64	6	16	NE	SR940 Br Nr Blakeslee	Tobyhanna Twp	Monroe	41.08470	-75.60540	01447720	120
147	Lackawaxen Rvr	STN	9/72	6	10	NE	SR590 Br	Lackawaxen Twp	Pike	41.48667	-74.99157	01432110	598
149	White Clay Crk	STN	11/72	6	15	SE	Creek Rd trail off S Bank Rd, below Confl of UNT; 1100ft dwnstr of gage	London Britain Twp	Chester	39.74472	-75.77308	01478245	60
150	Red Clay Crk	STN	11/72	6	15	SE	SR3013 (LR15037) Marshall'S Br	Kennett Twp	Chester	39.81635	-75.69150	01479820	28
154	Valley Crk	STN	11/72	6	10	SE	Wilson Rd Br	Tredyffrin Twp	Chester	40.08124	-75.45661	01473169	22
169	Little Neshaminy Crk	STN	11/72 - 1/88 & 10/20	6	10	SE	just dwnst of Almshouse Rd crossing	Warwick Twp	Bucks	40.23933	-75.06064	1464985	40
178	Pine Crk	REF	2/88-7/98 & 10/10	12	15	SC	Dwnstr of Lobachsville Rd (SR1023) Br	Pike Twp	Berks	40.40900	-75.73490	Manual	10
181	Little Bushkill Crk	REF	1/88-7/98 & 10/10	12	15	NE	Dwnstr of Sugar Mountain Rd (SR 2003) Br	Lehman Twp	Pike	41.09750	-75.00395	Manual	33
182	Delaware Rvr	STN	3/88	6	10	SE	2000 Yds Upstr of Buoy R6M-1000' above DE border	Marcus Hook	Delaware	39.80719	-75.40667	01463500	10425
183	E Br Dyberry Crk	REF	5/88-7/98 & 10/10	12	15	NE	Upstr of Rt 4007 (Dug Rd) Br; above confl with W Br Dyberry Crk	Dyberry Twp	Wayne	41.66161	-75.28816	Manual	17
185	Delaware Rvr	STN	8/88	6	616	NE	SR1020 (LR63027) Br Nr Callicoon NY	Damascus Twp	Wayne	41.76450	-75.06206	01427510	1713
191	Wild Creek	REF	8/98-12/04 & 10/15	12	15	NE	1st Br Upstr of Penn Forest Res T482	Penn Forest Twp	Carbon	40.94028	-75.58472	Manual	5
193	Wissahickon Crk	STN	1/02	6	10	SE	SR73 Br	Whitemarsh Twp	Montgomery	40.12393	-75.22030	01473900	41
194	Delaware Rvr	STN	11/02	6	10	NE	SR1004 Br @ Belvidere, NJ	Lower Mount Bethel Twp	Northampton	40.82880	-75.08470	01446500	4385
201	Susquehanna Rvr @ Marietta	SS	7/62	20	12	SC	SR462 Br	Hempfield Twp	Lancaster	40.02810	-76.51900	01576000	26004

202	Susquehanna Rvr @ Hburg	SS	5/62-2/95 & 10/12	20	612	SC	Market St Br	Harrisburg	Dauphin	40.25660	-76.88465	1570500	24083
203	Susquehanna Rvr @ Sunbury	SS	7/62	20	612	NC	Veterans Memorial Br btwn Sunbury & Shamokin Dam ; ST Hwy 61	Upper Augusta	Northumberland	40.85150	-76.80709	01554000	18299
204	Pequea Crk	SS	12/66	20	612	SC	SR324 Br @ Martic Forge	Conestoga Twp	Lancaster	39.90570	-76.32831	01576787	150
206	Chickies Crk	STN	7/62	6	12	SC	SR23 Br	E Donegal Twp	Lancaster	40.06291	-76.51534	01575900	108
210	W Conewago Crk	SS	6/62	20	12	SC	SR181 Br	Newberry Twp	York	40.08140	-76.71800	01574000	513
212	Yellow Breeches	SS	6/62	20	12	SC	Bridge St Br (Old US Rt 11) - New Cumberland	Fairview Twp	York	40.22419	-76.86056	01571500	218
214	Juniata Rvr	SS	6/62	20	12	SC	SR34 Br	Newport	Perry	40.47903	-77.12813	01567000	3352
217	Little Juniata Rvr	SS	3/50	20	612	SC	200 ft upstr of SR45 Br	Spruce Creek Twp	Huntingdon	40.60954	-78.13663	01558000	224
223	Raystown Br Juniata	SS	3/50	20	612	SC	SR913 Br W of Saxon	Liberty Twp	Bedford	40.21501	-78.26487	01562000	754
224	Frankstown Br Juniata	SS	6/62	20	612	SC	High Street bridge in Williamsburg	Williamsburg Boro	Blair	40.46301	-78.20008	01556000	293
226	Mahantango Crk	SS	7/62-11/87 & 1/12	20	12	SC	S Malta Rd (T482) Br	Upper Paxton Twp	Dauphin	40.61111	-76.91152	01555500	162
228	Middle Crk	STN	7/62	6	10	NC	SR11 Br (Old Rts 11&15)	Penn Twp	Snyder	40.77521	-76.86992	01555207	175
229	Penns Crk	SS	7/62	20	12	NC	SR104 Br - Village of Penns Crk	Centre Twp	Snyder	40.86700	-77.04927	01555000	306
243	Sherman Crk	SS	10/04	20	12	SC	Pisgah State Rd Crossing @ Dromgold	Carroll Twp	Perry	40.34420	-77.19435	01568000	188
249	Aughwick Crk	STN	1/72	6	10	SC	T403 Br - Aughwick Mills	Shirley Twp	Huntingdon	40.33480	-77.86000	01564512	320
252	Beaverdam Br Juniata Rvr	STN	9/72	6	610	SC	2000' upstr of mouth along SR2014 (T405)	Frankstown Twp	Blair	40.43066	-78.36441	01555858	87
256	Big Elk Crk	STN	11/72	6	10	SE	SR3006 (LR15016) Strickersville Rd Br	Franklin Twp	Chester	39.73012	-75.84789	01494990	41
259	Muddy Crk	SS	1/88-7/98 & 10/10	20	612	SC	Upstr of SR 2024 (Paper Mill Rd) Br	Peach Bottom Twp	York	39.77302	-76.31620	01577500	133
263	Octoraro Crk	SS	11/05	20	12	SC	US 1 @ Richardsmere, Md	Richardsmere, MD	Cecil	39.69368	-76.12473	01578475	189

269	Conewago Crk - Falmouth	SS	5/11	20	12	SC	Hillsdale Rd Br	Londonderry Twp	Dauphin	40.14931	-76.68775	01573710	47
271	Conodoguinet Crk	SS	10/04	20	12	SC	Sample Road Br	Silver Spring Twp	Cumberland	40.25546	-77.01854	01570000	467
272	Swatara Crk	SS	10/04	20	12	SC	Just upstr of Hanover St Br @ Swatara Crk Park	Derry Twp	Dauphin	40.28717	-76.67780	01573560	485
273	Conestoga Rvr	SS	10/04	20	12	SC	River Road Bridge (SR 3030) @ Safe Harbor	Manor Twp	Lancaster	39.93912	-76.38737	01576754	470
278	Conewago Crk - Bellaire	SS	10/11	20	12	SC	Prospect Rd Crossing Nr Lebanon/Lancaster County Border	Mount Joy Twp	Lancaster	40.19528	-76.56778	01573695	21
280	Big Spring Run	SS	10/11	20	12	SC	Along Gypsy Hill Rd about 0.5 miles from US 222	West Lampeter	Lancaster	39.99594	-76.26404	015765195	2
281	Paxton Crk	SS	1/12	20	12	SC	Kohn Rd crossing; 0.5 mi dwnstr of Progress Ave	Susquehanna Twp	Dauphin	40.30610	-76.85564	01571005	12
282	Kishacoquillas Crk	SS	1/12	20	12	SC	Bridge on Manns Narrows Rd	Derry Twp	Mifflin	40.65458	-77.58359	01565000	163
284	Pequea Crk	SS	11/12	20	12	SC	S Ronks Rd	Strasburg Twp	Lancaster	40.00917	-76.16222	01576767	70
285	Quittapahilla	SS	11/12	20	12	SC	Palmyra-Bellgrove Rd	N Annville Twp	Lebanon	40.34239	-76.56110	01573160	73
286	Codorus Crk	SS	11/12	20	12	SC	Codorus Furnace Rd (SR 1008)	E Manchester Twp	York	40.05226	-76.65445	01575585	277
287	Diehls Hollow	STN	10/20	6	15	NC	About 65 yds upstr of confl with Lick Run	Marion Twp	Centre	40.99975	-77.64590		1
288	Lick Run US	STN	10/20	6	15	NC	HW along Rt. 26	Marion Twp	Centre	40.98960	-77.64253		5
289	Lick Run DS	STN	10/20	6	15	NC	Along Rt. 26 dwnstr of Diehls Hollow	Howard Twp	Centre	41.00676	-77.64317		11
290	Sherman Creek	REF	10/20	12	15	NE	Along Big Valley Road (T-769)	Scott Twp	Wayne	41.97020	-75.44100		2
291	Faulkner Brook	REF	10/20	12	15	NE	Penn-York Road (T-780) 75 meters upstr of culvert	Scott Twp	Wayne	41.98350	-75.34450		2
292	Bowers Creek	REF	10/20	12	15	NE	Hypsy Gap Road (T-633), 75 meters upstr of culvert	Chestnut Hill Twp	Monroe	41.00270	-75.41010		1
294	Slate Run	REF	10/20	12	15	NC	About 500 ft upstr from confl with Pine Creek	Brown Twp	Lycoming	41.47152	-77.50557		45
295	Little Conewago Creek	SS	1/24	20	12	SW	6 yds upstrm of Gingrich Rd bridge	S Londonderry Twp	Lebanon	40.225273	-76.55458	01573684	5

296	Sibbald Run	REF	10/20	12	15	NW	60 yds upstr of Rt 62 rd crossing	Hickory Twp	Forest	41.53602	-79.43326		1
297	Stanton Brook	REF	10/20	12	15	NW	Stanton Brook Rd crossing	Hamlin Twp	McKean	41.73510	-78.55036		3
298	Hammer Creek	SS	1/24	20	12	SW	Dwnstr side of Obie Road Bridge	Heidelberg Twp	Lebanon	40.271083	-76.32172	01576381	12
301	Susquehanna Rvr - Danville	SS	7/62	20	612	NC	SR54 Br	Mahoning Twp	Montour	40.95770	-76.62120	01540500	11230
302	Susquehanna Rvr - Wilkes-Barre	SS	5/62	20	612	NE	Br East of US Rt 11 Nr Retreat State Prison	Newport Twp	Luzerne	41.18900	-76.08720	01536500	10155
305	Susquehanna Rvr - Towanda	SS	5/62	20	612	NC	James Street Br	Wysox Twp	Bradford	41.79159	-76.44291	01531500	7789
306	Susquehanna Rvr - Ny	STN	5/62	6	610	NE	SR11 Br Nr Great Bend	Great Bend Twp	Susquehanna	41.96382	-75.74190	01503000	2084
308	Fishing Crk	STN	5/62	6	16	NC	SR42 Br	Montour Twp	Columbia	40.99350	-76.47560	01539000	379
309	Nescopeck Crk	STN	5/62	6	10	NE	SR 339 Br	Nescopeck Twp	Luzerne	41.04703	-76.22105	01538600	172
313	Lackawanna Rvr	STN	5/62	6	610	NE	Bridge St Br	Old Forge	Lackawanna	41.35845	-75.74404	01536000	335
317	Tunkhannock Crk	SS	5/62	20	612	NE	SR6 Br	Tunkhannock Twp	Wyoming	41.55725	-75.89443	01534000	393
318	Towanda Crk	STN	5/62	6	610	NC	SR3006 (LR08193) Br Nr Monroeton	Monroe Twp	Bradford	41.70820	-76.48630	01532000	216
323	Susquehanna Rvr	STN	11/68	6	610	NE	SR92 Br - Falls	Exeter Twp	Wyoming	41.46199	-75.85433	01536500 & 01536000	9461
324	Tioga Rvr	STN	9/70	6	610	NC	450 ft dwnstr of T773 Br - Tioga Junction	Lawrence Twp	Tioga	41.95613	-77.11557	01518700	442
333	Sugar Crk	STN	1/88	6	610	NC	Pine Valley Rd Bridge	N Towanda Twp	Bradford	41.78131	-76.50258	01531488	184
334	Wyalusing Crk	STN	1/88	6	610	NC	SR706 Br - Merryall	Wyalusing Twp	Bradford	41.69740	-76.23060	01532950	211
341	Tioga Rvr	STN	10/10	6	610	NC	Along T754 (Brooklyn Rd)	Mansfield Boro	Tioga	41.79556	-77.07972	01516350	153
343	Cowanesque Rvr	STN	11/15	6	610	NC	State Highway 49 (E Main St) - Elkland, Pa	Elkland Boro	Tioga	41.98750	-77.30250	01519200	234
401	W Br Susquehanna Rvr - Lewisburg	SS	7/62	20	612	NC	SR45 Br	Lewisburg	Union	40.96690	-76.87872	01553500	6690

402	W Br Susquehanna Rvr - Williamsport	STN	3/50	6	610	NC	Maynard St Br	S Williamsport	Lycoming	41.22911	-77.01905	01551500	5668
404	W Br Susquehanna Rvr - Karthaus	SS	1/50-11/87 & 10/04	20	612	NC	SR 879 Br @ Karthaus	Karthaus Twp	Clearfield	41.11695	-78.10914	01542500	1393
406	W Br Susquehanna Rvr - Bower	STN	6/62	6	610	NC	T418 Br - Bower	Greenwood Twp	Clearfield	40.89719	-78.67752	01541000	315
408	Loyalsock Crk	STN	5/62	6	616	NC	SR973 Br Nr Loyalsockville	Eldred Twp	Lycoming	41.32519	-76.91134	01552000	437
409	Lycoming Crk	REF	5/62	12	15	NC	T840 Br - Camp Susque (Nr Gray)	Lewis Twp	Lycoming	41.41810	-77.03350	01550000	173
410	Pine Crk - Waterville	SS	5/62	20	612	NC	900 ft dwnstr of gage just upstr of island; along Rt 44	Cummings Twp	Lycoming	41.27218	-77.32695	01549700	946
413	Bald Eagle Crk	STN	5/62	6	610	NC	SR1003 (LR14010) Br - Curtin	Boggs Twp	Centre	40.97492	-77.74215	01547200	294
415	Spring Crk	STN	3/50	6	610	NC	SR3001 (LR14040) Hartles Br	Benner Twp	Centre	40.89002	-77.79431	01546500	86
418	Sinnemahoning Crk Nr Keating	STN	3/50	6	610	NC	SR4002 (LR18001) Br - Keating	E Keating Twp	Clinton	41.26114	-77.90717	01543500	1033
420	Driftwood Br Sinnemahoning	STN	3/50	6	616	NC	SR3002 (LR12004) Br - Sterling Run	Lumber Twp	Cameron	41.41351	-78.19698	01543000	272
422	Clearfield Crk	STN	3/50	6	610	NC	SR 2024 Old Erie Pike Road-Dimeling	Boggs Twp	Clearfield	40.97154	-78.40656	01541500	370
423	Beech Crk	STN	4/65	6	610	NC	SR150 Br - Beech Crk	Beech Creek Twp	Clinton	41.07486	-77.59240	01547950	171
429	Little Pine Crk	STN	9/72	6	616	NC	SR 44 Br	Cummings Twp	Lycoming	41.30971	-77.36285	01549590	180
433	Fishing Crk	STN	9/72	6	610	NC	SR2004 (LR18007) 2nd Br upstr of Cedar Run confl	Lamar Twp	Clinton	41.07536	-77.47784	01548075	137
434	Kettle Crk	STN	9/72	6	616	NC	Off SR4001 (LR18003) 3.2 Mi upstr of Pa Rt120	Noyes Twp	Clinton	41.31945	-77.87348	01545000	233
439	Bennetts Br Sinnemahoning	STN	8/76	6	610	NC	T343 Br Nr Castle Garden	Gibson Twp	Cameron	41.33397	-78.13570	01542790	366
444	Muncy Crk	STN	1/88	6	616	NC	SR2014 (Old Rt 147) Br	Muncy Creek Twp	Lycoming	41.21769	-76.78731	01553005	203
445	Bald Eagle Crk	SS	1/88	20	612	NC	SR2012 (LR18013) Br - Castanea	Castanea Twp	Clinton	41.12463	-77.43504	01548005(D) 01548085(WW)	769
448	W Br Susquehanna Rvr - Jersey Shore	SS	10/04	20	612	NC	SR 44 Br @ Jersey Shore	Jersey Shore Boro	Lycoming	41.20245	-77.25219	01549760	5230

449	Beech Crk	STN	10/10	6	610	NC	300 yds dwnstr of Iron Gate Rd Br	Liberty Twp	Centre	41.11139	-77.70194	01547950	153
451	E Fork Sinnemahoning	STN	10/10	6	610	NC	Upstr of Fork Rd Br Crossing	Wharton Twp	Potter	41.54250	-77.97861	01543693	48
452	Cedar Run	REF	1/05 - 9/10; 10/20	12	15	NC	0.5 mi upstr of SR 64 Br and 100 ft upstr of private BR	Lamar Twp	Clinton	41.07634	-77.48975		15
458	Hoagland Run	REF	10/10	12	15	NC	25 ft upstr of Rt 973	Lycoming Twp	Lycoming	41.32774	-77.12898	Manual	11
459	Kettle Crk	REF	10/10	12	15	NC	Just upstr of former Rt 318 Br @ Cross Fork	Leidy Twp	Clinton	41.47583	-77.82611	01544500	137
462	Chillisquaque Crk	SS	11/12	20	12	NC	Shakespeare Rd (SR1025)	E Chillisquaque Twp	Northumberland	40.97459	-76.80012	01553850	101
465	Carbaugh Run	REF	10/15	12	15	SC	250 m upstr of confl with Clear Run Tributary	Franklin Twp	Adams	39.89740	-77.45113	Manual	6
500	East Branch Sideling Hill Creek	REF	6/21	12	15	SC	Just upstr of Amaranth Rd	Monroe Twp	Bedford	39.80984	-78.33562		13
601	Lake Erie	STN	12/68	6	83	NW	City of Erie Water Intake (Chestnut St Plant)	Erie	Erie	42.15670	-80.15250	USCG601	
624	Lake Erie East	STN	10/15	6	83	NW	Eastern Basin of Lake Erie, approx 7 km N of Sixteenmile Crk	Erie	Erie	42.29425	-79.86684	N/A	
625	Lake Erie Central	STN	10/15	6	83	NW	Near PA-OH State Line in central basin of Lake Erie	Erie	Erie	42.11920	-80.51038	N/A	
632	Presque Isle Bay	STN	7/75	6	83	NW	W Front State St; NW of Public Dock; Inline W/ Harbr Channel (Mid)	Erie	Erie	42.14440	-80.08340	USCG632	
643	Conneaut Crk	STN	1/88	6	10	NW	SR3001 (LR25001) Br on Griffy Rd	Conneaut Twp	Erie	41.91811	-80.46889	04212945	150
644	Walnut Crk	STN	11/08	6	10	NW	Manchester Rd Br	Fairview Twp	Erie	42.07330	-80.23440	04213152	37
701	Monongahela Rvr	STN	4/50	12	610	SW	Rankin Bridge off SR837 Nr Lock & Dam 2	Braddock	Allegheny	40.40560	-79.88099	03075070 + 03083500	7338
702	Monongahela Rvr @ Charleroi	STN	4/50	12	610	SW	SR2018 Br (Lock St) - N Charleroi	N Charleroi	Washington	40.15201	-79.90412	03075070	1073
706	Youghiogheny Rvr @ Sutersville	STN	4/50	12	610	SW	SR3045 (LR 64226) Br	Sutersville	Westmoreland	40.24119	-79.80536	03083500	1712
709	Youghiogheny Rvr @ Confluence	STN	4/50	12	610	SW	SR281 Br	Confluence Boro	Somerset	39.80757	-79.36373	03077500	433
713	South Fork Tenmile	STN	4/50-1/88 & 10/10	12	610	SW	Rt 188 (Jefferson Rd) Br	Jefferson	Greene	39.92306	-80.07278	03073000	181

714	Dunkard Crk	STN	4/50	12	610	SW	SR2012 Br (Bobtown Hill Rd) Nr Bobtown	Dunkard Twp	Greene	39.76045	-79.97288	03072000	228
725	Monongahela Rvr @ Pt Marion	STN	4/75	12	610	SW	Dwnstr of Lock & Dam 8 @ Pt Marion	Point Marion	Greene	39.72680	-79.91180	03063000	2717
726	Casselman Rvr	STN	1/88	12	616	SW	SR2008 (LR55116) Br Nr Salisbury	Elk Lick Twp	Somerset	39.73238	-79.10055	03079000	73
728	Whiteley Crk	STN	1/88-10/98 & 10/10	12	610	SW	SR 19 Br @ Kirby	Whiteley Twp	Greene	39.80222	-80.11722	Manual	9
732	Monongahela Rvr @ Elizabeth	STN	10/10	12	610	SW	Rt 51 Br dwnstr of Dam No.3	Elizabeth Twp	Allegheny	40.27534	-79.88840	03075070	5340
733	Mill Run	REF	3/05	12	15	SW	50 m dwnstr of Quebec Rd	Wharton Twp	Fayette	39.76360	-79.66330	Manual	9
734	Jones Mill Run	REF	10/10	12	15	SW	Along Laurel Hill State Rd	Jefferson Twp	Somerset	40.00333	-79.23750	Manual	5
735	UNT House Run	REF	11/13	12	15	SW	Bristoria Rd Crossing (SR3020)	Jackson Twp	Greene	39.85390	-80.32990	Manual	2
736	N Fork Dunkard Fork	STN	10/15	6	610	SW	Riggs Road (T356) Br	Richhill Twp	Greene	39.87857	-80.42853	Manual	21
737	Enlow Fork	STN	10/15	6	610	SW	300' upstr of W Finley Rd/Ackley Crk Rd (SR4007) Br	West Finley Twp	Washington	39.96917	-80.44750	Manual	38
738	Tenmile Crk	STN	10/15	12	610	SW	Upstr of South Fork confl; dwnstr of Center Street Br	Clarksville	Washington	39.97521	-80.04179	Manual	135
739	Monongahela River @ Masontown	STN	10/15	12	610	SW	Rt 21 Br - Masontown	Monongahela Twp	Greene	39.85326	-79.92677	03072500	378
801	Allegheny Rvr @ Natrona	STN	3/50	12	610	SW	Hulton Highway Br	Oakmont	Allegheny	40.52710	-79.84640	03049500	11530
805	Allegheny Rvr @ W Hickory	STN	5/62	6	610	NW	SR127 Br - W Hickory	Harmony Twp	Forest	41.57002	-79.40560	03016000	3661
807	Allegheny Rvr @ Eldred	STN	5/62	6	610	NW	SR346 Br	Eldred	Mckean	41.96523	-78.38578	03010500	550
809	Kiskiminetas Rvr	STN	4/50 - 1/88 & 10/20	6	610	SW	Rt 56 Br	Vandergrift Boro	Westmoreland	40.60611	-79.56083	3048500	1527
810	Conemaugh Rvr	STN	3/50	6	610	SW	SR3003 (LR64269) Br Nr Tunnelton	Conemaugh Twp	Indiana	40.45407	-79.39084	03044000	1362
819	Mahoning Crk	STN	3/50-6/95 & 10/10	6	610	NW	T 748 Br; dwnstr from Mahoning Dam	Wayne Twp	Armstrong	40.92722	-79.29194	03036000	344
820	Redbank Crk	STN	3/50	6	610	NW	T468 Br - St Charles	Porter Twp	Clarion	40.99462	-79.39305	03032500	533

821	Clarion Rvr	STN	3/50-11/87 & 10/10	6	610	NW	Rt 854 Br	Piney Twp	Clarion	41.17000	-79.47250	03030515	969
822	Clarion Rvr	STN	3/50	6	610	NW	SR36 Br - Cooksburg	Farmington Twp	Clarion	41.33162	-79.20878	03029500	807
830	Tionesta Crk	STN	5/62	12	612	NW	SR1003 (LR27015) Br - Lynch	Howe Twp	Forest	41.60184	-79.04963	03017500	232
831	Brokenstraw Crk	STN	6/62	6	610	NW	SR6 Main St Br	Youngsville	Warren	41.85195	-79.31740	03015500	305
832	Conewango Crk	STN	6/62	6	610	NW	SR1012 (Liberty St) Br - Russell	Pine Grove Twp	Warren	41.93822	-79.13289	03015000	815
845	French Crk @ Sugarcreek	STN	2/73	6	610	NW	SR322 Br	Franklin	Venango	41.40140	-79.83080	03024000 & 03025000	1237
846	French Crk @ Meadville	STN	2/73	6	10	NW	SR2008 (LR20027) Br - Wilson Chutes Boat Launch	W Mead Twp	Crawford	41.58942	-80.15001	03023100	808
858	Tunungwant Crk	STN	3/73	6	610	NW	SR346 (LR42012) Br - Foster Brook	Foster Twp	Mckean	41.97699	-78.62451	03010956	123
860	Allegheny Rvr	STN	10/10	12	610	NW	Upstr of Rt 6 Br	Port Allegany Boro	Mckean	41.81800	-78.29320	03007800	252
861	Mahoning Crk	STN	1/88	12	610	NW	T374 Br - Sportsburg	Young Twp	Jefferson	40.92217	-79.00611	03034000	159
866	Allegheny Rvr Nr Warren	STN	1/88	12	610	NW	SR6 Br Nr Warren (Glade Br)	Mead Twp	Warren	41.82410	-79.11920	03012550	2220
867	Allegheny Rvr @ Kennerdell	STN	1/88	12	610	NW	SR3008 (LR60011) Br - Kennerdell	Clinton Twp	Venango	41.26390	-79.84120	03025500	6269
868	Oil Crk	STN	1/88	6	610	NW	SR8 Br N of Rouseville	Cornplanter Twp	Venango	41.48209	-79.69517	03020500	283
884	Allegheny Rvr @ Kittanning	STN	12/06	12	610	NW	US 422 Br, Kittanning, Pa	East Franklin	Armstrong	40.81260	-79.52260	03036500	8974
885	Piney Run	REF	6/21	12	015	SW	Beside Easy Camp Rd	Shade Twp	Somerset	40.14369	-78.74487		5
901	Ohio Rvr Nr East Liverpool	STN	5/62	12	610	SW	Newell Br Nr East Liverpool	E Liverpool, OH	Hancock, OH	40.61931	-80.59083	03086000 & 03107500 & 03108000	23533
902	Ohio Rvr @ Sewickley	STN	3/50	12	610	SW	SR4025 Sewickley Br	Sewickley	Allegheny	40.53337	-80.18756	03086000	19491
903	Raccoon Crk	STN	4/50	6	610	SW	SR3010 (LR04068) Br	Raccoon Twp	Beaver	40.62826	-80.33715	03108000	179
905	Beaver Rvr	STN	4/50	12	610	SW	SR588 Br	Eastvale	Beaver	40.76629	-80.31695	03107500	3114

906	Beaver Rvr	STN	4/50-9/03 & 10/10	12	610	NW	Dwnstr of SR288 Br	Wampum Boro	Lawrence	40.88861	-80.33600	03105500	2238
907	Connoquenessing Crk	STN	4/50	12	610	SW	SR1005 (LR04078) Br @ Hazen	Franklin Twp	Beaver	40.81676	-80.24214	03106000	356
909	Shenango Rvr @ New Castle	STN	5/62	6	610	NW	Washington St Br @ New Castle	New Castle	Lawrence	41.00003	-80.35053	03104500	794
910	Shenango Rvr @ Sharpsville	STN	4/50	6	610	NW	SR3025 (LR43081) Br	Sharpsville	Mercer	41.26764	-80.47012	03103500	589
911	Shenango Rvr Nr Pymatunning Dam	STN	5/62	6	610	NW	500' Dwnstr of Sugar Run; below Pymatuning Dam	W Shenango Twp	Crawford	41.49769	-80.46028	03101500	163
913	Little Shenango Rvr	STN	5/62	6	610	NW	SR4006 (LR43135) Williamson Rd Br N of Greenville	Hempfield Twp	Mercer	41.42598	-80.37392	03102500	108
915	Mahoning Rvr	STN	11/64	12	610	NW	SR224 Br - N Edinburg	Mahoning Twp	Lawrence	41.01847	-80.44041	03099600	1100
917	Connoquenessing Crk	STN	8/72	6	610	NW	SR3007 (LR10015) Br - Renfrew	Penn Twp	Butler	40.80601	-79.96528	03105810	137
922	Slippery Rock Crk	STN	3/73	6	610	NW	SR2005 (LR37083) Br - Camp Allegheny	Perry Twp	Lawrence	40.88409	-80.23372	03106500	401

STREAM NAME: **Br** = Branch **Crk** = Creek **Rvr** = River **Nr** = near **E** = East **W** = West
STATION TYPE: **REF** = Reference **SS** = Chesapeake Bay Nurient Loading **STN** = Standard
LOCATION: **Br** = bridge **confl** = confluence **dwnstr** = downstream **LR** = local route **m** = meters **RM** = river mile **Rt** = route **RXR** = railroad **SGL** = State Game Lands **SR** = State route **St** = street **upstr** = upstream

DEP WATER QUALITY NETWORK (WQN) – ACTIVE LAKE STATIONS REV. 11/2022

WQNL #	Lake Name	REG	Reachcode	Acres	Municipality	County	LAT	LONG	Period of Record
101	Green Lane Reservoir	SE	02040203008646	780	Upper Frederick	Montgomery	40.3416	-75.4794	2006-2010; 2021-2025
102	Hopewell Lake	SE	02040203001996	66	Union Twp	Berks	40.2024	-75.7823	2004-2008; 2021-2025
112	Minsi Lake	NE	02040105001626	115	Upper Mt Bethel Twp	Northampton	40.91204	-75.17046	2001-2005; 2021-2025
116	Struble Lake	SE	02040205001068	141	Honeybrook Twp	Chester	40.10787	-75.86419	2004-2008; 2021-2025
204	Canoe Creek Lake	SC	02050302000840	153	Frankstown Twp	Blair	40.4777	-78.28	7/96 - 8/00; 2021-2025
212	Kettle Dam Reservoir (Brush Mountain Dam)	SC	02050302000839	30	Tyrone Twp	Blair	40.5069	-78.3495	2006-2010; 2021-2025
213	Lake Perez	SC	02050302000825	67	Barree Twp	Huntington	40.65861	-77.91611	2006-2010; 2021-2025
312	Mountain Mud Pond	NE	02050107001705	30	Fell Twp	Lackawanna	41.591	-75.533	2021-2025
313	Belmont Lake	NE	02040103001169	174	Mount Pleasant Twp	Wayne	41.771	-75.445	2021-2025
402	Foster Joseph Sayers Lake	NC	02050204002478	2083	Liberty Twp	Centre	41.0478	-77.61	1991-1995; 2006-2010; 2021-2025
404	Kettle Creek Lake	NC	02050203000754	120	Leidy Twp	Clinton	41.36028	-77.92306	7/01 – 11/05; 2021-2025
711	Laurel Hill Lake	SW	05020006001941	62	Middlecreek Twp	Somerset	39.99735	-79.23541	2021-2025

D.3 ACTIVE BENTHIC MACROINVERTEBRATE STATIONS

The following table contains the locational information for the benthic macroinvertebrate sampling reach of active WQN stations. When a WQN station is created, the water chemistry sampling location is first evaluated to determine if it is also suitable for macroinvertebrate sampling. Streambed substrate, availability of riffle/run habitat, accessibility, and safety are assessed. If this location is appropriate for the collection protocol, the water chemistry location is the best choice for the macroinvertebrate sample. If the water chemistry location is not suitable, the nearest stream reach, meeting the needs of the sampling protocol and safety of the collector, to the water chemistry location is chosen. Once decided, the macroinvertebrate sampling location is a permanent location. The same reach should be sampled every time. If the waterbody is not suitable for the sampling protocol, then no macroinvertebrate samples are collected. If the macroinvertebrate location becomes unsuitable for the sampling protocol (i.e., bridge work, stream flow), no sample is collected and the WQD should be contacted to coordinate the next steps.

DEP WATER QUALITY NETWORK (WQN) – ACTIVE MACROINVERTEBRATE STATIONS REV. 11/2022

WQN	Stream Name	REG	Station Type	Sampling Frequency	Protocol	Season	Sampling Location	Lat	Long	Start Date (M/YY)
001	Genesee Rvr	NC	STN	even year	riffle-run	Nov-Apr	150 ft dwnstr of SR1010 (Commercial St)	41.99475	-77.87030	3/90
100	UNT Sixpenny	SE	REF	annual	riffle-run	even Fall/odd Spring	25 ft upstr of railroad tunnel.	40.24077	-75.77729	11/15
101	Delaware Rvr Nr Morrisville	SE	STN	2/yr	riffle-run	Sept & Nov	800 ft dwnstr of Calhoun St Br	40.21793	-74.77653	7/62
103	Delaware Rvr @ Port Jervis	NE	STN	even year	riffle-run	Aug-Sept	0.8 miles upstr of US Rts 6 & 209 Br	41.37982	-74.70929	5/62
104	W Br Delaware Rvr	NE	STN	even year	riffle-run	Nov-Apr	300 ft upstr of SR191 (Pennsylvania Ave Br)	41.95334	-75.29172	5/62
105	Brandywine Crk	SE	STN	even year	riffle-run	Nov-Apr	500 ft dwnstr of SR 1 Br	39.87054	-75.59501	7/62
110	Schuylkill Rvr	SE	STN	even year	riffle-run	Aug-Sept	Riffle dwnstr of Flat Rock Dan, 3.7 miles upstr from QW site	40.03905	-75.24720	7/62
111	Schuylkill Rvr	SE	STN	even year	riffle-run	Aug-Sept	Riffle 400 ft dwnstr of Rt 100 Br (2000 ft upstr of Hanover St Br)	40.24175	-75.65821	7/62
113	Schuylkill Rvr	SC	STN	even year	riffle-run	Aug-Sept	Riffle 1000 ft upstr of T954 (Fisher Dam Rd-Water St) Br	40.52468	-75.99563	7/62

WQN	Stream Name	REG	Station Type	Sampling Frequency	Protocol	Season	Sampling Location	Lat	Long	Start Date (M/YY)
115	Wissahickon Crk	SE	STN	even year	riffle-run	Nov-Apr	400 ft dwnstr of Forbidden Dr parking area	40.02681	-75.19367	7/62
116	Perkiomen Crk	SE	STN	even year	riffle-run	Nov-Apr	100 ft upstr of T325 Arcola Rd Br	40.15361	-75.45598	7/62
117	Tulpehocken Crk	SC	STN	even year	riffle-run	Nov-Apr	1100 ft dwnstr of Red Br Rd at County Park	40.36953	-75.97574	7/62
121	Neshaminy Crk	SE	STN	even year	riffle-run	Nov-Apr	800 ft upstr of SR213 Br	40.17611	-74.95683	7/62
123	Lehigh Rvr	NE	STN	even year	riffle-run	Aug-Sept	900 ft upstr of SR2012 Glendon Hill Rd Br - Hugh Moore Park	40.66748	-75.23761	7/62
126	Lehigh Rvr	NE	STN	even year	riffle-run	Aug-Sept	300 ft upstr of SR115 (Buck Blvd) Br	41.13094	-75.62516	5/62
130	Jordan Crk	NE	STN	even year	riffle-run	Nov-Apr	50 ft dwnstr of Martin Luther King Jr Blvd	40.60173	-75.46202	7/62
131	Aquashicola Crk	NE	STN	2/yr	riffle-run	Spring & Fall	600 ft upstr of Red Hill Dr	40.79674	-75.60813	7/62 - 12/87, 10/2018
137	Brodhead Crk	NE	STN	even year	riffle-run	Nov-Apr	25 ft upstr of SR2028 (LR45061- Logging Rd) Br	40.99359	-75.13779	5/62
138	Brodhead Crk	NE	STN	even year	riffle-run	Nov-Apr	350 ft dwnstr of SR 191 Br in Township Park	41.03615	-75.20918	5/62
139	Bushkill Crk	NE	STN	even year	riffle-run	Nov-Apr	300 ft dwnstr of T523 (Winona Falls Rd) Br	41.08811	-75.03690	5/62
141	Wallenpaupack Crk	NE	STN	even year	riffle-run	Nov-Apr	200 ft upstr of T353 (Creamery Rd)	41.33605	-75.33924	5/62
142	Tobyhanna Crk	NE	STN	even year	riffle-run	Nov-Apr	400 ft dwnstr of SR940 Br	41.08566	-75.60616	1/64
147	Lackawaxen Rvr	NE	STN	even year	riffle-run	Aug-Sept	100 ft dwnstr of SR590 Br (Hamlin Hwy)	41.48673	-74.99125	9/72
149	White Clay Crk	SE	STN	even year	riffle-run	Nov-Apr	Creek Rd Trail off S Bank Road; 1100 ft dwnstr USGS gage	39.74472	-75.77308	11/72
150	Red Clay Crk	SE	STN	even year	riffle-run	Nov-Apr	200 ft dwnstr of SR3013 (LR15037) Marshall Br Rd	39.81617	-75.69133	11/72

WQN	Stream Name	REG	Station Type	Sampling Frequency	Protocol	Season	Sampling Location	Lat	Long	Start Date (M/YY)
154	Valley Crk	SE	STN	even year	riffle-run	Nov-Apr	100 ft dwnstr of Wilson Rd Br	40.08138	-75.45621	11/72
169	Little Neshaminy Crk	SE	STN	even year	riffle-run	Nov-Apr	55 yds upstr of Almshouse Rd Br	40.23900	-75.06131	11/72 - 1/88 & 10/20
178	Pine Crk	SC	REF	annual	riffle-run	even Fall/odd Spring	100 ft dwnstr of Lobachsville Rd (SR1023) Br	40.40936	-75.73461	2/88-7/98 & 10/10
181	Little Bushkill Crk	NE	REF	annual	riffle-run	even Fall/odd Spring	Dwnstr of E Sugar Mountain Br crossing	41.09745	-75.00385	1/88-7/98 & 10/10
182	Delaware Rvr	SE	STN		No Bio Sampling		No Bio Sampling - Removing Plate Sampler Locations			3/88
183	E Br Dyberry Crk	NE	REF	annual	riffle-run	even Fall/odd Spring	Above confl with W Br Dyberry Crk	41.66161	-75.28832	5/88-7/98 & 10/10
185	Delaware Rvr	NE	STN	even year	riffle-run	Aug-Sept	3500 ft dwnstr of SR1020 (LR63027)(Callicoon Rd) Br on both sides of island	41.75643	-75.05655	8/88
191	Wild Creek	NE	REF	annual	riffle-run	even Fall/odd Spring	25 ft upstr of Hatchery Rd Br	40.94004	-75.58581	8/98-12/04 & 10/15
193	Wissahickon Crk	SE	STN	even year	riffle-run	Nov-Apr	200 ft upstr of SR 73 Br (E Skippack Pike)	40.12399	-75.21925	1/02
194	Delaware Rvr	NE	STN	even year	riffle-run	Aug-Sept	3.4 miles dwnstr of SR 1004 Br (Water St); upstr of RR Br near power plant	40.78958	-75.11581	11/02
201	Susquehanna Rvr @ Marietta	SC	SS	2/yr	riffle-run	Sept & Nov	just dwnstr of Rt 30	40.03270	-76.52480	7/62
202	Susquehanna Rvr @ Hburg	SC	SS	2/yr	riffle-run	Sept & Nov	150 ft upstr from Rockville train Br Nr boat ramp at Marysville STP.	40.33275	-76.91717	5/62-2/95 & 10/12
203	Susquehanna Rvr @ Sunbury	NC	SS	2/yr	semi-wadeable lg river	summer & fall	1300 ft dwnstr from Rt 61 Br	40.84764	-76.80479	7/62
204	Pequea Crk	SC	SS	annual	riffle-run	Nov-Apr	Riffle upstr of Coleman Covered Br	39.89874	-76.34206	12/66
206	Chickies Crk	SC	STN	even year	riffle-run	Nov-Apr	100 ft dwnstr of SR23 Br (Marietta Ave)	40.06265	-76.51567	7/62
210	W Conewago Crk	SC	SS	annual	riffle-run	Nov-Apr	250 ft dwnstr of Rt 181 Br	40.08160	-76.71693	6/62

WQN	Stream Name	REG	Station Type	Sampling Frequency	Protocol	Season	Sampling Location	Lat	Long	Start Date (M/YY)
212	Yellow Breeches	SC	SS	annual	riffle-run	Nov-Apr	950 ft upstr of Old US Rt. 11 (Bridge St) Br - New Cumberland	40.22208	-76.86201	6/62
214	Juniata Rvr	SC	SS	2/yr	semi-wadeable lg river	summer & fall	SR34 Br	40.47410	-77.12390	6/62
217	Little Juniata Rvr	SC	SS	annual	riffle-run	Nov-Apr	200 ft upstr of SR45 (Spruce Crk Rd) Br	40.60954	-78.13663	3/50
223	Raystown Br Juniata	SC	SS	annual	riffle-run	Nov-Apr	Riffle upstr of VFW ballfield	40.22042	-78.25401	3/50
224	Frankstown Br Juniata	SC	SS	annual	riffle-run	Nov-Apr	2000 ft dwnstr of abandon R&R	40.47578	-78.19135	6/62
226	Mahantango Crk	SC	SS	annual	riffle-run	Nov-Apr	Riffle dwnstr of S Malta Br	40.61118	-76.91190	7/62-11/87 & 1/12
228	Middle Crk	NC	STN	even year	riffle-run	Nov-Apr	100 ft upstr of SR11 Br (old Rts 11&15) S Market St Br	40.77482	-76.87020	7/62
229	Penns Crk	NC	SS	annual	riffle-run	Nov-Apr	Riffle dwnstr of Rt 104 Br	40.86658	-77.04846	7/62
243	Sherman Crk	SC	SS	annual	riffle-run	Nov-Apr	Pisgah State Rd Crossing @ Dromgold	40.34420	-77.19454	10/04
249	Aughwick Crk	SC	STN	even year	riffle-run	Nov-Apr	2000 ft dwnstr from Rt 522 (1.6 miles upstr from SR11 Br	40.31543	-77.87112	1/72
252	Beaverdam Br Juniata Rvr	SC	STN	even year	riffle-run	Nov-Apr	2000 ft upstr from mouth along SR2014 (T405)	40.43056	-78.36389	9/72
256	Big Elk Crk	SE	STN	even year	riffle-run	Nov-Apr	150 ft dwnstr of SR 3006 Strickersville Rd Br	39.73038	-75.84806	11/72
259	Muddy Crk	SC	SS	annual	riffle-run	Nov-Apr	Riffle 600 ft dwnstr of SR2024 (Paper Mill Rd) Br	39.77133	-76.31637	1/88-7/98 & 10/10
263	Octoraro Crk	SC	SS	annual	riffle-run	Nov-Apr	Riffle upstr of New Bridge /HorseShoe Rd	39.70677	-76.11520	11/05
269	Conewago Crk - Falmouth	SC	SS	annual	riffle-run	Nov-Apr	300 yds dwnstr of Hillsdale Rd Br	40.15111	-76.68974	5/11
271	Conodoguinet Crk	SC	SS	annual	riffle-run	Nov-Apr	Riffle dwnstr of WTP	40.25546	-77.01854	10/04

WQN	Stream Name	REG	Station Type	Sampling Frequency	Protocol	Season	Sampling Location	Lat	Long	Start Date (M/YY)
272	Swatara Crk	SC	SS	annual	riffle-run	Nov-Apr	Just upstr of Hanover St Br @ Swatara Crk Park	40.28717	-76.67780	10/04
273	Conestoga Rvr	SC	SS	annual	riffle-run	Nov-Apr	215 m dwnstr of River Road Br	39.93712	-76.38709	10/04
278	Conewago Crk - Bellaire	SC	SS	annual	riffle-run	Nov-Apr	Prospect Rd Crossing Nr Lebanon/Lancaster County Border	40.19528	-76.56778	10/11
280	Big Spring Run	SC	SS	annual	riffle-run	Nov-Apr	Along Gypsy Hill Rd about 0.5 miles dwnstr of US 222	39.99594	-76.26404	10/11
281	Paxton Crk	SC	SS	annual	riffle-run	Nov-Apr	Upstr of Kohn Rd	40.30584	-76.85421	1/12
282	Kishacoquillas Crk	SC	SS	annual	riffle-run	Nov-Apr	Upstr and dwnstr of Main Street Br	40.65479	-77.58422	1/12
284	Pequea Crk	SC	SS	annual	riffle-run	Nov-Apr	30 ft dwnstr of S Ronks Rd Br - Ronks	40.00921	-76.16234	11/12
285	Quittapahilla	SC	SS	annual	riffle-run	Nov-Apr	250 ft upstr of SR4008 (Palmra Bellgrove Rd) Br - Bellgrove	40.34232	-76.56106	11/12
286	Codorus Crk	SC	SS	annual	riffle-run	Nov-Apr	500 ft dwnstr of SR1008 (Codorus Furnace Rd) - Saginaw	40.05214	-76.65251	11/12
287	Diehls Hollow	NC	REF	2/yr	riffle-run	Spring & Fall	About 260 yds upstr of confl with Lick Run	40.99903	-77.64743	10/20
288	Lick Run US	NC	STN	annual	limestone	Jan-May	Headwaters along Rt. 26	40.98960	-77.64253	10/20
289	Lick Run DS	NC	STN	annual	limestone	Jan-May	Along Rt. 26 dwnstr of Diehls Hollow	41.00676	-77.64317	10/20
290	Sherman Creek	NE	REF	2/yr	riffle-run	Spring & Fall	Along Big Valley Road (T-769)	41.97020	-75.44100	10/20
291	Faulkner Brook	NE	REF	2/yr	riffle-run	Spring & Fall	Penn-York Road (T-780) 75 meters upstream of culvert	41.98350	-75.34450	10/20
292	Bowers Creek	NE	REF	2/yr	riffle-run	Spring & Fall	Just upstr Hypsy Gap Road (T-633)	41.00270	-75.41010	10/20
294	Slate Run	NC	REF	2/yr	riffle-run	Spring & Fall	Upstr of walking bridge; 0.25 mi from mouth of Slate Run	41.47100	-77.50663	10/20

WQN	Stream Name	REG	Station Type	Sampling Frequency	Protocol	Season	Sampling Location	Lat	Long	Start Date (M/YY)
296	Sibbald Run	NW	REF	2/yr	riffle-run	Spring & Fall	60 yds upstr of Rt 62 rd crossing	41.53602	-79.43326	10/20
297	Stanton Brook	NW	REF	2/yr	riffle-run	Spring & Fall	Stanton Brook Rd crossing	41.73510	-78.55036	10/20
301	Susquehanna Rvr - Danville	NC	SS	2/yr	semi-wadeable lg river	summer/fall	SR54 Br	40.95770	-76.62120	7/62
302	Susquehanna Rvr - Wilkes-Barre	NE	SS	2/yr	riffle-run	Sept & Nov	450 m upstr of N Street Br	41.25632	-75.87668	5/62
305	Susquehanna Rvr - Towanda	NC	SS	2/yr	riffle-run	Sept & Nov	Just upstream of Rt 6	41.76303	-76.43919	5/62
306	Susquehanna Rvr - Ny	NE	STN	2/yr	riffle-run	Sept & Nov	approx. 2.4 miles upstr of Rt 81	41.95720	-75.70630	5/62
308	Fishing Crk	NC	STN	odd year	riffle-run	Nov-Apr	1 mile upstr of SR42 Br (Mall Blvd); first riffle upstr of Railroad St Br	41.00390	-76.46131	5/62
309	Nescopeck Crk	NE	STN	odd year	riffle-run	Nov-Apr	700 ft upstr of SR339 Br	41.04561	-76.22166	5/62
313	Lackawanna Rvr	NE	STN	odd year	riffle-run	Nov-Apr	250 ft dwnstr from Bridge Street Br near gage	41.35907	-75.74490	5/62
317	Tunkhannock Crk	NE	SS	annual	riffle-run	Nov-Apr	350 ft dwnstr from SR 6 Br near USGS gage	41.55633	-75.89350	5/62
318	Towanda Crk	NC	STN	odd year	riffle-run	Nov-Apr	First riffle dwnstr of USGS gage	41.70667	-76.48460	5/62
323	Susquehanna Rvr	NE	STN	2/yr	riffle-run	Sept & Nov	900 m dwnstr of Rt 92	41.45426	-75.85116	11/68
324	Tioga Rvr	NC	STN	odd year	riffle-run	Nov-Apr	First riffle dwnstr from T773 Br (jct Cross Road)	41.95727	-77.11577	9/70
333	Sugar Crk	NC	STN	odd year	riffle-run	Nov-Apr	340 yds upstr of Pine Valley Rd Br	41.78365	-76.50214	1/88
334	Wyalusing Crk	NC	STN	odd year	riffle-run	Nov-Apr	250 ft dwnstr of SR 706 Br	41.69652	-76.23010	1/88
341	Tioga Rvr	NC	STN	odd year	riffle-run	Nov-Apr	Along T754 (Brooklyn Rd) at USGS gage; 0.6 miles upstr of Rt 6 Br	41.79699	-77.08020	10/10

WQN	Stream Name	REG	Station Type	Sampling Frequency	Protocol	Season	Sampling Location	Lat	Long	Start Date (M/YY)
343	Cowanesque Rvr	NC	STN	odd year	riffle-run	Nov-Apr	First riffle dwnstr of Rt 49 (Main St) Br	41.98789	-77.30247	11/15
401	W Br Susquehanna Rvr - Lewisburg	NC	SS	2/yr	semi-wadeable lg river	summer/fall	Just upstream of Rt 642 between Milton and West Milton	41.01892	-76.86078	7/62
402	W Br Susquehanna Rvr - Williamsport	NC	STN		No Bio Sampling		n/a			3/50
404	W Br Susquehanna Rvr - Karthaus	NC	SS	2/yr	semi-wadeable lg river	summer/fall	400 m dwnstr of Rt 879	41.11970	-78.10510	1/50-11/87 & 10/04
406	W Br Susquehanna Rvr - Bower	NC	STN	2/yr	semi-wadeable lg river	summer/fall	300 m upstr of T418 (Camp Corbly Rd) Br	40.89596	-78.67677	6/62
408	Loyalsock Crk	NC	STN	odd year	riffle-run	Nov-Apr	350 ft upstr of Rt 973	41.32618	-76.91227	5/62
409	Lycoming Crk	NC	REF	annual	riffle-run	even Spring/odd Fall	60 ft below T840 Br	41.41825	-77.03366	5/62
410	Pine Crk - Waterville	NC	SS	annual	riffle-run	Nov-Apr	First riffle dwnstr of Railroad Br	41.28364	-77.32072	5/62
413	Bald Eagle Crk	NC	STN	odd year	riffle-run	Nov-Apr	100 ft dwnstr of SR1003 (LR14010) Curtin Rd Br - Curtin	40.97503	-77.74225	5/62
415	Spring Crk	NC	STN	odd year	riffle-run	Nov-Apr	100 ft upstr of SR3001 (LR14040) (Hartles Br)	40.88963	-77.79375	3/50
418	Sinnemahoning Crk Nr Keating	NC	STN	odd year	riffle-run	Nov-Apr	2000 ft upstr from SR4002 (LR18001) Keating Mountain Rd Br	41.25978	-77.91439	3/50
420	Driftwood Br Sinnemahoning	NC	STN	odd year	riffle-run	Nov-Apr	100 ft dwnstr of SR3002 (LR12004) Sterling Run Rd Br	41.41290	-78.19706	3/50
422	Clearfield Crk	NC	STN	odd year	riffle-run	Nov-Apr	400 ft upstr of Old Erie Rd Br	40.97054	-78.40685	3/50
423	Beech Crk	NC	STN	odd year	riffle-run	Nov-Apr	350 ft dwnstr from SR150 N Eagle Valley Rd Br	41.07381	-77.59169	4/65
429	Little Pine Crk	NC	STN	odd year	riffle-run	Nov-Apr	3000 ft upstr from Rt 44 Br	41.31747	-77.36386	9/72
433	Fishing Crk	NC	STN	odd year	riffle-run	Nov-Apr	100 ft dwnstr of SR2004 (LR18007) 2nd Br upstr from Cedar Run	41.07493	-77.47791	9/72

WQN	Stream Name	REG	Station Type	Sampling Frequency	Protocol	Season	Sampling Location	Lat	Long	Start Date (M/YY)
434	Kettle Crk	NC	STN	odd year	riffle-run	Nov-Apr	Off SR4001 (LR18003) Kettle Creek Rd; 3.2 miles upstr from PA SR120	41.31944	-77.87385	9/72
439	Bennetts Br Sinnemahoning	NC	STN	odd year	riffle-run	Nov-Apr	100 ft upstr of T343 (Karthaus Mountain Rd) Br near Castle Garden	41.33355	-78.13578	8/76
444	Muncy Crk	NC	STN	odd year	riffle-run	Nov-Apr	200 ft dwnstr of USGS Gage	41.20802	-76.75305	1/88
445	Bald Eagle Crk	NC	SS	annual	riffle-run	Nov-Apr	Riffle between bridges	41.13241	-77.40634	1/88
448	W Br Susquehanna Rvr - Jersey Shore	NC	SS	2/yr	semi-wadeable lg river	summer & fall	Approx 300 m dwnstr of SR 44 Br	41.20400	-77.25200	10/04
449	Beech Crk	NC	STN	odd year	riffle-run	Nov-Apr	900 ft dwnstr of Iron Gate Rd Br	41.11140	-77.70187	10/10
451	E Fork Sinnemahoning	NC	STN	odd year	riffle-run	Nov-Apr	350 ft upstr SR3001 (E Fork Rd) Br	41.54216	-77.97867	10/10
452	Cedar Run	NC	REF	annual	limestone	Jan-May	0.5 miles upstr of SR 64 Br and 100 ft upstrm of private Br	41.07634	-77.48975	1/05 - 9/10; 10/20
458	Hoagland Run	NC	REF	annual	riffle-run	even Spring/odd Fall	25 ft upstr of Rt 973	41.32774	-77.12898	10/10
459	Kettle Crk	NC	REF	annual	riffle-run	even Spring/odd Fall	50 ft above gaging station	41.47591	-77.82580	10/10
462	Chillisquaque Crk	NC	SS	annual	riffle-run	Nov-Apr	300 ft dwnstr of SR 1025 Shakespeare Rd Br near Potts Grove	40.97444	-76.80124	11/12
465	Carbaugh Run	SC	REF	3/yr	riffle-run	Spring, Summer, & Fall	250 m upstr of Clear Run tributary	39.89740	-77.45113	10/15
500	East Branch Sideling Hill Creek	SC	REF	annual	riffle-run	even Fall/odd Spring	Above and below Amarnth Rd crossing	39.80976	-78.33564	6/21
601	Lake Erie	NW	STN	n/a	n/a	n/a	n/a			12/68
624	Lake Erie East	NW	STN	n/a	n/a	n/a	n/a			10/15
625	Lake Erie Central	NW	STN	n/a	n/a	n/a	n/a			10/15

WQN	Stream Name	REG	Station Type	Sampling Frequency	Protocol	Season	Sampling Location	Lat	Long	Start Date (M/YY)
632	Presque Isle Bay	NW	STN	n/a	n/a	n/a	n/a			7/75
643	Conneaut Crk	NW	STN	odd year	riffle-run	Nov-Apr	300 ft upstr from SR3001 (LR25001) Br (Griffey Rd)	41.91806	-80.46689	1/88
644	Walnut Crk	NW	STN	odd year	riffle-run	Nov-Apr	250 ft dwnstr of Manchester Rd Br at the USGS gage	42.07393	-80.23511	11/08
701	Monongahela Rvr	SW	STN		No Bio Sampling		No Bio Sampling - Removing Plate Sampler Locations			4/50
702	Monongahela Rvr @ Charleroi	SW	STN		No Bio Sampling		No Bio Sampling - Removing Plate Sampler Locations			4/50
706	Youghiogheny Rvr @ Sutersville	SW	STN	odd year	riffle-run	Aug-Sept	400 ft dwnstr of 1st Street Br	40.24146	-79.80357	4/50
709	Youghiogheny Rvr @ Confluence	SW	STN	odd year	riffle-run	Aug-Sept	350 ft dwnstr of dam	39.80288	-79.36693	4/50
713	South Fork Tenmile	SW	STN	odd year	riffle-run	Nov-Apr	Riffle upstr from Rt 188 Jefferson Rd Br	39.92253	-80.07229	4/50-1/88 & 10/10
714	Dunkard Crk	SW	STN	odd year	riffle-run	Nov-Apr	350 ft upstr from SR2012 (Bobtown Hill Rd) Br	39.76092	-79.97164	4/50
725	Monongahela Rvr @ Pt Marion	SW	STN		No Bio Sampling		No Bio Sampling - Removing Plate Sampler Locations			4/75
726	Casselman Rvr	SW	STN	odd year	riffle-run	Nov-Apr	Casselman River Br St. Park in Grantsville (Rt 40)	39.69656	-79.14338	1/88
728	Whiteley Crk	SW	STN	odd year	riffle-run	Nov-Apr	Riffle 150 ft upstr of Rt 19 Br	39.80223	-80.11778	1/88-10/98 & 10/10
732	Monongahela Rvr @ Elizabeth	SW	STN		No Bio Sampling		No Bio Sampling - Removing Plate Sampler Locations			10/10
733	Mill Run	SW	REF	annual	riffle-run	even Spring/odd Fall	150 ft dwnstr of Quebec Rd	39.76334	-79.66359	3/05
734	Jones Mill Run	SW	REF	2/yr	riffle-run	Spring & Fall	300 ft upstr of T551 (Laurel Hill Park Rd) Br - Bakersville	40.00346	-79.23851	10/10
735	UNT House Run	SW	REF	annual	riffle-run	even Spring/odd Fall	Upstr from State Hwy 3020 (Bristoria Rd)	39.85401	-80.32995	11/13

WQN	Stream Name	REG	Station Type	Sampling Frequency	Protocol	Season	Sampling Location	Lat	Long	Start Date (M/YY)
736	N Fork Dunkard Fork	SW	STN	odd year	riffle-run	Nov-Apr	15 ft upstr of Riggs Rd Br	39.87857	-80.42853	10/15
737	Enlow Fork	SW	STN	odd year	riffle-run	Nov-Apr	500 ft upstr of W Finley Rd Br	39.969352	-80.447611	10/15
738	Tenmile Crk	SW	STN	odd year	riffle-run	Nov-Apr	Dwnstr of Center St Br; upstr of South Fork confl	39.97521	-80.04179	10/15
739	Monongahela River @ Masontown	SW	STN		No Bio Sampling		No Bio Sampling - Removing Plate Sampler Locations			10/15
801	Allegheny Rvr @ Natrona	SW	STN		No Bio Sampling		No Bio Sampling - Removing Plate Sampler Locations			3/50
805	Allegheny Rvr @ W Hickory	NW	STN	even year	riffle-run	Aug-Sept	300 ft dwnstr of SR 127 Br along LEW	41.56908	-79.40444	5/62
807	Allegheny Rvr @ Eldred	NW	STN	even year	riffle-run	Aug-Sept	600 ft upstr from SR 346 (Prentisvale Rd) Br	41.96330	-78.38630	5/62
809	Kiskiminetas Rvr	SW	STN	even year	riffle-run	Nov-Apr				4/50 - 1/88 & 10/20
810	Conemaugh Rvr	SW	STN	even year	riffle-run	Nov-Apr	1000 ft dwnstr of Tunnelton Rd Br	40.45589	-79.39442	3/50
819	Mahoning Crk	SW	STN	even year	riffle-run	Nov-Apr	400 ft dwnstr from T748 Br along LEW	40.92738	-79.29196	3/50-6/95 & 10/10
820	Redbank Crk	NW	STN	even year	riffle-run	Nov-Apr	400 ft dwnstr from T468 (St. Charles Rd) Br - St. Charles	40.99499	-79.39450	3/50
821	Clarion Rvr	NW	STN	even year	riffle-run	Nov-Apr	USGS Gage 1800 ft below dam	41.19278	-79.44056	3/50-11/87 & 10/10
822	Clarion Rvr	NW	STN	even year	riffle-run	Nov-Apr	150 ft upstr from SR36 Br	41.33148	-79.20816	3/50
830	Tionesta Crk	NW	STN	even year	riffle-run	Nov-Apr	500 ft dwnstr from SR1003 (LR27015) Blue Jay Rd Br	41.60059	-79.05019	5/62
831	Brokenstraw Crk	NW	STN	even year	riffle-run	Nov-Apr	500 ft upstr from Main Street Br - Youngsville	41.85100	-79.31931	6/62
832	Conewango Crk	NW	STN	even year	riffle-run	Nov-Apr	400 ft dwnstr from SR1012 (Akeley Hollow/Hungry Hollow Rd) Br - Russell	41.93761	-79.13430	6/62

WQN	Stream Name	REG	Station Type	Sampling Frequency	Protocol	Season	Sampling Location	Lat	Long	Start Date (M/YY)
845	French Crk @ Sugarcreek	NW	STN	even year	riffle-run	Nov-Apr	300 ft dwnstr from SR322 (13th St Br)	41.40142	-79.82966	2/73
846	French Crk @ Meadville	NW	STN	even year	riffle-run	Nov-Apr	just dwnstr of Wilson Chutes Rd Br (SR2008)	41.58917	-80.15028	2/73
858	Tunungwant Crk	NW	STN	even year	riffle-run	Nov-Apr	200 ft dwnstr from SR346 (LR42012) Bolivar Drive Br	41.97759	-78.62459	3/73
860	Allegheny Rvr	NW	STN	even year	riffle-run	Nov-Apr	E Mill St Br	41.80778	-78.28806	10/10
861	Mahoning Crk	NW	STN	even year	riffle-run	Nov-Apr	600 ft dwnstr from Gresock Rd Br - Sportsburg	40.92261	-79.00831	1/88
866	Allegheny Rvr Nr Warren	NW	STN	even year	riffle-run	Aug-Sept	SR6 Br Nr Warren (Glade Br)	41.82339	-79.11632	1/88
867	Allegheny Rvr @ Kennerdell	NW	STN	even year	riffle-run	Aug-Sept	750 ft dwnstr from Kenerdell Rd Br on LEW	41.26293	-79.83829	1/88
868	Oil Crk	NW	STN	even year	riffle-run	Nov-Apr	300 ft upstr from SR 8 (William Flinn Hwy) Br	41.48283	-79.69417	1/88
884	Allegheny Rvr @ Kittanning	SW	STN		No Bio Sampling		No Bio Sampling - Removing Plate Sampler Locations			12/06
885	Piney Run	SW	REF	annual	riffle-run	even Fall/odd Spring	Dwnstr of the bridge/culvert crossing	40.14449	-78.74025	6/21
901	Ohio Rvr Nr East Liverpool	SW	STN		No Bio Sampling		No Bio Sampling - Removing Plate Sampler Locations			5/62
902	Ohio Rvr @ Sewickley	SW	STN		No Bio Sampling		No Bio Sampling - Removing Plate Sampler Locations			3/50
903	Raccoon Crk	SW	STN	odd year	riffle-run	Nov-Apr	300 ft dwnstr from Moffett Run Rd Br	40.62841	-80.33818	4/50
905	Beaver Rvr	SW	STN		No Bio Sampling		No Bio Sampling - Removing Plate Sampler Locations			4/50
906	Beaver Rvr	NW	STN	odd year	riffle-run	Aug-Sept	300 ft dwnstr from State Highway 288 (Wampum Rd) Br	40.88838	-80.33554	4/50-9/03 & 10/10
907	Connoquenessing Crk	SW	STN	odd year	riffle-run	Aug-Sept	350 ft dwnstr from SR 1005 (Cella Rd) Br	40.81722	-80.24310	4/50

WQN	Stream Name	REG	Station Type	Sampling Frequency	Protocol	Season	Sampling Location	Lat	Long	Start Date (M/YY)
909	Shenango Rvr @ New Castle	NW	STN	odd year	riffle-run	Aug-Sept	500 ft dwnstr from W Washington St Br	40.99825	-80.35059	5/62
910	Shenango Rvr @ Sharpsville	NW	STN	odd year	riffle-run	Nov-Apr	500 ft upstr from SR388 Kelly Rd/E High St Exd Br	41.26693	-80.47108	4/50
911	Shenango Rvr Nr Pymatunning Dam	NW	STN	odd year	riffle-run	Nov-Apr	small area of cobble just below the dam outflow	41.49727	-80.46125	5/62
913	Little Shenango Rvr	NW	STN	odd year	riffle-run	Nov-Apr	50 ft dwnstr from SR4006 (LR43135) Williamson Rd Br	41.42603	-80.37368	5/62
915	Mahoning Rvr	NW	STN	odd year	riffle-run	Nov-Apr	900 ft dwnstr from SR 224 (West State St) Br	41.01748	-80.43773	11/64
917	Connoquenessing Crk	NW	STN	odd year	riffle-run	Nov-Apr	1000 ft dwnstr from Meridian Rd Br	40.80738	-79.96797	8/72
922	Slippery Rock Crk	NW	STN	odd year	riffle-run	Nov-Apr	700 ft upstr from Van Gorder Mill /Camp Allegheny Rd Br	40.88484	-80.23147	3/73

D.4 DISCONTINUED STATIONS

DEP WATER QUALITY NETWORK (WQN) – DISCONTINUED STATIONS REV. 11/2022

WQN	Stream Name	Station Type	Period of Record (M/YY)	SAC	REG	Year Inac-tivated	Location	Municipality	County	Lat	Long	USGS Gage	Drain Area (Sq Mi)
102	Delaware Rvr		7/62 - 7/87		NC	1987	Suspension Br - Riegelsville	Riegelsville	Bucks	40.59389	-75.19111	1457500	6178
106	W Br Brandywine Crk		7/62 - 11/87		SE	1987	Northbrook Rd Br	W Bradford Twp	Chester	39.92306	-75.68806	1480617	125
107	E Br Brandywine Crk		7/62 - 12/73		SE	1973	Shaws Br (Rt 842) 0.5 miles NE of Wawaset	Wawaset	Chester	39.92528	-75.64861	1480950	123
108	E Br Brandywine Crk		7/62 - 11/87		SE	1987	25 yds dwnstr of LR15141 Br	Downingtown	Chester	40.03472	-75.70861	1480700	60
109	Chester Crk		7/62 - 10/74		SE	1974	Jct of Bridgewater & Creek Rds	Chester	Delaware	39.86417	-75.39750	1477010	61
112	Schuylkill Rvr		7/62 - 1/88		SE	1988	Bingamen St Br	Reading	Berks	40.32528	-75.93333	1471530	907
114	Schuylkill Rvr		7/62 - 12/87		NE	1987	Palo Alto Br - Pottsville	Pottsville	Schuylkill	40.68361	-76.18667	1467500	54
118	Maiden Crk		7/62 - 2/95		SC	1995	SR061 Br Nr Reading Water Works	Ontelaunee Twp	Berks	40.42444	-75.94444	1470759	216
119	Little Schuylkill Rvr		7/62 - 12/87		NE	1987	Rt 443 Br - 0.3 miles W of Rt 29	Walker Twp	Schuylkill	40.75444	-75.94667	1469710	69
120	W Br Schuylkill Rvr		7/62 - 12/87		NE	1987	Rt 183 Br	Cressona	Schuylkill	40.63417	-76.19194	1467950	49
122	Tohickon Crk		7/62 - 11/87		SE	1987	Dwnstr Fr Stover Mill on T413	Bedminster Twp	Bucks	40.43361	-75.11694	1459500	98
124	Lehigh Rvr		7/62 - 12/87		NE	1987	Hill To Hill Br on Rt 191	Bethlehem	Northampton	40.61444	-75.38472	1453000	1227
125	Lehigh Rvr	STN	7/62 - 8/18	10	NE	2018	Rt 145 Br - Treichlers	Walnutport	Northampton	40.75307	-75.60262	01451000	888
127	Saucon Crk		7/62 - 12/87		NE	1987	LR48010 Br	Bethlehem	Northampton	40.61972	-75.33639	1454653	58
128	Monocacy Crk		7/62 - 11/87		NE	1987	Br @ mouth on West Lehigh St	Bethlehem	Northampton	40.61694	-75.38111	1452600	49
129	Little Lehigh Crk		7/62 - 12/95		NE	1995	Robin Hood Br - Little Lehigh Parkway	Allentown	Lehigh	40.58222	-75.48361	1451500	82
132	Pohopoco Crk		7/62 - 11/87		NE	1987	T163 Spur Br @ mouth	Parryville	Carbon	40.81583	-75.67250	1450020	111
133	Nesquehoning Crk		7/62 - 11/87		NE	1987	Mouth 1.5 miles below Rt 29	Mauch Chunk Twp	Carbon	40.87528	-75.76306	1448901	34
134	Black Crk		7/62 - 11/87		NE	1987	RXR Br below confl with Quakake Crk	Weatherly	Carbon	40.93111	-75.82222	1448850	55
135	Bushkill Crk		7/62 - 12/87		SE	1987	Rt 611 Br	Easton	Northampton	40.69583	-75.20500	1446990	80
136	Martins Crk		7/62 - 11/87		SE	1987	RXR Br @ mouth	Lower Mt Bethel	Northampton	40.77833	-75.17667	1446655	45
140	Lackawaxen Rvr		5/62 - 11/87		NE	1987	Park St Br	White Mills	Wayne	41.52500	-75.20528	1430200	197
143	Schuylkill Rvr		7/72 - 2/76		SE	1976	Strawberry St Br Nr Belmont Filter Plant	Philadelphia	Philadelphia	39.99500	-75.19444	1474500	1883
144	Cacoosing Crk		10/72 - 12/87		SE	1987	T501 Br @ mouth	Spring Twp	Berks	40.36611	-75.99472	1470982	22
145	Saucon Crk		10/72 - 12/87		SE	1987	LR48001 Br	Lower Saucon Twp	Northampton	40.55778	-75.36222	1454510	30

WQN	Stream Name	Station Type	Period of Record (M/YY)	SAC	REG	Year Inac-tivated	Location	Municipality	County	Lat	Long	USGS Gage	Drain Area (Sq Mi)
146	Monocacy Crk		10/72 - 11/87		SE	1987	Saint Anne Shrine PA Rt 191	Lower Nazareth Twp	Northampton	40.68694	-75.34056	1452400	33
148	Delaware Rvr		1/76 - 11/02		NE	2002	RXR Br @ PPL Martin's Crk Power Plant	Lwr Mt Bethel Twp	Northampton	40.78917	-75.11639	1446500	4552
151	Brandywine Crk		11/72 - 8/73		SE	1973	Along dirt Rd approx .25 miles upstr Fr Pa Rt 100 Br	Pennsburg Twp	Chester	39.86917	-75.59306		292
152	E Br Brandywine Crk		4/73 - 11/87		SE	1987	10 yds dwnstr Fr Jefferies Br	East Bradford Twp	Chester	39.93889	-75.63694	1480870	121
153	W Br Brandywine Crk		5/73 - 8/73		SE	1973	Northbrook Rd Br	W.bradford Twp	Chester	39.92611	-75.66306		135
155	French Crk		11/72 - 12/87		SE	1987	20 yds upstr Fr Br; 0.25 miles dwnstr Fr Wilson's Corner	E Vincent Twp	Chester	40.15139	-75.60167	1472157	59
156	French Crk	REF	1/00-12/04		SE	2004	30 yds dwnstr of Coventry Rd Br	East Nantmeal Twp	Chester	40.17139	-75.69056		37
157	E Br Chester Crk		11/72 - 11/87		SE	1987	10 yds upstr Fr Br on Cheyney Woods Rd	Thornbury Twp	Delaware	39.92917	-75.53333	1476848	20
158	Ridley Crk		11/72 - 11/87		SE	1987	150 yds dwnstr Fr jct with waterville Rd & Cobblestone Rd	Chester Twp	Delaware	39.87444	-75.38222	1476510	34
159	Darby Crk		4/73 - 11/87		SE	1987	15 yds dwnstr Fr Watertower @ Garret Tract	Upper Darby Twp	Delaware	39.93444	-75.30000	1475470	35
160	Wissahickon Crk		4/73 - 12/87		SE	1987	85 yds dwnstr Fr Mt Pleasant Ave Br	Whitpain Twp	Montgomery	40.15806	-75.23306	1473895	19
161	Perkiomen Crk		4/73 - 5/74		SE	1974	20 yds dwnstr Fr dam along Rt 29; 0.75 miles N of Rt 422	Perkiomen Twp	Montgomery	40.22944	-75.45194	1473000	279
162	E Br Perkiomen Crk		4/73 - 11/87		SE	1987	20 yds dwnstr Fr Gardes Rd Br	Lower Salford Twp	Montgomery	40.25583	-75.44417	1472820	61
163	Skippack Crk		4/73 - 11/87		SE	1987	20 yds dwnstr Fr old abutment to Br on Arcola Rd	L Providence Twp	Montgomery	40.15028	-75.44611	1473140	56
164	Frankford Crk		11/72 - 11/87		SE	1987	120 yds upstr Fr Castor Ave Br	Philadelphia	Philadelphia	40.01667	-75.11139	1467087	30
165	Pennypack Crk		11/72 - 1/88		SE	1988	70 yds upstr Fr Frankford Ave Br	Philadelphia	Philadelphia	40.04389	-75.02083	1467048	54
166	Pennypack Crk		4/73 - 1/88		SE	1988	100 yds dwnstr Fr Rt 63 Br (Welsh Rd)	Lower Moreland Twp	Montgomery	40.12056	-75.07250		28
167	Neshaminy Crk		1/73 - 1/88		SE	1988	50 yds dwnstr Fr Br on Alm House Rd	Doylestown Twp	Bucks	40.28667	-75.16056	1465500	69
168	Neshaminy Crk		1/73 - 8/73		SE	1973	Rt 213 Br	Langhorne	Bucks	40.17389	-74.95722	1465500	209
170	Tohickon Crk		1/73 - 8/73		SE	1973	Dwnstr Fr Stover Mill on T413	Bedminster Twp	Bucks	40.43361	-75.11694	1459500	98
171	Tohickon Crk		1/73 - 11/87		SE	1987	Upstr Fr covered Br on Covered Br Rd	Haycock Twp	Bucks	40.45500	-75.27944	1459182	34
172	Chester Crk		4/73 - 1/88		SE	1988	35 yds dwnstr Fr Dutton Mills Rd Br	Chester Twp	Delaware	39.86750	-75.40861	1477000	61
173	Wissahickon Crk		6/86 - 6/86		SE	1986	Bells Mill Rd Br	Philadelphia	Philadelphia	40.07917	-75.22861		53
174	Delaware Rvr		2/76 - 7/88		NE	1988	SR1023 Nr Lordville	Manchester Twp	Wayne	41.86750	-75.21500	1427510	748
175	Tulpehocken Crk		11/75 - 12/87		SE	1987	Off T501 (Water Rd); 1.1 miles upstr Fr Plum Crk	L Heidelberg Twp	Berks	40.36833	-76.02278	1470960	179
176	Delaware Rvr		7/79 - 2/96		NE	1996	SR080 (I80) Br Nr Del Water Gap	Smithfield Twp	Monroe	40.97722	-75.13583	1440200	3845
177	Northkill Crk	REF	1/88 - 7/98		SC	1998	T528/720 (Northkill Rd) Nr Shartlesville	Upper Bern Twp	Berks	40.53389	-76.12528		2

WQN	Stream Name	Station Type	Period of Record (M/YY)	SAC	REG	Year Inac-tivated	Location	Municipality	County	Lat	Long	USGS Gage	Drain Area (Sq Mi)
179	E Br White Clay Crk	REF	1/88 - 7/98		SE	1998	T355 (Spencer Rd.) Nr London Grove	W Marlborough Twp	Chester	39.85833	-75.78361		3
180	Lizard Crk		1/88 - 3/88		NE	1988	T334 Nr Germans	E Penn Twp	Carbon	40.76278	-75.76333		35
184	Brushy Run	REF	5/88 - 9/97		NE	1997	Along private Rd; 3500' above mouth	Walker Twp	Schuylkill	40.73750	-75.99194		1
186	Tinicum Crk	REF	8/98-12/04		SE	2004	Headquarters Rd; SR1012	Tinicum Twp	Bucks	40.47056	-75.13722	1458900	15
187	Cooks Crk	REF	8/98-12/04		SE	2004	Rattlesnake Rd & Durham Rd; Rt212 lower intersection	Durham Twp	Bucks	40.58139	-75.20528		29
188	Baldwin Run	REF	10/98 - 12/99		SE	1999	Concord Rd	Aston/chester Twp	Delaware	39.85611	-75.40583		1
189	Johnson Crk	REF	8/98 - 10/00		NE	2000	Along Crossman Rd 0.5 miles S of SR371	Mount Pleasant Twp	Wayne	41.73111	-75.38417		10
190	Choke Crk	REF	8/98-12/04		NE	2004	LR35086; Thornhurst Rd Br – Luz/Lack Co line	Lehigh Twp	Lackawanna	41.16167	-75.60278		2
192	Adams Crk	REF	8/98 - 12/04		NE	2004	US Rt 209 Br	Delaware Twp	Pike	41.23944	-74.86722		8
195	Rock Run	REF	3/05 - 9/10		SE	2010	Old SR23 Br	South Coventry Twp	Chester	40.17372	-75.69600		3
196	W. Br Perkiomen Crk	REF	3/05 - 9/10		SC	2010	250 M upstr of Anthony's Mill Rd Br; along Forgedale Rd	Washington Twp	Berks	40.40516	-75.61370		12
197	Blooming Grove Crk	REF	3/05 - 9/10		NE	2010	300 m upstr of T 494 Br	Lackawaxen Twp	Pike	41.43631	-75.10516		23
198	Tunkhannock Crk	REF	3/05 - 9/10		NE	2010	SR 4001 Br	Tunkhannock Twp	Monroe	41.03450	-75.46000		11
199	Kintz Crk	REF	3/05 - 9/10		NE	2010	20 m upstr of conf w/ Mozette Ck	Dennison Twp	Pike	41.28050	-75.24210		4
205	Conestoga Crk		12/66 - 12/87		SC	1987	500 ft below Water Works	Lancaster	Lancaster	40.05000	-76.27750	1576500	322
207	Codorus Crk		11/62 - 2/95		SC	1995	SR1012 (IR66021) Br Mundis Mill Rd)	Manchester Twp	York	40.01083	-76.71056	1575500	260
208	W Br Codorus Crk		6/62 - 12/87		SC	1987	T488 Br @ Martin	North Codorus Twp	York	39.88722	-76.83583	1574520	76
209	S Br Codorus Crk		6/62 - 12/87		SC	1987	York Water Co Pump Station	Spring Garden Twp	York	39.92056	-76.74917	1575000	117
211	Swatara Crk		7/62 - 8/04		SC	2004	SR441 Br	Middletown	Dauphin	40.19115	-76.73095	1573560	571
213	Conodoguinet Crk		6/62 - 2/95		SC	1995	SR1009 Br	Silver Spring Twp	Cumberland	40.25861	-77.07778	1570000	435
215	Juniata Rvr		7/62 - 11/87		SC	1987	LR31084 Br 0.3 miles upstr Fr USGS Gage	Mapleton	Huntingdon	40.39500	-77.94000	1563500	2027
216	Juniata Rvr		3/50 - 11/87		SC	1987	Twp Rd A-135 Br	Huntingdon	Huntingdon	40.48472	-78.01917	1559000	817
218	Little Juniata Rvr		3/50 - 12/87		SC	1987	Rt 220 Br	Antis Twp	Blair	40.62750	-78.29500	1556480	94
219	Bald Eagle Crk		3/50 - 11/87		SC	1987	0.2 miles upstr Fr Westvaco Paper	Snyder Twp	Blair	40.66694	-78.23389	1557550	161
220	Kishacoquillas Crk		6/62 - 11/87		SC	1987	SR522 Br (Walnut St)	Lewistown	Mifflin	40.60611	-77.56528	1565000	190
221	Aughwick Crk		1/50 - 4/71		SC	1971	Rt 994 Br above confl with Three Springs Crk	Cromwell Twp	Huntingdon	40.21250	-77.92556	1564500	174
222	Raystown Br Juniata Rvr		3/50 - 1/73		SC	1973	Br @ Jct of Twp Rd 430/431	Juniata Twp	Huntingdon	40.42639	-78.02972	1563000	8

WQN	Stream Name	Station Type	Period of Record (M/YY)	SAC	REG	Year Inac-tivated	Location	Municipality	County	Lat	Long	USGS Gage	Drain Area (Sq Mi)
225	Wiconisco Crk		7/62 - 11/87		SC	1987	Rt 147 Br Nr mouth	Millersburg	Dauphin	40.53722	-76.96083	1555600	116
227	Mahanoy Crk		7/62 - 8/75		NC	1975	Rt 147 Br Nr mouth	Jackson Twp	Northumberland	40.72611	-76.83778	1555252	157
230	Shamokin Crk		7/62 - 11/87		NC	1987	Foot Br @ USGS Gage	Shamokin Twp	Northumberland	40.81028	-76.58444	1554500	55
231	Conestoga Rvr		6/62 - 8/04		SC	2004	T561 Iron Br Nr Rock Hill	Manor Twp	Lancaster	39.96139	-76.36583	1576754	402
232	Susquehanna Rvr		9/70 - 12/75		SC	1975	Rt 372 Br - Holtwood	Martic Twp	Lancaster	39.81694	-76.32333	1576990	26799
233	Standing Stone Crk		3/50 - 6/61		SC	1961	Old Rt 22 below Water Treatment Plant (SR1010)	Huntingdon	Huntingdon	40.52361	-77.97083	1559500	129
234	Dunning Crk		3/50 - 5/61		SC	1961	N of Jct LR05051 & T478 Nr Belden	Bedford Twp	Bedford	40.07167	-78.49278	1560000	172
235	Brush Crk		3/50 - 5/61		SC	1961	@ Gapsville	E Providence Twp	Bedford	39.95556	-78.25417	1561000	37
236	Great Trough Crk		3/50 - 6/61		SC	1961	Forest Rd Nr mouth (Rothrock State Forest)	Todd Twp	Huntingdon	40.35000	-78.13056	1562500	85
237	Mill Crk		10/72 - 12/87		SC	1987	LR36009 Br (Mascot)	Leacock Twp	Lancaster	40.06278	-76.15694	1576515	20
238	Quittapahila Crk		10/72 - 12/87		SC	1987	LR38003 Br	N Annville Twp	Lebanon	40.35056	-76.61444	1573205	77
239	Mountain Crk		3/73 - 11/87		SC	1987	Confl with Yellow Breeches Crk	S Middleton Twp	Cumberland	40.14472	-77.17861	1571197	63
240	Conodoguinet Crk		3/73 - 08/04		SC	2004	SR11 (PA Rts 11&15) Br Nr mouth	West Fairview Twp	Cumberland	40.27111	-76.91472	1570280	507
241	Middle Spring Crk		3/73 - 11/87		SC	1987	T312 Br	Southampton Twp	Cumberland	40.08528	-77.54306	1569320	43
242	Middle Crk		9/72 - 11/87		NC	1987	Rt 522 & 104 Br	Middleburg	Snyder	40.78861	-77.01167	1555205	128
243a	Sherman Crk	REF	3/73 - 7/98		SC	1998	SR2002 (IR50014) Br	Penn Twp	Perry	40.37861	-77.08222	1568000	
244	Little Juniata Rvr		3/73 - 11/87		SC	1987	Confl with Susquehanna Rvr	Duncannon	Perry	40.38889	-77.03222	1567350	30
245	Tuscarora Crk	REF	1/72 - 7/98		SC	1998	SR075 Br	Port Royal	Juniata	40.52778	-77.38389	1566000	270
246	Jacks Crk		11/72 - 11/87		SC	1987	SR022 Br	Lewistown	Mifflin	40.58528	-77.55750	1565515	60
247	Kishacoquillas Crk		11/72 - 11/87		SC	1987	LR44002 Br	Derry Twp	Mifflin	40.63111	-77.56694	1565300	165
248	Honey Crk		11/72 - 11/87		SC	1987	@ mouth	Reedsville	Mifflin	40.66222	-77.59444	1564995	94
250	Dunning Crk		10/72 - 11/87		SC	1987	Rxr Br off T-477 Nr mouth	Bedford Twp	Bedford	40.02389	-78.47750	1560000	197
251	Bobbs Crk		10/72 - 11/87		SC	1987	LR05060 Br - Reynoldsville	E St Clair Twp	Bedford	40.14722	-78.55583	1559920	65
253	Kishacoquillas Crk		11/72 - 11/87		SC	1987	Rt 322 Br	Brown Twp	Mifflin	40.65472	-77.58333	1565000	163
254	Deer Crk		3/73 - 11/75		SC	1975	T542 Br	Shrewsbury Twp	York	39.72306	-76.61278	1578320	12
255	Ebaughs Crk		11/74 - 12/87		SC	1987	4200 ft dwnstr Fr The Stewardstown STP	Hopewell Twp	York	39.74556	-76.60556	1579850	5
257	Swatara Crk		2/76 - 11/87		NE	1987	Spittler Rd Br 0.1 miles E of Rt 125	Pine Grove Twp	Schuylkill	40.57500	-76.40278	1571824	44

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258	Mahonoy Crk		11/75 - 11/87		NC	1987	Br on City Rd 1.8 miles NW of Herndon	Jackson Twp	Northumberland	40.72444	-76.81583	1555251	155
260	Conodoguinet Crk		1/88 - 2/95		SC	1995	T427 Br dwnstr Fr Greider	L Frankford Twp	Cumberland	40.22111	-77.25861	1569500	347
261	W Br Octoraro Crk	REF	1/88 - 2/95		SC	1995	SR472 Br Nr Black Rock	Colerain Twp	Lancaster	39.85972	-76.11639		24
262	Mountain Crk	REF	1/88 - 7/98		SC	1998	T340 @ Pine Grove Furnace	Cooke Twp	Cumberland	40.02556	-77.31806		13
264	Kishacoquillas Creek		1/88 – 2/95		SC	1995	SR3001 (Main St) Bridge	Lewistown Boro	Mifflin	40.59599	-77.57317		191
265	Rambo Run	REF	8/98 - 9/03		SC	2003	T632 Crossing	E Hopewell Twp	York	39.82194	-76.53306		8
266	Little Conestoga Crk	REF	8/98 - 1/00		SC	2000	Br on SR3027	Manor Twp	Lancaster	39.97111	-76.37556		62
267	Conewago Crk	REF	8/98 - 12/04		SC	2004	0.7 miles S of Rt234 on Bottom Rd; T363	Franklin Twp	Adams	39.93667	-77.36583		7
268	Penns Crk	REF	9/98 - 9/03		NC	2003	SR2012 Br @ Farmers Mills	Gregg Twp	Centre	40.86556	-77.60944		14
270	Letort Spring Run	REF	12/99 - 9/10		SC	2010	Bonny Brook Rd Br	South Middleton Twp	Cumberland	40.17585	-77.18588		4
274	Sinking Run	REF	3/05 - 9/10		SC	2010	First Br below Sinking Valley Church on SR1013	Tyrone Twp	Blair	40.61150	-78.18750		28
275	Conodoguinet Ck	REF	3/05 - 9/10		SC	2010	650 m upstr of SGL 76 Boundary	Letterkenny Twp	Franklin	40.06955	-77.75664		22
276	E Licking Crk	REF	3/05 - 9/10		SC	2010	First Br above Clearview Res on SR4002	Milford Twp	Juniata	40.54760	-77.52600		22
277	Elk Crk	REF	1/05 - 9/10		NC	2010	Upstr of UNT @ Spring Bank	Miles Twp	Centre	40.92449	-77.47575		31
279	Bobs Crk	REF	3/06 - 9/20	13	SC	2020	SR869 dwnstr of confl with Wallack's Br	Union Twp	Beford	40.26310	-78.59090	MANUAL	22
283	Spring Crk		11/12 - 5/13		SC	2013	Incinerator access Rd Nr intersection with S Cameron St	Harrisburg	Dauphin	40.24276	-76.85709	01571104	11
293	Cross Keys Run	REF	10/20 – 3/22	122	NE	2023	150 meters upstr of confl with Tobyhanna Crk	Coolbaugh Twp	Monroe	41.16320	-75.45450		2
303	Susquehanna Rvr		5/62 - 3/92		NE	1992	Coxton Rxr Br off SR 92; upstr Fr Scovell Island	Exeter Twp	Luzerne	41.35000	-75.80139	1536500	9904
304	Susquehanna Rvr		5/62 - 11/87		NE	1987	LR65041 Br E of Rt 6 & 309	Braintrim Twp	Wyoming	41.65000	-76.16111	1533205	8554
307	Catawissa Crk		5/62 - 1/88		NC	1988	Rt 42 Br	Catawissa Twp	Columbia	40.94722	-76.45583	1540348	150
310	Wapwallopen Crk	REF	5/62 - 10/00		NE	2000	RXR Br dwnstr Rt 239	Conyngham Twp	Luzerne	41.07139	-76.13389	1538000	53
311	Solomon Crk		5/62 - 11/87		NE	1987	Army Eng Flood Control Pumping Sta Nr Fallow St	Hanover Twp	Luzerne	41.23750	-75.94389	1537510	469
312	Toby Crk		5/62 - 12/87		NE	1987	Grove St Br Nr Rt 309	Pringle Boro	Luzerne	41.28083	-75.89611	1537000	33
314	Lackawanna Rvr		5/62 - 11/87		NE	1987	Ridge St Br Nr Main St	Archbald	Lackawanna	41.50417	-75.54306	1534520	109
315	Lackawanna Rvr		5/62 - 11/87		NE	1987	Wooden platform above spillway N of Rt 171 Br	Clifford Twp	Susquehanna	41.69750	-75.48556	1534300	36
316	Legetts Crk		5/62 - 11/87		NE	1987	Btwn Main St Br & Rxr Br	Scranton	Lackawanna	41.45417	-75.66222	1534850	15
319	Tioga Rvr		5/62 - 12/87		NE	1987	T667 Br	Tioga Twp	Tioga	41.90833	-77.12972	1518000	280

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320	Cowanesque Rvr	STN	5/62-9/03 & 10/04-9/15	610	NC	2015	US 15 Br N of Lawrenceville, NY	Lindley Twp	Steuben	42.00140	-77.12780	01520000	300
321	Crooked Crk		5/62 - 11/87		NE	1987	LR58113 Br	Tioga Twp	Tioga	41.91056	-77.13778	1518600	130
322	Susquehanna Rvr		11/68 - 12/87		NE	1987	SR309 Br	Tunkhannock	Wyoming	41.53472	-75.94861	1534060	8898
325	W Br Fishing Crk	REF	1/05 - 9/10		NC	2010	360 m upstr of SGL 13 boundary	Davidson Twp	Sullivan	41.30560	-76.43090		20
325a	Wapwallopen Crk		11/72 - 12/87		NE	1987	LR40022 Br	Dorrance Twp	Luzerne	41.09343	-76.00456	1537980	
326	Harveys Crk		8/72 - 12/87		NE	1987	SR11 Br	West Nanticoke	Luzerne	41.22111	-76.00694	1537650	46
327	Roaring Brook		9/72 - 11/87		NE	1987	Cedar Ave Br	Scranton	Lackawanna	41.40444	-75.65194	1535060	58
328	Bowman Crk		10/72 - 11/87		NE	1987	LR65005 Br	Eaton Twp	Wyoming	41.51472	-75.96139	1534055	118
329	S Br Tunkhannock Ck		10/72 - 11/87		NE	1987	Nr confl with Tunkhannock Crk	Tunkhannock Twp	Wyoming	41.56333	-75.87500	1533992	293
330	Lackawanna Rvr		2/76 - 11/87		NE	1987	Rxr Br Nr mouth	Pittston Twp	Luzerne	41.34389	-75.78694	1536103	348
331	Black Crk		2/76 - 11/87		NE	1987	Br upstr Fr confl with Nescopeck Crk	Black Creek Twp	Luzerne	41.00750	-76.16667	1538590	62
332	Chemung Rvr		2/76 - 11/05		NC	2005	SR4022 Br	Athens Twp	Bradford	41.98611	-76.55472	1531000	1195
335	Wysox Crk	REF	1/88 - 8/98		NC	1998	SR1027 Br	Wysox	Bradford	41.80250	-76.37444		95
336	Dyberry Crk	REF	2/88 - 8/88 3/01 - 12/04		NE	2004	SR4007 Br (Dug Rd) Nr Tanners Falls	Dyberry Twp	Wayne	41.65309	-75.28205		47
337	Bowman Crk	REF	1/88 - 7/98		NE	1998	SR3002 1.3 miles N of Stull	Noxen Twp	Wyoming	41.41583	-76.09083		35
338	Riley Crk	REF	10/98 - 12/99		NE	1999	Br on T309	Auburn Twp	Susquehanna	41.64917	-76.00750		16
339	Little Fishing Crk	REF	8/98 - 12/04		NC	2004	SR4033 Br; 200 ft upstr Fr Wolfhouse Run	Pine Twp	Columbia	41.16583	-76.50361		21
340	Unt Tunkanock Crk	REF	12/99 - 12/04		NE	2004	SR2046 Br - Gelatt	Gibson Twp	Susquehanna	41.80861	-75.58111		9
342	Wysox Crk	REF	10/10-9/15	13	NC	2015	Mercur Hill Rd Br	Wysox Twp	Bradford	41.78670	-76.38328	Manual	100
405	W Br Susquehanna Rvr		12/48 - 12/87		NC	1987	Rt 453 Br	Curwensville	Clearfield	40.96139	-78.51944	1541200	367
407	Muncy Crk		5/62 - 11/87		NC	1987	SR220 Br	Wolf Twp	Lycoming	41.24861	-76.71750	1552500	92
411	Pine Crk	REF	5/62-11/87 & 10/10-9/15	13	NC	2015	T762 Br - Cedar Run	Brown Twp	Lycoming	41.52089	-77.44781	01548500	601
412	Bald Eagle Crk		5/62 - 11/87		NC	1987	First Br approx 1 mile dwnstr Fr Foster Sayers Dam	Liberty Twp	Centre	41.05861	-77.59556	1547500	384
414	Marsh Crk		5/62 - 12/81		NC	1981	SR220 Br	Liberty Twp	Centre	41.06111	-77.61694	1547400	43
416	Logan Br Spring Crk		5/62 - 12/87		NC	1987	Willowbank St Br - Bellefonte	Bellefonte	Centre	40.90750	-77.78250	1546750	23
417	Kettle Crk		3/50 - 9/74		NC	1974	Rt 120 Br - Westport	Westport	Clinton	41.30056	-77.84111	1545020	247

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419	Frst Fork Sinnemahoning		5/62 - 1/05		NC	2005	SR120 Br Nr Sinnemahoning	Grove Twp	Cameron	41.32000	-78.08083	1544000	267
421	Moshannon Crk		3/50 - 11/87		NC	1987	Rxr Br upstr Fr SR970	Oceola	Clearfield	40.84944	-78.26806	1542000	69
424	Bald Eagle Crk		3/50 - 4/76		NC	1976	T325 Br	Beech Creek	Clinton	41.06528	-77.56750	1548000	558
425	Chillisquaque Crk		2/70 - 1/88		NC	1988	Rt 54 Br N of Washingtonville	Washingtonville	Montour	41.06111	-76.68056	1553700	52
426	Buffalo Crk		9/72 - 11/87		NC	1987	Rt 15 Br	Lewisburg	Union	40.97194	-76.89167	1553480	134
427	White Deer Crk	REF	9/72 - 8/98		NC	1998	SR1011 Br - White Deer	White Deer Twp	Union	41.07556	-76.87278	1553150	44
428	Loyalsock Crk	REF	10/72-8/98 & 10/10-9/15	15	NC	2015	SR 87 N of Hillsgrove	Hillsgrove Twp	Sullivan	41.45642	-76.68931	Manual	288
430	Pine Crk	REF	10/72 - 8/98		NC	1998	Off SR362 @ mouth of Darling Run	Shippen Twp	Tioga	41.73833	-77.43028	1548248	361
431	Pine Crk		10/72 - 12/87		NC	1987	0.5 miles dwnstr Fr LR52061 Br Nr Galeton	Pike Twp	Potter	41.73861	-77.62611	1548340	178
432	Chatham Run		9/72 - 8/75		NC	1975	First Br upstr Fr Rt 220 Br	Dunnstable Twp	Clinton	41.17278	-77.36694	1548095	14
435	Sandy Run		9/72 - 3/74		SW	1974	Nr mouth	Adams Twp	Cambria	40.34750	-78.78333	3040510	9
436	Chest Crk		10/72 - 12/87		NC	1987	Br on T324	Mahaffey	Clearfield	40.86833	-78.72056	1540823	128
437	Driftwood Br Sinnemahoning		3/73 - 11/87		NC	1987	500 yds upstr Fr Br on Rt 555	Driftwood Boro	Cameron	41.33806	-78.13583	1543000	317
438	Alder Run		5/76 - 11/87		NC	1987	Br on Co Rd - 2.2 miles NW of Keplertown	Graham Twp	Clearfield	41.01389	-78.19972	1541800	13
440	Anderson Crk		5/76 - 11/87		NC	1987	On Meadow St Br	Curwensville	Clearfield	40.97528	-78.53056	1541248	77
441	Moshannon Crk		5/76 - 11/87		NC	1987	Rt 53 Br 3 miles W of Moshannon	Snow Shoe Twp	Centre	41.03667	-78.05778	1542310	263
442	Marsh Crk		2/76 - 12/87		NC	1987	Upstr Fr confl with Pine Crk - Ansonia	Shippen Twp	Tioga	41.74639	-77.42722	1538310	81
443	Young Womans Crk	REF	7/80-8/98 & 10/10-9/15	15	NC	2015	0.3 miles dwnstr of Laurelly Fork	Chapman Twp	Clinton	41.38930	-77.69090	01545600	46
446	Muncy Creek	REF	8/98 - 12/04		NC	2004	SR2002 Br Nr mouth of Slip Run	Davidson Twp	Sullivan	41.35694	-76.53500		23
447	Spruce Run	REF	8/98 - 12/04		NC	2004	SR1001 Br 3.5 miles upstr Fr Res	West Buffalo Twp	Union	41.02389	-77.06528		7
450	Fishing Crk Nr Nittany	REF	1/05 - 9/10		NC	2010	1.2 miles upstr of Lower SR 2002 Br	Porter Twp	Clinton	40.98366	-77.51662		72
453	Cedar Run @ Mine Hole Run	REF	1/05 - 9/10		NC	2010	Most southern crossing of SR 3001	Morris Twp	Tioga	41.55490	-77.45900		26
454	First Fork Sinnemahoning Crk	REF	1/04 - 9/10		NC	2010	At Potter/Cameron Co Line	Wharton Twp	Potter	41.47539	-78.05051		205
455	Killbuck Run	REF	3/06 - 9/10		SW	2010	0.85 strm miles upstrm of SR1021 Br	Chest Twp	Cambria	40.66470	-78.58820		7
456	W Br Pine Crk	REF	10/10-9/15	15	NC	2015	Union St Br @ Galeton	Galeton Boro	Potter	41.73187	-77.65020	Manual	71
457	Blockhouse Crk	REF	10/10-9/15	15	NC	2015	SR 284 Nr English Center	Pine Twp	Lycoming	41.47367	-77.23083	01549500	38

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460	Mosquito Crk	REF	10/10-9/15	15	NC	2015	Upstr of Quehanna Hwy Br @ Karthaus	Karthaus Twp	Clearfield	41.12889	-78.13444	Manual	64
461	White Deer Crk	REF	10/10-9/15	15	NC	2015	Upstr of Rt 15 Br	White Deer Twp	Union	41.07472	-76.87278	01553140	44
463	Rock Run	REF	10/15-9/20	87, METH, SSC	NC	2020	800 m upstr of confl with Lycoming Crk	Mcintyre Twp	Lycoming	41.50202	-76.94449	Manual	28
464	Hyner Run	REF	10/15-9/20	87, METH, SSC	NC	2020	200 m upstr of Log Rd Hollow and 100 m dwnstr E Br Hyner	Chapman Twp	Clinton	41.36047	-77.62272	Manual	25
466	Porcupine Crk	REF	10/15-12/20	15	NW	2020	President Rd - President Township	President Twp	Venango	41.43929	-79.54554	Manual	12
467	Corey Crk	STN	12/15-9/20	610	NC	2020	50 meters upstr of Harris Rd (T818)	Sullivan Twp	Tioga	41.79088	-77.01507	01516500	12
501	Conococheague Crk	SS	6/62-9/14	12	SC	2014	Along MD SR494 approx 3 miles dwnstr Fr PA/MD border	Fairview	Washington Md	39.70810	-77.83330	01614500	499
502	Conococheague Crk		9/70 - 11/73		SC	1973	Rt 16 Br W of Greencastle	Greencastle Boro	Franklin	39.79194	-77.75444	1614170	249
503	Rock Crk		3/73 - 11/87		SC	1987	SR2035 Br W of Lake Heritage	Cumberland Twp	Adams	39.80472	-77.21167	1638890	23
504	E Br Antietam Crk		3/73 - 12/87		SC	1987	0.9 milea upstr Fr confl with Antietam Crk	Washington Twp	Franklin	39.72611	-77.59389	1618950	52
505	Licking Crk		1/88 – 1/05		SC	2005	SR456 Br – PA/MD border	Warren Twp	Franklin	39.72306	-78.06028	1613500	159
506	Little Wills Crk	REF	1/88 - 7/98		SC	1998	SR096 Br @ Bard	Harrison Twp	Bedford	39.92694	-78.66000	1600700	10
507	Rock Crk		1/88 - 2/95		SC	1995	SR3002 (IR01066) Br Nr Harney	Cumberland Twp	Adams	39.73000	-77.23028	1638950	60
508	Falling Spring Br	REF	8/98 - 12/99		SC	1999	Crossing of T515	Guilford Twp	Franklin	39.91309	-77.616322		4
509	Licking Crk	SS	12/04-9/14	12	SC	2014	Pectonville Rd Br	Pectonville, Md	Washington	39.67596	-78.04249	01613525	194
510	Tonoloway Crk	SS	4/05-9/14	12	SC	2014	300 yds upstr of Timber Ridge Rd (SR 2005) Br	Thompson Twp	Fulton	39.72833	-78.15194	01613095	111
511	Antietam Crk	SS	3/05-9/14	12	SC	2014	Upstr of Millers Church Rd crossing @ Rocky Forge, Md	Rocky Forge, Md	Washington	39.71639	-77.60730	01619000	94
512	Sideling Hill Crk	SS	11/04-9/14	12	SC	2014	Zeigler Rd Br; 4 miles S of Bellegrove, Md	Bellegrove, Md	Washington	39.64953	-78.34414	01610155	103
513	Town Crk	SS	10/06-9/14	12	SC	2014	Pack Horse Rd crossing; Nr Oldtown, Md	Cumberland, Md	Allegheny	39.55320	-78.55500	01609000	149
514	Sideling Hill Crk	REF	10/10-5/21	15	SC	2021	Upstr of Hixon Rd Crossing	Union Twp	Fulton	39.73250	-78.34638	Manual	72
602	Sixteen-Mile Crk		3/73 – 11/87		NW	1987	220 yds dwnstr of Rt 5 Brdg	North East Twp	Erie	42.23820	-79.83210	4213294	19
603	Walnut Crk		3/73 - 11/87		NW	1987	40 yds upstr Fr LR25025 Br - Last Br upstr Fr mouth	Fairview Twp	Erie	42.07306	-80.23472	4213150	37
604	Elk Crk		3/73 - 11/87		NW	1987	250 yds dwnstr Fr Rt 5 Br	Girard Twp	Erie	42.02056	-80.36139	4233085	98
612	Lake Erie		7/75 - 8/78		NW	1978	500 yds N of Beach 5 Presque Isle (mid-depth)	Millcreek Twp	Erie	42.16528	-80.14778	4213162	
613	Lake Erie		7/75 - 8/78		NW	1978	500 yds N of Beach 5 Presque Isle (bottom)	Millcreek Twp	Erie	42.14861	-80.14778	4213163	
622	Lake Erie	STN	7/75 - 5/2020	10	NW	2020	Off of Hammermill Cribs Island	Erie	Erie	42.15444	-80.05830	USCG622	

WQN	Stream Name	Station Type	Period of Record (M/YY)	SAC	REG	Year Inac-tivated	Location	Municipality	County	Lat	Long	USGS Gage	Drain Area (Sq Mi)
623	Lake Erie		7/75 - 8/78		NW	1978	1000 yds N of Gilson Ave (bottom)	Millcreek Twp	Erie	42.15444	-80.05833	4213166	
626	N Br Sugar Run	REF	10/15-12/20	15	NW	2020	Approx. 0.5 miles upstr of SR 321, along Anf Rd	Corydon Twp	Mckean	41.90080	-78.88280	Manual	11
627	Buck Run	REF	10/15-12/20	15	NW	2020	Approx. 100 m dwnstr of Lanigan Brook	Sergeant	Mckean	41.64030	-78.68440	Manual	7
628	Kitchen Crk	REF	10/15-11/20	15	NE	2020	Approx. 200 m upstr of SR 118	Fairmount	Luzerne	41.30143	-76.27433	Manual	9
629	Stony Brook	REF	10/15-11/20	15	NE	2020	Approx. 150 m upstr of SR 3001 Br (Windy Valley Rd)	Forkston	Wyoming	41.46771	-76.16127	Manual	9
630	E Br Hemlock Crk	REF	10/15-11/20	15	NE	2020	Approx. 15 m upstr of Putman Hill Rd Culvert on SGL 70	Harmony Twp	Susquehanna	41.96200	-75.49067	Manual	5
631	Presque Isle Bay		8/81 - 8/81		NW	1981	Bay midway btw the public dock & Baymouth (surf)	Millcreek Twp	Erie	42.14444	-80.09444	4213167	
633	Presque Isle Bay		7/75 - 9/76		NW	1976	Bay midway btw the public dock & Baymouth (bottom)	Millcreek Twp	Erie	42.14444	-80.09444	4213169	
634	Crooked Crk	REF	10/15-11/20	15	NE	2020	20 m upstr of stream ford coming from SGL 159 parking area along Crooked Crk Rd (T-724)	Buckingham Twp	Wayne	41.79167	-75.27391	Manual	6
635	Dixie Run	STN	10/15-9/20	87, METH	NC	2020	100 m upstr of Love Hollow Rd at Tioga State Forest boundary	Morris Twp	Tioga	41.55813	-77.33044	Manual	4
641	Twelvemile Crk	REF	8/88 – 12/04		NW	2004	100 yds below SR 5 Br	Harborcreek Twp	Erie	42.20972	-79.91500	4213273	13
642	E Br Conneaut Crk		1/88 - 8/89		NW	1989	T883 Br SGL #101	Beaver Twp	Crawford	41.84583	-80.47222		8
682	Lake Erie		8/76 - 8/78		NW	1978	1.5 miles N/NE of Shades Beach (mid-depth)	Northeast Twp	Erie	42.21167	-79.95667		
692	Lake Erie		6/76 - 8/78		NW	1978	NE of Presque Isle Beach 10 beyond mixing zone (mid-depth)	Northeast Twp	Erie	42.18750	-80.05306		
693	Lake Erie		6/76 - 8/78		NW	1978	NE of Presque Isle Beach 10 beyond mixing zone (bottom)	Northeast Twp	Erie	42.18750	-80.05306		
703	Monongahela River	STN	4/50-6/88	10		1988	Gaging Station above Lock Dam 7	Greensboro	Greene	39.78750	-79.92083	03072500	4383
704	Turtle Crk		6/62 - 6/95		SW	1995	2nd RXR Br dwnstr Fr SR993	Trafford	Westmoreland	40.38556	-79.76472	3084400	56
705	Abers Crk		6/62 - 11/87		SW	1987	Rt 286 Br	Plum Boro	Allegheny	40.45028	-79.71389	3084000	4
707	Youghiogheny Rvr		4/50 - 1/88		SW	1988	Crawford Ave Br	Connellsville	Fayette	40.01750	-79.59389	3082500	1324
708	Youghiogheny Rvr		4/50 - 11/75		SW	1975	SR381 Br	Ohiopyle	Fayette	39.87083	-79.49306	3081500	1061
710	Casselman Rvr		4/50 - 6/95		SW	1995	SR3011 (IR 55029) Br - Markleton	Upr Turkeyfoot Twp	Somerset	39.85972	-79.22833	3079000	383
711	Laurel Hill Crk		4/50 - 1/73		SW	1973	PA Rt 281 Br	Lwr Turkeyfoot Twp	Somerset	39.82139	-79.32111	3080000	121
712	Redstone Crk		6/62 - 1/88		SW	1988	LR26026 Br - Waltersburg	Franklin Twp	Fayette	39.98000	-79.76444	3074500	74
715	Sewickley Crk		9/63 - 6/95		SW	1995	SR3014 (IR64165) Br	Hunker	Westmoreland	40.21250	-79.61583	3083250	81
716	Southfork Tenmile Crk		9/72 - 12/75		SW	1975	Br off LR30051	Clarksville	Greene	39.97250	-80.04583	3073535	199
717	Tenmile Crk	STN	8/72 - 6/95	610	SW	1995	SR088 Br - Millsboro	E. Bethlehem Twp.	Washington	39.98333	-80.01056	3072845	338

WQN	Stream Name	Station Type	Period of Record (M/YY)	SAC	REG	Year Inac-tivated	Location	Municipality	County	Lat	Long	USGS Gage	Drain Area (Sq Mi)
718	Pigeon Crk		8/72 - 11/87		SW	1987	T854 Br Nr Rt 481	Carroll Twp	Washington	40.19056	-79.93056	3075040	59
719	Peters Crk		8/72 - 11/87		SW	1987	Rt 51 Br	Jefferson Boro	Allegheny	40.29083	-79.91806	3075090	43
720	Turtle Crk		8/72 - 11/75		SW	1975	Rt 22 Br - Murrysville	Franklin Twp	Westmoreland	40.42694	-79.69861	3083975	18
721	Jacobs Crk		9/72 - 12/87		SW	1987	LR26131 Br	Scottdale	Westmoreland	40.09222	-79.59278	3083038	59
722	Casselman Rvr		2/73 - 12/75		SW	1975	Rt 53 Br	Addison Twp	Somerset	39.79556	-79.32333	3079200	433
723	Big Sandy Crk	REF	8/72-11/87 & 10/10-9/15	13	SW	2015	SR2004 (Quebec Rd) Br @ Elliottsville	Wharton Twp	Fayette	39.76944	-79.62689	03070400	16
724	Laurel Hill Crk	REF	10/72-11/87 & 10/10-9/15	13	SW	2015	SR 281 Br @ Ursina	Lower Turkeyfoot Twp	Somerset	39.82291	-79.32075	03080000	121
727	Cheat Rvr	STN	1/88-9/15	610	SW	2015	SR119 Br - Pt Marion	Springhill Twp	Fayette	39.74160	-79.90009	03071600	1423
729	Indian Crk	REF	1/88 - 10/98		SW	1998	SR381 Br upstr Fr Jones Mill	Donegal Twp	Westmoreland	40.08417	-79.34028		18
730	Kooser Run	REF	8/98 - 9/03		SW	2003	Br in Kooser St Pk above lake	Jefferson Twp	Somerset	40.06000	-79.22806		3
731	S Fork Ten Mile Crk	REF	8/98 - 9/03		SW	2003	T436 Br W of Rogersville	Center Twp	Greene	39.88250	-80.31750		19
802	Allegheny Rvr		3/50 - 12/06		SW	2006	SR 128 Br	North Buffalo Twp	Armstrong	40.76111	-79.54583	3036500	8991
803	Allegheny Rvr	REF	2/62 – 12/87 8/98 – 12/04		SW	2004	Rt 368 Br Nr Parkers Landing	Hovey Twp	Armstrong	41.10194	-79.67944	3031500	7663
804	Allegheny Rvr		3/50 - 12/87		NW	1987	SR322 Br - Franklin	Franklin	Venango	41.38944	-79.81944	3025500	5960
806	Allegheny Rvr		6/62 - 12/87		NW	1987	Rt 62 Br below Warren	Brokenstraw Twp	Warren	41.84056	-79.25417	3015320	3154
808	Buffalo Crk		3/50 - 11/87		SW	1987	LR3102 @ Iron Br	S Buffalo Twp	Armstrong	40.73500	-79.67861	3048900	125
811	Conemaugh Rvr		3/50 - 12/87		SW	1987	Rt 56 Br	Seward	Westmoreland	40.41917	-79.02639	3041500	720
812	Loyalhanna Crk		4/50 - 6/95		SW	1995	SR1046 (IR64185) Br @ Dam	Loyalhanna Twp	Westmoreland	40.46472	-79.45194		291
813	Loyalhanna Crk		4/50 - 11/87		SW	1987	Concrete fording point off Rt 30	Unity Twp	Westmoreland	40.28639	-79.31972	3044997	166
814	Blacklick Crk		4/50 - 6/95		SW	1995	T660 Br Nr Josephine	Burrell Twp	Indiana	40.47333	-79.18361	3042000	193
815	Two Lick Crk		6/62 - 11/87		SW	1987	T598 Br	Center Twp	Indiana	40.51278	-79.16944	3042500	172
816	Little Conemaugh Rvr		3/50 - 1/88		SW	1988	Rt 271 Br btw Franklin & Johnstown	Johnstown	Cambria	40.32833	-78.92083	3041025	190
817	Stony Crk		3/50 - 1/88		SW	1988	Rt 53 Br	Ferndale	Cambria	40.29361	-78.91889	3040000	454
818	Crooked Crk		3/50 - 6/95		SW	1995	SR066 Br	Bethel Twp	Armstrong	40.71806	-79.53667		281
823	Clarion Rvr		5/62 - 1/76		NW	1976	Grant St Br	Johnsonburg	Elk	41.48611	-78.67861	3028500	204
824	W Br Clarion Rvr		5/62 - 12/87		NW	1987	LR24011 Br - Wilcox	Jones Twp	Elk	41.57528	-78.69250	3028000	63

WQN	Stream Name	Station Type	Period of Record (M/YY)	SAC	REG	Year Inac-tivated	Location	Municipality	County	Lat	Long	USGS Gage	Drain Area (Sq Mi)
825	E Br Clarion Rvr		3/50 - 12/87		NW	1987	LR24013 Br above Glen Hazel	Jones Twp	Elk	41.54444	-78.61389	3027545	80
826	French Crk		6/62 - 11/87		NW	1987	LR60019 Br	Utica	Venango	41.43750	-79.95611	3024000	1030
827	French Crk		6/62 - 10/90		NW	1990	T700 Br	Amity Twp	Erie	41.95639	-79.87722	3021500	208
828	Oil Crk		5/62 - 10/86		NW	1986	Rt 8 Br N of Rouseville	Cornplanter Twp	Venango	41.48167	-79.69556	3020500	283
829	Tionesta Crk		5/62 - 11/87		NW	1987	LR27001 Br	Tionesta Twp	Forest	41.48500	-79.45000	3020050	479
833	Clarion Rvr		3/50 - 12/87		NW	1987	PA Rt 948 Br	Ridgway	Elk	41.42083	-78.73611	3029000	306
834	Clarion Rvr		3/50 - 5/61		NW	1961	Off T315 S of St Petersburg	Richland Twp	Clarion	41.14917	-79.66028	3031000	1246
835	Mahoning Crk		3/50 - 5/61		NW	1961	Rt 436 Br	Punxsutawney	Jefferson	40.93917	-79.00861	3034000	155
836	Little Mahoning Crk		3/50 - 1/61		SW	1961	LR32101 Br	South Mahoning Twp	Indiana	40.83611	-79.11028	3034500	88
837	Crooked Crk		3/50 - 4/61		SW	1961	PA Rt 210 Br - Idaho	South Bend Twp	Armstrong	40.65472	-79.34889	3038000	191
838	Pine Crk		8/72 - 11/87		SW	1987	LR02136 Br - Bryant	Hampton Twp	Allegheny	40.57833	-79.96972	3049750	37
839	Deer Crk		10/72 - 11/87		SW	1987	Rt 910 Br Nr LR02150	West Deer Twp	Allegheny	40.61750	-79.90222	3049645	11
840	Buffalo Crk		8/72 - 11/75		NW	1975	T664 Br	Clearfield Twp	Butler	40.86528	-79.69472	3048850	42
841	Cowanshannock Crk		8/72 - 11/87		SW	1987	T612 Br off Rt 85	Valley Twp	Armstrong	40.80528	-79.38389	3106005	37
842	Mahoning Crk		8/72 - 11/75		SW	1975	0.1 mile E on T743 Fr Jct with T691	Pine Twp	Armstrong	40.93278	-79.44500	3036100	422
843	Clarion Rvr	STN	3/73-9/20	610	NW	2020	SR58 Br	Callensburg	Clarion	41.12991	-79.55423	03030852	1161
844	Elk Crk		3/73 - 11/87		NW	1987	Rt 120 Br	Ridgway	Elk	41.42528	-78.72722	3028900	61
847	French Crk		2/73 - 12/87		NW	1987	First Br S of Saegertown on Rt 19	Woodcock Twp	Crawford	41.70778	-80.14583	3022505	631
848	Lake Crk		2/73 - 11/75		NW	1975	T364 Br	Jackson Twp	Venango	41.55000	-79.90389	3024230	26
849	Woodcock Crk		2/73 - 11/87		NW	1987	LR20141 Br	Woodcock Twp	Crawford	41.71028	-80.13944	3022600	51
850	Conneauttee Crk		11/72 - 11/83		NW	1983	T319 Br SE of Edinboro	Washington Twp	Erie	41.86278	-80.12500	3021670	17
851	Muddy Crk		11/72 - 11/87		NW	1987	LR20098 Br	Steuben Twp	Crawford	41.69111	-79.87333	3021600	8
852	Oil Crk		2/73 - 12/87		NW	1987	PA Rt 8 Br - Oil City	Oil City	Venango	41.43389	-79.70972	3020500	320
853	Tionesta Crk		2/73 - 11/75		NW	1975	250 yds dwnstr Fr Rt 36 Br	Tionesta Twp	Forest	41.48694	-79.45500	3020050	479
854	Brokenstraw Crk		3/73 - 12/75		NW	1975	RXR Br off Old Rt 6 - Irvine	Brokenstraw Twp	Warren	41.83972	-79.26417	3015530	328
855	Kinzua Crk		3/73 - 12/87		NW	1987	Rt 219 Br - Tally Ho	Lafayette Twp	Mckean	41.76639	-78.71861	3011800	39
856	Potato Crk		3/73 - 11/87		NW	1987	T366 Br - Farmers Valley	Keating Twp	Mckean	41.84139	-78.42917	3009700	166

WQN	Stream Name	Station Type	Period of Record (M/YY)	SAC	REG	Year Inac-tivated	Location	Municipality	County	Lat	Long	USGS Gage	Drain Area (Sq Mi)
857	Allegheny Rvr		3/73 - 11/87		NC	1987	0.5 miles dwnstr Fr LR52062 Br	Eulalia Twp	Potter	41.75778	-78.05306	3007000	91
859	Little Toby Crk		2/76 - 11/87		NW	1987	RXR Br 0.1 miles upstr Fr Rt 949 Nr Portland Mills	Spring Creek Twp	Elk	41.36472	-78.82278	3029170	124
862	Blue Eye Run	REF	1/88 - 7/98		NW	1998	SR027 Br Nr Garland	Pittsfield Twp	Warren	41.81861	-79.42917		10
863	Cathers Run	REF	1/88 - 7/98		NW	1998	T634 Br Nr mouth	Millcreek Twp	Clarion	41.31472	-79.23083		18
864	S Br Two Lick Crk	REF	1/88 - 10/98		SW	1998	SR1014 Br upstr Fr Wandin	Green Twp	Indiana	40.67472	-78.94444		20
865	Mclaughlin Crk	REF	9/89 - 7/98		NW	1998	T639 Br Nr Hydetown	Oil Creek Twp	Crawford	41.68417	-79.72444		9
869	French Crk	REF	12/94 – 12/04		NW	2004	SR1002 Br	Venango Twp	Crawford	41.77167	-80.10750	3022000	599
870	Clear Shade Crk	REF	8/98-12/04 & 10/10-5/21	15	SW	2021	250 ft upstr of SR160 (Dark Shade Rd) Br - Cairnbrook	Ogle Twp	Somerset	40.14874	-78.81639	Manual	32
871	Minister Crk	REF	8/98 – 12/04		NW	2004	SR666 Br – Minister	Howe Twp	Forest	41.62109	-79.15329		11
873	W Br Caldwell Crk	REF	8/98-12/04 & 10/10-12/20	15	NW	2020	250 m upstr of Flat Rd (T304) Br NE of Selkirk	Southwest Twp	Warren	41.69678	-79.57235	03020449	19
875	Silver Crk	REF	8/98 - 3/02		NW	2002	Snake Rd Br (T638)	Parker Twp	Butler	41.03485	-79.74667		7
876	Silver Crk	REF	5/02 - 12/04		NW	2004	SR1004 Br - Walley Mill	Parker Twp	Butler	41.04500	-79.77694		6
877	East Hickory Crk	REF	1/05 - 9/10		NW	2010	Upstr of Forest Service Rd 119 Nr Queen, PA	Limestone Twp	Warren	41.64198	-79.33797		20
878	Mill Crk	REF	1/05-9/15	15	SW	2015	Roberts Rd (T 984) Br	Ligonier Twp	Westmoreland	40.25169	-79.15547	MANUAL	12
879	Woodcock Crk	REF	1/05 - 9/10		NW	2010	50 m upstr of SR 2036 Br	Randolf Twp	Crawford	41.64394	-79.96688		6
880	Quemahoning Crk	REF	1/05 - 9/10		SW	2010	50 m dwnstr Fr SR4015 Br	Lincoln Twp	Somerset	40.06896	-79.10914		5
881	Havens Run	REF	1/05 - 9/10		NW	2010	600 m upstr of SGL 30 Rd	Norwich Twp	Mckean	41.64810	-78.36240		6
882	South Fork Pine Crk	REF	3/06-9/15	13	SW	2015	T 738 Br Nr Echo, PA	Valley Twp	Armstrong	40.84730	-79.36370	MANUAL	23
883	Little Yellow Crk	REF	3/06-9/15	15	SW	2015	Upstr of SR2027 Br	Brush Valley Twp	Indiana	40.55650	-79.00580	MANUAL	15
904	Beaver Rvr		5/62 - 1/88		SW	1988	Old Rt 51 Br	Rochester	Beaver	40.70139	-80.29583	3107615	3158
908	Slippery Rock Crk		4/50 - 12/75		NW	1975	LR37083 Br	Perry Twp	Lawrence	40.88389	-80.23389	3106500	401
912	Pymatuning Crk		5/62 - 8/64		NW	1964	PA Rt 846 Br	S Pymatuning Twp	Mercer	41.30944	-80.47444		169
914	Chartiers Crk		4/50 - 6/95		SW	1995	LR02037 Br	Carnegie	Allegheny	40.40000	-80.09861	3085500	256
916	Chartiers Crk		8/72 - 12/87		SW	1987	T748 Br	Peters Twp	Washington	40.27583	-80.14361	3085260	92
918	Two Mile Run		8/72 - 1/88		SW	1988	L St Br	Vanport Boro	Beaver	40.68944	-80.32778	3107620	5
919	Brush Crk		8/72 - 11/75		SW	1975	T613 Br off Rt 588	Marion Twp	Beaver	40.80389	-80.24028	3106018	52

WQN	Stream Name	Station Type	Period of Record (M/YY)	SAC	REG	Year Inac-tivated	Location	Municipality	County	Lat	Long	USGS Gage	Drain Area (Sq Mi)
920	Glade Run		8/72 - 12/75		NW	1975	T423 Br	Forward Twp	Butler	40.75806	-79.99222	3105850	35
921	Slippery Rock Crk		8/72 - 12/87		NW	1987	LR10048 Br	Worth Twp	Butler	41.03639	-80.10889	3106153	263
923	N Fork Little Beaver Crk		3/73 - 12/87		NW	1987	LR37004 Br Nr Enon Valley	Little Beaver Twp	Lawrence	40.85778	-80.44083	3109390	56
924	Big Run		3/73 - 11/75		NW	1975	Rt 18 Br	New Castle	Lawrence	40.98444	-80.35000	3105248	28
926	Slippery Rock Crk		2/76 - 11/87		NW	1987	LR10063 Br Nr Boyers	Marion Twp	Butler	41.10944	-79.90833	3106030	28
927	Shenango Rvr		1/76 - 1/88		NW	1988	Ohio St Br Nr Sharon; 1.3 miles dwnstr Fr Ltl Yankee Run	Hickory Twp	Mercer	41.19889	-80.50583	3103500	702
928	Traverse Crk	REF	1/88 - 10/98		SW	1998	SR018 Br @ Raccoon Crk State Park	Hanover Twp	Beaver	40.50306	-80.42389		15
929	Thorn Crk	REF	1/88 - 7/98		NW	1998	T556 Br W of Saxonburg	Jefferson Twp	Butler	40.75083	-79.82833		13

D.5 WQN OBJECTIVES

pennsylvania
DEPARTMENT OF ENVIRONMENTAL PROTECTION

OFFICE OF WATER PROGRAMS

BUREAU OF CLEAN WATER

WATER QUALITY NETWORK OBJECTIVES

2020

Appendix D Pennsylvania's Surface Water Quality Monitoring Network (WQN) Manual

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2020

OBJECTIVES OF THE WATER QUALITY NETWORK

The Pennsylvania Water Quality Network (WQN) is a statewide, fixed-station sampling network that has been gathering important surface water quality data across the Commonwealth for decades. The design of the network, the design of sampling plans in the network, and the decades of data available at some stations have allowed WQN data to be used for many purposes by the Pennsylvania Department of Environmental Protection (DEP), other state and federal agencies, and other interested parties. This document describes the key uses of WQN water chemistry and macroinvertebrate data. For more information on this sampling network, please visit the WQN webpage at DEP Water Quality Network.

Trend Analysis

Trend analysis is one of the core functions of the WQN. The WQN's fixed-station monitoring strategy is uniquely suited for measuring changes in water quality through time. Trend data collected in the WQN is used to monitor incremental progress in water quality, to calibrate Total Maximum Daily Load (TMDL) models, and to track sources and timing of potential impacts to water quality. Analyses of trend data are also one of the most efficient and accessible ways to communicate vast amounts of water quality data to a variety of stakeholders, including the public, which promotes transparency.

Example

As part of a joint effort, DEP partners with the Susquehanna River Basin Commission (SRBC), the United States Geological Survey (USGS), and the Chesapeake Bay Program (CBP) in a rigorous sampling program to measure nutrient and sediment concentrations at strategic locations within the Susquehanna River basin to better understand nutrient and sediment inputs to the Chesapeake Bay. This monitoring network is incorporated into the WQN and consists of six mainstem river stations and thirty tributary stations. From this extended WQN program, SRBC reports on trends in nutrient and suspended sediment concentrations, loads, and yields in the Susquehanna River basin. Information from these reports helps managers from multiple agencies create and adapt appropriate action plans.

Permitting

Data from the WQN informs the evaluation and development of water quality-based effluent limitations (WQBEL) in National Pollutant Discharge Elimination System (NPDES) permits which control the discharge of pollutants from point sources into surface waters. WQBELs, as defined in 25 Pa. Code section 96.1, are effluent limitations based on the need to support the water quality criteria and to assure protection of existing and designated uses. DEP often uses WQN data as background data for naturally occurring pollutants when determining whether a stream has assimilative capacity to accept a pollutant limit and in developing WQBELs where there is reasonable potential to exceed water quality criteria.

WQN data also provide important background data when permitting discharges in High Quality Waters (HQ) and Exceptional Value Waters (EV) where existing water quality is protected and maintained. In permitting discharges to these special protection waters, data from a WQN station

near the proposed or existing discharge site, or from a WQN station that is representative of the proposed or existing discharge site, can be used to develop non-degrading effluent limitations. Additionally, since the toxicity of several pollutants vary with hardness (e.g., copper, lead, nickel, silver, and other metals) or pH (e.g., ammonia), many Chapter 93 water quality criteria require background hardness or pH to use in calculating criteria values. In these situations, WQN data can be used to eliminate the use of default hardness or pH values, or the need for additional data collection to calculate appropriate water quality criteria values.

Example
DEP permit writers can use WQN data to develop non-degrading effluent permit limits for discharges to HQ and EV streams. A DEP permit writer used water chemistry data collected at WQN station 195 on Rock Run to calculate the non-degrading effluent limitations for an Exceptional Value tributary to French Creek in Chester County. The WQN station allowed the permit writer to use data that is representative of the watershed containing the permitted discharge, instead of default data values or requiring the need to collect additional data.

Water Quality Assessments for Federal Clean Water Act Sections 303(d) and 305(b)
The WQN is crucial for Pennsylvania to meet federal mandates to monitor water quality and to assess monitored water quality against water quality standards. Depending on the type of WQN station, certain chemical parameters are collected frequently enough to determine if water quality criteria are being met. This information is one part of the overall surface water monitoring program that helps build the Pennsylvania Integrated Water Quality Monitoring and Assessment Report, which is required by the federal Clean Water Act on a biennial basis. In addition, biological assessment determinations can also be made at WQN stations where biological data are collected using current assessment methodologies.

Example
Historical and current data for nitrates collected at the WQN station on Connoquenessing Creek located downstream of an industrial discharge in Butler County was instrumental in documenting the impairment and subsequent restoration of the creek’s Potable Water Supply (PWS) use. The PWS use of Connoquenessing Creek downstream of the discharge was listed as impaired on the 303(d) list for exceeding the nitrite plus nitrate PWS criterion in 25 Pa. Code § 93.7 as documented in historical WQN data. After the industrial discharger discontinued nitric acid pickling, WQN data documented water quality in Connoquenessing Creek downstream of the discharge as no longer exceeding the nitrite plus nitrate PWS criterion. In this instance, WQN data was central to both the listing and subsequent delisting of Connoquenessing Creek.

Data to Inform Permits
The WQN is specifically designed to evaluate both the quality of Pennsylvania's surface waters and the effectiveness of DEP’s water quality management program. Identifying changes in water quality at certain locations over time helps ensure permits are written accurately and are protective of water

quality standards. Additionally, the WQN serves not only to help inform permitting, but also help operators describe in permit applications how they are able to comply with water quality standards.

Example

The WQN data was essential in helping show compliance with water quality standards as discussed in the industrial discharge example above. Although the WQN data initially documented the impairment, WQN data also helped the operator demonstrate compliance with water quality standards after the process change at the plant.

Threatened and Endangered Species Protection

Threatened and endangered species are an ongoing concern related to NPDES discharges. The United States Fish and Wildlife Service (USFS) has recommended stringent criteria for multiple pollutants including ammonia-nitrogen, chloride, total dissolved solids (TDS), and nickel to protect threatened and endangered mussel species. As noted in the permitting discussion above, WQN data are essential in accurately evaluating appropriate criteria for some of these pollutants since the availability of background data can have a significant impact on these evaluations. Not only is it critical to have accurate background data of the pollutants of concern but it is also critical to have accurate hardness and pH background data to calculate appropriate pollutant criteria and associated potential impact areas.

Example

Endangered mussels are of special concern in the Allegheny River, French Creek, Shenango River, and many tributaries to these surface waters. Segments of all three main streams have been designated as "critical habitat" for the rabbitsfoot mussel, and many other threatened and endangered mussel species. Numerous NPDES permits in this region of Pennsylvania have been issued with concurrence by USFS as protective of endangered species, using a permitting strategy that includes a robust evaluation of WQN data.

Emerging Science

Due to the years- to decades-long historical records of physical, chemical, and biological data collected at many stations, WQN sites often serve as prime locations to collect new data (e.g., contaminants of emerging concern) and to develop new collection protocols (e.g., periphyton monitoring protocols). The ability to contextualize new kinds of data in robust historical records characterizing particular sites across a range of parameters and assemblages can greatly reduce the cost and time needed to expand monitoring approaches when novel environmental concerns arise.

Example

During an intensive study of the Susquehanna River, WQN stations were almost exclusively used when selecting locations to place passive chemical samplers designed to detect hundreds of contaminants of emerging concern. The combination of this new data and the existing WQN data substantially reduced the overall cost of the project, compared with project costs if the passive samplers were deployed in locations without robust water quality network data.

D.6 FIELD PREPARATION AND FIXATIVE CHART

Standard Analysis Code (SAC)	Parameters	Bottle Ware	Field Prep/Fixative
010	general chemistry	1 - 500 mL HDPE plastic bottle	iced to 4°C
	N/P	1 - 500 mL HDPE plastic bottle	10% H2SO4 to pH <2; iced to 4°C
	metals, total	1 - 125 mL HDPE plastic bottle	1:1 HNO3 to pH <2; iced to 4°C
012	general chemistry	2 - 500 mL HDPE plastic bottle	iced to 4°C
	general chemistry, dissolved	1 - 125 mL HDPE plastic bottle	field filtered; iced to 4°C
	N/P	1 - 500 mL HDPE plastic bottle	10% H2SO4 to pH <2; iced to 4°C
	N/P, dissolved	1 - 125 mL HDPE plastic bottle	field filtered; 10% H2SO4 to pH <2; iced to 4°C
	metals, total	1 - 125 mL HDPE plastic bottle	1:1 HNO3 to pH <2; iced to 4°C
	TOC	2 - 40 mL amber VOA vials	10% H2SO4 to pH <2; iced to 4°C
015	general chemistry plus CBOD	3 - 500 mL HDPE plastic bottle	iced to 4°C
	general chemistry, dissolved	1 - 125 mL HDPE plastic bottle	field filtered; iced to 4°C
	N/P	1 - 125 mL HDPE plastic bottle	10% H2SO4 to pH <2; iced to 4°C
	N/P, dissolved	1 - 125 mL HDPE plastic bottle	field filtered; 10% H2SO4 to pH <2; iced to 4°C
	metals, total	1 - 125 mL HDPE plastic bottle	1:1 HNO3 to pH <2; iced to 4°C
	metals, dissolved	1 - 125 mL HDPE plastic bottle	field filtered; 1:1 HNO3 to pH <2; iced to 4°C
	Organic Carbon, Total	2 - 40 mL amber VOA vials	10% H2SO4 to pH <2; iced to 4°C
	Organic Carbon, Dissolved	2 - 40 mL amber VOA vials	field filtered: 10% H2SO4 to pH <2; iced to 4°C
016	general chemistry plus CBOD	2 - 500 mL HDPE plastic bottle	iced to 4°C
	N/P	1 - 500 mL HDPE plastic bottle	10% H2SO4 to pH <2; iced to 4°C
	metals, total	1 - 125 mL HDPE plastic bottle	1:1 HNO3 to pH <2; iced to 4°C
	metals, dissolved	1 - 125 mL HDPE plastic bottle	field filtered; 1:1 HNO3 to pH <2; iced to 4°C
083	general chemistry	2 - 500 mL HDPE plastic bottle	iced to 4°C
	N/P	1 - 125 mL HDPE plastic bottle	10% H2SO4 to pH <2; iced to 4°C
	metals, total	1 - 125 mL HDPE plastic bottle	1:1 HNO3 to pH <2; iced to 4°C
122	general chemistry plus CBOD	3 - 500 mL HDPE plastic bottle	iced to 4°C
	general chemistry, dissolved	1 - 125 mL HDPE plastic bottle	field filtered; iced to 4°C
	N/P	1 - 125 mL HDPE plastic bottle	10% H2SO4 to pH <2; iced to 4°C
	N/P, dissolved	1 - 125 mL HDPE plastic bottle	field filtered; 10% H2SO4 to pH <2; iced to 4°C

	metals, total	1 - 125 mL HDPE plastic bottle	1:1 HNO3 to pH <2; iced to 4°C
	metals, dissolved	1 - 125 mL HDPE plastic bottle	field filtered; 1:1 HNO3 to pH <2; iced to 4°C
	Organic Carbon, Total	2 - 40 mL amber VOA vials	10% H2SO4 to pH <2; iced to 4°C
	Organic Carbon, Dissolved	2 - 40 mL amber VOA vials	field filtered: 10% H2SO4 to pH <2; iced to 4°C
610 *all SAC010 bottles plus	general chemistry	1 - 500 mL HDPE plastic bottle	iced to 4°C
612 *all SAC012 bottles plus	general chemistry	1 - 500 mL HDPE plastic bottle	iced to 4°C
616 *all SAC016 bottles plus	general chemistry	1 - 500 mL HDPE plastic bottle	iced to 4°C
B021	fecal coliform, E.coli	1 - 125 mL plastic bottle sterilized & pretreated with sodium thiosulfate	iced to ≤ 6°C
RAD99	gross alpha, gross beta	2 - 500 mL glass bottle	iced to 4°C
B029	chlorophyll a	1 - 47 mm glass fiber filter in petri dish	wrap in aluminum foil and place on dry ice or ice to 4°C & freeze within 24 hrs
--	suspended sediment	1 - 500 mL plastic bottle	store in cool dark place

All WQN stations require the following STANDARD FIELD ANALYSES: Temp (°C), pH, Dissolved Oxygen, and Specific Conductance. These results should be recorded on the Sample Submission Sheet.

* Must be shipped to USGS lab in Louisville, KY.

D.7 STREAM FLOW MEASUREMENT PROTOCOLS

Wire-Weight Gages

A wire-weight (WW) gage consists of a drum wound with a single layer of cable, a bronze weight attached to the end of the cable, a graduated disc and counter, and a check bar of known elevation. The gage is housed within a small cast aluminum lockable box and generally mounted to a rigid structure (i.e., bridge or trestle) directly above the water surface. The graduated disc is permanently connected to the counter and to the shaft of the drum. The drum reel is equipped with pawl and ratchet for holding the suspended weight at any desired elevation.

The check-bar for the WW gage is set by levels as a reference elevation to gate datum. The gage is set so that when the bottom of the suspended weight is at the water surface, the gage height is indicated by the combined readings of the counter and the graduated disc.

Reading Instructions

- Unlock the case and gently open the cover. If the cover jams and will not open freely, do not force it open. The cover has probably become jammed against the crank handle. Try to move the handle into a position that will allow the cover to open. At this point, make sure the pawl is engaged or that you can hold onto the handle to prevent the weight falling to the stream bed and unspooling the cable.
- Inspect the cable. It should be tightly wound with windings that touch each other, with no overlaps. If cable is not properly wound, check-bar and water-surface readings will be incorrect. If necessary, unwind the cable and utilizing the level-wind pulley and gloves, rewind the cable onto the reel. (This should only be necessary in extreme situations.)
- The check-bar is found directly under the weight as it hangs in the box and has two positions, the forward position (away from you) which prevents the weight from falling to the bottom of the box while locked (and into the stream when opened), and a rear position (toward you) which allows the weight to be lowered to the stream. You should find the check-bar in the forward position. Elevations are run to the check-bar in the forward position and the gage is set to read this elevation when the weight just makes contact with the check-bar.
- In order to determine the check-bar reading,
    - Make sure the check-bar is in the forward position.
    - Lower the weight until the weight just lightly touches the check-bar.
    - Record this reading to three decimal places. This will involve estimating the last digit.
- Raise the weight and slide the check-bar to the rear position. Lower the weight until it just lightly touches the surface of the water and record your reading to two decimal places. Average the peaks and troughs of elevation if the water surface is surging. You should repeat this process at least once. If the air temperature is below freezing, the first time the weight touches the water, a layer of ice will form on the bottom of the weight causing it to become slightly longer. Repeated contacts with the water surface continues to add layers of ice to the bottom of the weight. For this reason, water surface elevations should be made as accurately as possible on the first or second try.

- Engage the pawl (this will prevent the weight from falling and unspooling the cable should the crank slip from your hand while rewinding) and crank the weight to its original position. Slide the check-bar to its forward notch. The crank handle should be located in the rear position, allowing the cover to close without touching the crank handle.
- Close the cover and lock the case.

Electric Tape Gages

The electric tape (ET) gage consists of a steel tape graduated in feet and hundredths, to which is fastened a cylindrical weight (usually brass), a reel in a frame for the tape, a 4½-volt battery and a voltmeter. One terminal of the battery is attached to a ground connection and the other to one terminal of the voltmeter. The other terminal of the voltmeter is connected through the frame, reel and tape, to the weight. The weight is lowered until it contacts the water surface. This contact completes the electric circuit and produces a signal on the voltmeter. With the weight held in the position of first contact, the water surface elevation reading is observed at the tape index (a black line indented just below the voltmeter and usually immediately above the shelf) provided on the reel mounting. ET gages are installed in a manner that will produce a dependable circuit, yielding a strong signal when the weight touches the water surface.

Reading Instructions

The dog on the left side of the reel is disengaged by lifting the rear portion in an upward direction while holding onto the handle on the right side. This allows the weight and tape to be lowered until it contacts the water surface. This contact completes the electric circuit and produces a fluctuation on the voltmeter on the front of the tape reel frame. Once a contact with the water surface has been made, the weight is raised slightly until contact is broken, and then lowered very slowly until the water surface is again contacted. This procedure is repeated until a consistent reading at the index line is produced. This reading may be confirmed visually by shining a flashlight into the well at the spot where the weight will make contact with the surface of the water and lowering the weight very slowly until contact with the surface is visually confirmed (the voltmeter needle should also deflect at this time if working correctly). The reading of the water surface elevation is then taken by observing the reading of the steel tape at the index line on the gage. NOTE: HAZARD!! In order to make a visual observation of the weight touching the water surface in the well, it is often necessary to raise the door (located on the floor) to the well. Once the door is opened, extreme care should be exercised to ensure that nothing is dropped into the well. This type of reading is best left to experienced personnel.

Possible Interferences

- If the weight touches any grounded item (steel piping or the steel side of an oil tube or a steel pipe well) the circuit will be completed, and the voltmeter will deflect as if the water surface has been contacted.
  Solution: Physically move the tape past any items in the well that will produce a false reading. Keep in mind that the tape should be kept as vertical as possible for accurate readings.

- If any oil has seeped into the well onto the water surface from the oil tube, the needle will not deflect upon hitting the oil, but will deflect when weight is lowered through the oil and contacts the water surface (this will not be an accurate water elevation).
  Solution:
  - Visually determine elevation of the top of the oil surface using the procedure described above for visual readings and record this number.
  - From the top of the oil very slowly lower the weight until a deflection of the voltmeter occurs. This is the top of the water surface.
  - Subtract this reading from the one made in step 1. and multiply the answer by 0.2.
  - Subtract this value from the top of oil reading determined in step 1. This is the actual surface elevation.

    Example: 5.28 surface of oil
    surface of water
    0.05 difference
    X.20 = .0100
    5.28-.01 = 5.27 (actual water surface elevation)
- If the bottom of the weight is corroded, even slightly, the meter will not deflect until the water in the well encounters a portion of the weight free of corrosion, completing the circuit. This produces an erroneous reading as the circuit is not completed at the bottom of the weight, but at some point along the side.
  Solution: Using a piece of emery paper rub lightly over the bottom of the weight to remove corrosion. It is also acceptable to lightly scrape the edge of a knife across the bottom of the weight. Care must be taken to avoid removing any brass from the bottom of the weight as repeated cleanings over years may change the weight length.
- If the bottom of the weight is corroded, even slightly, the meter will not deflect until the water in the well encounters a portion of the weight free of corrosion, completing the circuit. This produces an erroneous reading as the circuit is not completed at the bottom of the weight, but at some point along the side.
  Solution: Using a piece of emery paper rub lightly over the bottom of the weight to remove corrosion. It is also acceptable to lightly scrape the edge of a knife across the bottom of the weight. Care must be taken to avoid removing any brass from the bottom of the weight as repeated cleanings over years may change the weight length.
- The cleaning of the weight is a delicate procedure as the weight must first be lowered a few feet, so it may be grasped through the shelf door or opening. Very often the tape may be very rusted at the point the tape enters the weight or just below that point. "Any twisting or bending will break the tape off the weight." Should this happen, lay the weight on the shelf and contact the USGS as soon as possible.
- Ice in the well.
  Solution: Ice must be broken loose and, in some cases, removed. This is a solution that is best left to experienced USGS technicians, and in no instance should DEP personnel enter the well.

Staff Gages

Staff gages are simply rods or boards that are securely attached to a backing or permanent foundation. These non-recording gages are graduated, and stage can be directly read. Vertical staff gages are used as reference gages within stilling wells, and both vertical and inclined staff gages may be used as outside reference gages. Lake height measurements for the WQN are obtained from staff gages.

Digital Recorders

Digital recorders are slow speed, battery operated instruments which record information in the binary decimal code form by use of a paper-tape punch mechanism. Sixteen channel paper-tape is punched with a four-digit number at preselected time intervals of 5, 15, 30 or 60 minutes. Stage heights of zero to 99.99 feet can be recorded in increments of hundredths of a foot. A battery powers the sequencing camshaft and electro-mechanical timing devices. Pins on the punch block are forced against the paper-tape and code discs by spring action thereby recording the position of the discs at that moment. After the paper-tape is punched, it is advanced, and the punch spring is compressed for the next readout cycle. The tape can be either manually read to determine stage, or electronic translators can then convert the punch-tape records to a magnetic tape form that is utilized to compute daily mean gage height and daily stream discharge via digital computers.

Manual Measurement

A full stream width transect is required to manually measure stream flows. Total stream flow must be calculated across this transect using the procedure of Buchanan and Somers (1969) described briefly below. Record the depths and velocities for each of a minimum of 20 equally spaced points along the transect, thereby constructing a stream channel profile and accompanying current velocity measurements. Sum the products of each cell area calculation multiplied by velocity to determine total flow (cfs) across the transect.

Telemark

Some gages are equipped with telecommunication capabilities that provide for their remote reading via the internet.

APPENDIX E: QUALITY ASSURANCE PROJECT PLAN (QAPP): DATA COLLECTION FOR THE PURPOSE OF EXISTING USE EVLUATIONS, PROTECTED USE ASSESSMENT, AND EVALUATIONS OF POINT AND NONPOINT SOURCE IMPACTS TO SURFACE WATERS

Appendix E Quality Assurance Project Plan (QAPP)

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2021

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2023

Disclaimer:
Appendix E is a modified version of a QAPP approved by EPA in July 2021. An official, signed copy of the July 2021 QAPP is available upon request.

PENNSYLVANIA DEPARTMENT OF ENVIRONMENTAL PROTECTION
Bureau of Clean Water (BCW)
Water Quality Division (WQD)

SECTION A: PROJECT MANAGEMENT

A1. Title Page and Approval Sheet

TITLE: Quality Assurance Project Plan (QAPP): Data collection for the purpose of existing use evaluations, protected use assessments, and evaluations of point and nonpoint source impacts to surface waters

EFFECTIVE DATE: August 1, 2021; modified September 2023

AUTHORITY: Pennsylvania Clean Streams Law, 35 P.S. §§ 691.1 et. seq., the Federal Clean Water Act, 33 U.S.C.A §§ 1251 et. seq., 25 Pa. Code Chapters 93 and 96.

POLICY: This guidance provides the established QAPP for water quality data collection in surface waters of Pennsylvania

PURPOSE: The guidance was developed to establish and standardize the Department of Environmental Protection's (DEP) QAPP procedures for data collection for existing use evaluations, protected use assessments, and evaluations of point and nonpoint source impacts to surface waters.

DISCLAIMER: The policies and procedures outlined in this guidance document are intended to supplement existing requirements. Nothing in the policies or procedures shall affect regulatory requirements.

The policies and procedures herein are not an adjudication or a regulation. There is no intent on the part of DEP to give these rules that weight or deference. This document establishes the framework within which DEP will exercise its administrative discretion in the future. DEP reserves the discretion to deviate from this policy statement if circumstances warrant.

PAGE LENGTH: 37 Pages

DEP Project Manager's Signature: _____

DEP Project Manager: Michael (Josh) Lookenbill

DEP Project Quality Assurance Manager's Signature: _____

DEP Project Quality Assurance Manager: Erika Arnold

EPA Project Manager's Signature: _____

Note: This approval action represents EPA's determination that the document(s) under review comply with applicable requirements of the EPA Region 3 Quality Management Plan [https://www.epa.gov/sites/production/files/2020-06/documents/r3qmp-final-r3-signatures-2020.pdf] and other applicable requirements in EPA quality regulations and policies [https://www.epa.gov/quality]. This approval action does not represent EPA's verification of the accuracy or completeness of document(s) under review, and is not intended to constitute EPA direction of work by contractors, grantees or subgrantees, or other non-EPA parties.

EPA Project Manager: Steve Hohman

EPA Project Quality Assurance Manager's Signature: _____

Note: This approval action represents EPA's determination that the document(s) under review comply with applicable requirements of the EPA Region 3 Quality Management Plan [https://www.epa.gov/sites/production/files/2020-06/documents/r3qmp-final-r3-signatures-2020.pdf] and other applicable requirements in EPA quality regulations and policies [https://www.epa.gov/quality]. This approval action does not represent EPA's verification of the accuracy or completeness of document(s) under review, and is not intended to constitute EPA direction of work by contractors, grantees or subgrantees, or other non-EPA parties.

EPA Project Quality Assurance Officer: Elizabeth Gaige

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A3. Distribution List

This QAPP will be appended to the latest edition of the DEP *Water Quality Monitoring Protocols for Surface Waters* (Lookenbill and Arnold 2023), which is available on the DEP Water Quality Division website at: https://www.dep.pa.gov/Business/Water/CleanWater/WaterQuality/Pages/Data-Collection-Protocols.aspx.

It will also be placed electronically in in-house records at the DEP. It can be made available upon request. QAPP revisions will also be re-appended to DEP monitoring protocols. In addition, this QAPP will be distributed to each of the DEP Regions and cooperating programs who are involved with surface water and data collection. This will ensure data will be collected according to the quality addressed in this QAPP. Any revisions to this document will also be sent to those on the distribution list. The following is the distribution list of current staff that oversees coordination in each region and each respective agency:

- DEP Northwest Region – Joseph Brancato, Aquatic Biologist Supervisor
- DEP Southwest Region – Richard Spear, Aquatic Biologist Supervisor
- DEP Northcentral Region – Anne Hughes, Environmental Group Manager
- DEP Southcentral Region – Kristen Bardell, Aquatic Biologist Supervisor
- DEP Northeast Region – Walter Holtsmaster, Aquatic Biologist Supervisor
- DEP Southeast Region – Steven Unger, Aquatic Biologist Supervisor
- DEP Central Office –Erika Arnold, Environmental Group Manager - Monitoring
- Department of Natural Resources and Conservation (DCNR) – Nick Decker, Resource Manager
- Bureau of Laboratories (BOL) – Pamela Higgins, Bureau Director

A4. Project Organization and Task Responsibility

The following is a list of key project personnel and their corresponding responsibilities. Due to staff turnover, the list provided gives a general description of those involved. See Figure 1 – Organizational Chart to better define their relationships.

- Sampling Operations – WQD Central Office Biologists, DEP Regional Biologists
- Sampling Quality Control (QC) – DEP Project Manager
- Laboratory Analysis – DEP Bureau of Laboratories (BOL), Outside laboratories as needed
    - Inorganic Division – Trace Metals & Sample Receiving Section and Automated Analysis & Biochemistry Analytical Section
    - Organic Division – Organic Chemistry Section
    - Biological Services Division – Microbiology Lab, Giardia Lab and Microbial Source Tracking Lab
- Laboratory Quality Control – BOL Quality Assurance and Safety Section
- Data and QC Processing – DEP Project Manager, WQD Central Office Biologists, DEP Regional Biologists
- Data Review and Review QC – Falls to staff responsible for survey, could include WQD Central Office Biologist or DEP Regional Biologist
- Performance and Systems Auditing – WQD, Monitoring Section and Assessment Section
- Overall Project Coordination and QA – WQD, Monitoring Section
- Maintenance and Distribution of Officially Approved QAPP – DEP Project Quality Assurance Manager

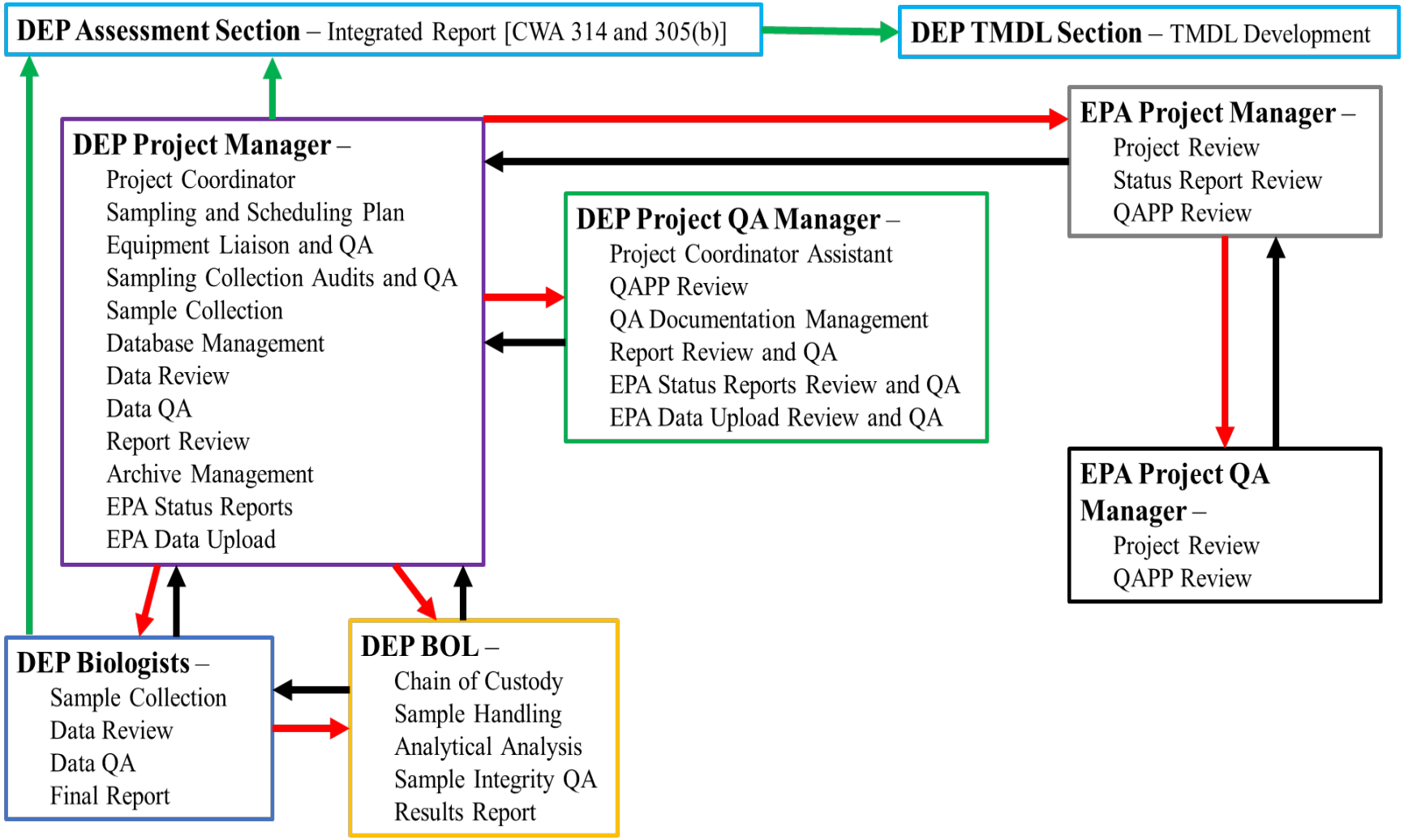

Figure 1. Organizational Chart

A5. Problem Background

The primary purpose for monitoring surface waters is to assess and evaluate protected uses and to broadly evaluate point and nonpoint sources and associated impacts on surface waters in accordance with 25 Pa. Code §§ 93 and 96 of DEP's regulations. Monitoring is driven by the following primary objectives:

Assessment of Protected Water Uses

To investigate and determine if the receiving waterbody is supporting or impaired for water quality standards (WQS) thresholds (thresholds for supporting the protected uses) and possible sources and causes of impairment from point or non-point sources of conventional pollutants and known or suspected water quality problems in surface waters. These surveys are performed to reevaluate existing assessments and identify additional sources and causes of water quality impairments identified by previous assessment efforts. Chemical and bacteriological data are typically compared to water quality criteria while considering frequency and duration with which a given criterion was developed according to supporting technical documentation and assessment methods.

- Pennsylvania surface water quality criteria can be found in 25 Pa. Code § Chapter 93 (https://www.pacodeandbulletin.gov/Display/pacode?file=/secure/pacode/data/025/chapter93/chap93toc.html)

- Technical documentation (https://www.dep.pa.gov/Business/Water/CleanWater/WaterQuality/Pages/Macroinvertebrates.aspx)

- Assessment methods (https://www.dep.pa.gov/Business/Water/CleanWater/WaterQuality/Pages/Assessment-Methodology.aspx)

Biological data (macroinvertebrates and fish) are used to calculate indices that are compared to supporting water quality standards thresholds described in the assessment methods (Shull and Whiteash 2023) and accompanying technical documentation.

Data Usage

Data are used for generating the Integrated Water Quality Report, listing impaired waterbodies as required by Section 303(d), and to support the compliance and permitting programs by defining the impact of specific discharges or land-based activities on receiving waters. Physical, chemical and/or biological data collected during surveys are generally evaluated using non-parametric, classification type analyses designed to display differences or similarities between sampling stations and metric thresholds.

Stream Redesignation and Existing Use Evaluations

To review and revise, if necessary, WQS to ensure that protected uses are accurate.

Data Usage

Data will be used to evaluate the need for antidegradation protection in selected Pennsylvania waters. The evaluation will consider biotic and abiotic components of the watershed ecosystem. Features such as water quality sensitivity, land use activities, and the environmental and ecological significance of the watershed are evaluated in an effort to determine the correct protected use per the requirements in 25 Pa. Code § 93.4b.

Maintenance of fixed station Water Quality Network (WQN)

To establish and maintain a database to assist in a) describing water quality conditions across Pennsylvania surface waters; b) identifying long-term trends of improvement or degradation in major waterbodies; c) determining the suitability of waterbodies for water supply, recreation, fish, aquatic life, and other uses; d) providing a limited measure of “background” water quality throughout Pennsylvania’s Ecoregions; and e) providing a basis for evaluating success of the water quality management program.

Data Usage

WQN data will be used to generate basic statistics to generally characterize water quality by station; these values can then be compared to established standards and criteria to determine the effectiveness of water quality management programs. Furthermore, seasonally adjusted Kendall tests are applied to historic WQN data to assess long-term trends in water quality using a Weighted Regressions on Time, Discharge, and Season (WRTDS) model (Hirsch et al. 2010). Additionally, collection of data at “ambient” stations provides a measure of background conditions at unaffected or minimally affected sampling locations. These data provide both a measure of long-term water quality trends resulting from airborne contaminants and background water quality as identified by ecoregion. This information can then be used to develop permits by the permitting programs or to determine the potential use status (impaired or supporting) of nearby streams degraded by anthropogenic inputs.

Other Objectives

To include cause and effect monitoring, point of first use determinations, and protocol, method, and standards development.

Date Usage

Cause and effect monitoring data will be employed to investigate possible relationships between point or nonpoint sources of conventional pollutants and known or suspected water quality problems through the collection and analysis of biological, physical, and chemical data. These surveys are designed primarily to monitor the effectiveness of permitted treatment facilities but are also used to

investigate reported or suspected water quality impacts for nonpoint source and other pollution sources.

Point of first use surveys are completed as part of the evaluation and permitting of wastewater discharges to intermittent and ephemeral streams, drainage channels and swales, and storm sewer. The point of first surface water use establishes where Chapter 93 WQS must be supported.

Protocol, method, and standard development data collections will be used to implement the Pennsylvania Clean Streams Law and the Federal Clean Water Act. To achieve this, at a statewide level, WQS are developed to establish the protected uses, criteria, and antidegradation requirements that surface waters must meet.

A6. Project Task Description

Routine surface water data collections for the purpose of evaluating protected uses (existing and designated), assessing protected uses, and investigating point and nonpoint source impacts will be conducted. Products include existing use evaluations, redesignation recommendations, protected use assessments, and evaluations of point and nonpoint source discharges or impacts. DEP implements a hybrid approach with respect to selecting monitoring locations. One component includes a fixed station, Pennsylvania Water Quality Network (WQN) where stations are established indefinitely for the purpose of developing long-term datasets that allow for the development of water quality trends, the ability to measure the effectiveness of water management programs, the ability to develop water quality assessment methods and standards, and to provide data that will inform permitted activities. The WQN also includes locations where data are collected at fixed intervals of five years. Every five years data are evaluated to determine if the data are serving the previously identified objectives. After each five-year cycle new stations may be added, stations may be discontinued, and some stations may be modified.

In addition to the WQN, fixed station approach, more intense short-term locations are established to evaluate protected uses, assess protected uses, and to evaluate point and nonpoint source impacts. Short-term stations are chosen to ensure that data representative of conditions in a given stream reach will be obtained. Factors considered in locating these stations include watershed land uses, volume and chemical characteristics of known point source wastewater discharges, physiographic and demographic conditions that contribute to non-point source problems, and stream hydrology. Every effort is made to sample representative, homogeneous, low-flow water columns at comparable locations. However, because weather, stream flow conditions, and accessibility vary, observations of instream and riparian physical characteristics are recorded for reference in estimating water quality under various conditions. Data collection includes biological, chemical, and physical data.

Frequency of sampling varies depending on sampling objectives and are described in *Pennsylvania's Surface Water Quality Monitoring Network Manual* (Pulket and Arnold 2023). At WQN stations, waters samples are collected bimonthly (water samples at ambient and bay loading stations are collected monthly) as discrete samples at mid-channel, mid-depth on small streams and laterally composited, depth integrated samples on larger streams or rivers. Semi-quantitative benthic macroinvertebrate samples are collected annually according to DEP data collection protocols. At WQN lake stations, an annual qualitative plankton tow is substituted for semi-quantitative macroinvertebrate sampling. Lake water samples (except Lake Erie) are collected once a year during summer stratification and consist of a sample collected one meter below the surface and a sample collected one meter off the bottom at the deepest part of the lake. Sampling schedules for short-term stations are either limited to one-time collections and/or governed by protocol and the water quality criteria evaluation needs. Since data collection is ongoing, only a general timeline is provided below (Table 1). Sample collection and reporting requirements are further described in Section B of this QAPP. WQN stations and sampling objectives can be found appended to DEP's Monitoring Book as the WQN Manual (Pulket and Arnold 2023).

Table 1. General timeline schedule

Month (represents monthly sequence from project start and not calendar months)												
Activity	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
File Search												
Field Recon.												
Field Survey												
Laboratory Work-up												
Report Writing												

Project fiscal information is described as follows:

Data Collection

Office – Planning, file searches, contacts with resource persons	2 workdays
Field – Reconnaissance, water sample collection, field observations	6 workdays
Taxonomic Identifications – Biological samples	4 workdays
Laboratory Support – Chemical/physical/biological analyses	2 workdays
Report Preparation – Data compilation, analysis, and interpretation, recommendations, draft report preparation, comment review, revisions	10 workdays
TOTAL	24 workdays

A7. Quality Objectives and Criteria

To meet project objectives, biological, physical, chemical, or a combination of the three types of data are collected. Biological data collected for evaluation and assessment purposes includes macroinvertebrate, fish, fish tissue, mussels, algae/periphyton, aquatic macrophytes, and bacteria data. The collection of physical characteristics in the form of habitat evaluations can also be used to make evaluations and assessments. Stream flow and discharge can also be collected as supplemental information to make an informed decision.

DEP data collection protocols and assessment methods are part of a larger conceptual framework that DEP uses to collect quality data and make decisions on various surface water matters across Pennsylvania. This larger conceptual framework begins with technical documentation that contains the technical support information and literature to ensure accurate and precise data analysis and interpretation.

DEP technical documentation can be found at:
https://www.dep.pa.gov/Business/Water/CleanWater/WaterQuality/Pages/Macroinvertebrates.aspx

DEP technical documentation includes characterizations of data quality and quantity to characterize water quality, biological assemblages, or other parameters with minimal uncertainty in comparison to relevant WQS. It also includes performance criteria that data need to achieve in order to make accurate protected use (designated and existing) evaluations and assessments. General quality control specifications including the frequency of blanks and replicates are described throughout this QAPP, and more specifically in the technical documentation, data collection protocols, and as appropriate, in the assessment methods. Laboratory quantitation limits can be found in the *Quality Assurance Manual for the PA DEP Bureau of Laboratories* (DEP 2019). Representativeness is addressed in the Monitoring Book *Chapter 2, Sampling Design and Planning* (Lookenbill et al. 2023) and more specifically, within the appropriate data collection protocols. Precision estimates for biological methods are described in detail in respective technical documentation as is detailed development efforts that resulted in these precision statistics.

Use (designated and existing) evaluations and use assessments based on physicochemical data can be collected via discrete chemistry data collection or continuous instream monitoring (CIM). Sediment samples, passive samplers and in-situ discrete field observations may also be collected to provide additional insight into water quality conditions. To ensure discrete chemistry samples are collected with the analytes of interest, the DEP provides specific Standard Analysis Codes (SAC) which collectors are required to use for chemical and biological samples submitted to DEP Bureau of Labs. Any deviation from the SACs provided should be approved by the DEP Project Manager. In addition, at the discretion of WQD, analytes can be added or removed at any time. Addition of analytes may be warranted if limiting conditions are met and an analysis to include a more robust suite is needed (e.g. the addition of dissolved constituents, the addition of phaeophytin, etc.).

All data collection will be conducted according to DEP reviewed and approved monitoring protocols. Protocols specific to these projects are found in *Water Quality Monitoring Protocols for Surface Waters* (Monitoring Book, Lookenbill and Arnold 2023) and DEP's *Evaluations of Phosphorus Discharges to Lakes, Ponds, and Impoundments* (DEP 2013).

Quality control efforts specific to these projects will be further described in Section B of this QAPP and are briefly described herein. All biological and chemical samples shall undergo the collection of replicates every 20 samples or once per project. Chemical samples shall also undergo collection of blanks every 20 samples or once per project. Additional equipment blanks and source water blanks are recommended if equipment and/or blank water is suspect of contamination. Furthermore, if sampling equipment is noted as malfunctioning at time of collection, it should be noted in a field notebook or on a field data sheet and the DEP project manager should be informed. If corrective action can be made in the field, the collector should take the necessary actions to problem-solve. If a solution cannot be determined in the field, field work should cease and be rescheduled until the problem is solved. For instance, if the chemistry filtering equipment breaks or malfunctions, no samples should be taken and sampling should be rescheduled; or for instance, if a field meter probe gives erroneous readings the collector should determine if calibration drifted, requiring equipment recalibration. Further information regarding probe accuracies warranting recalibration are outlined in Hoger and Arnold 2023a; criteria are summarized in Table 2. Additionally, assurance of sample integrity is required of any collector. Steps should be taken to avoid contamination of the sample by an outside source; if at any point during sample processing contamination is observed, the sample must be properly disposed of and recollected.

Table 2. Calibration criteria for field measured parameters

Field Parameter	*Calibration Criteria Warranting Equipment Recalibration*
Temperature	± 0.2°C
Specific Conductance	± 5 µS/cm or 3%, whichever is greater
pH	± 0.2 units
Dissolved Oxygen	± 0.3 mg/L
Turbidity	± 0.5 FNU or ± 5%, whichever is greater

Furthermore, chemical samples go through an accuracy check by requiring spikes, duplicate, and blank analyses. Accuracy is determined by routine laboratory protocol described in the *Quality Assurance Manual for the PA Department of Environmental Protection Bureau of Laboratories* (DEP 2019) and in test specific Standard Operating Procedures (SOP). The BOL Quality Assurance Manual is provided in Supplemental Document # 1 and SOPs can be made available upon request.

Sensors used for field chemistry measures, whether discrete readings or continuous deployments, are selected that have a range that will cover expected environmental conditions for the project, with sufficient precision and accuracy. For all continuous deployments and preferably for all discrete readings as well, optical DO sensors will be used as they have higher degrees of accuracy and hold calibration much better. Further details on sensor selection can be found in the Sensor Selection section of Hoger and Arnold (2023a).

A8. Training and Certifications

All data collectors need to be properly trained prior to involvement in data collections and data management. Ensuring data collectors are properly trained is the responsibility of the quality assurance manager and/or project manager. Training involves reading DEP's protocols and training by DEP senior staff or program specialists. New collectors will go through a training period at the discretion of the supervising biologist or water program specialist. All training events are to be tracked by the new collector and their supervisor. When an audit is performed documentation is to be summarized in an audit report. The audit report will contain all pertinent information to the audit such as name and contact information of collector, name and contact information of auditor, date of audit, monitoring objectives, a brief description of the sampling plan, data protocol(s) being audited, details regarding implementation of protocols, a summary and conclusion of the audit, and any recommendations the auditor has as a result of the collector's performance. The audit report is to be signed by both the collector and auditor and maintained as a permanent record.

DEP staff who perform macroinvertebrate identification are required to maintain Society for Freshwater Science Ephemeroptera Plecoptera Trichoptera (EPT) East Genus-Level Certification. Any new biologist will endure quality assurance audits to ensure adherence to protocols. In addition,

an auditor shall accompany an individual collector on at least one survey at a minimum of every five years. The auditor shall also randomly select any documentation to verify that data are accurate and complete, and that appropriate analytical techniques are used. The auditor will maintain records for each individual to include: (1) the date of the audit; (2) a list of protocols for which the individual was evaluated; (3) any deficiencies noted; and (4) recommendations offered and corrective actions taken. Training and quality assurance records will be maintained in DEP electronic training record files.

All taxonomy should be completed by specialized and/or certified taxonomists. Taxonomists may be in-house DEP biologist or be contracted out to recognized laboratories. Taxonomists should use the most updated resources to aid in taxonomic identifications. Furthermore, taxonomists should undergo QA/QC checks on 10% of samples identified by a certified taxonomist. Biologists who are involved with macroinvertebrate subsampling will need to be properly trained with documentation that tracks sorting error as is described in the QAPP, Appendix E.1.

The laboratories used for sample analyses must be accredited. The DEP Bureau of Laboratories (BOL) will primarily be used for laboratory analyses. BOL is accredited by the National Environmental Laboratory Accreditation Program (NELAP) through the New Jersey and Pennsylvania Department of Environmental Protection (DEP) accreditation bodies. If at any time during the project a new laboratory needs selected to process samples, the laboratory needs to be a DEP contracted lab and accredited by bodies within the NELAP.

A9. Documents and Records

Historic and current QAPP documents are housed in WQD's shared electronic files that are backed up according to DEP's information technology security protocols. Documents are to be maintained and retained permanently. This QAPP will be internally reviewed annually and will be submitted to USEPA for reapproval if material changes are made. Any amendments or updates to the document should be labeled as such. Every 5 years and as needed, this QAPP should be updated, revised and approved as a new document that designates signatures and the date of final approval. This will aid in keeping track of 5-year QAPP resubmission as required by the USEPA. Updated versions will be shared with those on the distribution list given above. Table 3 characterizes changes to this controlled, QAPP over time. The most recent version is presented in the top row of the table. Quality assurance data can be obtained through a request to the DEP Quality Assurance Officer.

Table 3. QAPP document control

Document Control Version #	Title	History/Changes	Effective Date
TBD	QAPP: Data collection for the purpose of existing use evaluations, protected use assessments, and evaluations of point and nonpoint source impacts to surface waters	Previous QAPPs updated and consolidated into Programmatic QAPP	August 1, 2021
391-3200-002	Fixed Station Surface Water Quality Network Monitoring		September 22, 2014
384-3200-001	Instream Comprehensive Surveys		September 22, 2014

To further describe documents and record keeping, each step of the data collection and interpretation process is outlined below. Descriptions include creation, maintenance and retention of records and responsible persons.

Data Collection

Data collected should be recorded on 'Data Collection Forms' provided in the Appendices of DEP's Monitoring Book (Lookenbill and Arnold 2023). Additional field notes can be written on the 'Data Collection Forms' or documented in a field notebook. These 'Data Collection Forms' and notes will be used to ascribe in-situ water quality conditions associated with biological and analytical chemistry sample results.

All benthic macroinvertebrate and fish samples should be given a unique station identification (ID) number that includes data collected, time collected, and collector (yyyymm-HHMM-collector). This station ID shall be retained with each sample through the entire sampling and subsampling processes. Benthic macroinvertebrate samples and associated data should be entered into DEP's Streams and Lakes Integrated Management System (SLIMS) database. Fish tissue data will be entered in DEP's Samples Information System (SIS); and, fish, mussel and periphyton community data are stored in Excel or Access database spreadsheets. Aquatic Macrophyte vouchered specimens will be given a unique voucher ID number that includes data, lake alias, rake number and user (yyyymmdd-LakeAlias-R#-user).

For water samples, the field data, sample and station descriptions, and sample numbers should be entered into SIS according to Shull, 2017 and retained with the collector until the report is final. DEP databases reside on enterprise data systems that are backed up and protected according to DEP information technology security protocols. Record retention for water quality data is permanent.

At the end of each sampling season, all 'Data Collection Forms' should be filed and retained for future reference. A filing system will be maintained by each collector. It is recommended that the collector keep hard copies of completed 'Data Collection Forms' as well as maintain specific folders on their computer as an electronic backup labeled for each data type as a repository for all 'Data Collection Forms' (i.e. Macroinvertebrate Data, Fish Data, CIM Data, WQN Data, etc.). It is further recommended to help facilitate order that all 'Data Collection Forms' be arranged by date and scanned by year to be placed in a folder that is labeled as 'YYYY Fish – 'Field Data Sheets.' Paper copies can be archived at the discretion of the field collector and field notebooks should be retained by the collector.

Calibration logs and calibration equipment checks should be maintained similar to 'Data Collection Forms'. It is recommended that calibration logs be kept with each unique piece of equipment. At the end of the sampling season, the calibration logs shall be scanned into an electronic file and paper copies archived. Archives are stored in DEP electronic files that are backed up and protected according to DEP information technology security protocols. Paper copies can be archived at the discretion of the equipment manager.

Data Processing

Biological samples that have been processed and identified will be enumerated on bench sheets found in Appendices of DEP's Monitoring Book (Lookenbill and Arnold 2023) Benthic macroinvertebrate taxonomic data will be entered into DEP's SLIMS database. Fish, mussel, periphyton, plankton, and aquatic macrophyte community data will be entered into Excel and Access databases.

All chemical and physicochemical data with the exception of continuous instream monitoring data are entered into DEP's SIS database (Shull 2017). Date, time, location, and the unique sample identifier will be stored in the SIS database. This database will be used by the BOL to import their analytical results to match field information; BOL will do this according to the directive found in BOL's Quality Assurance Manual (DEP 2019). Each collecting biologist is responsible for following-up on the status of samples submitted. This includes ensuring samples are analyzed, cross checking samples with replicate and blank samples and exporting the data from SIS for interpretation.

Continuous instream monitoring data will be housed in a database capable of handling large continuous files. DEP uses AQUARIUS database and software to store, manage, and visualize continuous water quality data. AQUARIUS allows for documenting and performing corrections of the data according to *Continuous Physicochemical Data Collection Protocol* (Hoger and Arnold 2023a).

In preparation for data interpretation and report writing, formatted templates containing formulas and report requirements are provided to collectors. These templates or another suitable data organization

matrix may be used. If the latter option is used by the biologist, they must ensure all calculations and visual displays maintain integrity. This option requires additional quality assurance by a second (or third party if needed) and the Project Manager before contents can be used to generate reports.

Reports

Data will be reported in the form of tables, figures, maps and/or charts. If warranted, a full report will be written. Scenarios in which a full report is needed include but is not limited to redesignation recommendations, integrated report delistings, and phosphorus control recommendations. Redesignation report guidance can be found in DEP's *Water Quality Antidegradation Implementation Guidance, Document Number 391-0300-002* (DEP 2003). Delisting requirements can be found in DEP's *Assessment Determination and Delisting Methods* (Shull and Pulket 2021). Phosphorus control reports will follow a general format as described in DEP's *Evaluations of Phosphorus Discharges to Lakes, Ponds, and Impoundments* (DEP 2013). Continuous instream monitoring reports are posted on the DEP website at: https://www.dep.pa.gov/Business/Water/CleanWater/WaterQuality/Pages/CIMReports.aspx.

Reports are not limited to those items described in the guidance and methods documents, some projects provide for special cases that require additional analyses and discussions; these additions can be included at the discretion of the reporting supervisor.

Reports should go through a review process by a second party to address any grammar or reporting errors/discrepancies. Corrective action should be taken by the report writer and reviewer; if no agreement can be made, the reviewing party should reach out to the DEP Project Manager for an additional review. Once finalized, a copy should be dated and filed appropriately. For final filing the document should be converted from a .docx file to a .pdf file.

Final Filing

Reports which have been reviewed and finalized should be saved in DEP's electronic stream files. Stream files are organized by a unique 5-digit code, which is associated with DEP's historic stream codes associated with the NHD Flowline flowing through the waterbody, and the stream, river or lake name. Reports shall remain in the stream files permanently.

Management of QA Record

QA records including training records, quality assurance audits, and corrective actions will be maintained in DEP electronic training record files permanently.

SECTION B: DATA GENERATION AND ACQUISITION

B1. Experimental Design

Project design will be applied to surface waters statewide. The type of experimental project design used is based off data of interest. Collectors should use DEP's Monitoring Book (Lookenbill and Arnold 2023) to develop a sampling plan conducive to each project's objective. The sampling plan will be objective specific but typically include a combination of:

- Chemical Data Collection
    - In-Situ Field Meter, Transect, and Profile Data Collection
    - Discrete Water Chemistry
    - Continuous Water Chemistry
- Physical Data Collection
    - Habitat Evaluation
    - Water Flow Data Collection
- Biological Data Collection
    - Benthic Macroinvertebrates
    - Fish
    - Bacteria

Site location selection is fluid from year to year based on regional and state priorities. In addition, environmental factors play a role in selecting sites year to year. For this reason, site specific maps are not provided in this QAPP. Site locations are documented as locational attributes in the SLIMS and SIS applications. Locational attributes will also be documented in the Excel and Access Databases used for storage and maintenance of data.

Biological samples are collected across a transect or throughout a large portion of the waterbody to ensure inclusion of all available habitat. In flowing waterbodies water samples are collected at mid-channel, mid-depth unless stream width, hydrology, discharge locations or volumes, or observed biological conditions indicate stratification of flow than integrated type sampling is used. Lake water samples are collected with the use of a Van Dorn or Kemmerer sampler at a depth of one meter below the surface and one meter above the lake's bottom. Parameters frequently analyzed are listed in Table 3. All water chemistry samples are cooled to less than or equal to 6ºC without freezing and shipped to the laboratory. Additional parameters may be required based on the specific nature of the waterbody survey; details regarding field collection requirements and laboratory test methods can be obtained upon request.

Table 4. Analyte Tests (commonly used Standard Analysis Code 087)

Test Codes	Test Description	Test Method
410	ALKALINITY AS CaCO3 @ pH 4.5	SM 2320B
01106H	ALUMINUM, DISSOLVED (WATER & WASTE) BY ICPMS	EPA 200.8
01105H	ALUMINUM, TOTAL (WATER & WASTE) ICPMS	EPA 200.8
00608A	AMMONIA DISSOLVED AS NITROGEN	EPA 350.1
00610A	AMMONIA TOTAL AS NITROGEN	EPA 350.1
01007A	BARIUM, TOTAL (WATER & WASTE) BY ICP	EPA 200.7
01022K	BORON, TOTAL (WATER & WASTE) BY ICP	EPA 200.7
01025H	CADMIUM, DISSOLVED (WATER & WASTE) BY ICPMS	EPA 200.8
00916A	CALCIUM, TOTAL (WATER & WASTE) BY ICP	EPA 200.7
01040H	COPPER, DISSOLVED (WATER & WASTE) BY ICPMS	EPA 200.8
01042H	COPPER, TOTAL (WATER & WASTE) BY ICPMS	EPA 200.8
00631A	DISSOLVE NITRATE & NITRITE NITROGEN	EPA 353.2
00671A	DISSOLVE ORTHO PHOSPHORUS	EPA 365.1
00602A	DISSOLVED NITROGEN AS N	SM 4500-NC
00666A	DISSOLVED PHOSPHORUS AS P	EPA 365.1
900	HARDNESS, TOTAL (CALCULATED)	SM 2340 B
01046A	IRON, DISSOLVED (WATER & WASTE) BY ICP	EPA 200.7
01045A	IRON, TOTAL (WATER & WASTE) BY ICP	EPA 200.7
01049H	LEAD, DISSOLVED (WATER & WASTE) BY ICPMS	EPA 200.8
01051H	LEAD, TOTAL (WATER & WASTE) BY ICPMS	EPA 200.8
01130A	LITHIUM, DISSOLVED (WATER &WASTE) BY ICP	EPA 200.7
01132A	LITHIUM, TOTAL (WATER & WASTE) BY ICP	EPA 200.7
99020	LOW BROMIDE BY IC	EPA 300.1 B
00927A	MAGNESIUM, TOTAL (WATER & WASTE) BY ICP	EPA 200.7
01056A	MANGANESE, DISSOLVED (WATER & WASTE) BY ICP	EPA 200.7
01055A	MANGANESE, TOTAL (WATER & WASTE) BY ICP	EPA 200.7
01065A	NICKEL, DISSOLVED (WATER & WASTE) BY ICP	EPA 200.7
01067A	NICKEL, TOTAL (WATER & WASTE) BY ICP	EPA 200.7
82550	OSMOTIC PRESSURE, MOS/KG	BOL 3003
403	pH, LAB (ELECTROMETRIC)	SM 4500-H+ B
00937A	POTASSIUM, TOTAL (WATER & WASTE) BY ICP	EPA 200.7
01147H	SELENIUM, TOTAL (WATER & WASTE) BY ICPMS	EPA 200.8
00929A	SODIUM, TOTAL (WATER & WASTE) BY ICP	EPA 200.7
95	SPECIFIC CONDUCTIVITY @ 25.0 C	SM 2510B
01082A	STRONTIUM, TOTAL (WATER & WASTE) BY ICP	EPA 200.7
00403T	TEMPERATURE AT WHICH PH IS MEASURED	SM 4500-H+ B
940	TOTAL CHLORIDE-ION CHROMATOGRAPH	EPA 300.0
70300U	TOTAL DISSOLVED SOLIDS @ 180C BY USGS-I-1750	USGS I-1750
00630A	TOTAL NITRATE & NITRITE NITROGEN	EPA 353.2
00600A	TOTAL NITROGEN AS N	SM 4500-NC
680	TOTAL ORGANIC CARBON	SM 5310 C
70507A	TOTAL ORTHO PHOSPHORUS AS P	EPA 365.1
00665A	TOTAL PHOSPHORUS AS P	EPA 365.1
945	TOTAL SULFATE-ION CHROMATOGRAPH	EPA 300.0
530	TOTAL SUSPENDED SOLIDS	USGS I-3765
01090A	ZINC, DISSOLVED (WATER & WASTE) BY ICP	EPA 200.7
01092A	ZINC, TOTAL (WATER & WASTE) BY ICP	EPA 200.7

B2. Sampling Protocols

Biological data collection will be conducted according to protocols described in Chapter 3 of DEP's Monitoring Book (Lookenbill and Arnold 2023). Depending on waterbody classification, benthic macroinvertebrate data collection shall follow one of the four stream macroinvertebrate data collection protocols (riffle-run, limestone, multihabitat or semiwadeable large river streams). Regardless of macroinvertebrate protocols used, all samples will be processed according to *Macroinvertebrate Laboratory Subsampling and Identification Protocol* (Brickner 2020). Fish tissue will be collected according to DEP's *Fish Tissue Data Collection Protocol* (Wertz 2021). Bacteriological data will be collected according to DEP's *Bacteriological Data Collection Protocol* (Miller 2023). These three types of biological data will be used for the purpose of conducting use evaluations and use assessments.

Other biological data that may be collected for purposes of describing water quality conditions and supporting evaluation and assessment decisions are briefly described herein. Fish community data will be collected according to DEP's *Fish Data Collection Protocol* (Wertz and Arnold 2023) and mussel community data will be collected according to DEP's *Mussel Data Collection Protocol* (Spear et al. 2017). Periphyton and chlorophyll-a samples from streams and rivers will be collected according to DEP's *Periphyton Data Collection Protocol* (Butt 2017). Plankton, macrophyte and chlorophyll-a samples from lakes will be collected according to DEP's *Plankton Data Collection Protocol* (Arnold 2023b), *Aquatic Macrophtye Data Collection Protocol* (Arnold 2023a), and *Chloprphyll-a, Phycocyanin, and Ash Free Dry Mass Data Collection Protocol* (Arnold and Butt 2023a) respectively.

Physical data collection in streams and rivers can include stream habitat assessments and water flow data collection. Descriptions of protocols to collect physical data are found in Chapter 5 of DEP's Monitoring Book (Lookenbill and Arnold 2023). Physical habitat assessment data collection will be completed when employing all flowing waterbodies biological data collection protocols.

Water samples will be collected according to Shull and Arnold, 2023; modifications may be made depending on waterbody type and size. All sampling equipment should be rinsed with surface water three times prior to collection and/or transfer. Most water samples are collected into HDPE plastic bottles with the exception of organic chemistry samples which are to be collected in amber glass bottleware. Water samples containing water for metal analyses should be fixed with HNO_3 to a pH of <2. Water samples containing water for ammonia, phosphorus and organic carbon analyses should be fixed with H_2SO_4 to a pH of <2. Samples are immediately placed on ice until submission to BOL. Samples should be submitted the day of sampling or the morning after the sampling event to ensure 48-hour holding times are not exceeded. Analytes with 48-hour holding times are nitrate, phosphorus and turbidity. Alkalinity has a holding time of 14 days. Specific conductivity, bromide, sulfate and total nitrogen have 28-day holding times; all other parameters have a holding time of six months or longer. After each use, water sampling equipment should be thoroughly rinsed with deionized water and cleaned according to protocols in DEP's Monitoring Book (Lookenbill and Arnold 2023). All other

chemistry collections, such as sediment sampling and passive sampler analysis should follow methodologies found in Chapter 4 of DEP's Monitoring Book (Lookenbill and Arnold 2023).

With any data collection protocol used during a sampling project, it is recommended that in-situ field meter water quality readings be taken. Measurements could be a single discrete reading to describe current water quality conditions, transect readings across the width of the stream or river to verify homogeneity of the water system, or profile depth reading to determine stratification of larger bodies of water such as impoundments and lakes. To collect in-situ readings, a calibrated field meter is used. At a minimum, temperature, pH, dissolved oxygen (DO), and specific conductance measurements are recorded; other parameters are optional. The field meter should be calibrated according to procedures outlined in Hoger and Arnold, 2023b and guidelines referenced from the equipment's operation manual. A summary of calibration recommendations is provided in Table 5. DEP does not use any one specific field meter, to that end specific guidance on calibration operating procedures cannot be made. At the end of the field day, calibration checks are recommended as a check against standards to ensure readings are within the criteria found in Table 2. The collector should also perform calibration checks anytime field parameters do not make sense for expected conditions. At the end of the day, the collector should ensure the field meter is free from debris prior to storage. To clean the field meter equipment, run clean tap water over the probes to rinse large particles; if a more thorough cleaning is required use a small amount of soap and a soft bristled brush on the probes. For storage, a small amount of tap water or pH 4 standard should be placed in the storage cup; probes should not be submerged for overnight or long-term storage.

In addition to discrete physicochemical data, monitoring objectives may require the collection of continuous physicochemical data according to Hoger and Arnold 2023b. Discrete physicochemical data typically includes at least four parameters: water temperature, specific conductance, pH, and DO. Continuous water quality monitors should be calibrated according to procedures outlined in Hoger, 2020 and guidelines referenced from the equipment's operation manual. A summary of calibration recommendations is provided in Table 5. Calibration follows guidelines referenced from the equipment's operation manual. DEP does not use any one specific continuous water quality monitor, to that end specific guidance on calibration operating procedures cannot be made. Field meters are used during servicing of a continuous water quality monitor (1) as a general check of reasonableness of monitor readings, (2) as an independent check of environmental changes during the service interval, and (3) to make cross-section surveys or vertical profiles in order to verify the representativeness of the location of the sonde in the aquatic environment. Data collected by the field meter should not be used to calibrate the instream monitor. However, some instream monitors cannot be calibrated, in which case the calibrated field meter must be used in the calibration correction of monitor records. In addition, DEP may use a calibrated field meter measurement for undocumented data error corrections (a potential result from the DEP deployment method, discussed in detail Hoger and Arnold 2023b). Independent field measurements must be made before, during, and after servicing the monitor in order to document environmental changes during the service. Side-by-side

measurements between the field meter and the monitor are made by placing the calibrated field meter as close to the monitor sensors as possible. The field meter should refresh data on the display at 10 second intervals, or more frequently, to ensure the most accurate data are recorded during measurements.

B3. Sample Handling and Custody

For biological samples, macroinvertebrates and fishes, samples can be processed for enumeration by the collector or by other designated staff. Sample containers are labeled with date, time, and collector (yyyymm-HHMM-collector), stream name, location, and number of containers per sample. A database and archive system shall be maintained for all samples in which identifications have been completed. Each Regional Office shall maintain their own archiving system; Central Office will maintain their own archiving system which can be used by Regional Offices if requested.

Sample handling and chain of custody for chemistry samples should be followed according to the description provided in Shull and Arnold 2023. Steps are summarized herein. The collector will use a unique identifier, a seven-digit number that contains a four-digit collector number and a three-digit sample number. The collector is responsible for recording locational information and the date and time of the sample. The above information should be recorded on a sample submission form which is submitted to BOL at time of relinquishment. Samples can be submitted one of two ways. The collector can hand deliver samples to BOL's sample receiving. Under this option, the collector unloads cooler contents and places samples in designated refrigerators and/or freezers; the sample submission form remains with the samples. The collector also has the option of using a courier service contracted by the DEP. Under this option the collector will drop coolers at a designated location, located throughout the state, for courier pick-up and delivery to BOL. The collector should insert their sample submission form into a plastic sealable bag or whirl-pack (preferably double bagged) and tape it to the inside lid of the cooler. It is recommended that collectors either scan or take a picture of sample submission forms for their records. Upon receipt by BOL staff, the temperature of each sample bottle is measured using a calibrated infrared thermometer to ensure proper shipping, handling and holding requirements. If samples are found to be above 6°C, BOL will flag the sample and contact the collector. The flag will remain with the sample and the reporting biologist shall qualify the data.

Continuous physicochemical data, including operational and maintenance records, are recorded on portable electronic devices and transferred to AQUARIUS.

Table 5. Summary of Calibration Recommendations

Parameter	Calibration	Notes
Temperature	Check against NIST certified equipment	
pH	Three-point calibration (4,7,10)	Two-point calibration acceptable if conditions are known
Dissolved Oxygen (DO)	100% water-saturated air: • Water in bottom of calibration cup (do not submerge sensor) • Calibration cup on loosely to prevent pressure buildup • Run a wipe cycle, if possible, to remove water droplets on sensor	• Calibration **onsite** to avoid influence of barometric pressure differences. • Check throughout the day. • Allow plenty of time in cold weather.
Specific Conductance	One-point calibration with checks • Calibrate at a high value (1000 µS/cm) • Check at a low value (100 µS/cm) • Rinse with Deionized (DI) water and check in air (should be 0 µS/cm)	If values above 1000 µS/cm are expected, also check in a higher standard.

B4. Analytical Methods

Table 3 above references analytical methods used in analyses of discrete chemistry samples. The BOL is responsible for setting SOPs for analytical analyses. BOL SOPs are currently not housed with the WQD but can be obtained upon request. If at any time during lab analyses sample integrity is compromised, the chemist is responsible for documenting the flag in the final BOL results report. Occasionally, holding times are exceeded or samples are incorrectly preserved; if so, BOL notifies the collector immediately. The collector must then respond to BOL within 10 days to request or decline results. The collector's supervisor and any other interested parties must be copied on their response. If analytes used to make assessment decisions are impacted by either lab equipment failure or collection error, the sample should be recollected. While not ideal, the collector may accept the data for the analytes; though, results should not be used to make assessment determinations.

Other data including biological, physical, field meter, and continuous water quality data that is not submitted to BOL are analyzed according to specific data collection protocols and assessment methods.

B5. Quality Control

DEP refers to the U.S. Geological Survey Techniques and Methods – *Design, Analysis, and Interpretation of Field Quality-Control Data for Water-Sampling Projects* (Mueller et al. 2015) to define and implement quality control samples with some exceptions, which are noted below.

Field Quality Control Samples

Two categories of field quality control samples, blanks and replicates, are implemented with data collection protocols. Generally, replicates are applicable to biological, chemical, and physical data collection protocols; while, with some exceptions, blanks typically are applied to chemical data collection protocols. While some protocols follow specific QC schedules; QC sample definitions are to be consistently maintained. Definitions for QC samples are described in Table 6.

Field blanks and sequential replicates are the most common quality control samples collected. Generally, for every type of sample collected, field blanks (if applicable) and sequential replicates are to be collected every 20th sample or once per project at a minimum. Split replicates are strongly encouraged to be submitted as part of the collector's routine QC schedule, additional split replicates can also be sample design and protocol dependent. Field blanks are to be collected stream side or lake side. Equipment blanks are recommended on any new sampling equipment and at the start of each field season or on a case by case basis. Equipment blanks are to be collected in a controlled environment like the office, lab, or warehouse. When collecting blank QC samples, Ultra Purified water is to be used. Ultra Purified water can be obtained from the BOL, CO, or another supplier. When implementing QC sampling, the same sampling procedures that are or would be used to collect an environmental sample will be used to collect the QC sample. Protocols were described in section B2 and detailed procedures are outlined in the Monitoring Book (Lookenbill and Arnold 2023)

Table 6. Field Quality Control Samples

Category	Type	Subtype	Purpose
Blank	Equipment	Equipment	To detect if cleaning of the sampling equipment system is adequate.
		Sampler/Tools	Conducted if sampler is thought to be source of contamination.
		Filtration	Conducted if the filtration pieces are thought to be source of contamination.
		Source	To detect if the blank water source has been contaminated.
	Field	Field	To detect if the field process or environmental conditions introduce contamination.
Replicate	Sequential	In-Situ	To determine if field techniques were properly executed.
		Intra-Site	To determine spatial variability within the sampling reach (variability within a short distance).
		Inter-Site	To determine variability between a sampling reach (variability between a larger distance).
	Split	Field	To determine analytical precision and variability of sample (split prior to preservation).
		Lab	To determine analytical precision and variability of laboratory processes (split after preservation).
	Concurrent	Equipment	To determine sampling equipment precision and variability.

Biological Samples

All biological field data collections will incorporate the collection of replicates. The same equipment and crew will be used to collect the sequential sample. Sequential replicate benthic macroinvertebrate samples shall be collected within the same reach as the initial sampling. Habitat, which was sampled previously should not be resampled, but rather other similar and suitable habitat should be sampled. For semi-wadable benthic macroinvertebrate collections of replicates shall be collected as a site replicate; this means if it was determined that there were three water quality zones and three samples across the width of the river need be collected, the replicate should also contain three samples each in the same reach as the respective water quality zones. In addition, it is recommended to collect intra-site and inter-site replicates to discriminate against variation within and between sites in the same stream reach. Fish and plankton samples should undergo, a similar replicate collection schedule of benthic macroinvertebrate samples. Periphyton sampling QC is detailed more extensively in *Periphyton Data Collection Protocol* (Butt 2017) and briefly described below.

Since aquatic macrophyte surveys and mussel surveys take a significant amount of time and require many moving parts, crews only have to perform a replicated effort every 5 years. For aquatic macrophyte surveys, the community composition portion of the data collection efforts will only be replicated. This requires two sets of evenly spaced sample points be determined using the procedures described in *Aquatic Macrophyte Data Collection Protocols* (Arnold 2023a).

Bacteria collections will, in addition to field blanks and sequential replicates, incorporate split replicates. For bacteria, field blanks are required every 20th sample or for each sampling trip/day. When collecting a bacteria field blank, reagent free sterile (autoclaved) water will be used instead of Ultra Purified water. The autoclaved water is obtained from BOL or the laboratory analyzing the samples. Bacteria sequential and split replicates should also be collected to gauge testing variability, potential sources of contamination due to collection procedures, and precision of the sampling process. As part of the bacteriological source tracking, qPCR sequential and split replicates will additionally be collected at the start, middle, and end of the sampling event.

Similarly, algal biomass and Harmful Algal Bloom (HAB) data collections will incorporate sequential replicates, split replicates, and field blanks. For benthic periphyton and algal biomass samples, field sequential replicates are to be collected every 20th sample or once per basin/project; split replicate samples are already built into the protocol and detailed procedures are found in *Periphyton Data Collection Protocol* (Butt 2017), *Chlorophyll-a, Phycocyanin, and Ash Free Dry Mass Data Collection Protocol* (Arnold and Butt 2023a), and *Harmful Algal Bloom (HAB) Data Collection Protocol* (Arnold and Butt 2023b). When collecting surface water algal biomass and HAB samples, sequential replicates and field blanks are to be collected every 20th sample. Split replicates should also be collected when concentrations are anticipated to be high. To collect a split surface water algal biomass or HAB samples, double the volume of water will be collected. This volume will be aliquoted on two filters or into two bottles respectively and submitted as separate samples.

Since bacteria, periphyton, algal biomass, and HAB sample collections require a strict QC schedule, it is recommended that field QC be carried out in the following order. The environmental sample should be collected first, then the sequential replicate, then split replicate bottle or filter A (SA), then split replicate bottle or filter B (SB), and finally the field blank. Following sequential order, these samples should all be identified with a unique sequence number, date, and time combination (Table 7).

Table 7. Sequencing environmental, duplicate, split, and blank samples.

Sample Type	Sequence	Date	Time
Environmental	100	6/30/2021	1000
Sequential Replicate	101	6/30/2021	1002
Split Replicate (SA)	102	6/30/2021	1004
Split Replicate (SB)	103	6/30/2021	1006
Blank	104	6/30/2021	1008

Chemical Samples

Equipment blanks are recommended once per field season, typically at the start of the field season and incorporates all components of the data collection process from sample collection to sample filtration. To collect equipment blanks, data collection protocols will be followed in a controlled setting like a lab space or warehouse workstation. Sampler/tools, filtration, and source equipment blanks are to be collected on an as needed basis if these specific components are the suspected contamination source. To collect sampler/tool or filtration blanks, the equipment will be rinsed with blank water three times; the rinsate is to be saved and analyzed for the parameters of concern. To collect a source blank, a sample is to be taken directly from the spigot of the source water.

Discrete physicochemical and sediment collections require at a minimum in-situ sequential replicates be collected every 20th sample. Sequential replicates are to be sampled in the same manner as the environmental sample. Split replicates should also be collected when concentrations are anticipated to be high. To collect a split replicate, double the volume of water or sediment will be collected. This volume will be aliquoted on two filters or into two bottles respectively and submitted as separate samples and will follow the labeling order is outlined in Table 7. For discrete physicochemical samples, field blanks are to be collected at minimum every 20th sample or once per project. Equipment blanks (sampler/tools or filtration) will be collected as needed at highly-impacted sites. This is particularly important for sediment sampling at highly-impacted sites, where it is recommended that an equipment tool blank be collected. Source blanks are to be collected as needed.

When employing the passive sampler data collection protocol, replicates are not required due to the expense of the equipment; however, a recommendation of at least one duplicate per set of around 10 is given. Field blanks are required for all passive sampler deployments. Blanks are just regular samplers that will be exposed to air in the field while the passive samplers are being deployed and retrieved from the water. Any air contamination will be picked up and measured by these blanks. Air contamination is common, particularly with Semi-Permeable Membrane Devices (SPMDs), depending on the compounds measured. Industrial areas and cities may produce airborne contaminants that are easily picked up by SPMDs. The number of blanks implemented is up to the collector's discretion – DEP normally uses one blank per three sites/deployments, but more blanks are always optimal. The only issue with this procedure is that any contamination that results will be unable to be pinpointed to any one site or sampler.

Protocols that require the use of field meters and continuous instream monitoring equipment are required to be calibrated before use. Proper calibration should follow manufacturers' guidelines. In addition to these guidelines, DEP requires several procedures to further ensure reliability and defensibility of data. Field meter calibration will be checked before each day of use to ensure that calibration is within the guidelines. All calibration activity, including checks, need to be logged and records maintained for quality assurance.

Quality control of CIM data incorporates multiple field visits throughout the sampling period. It is recommended that data be downloaded, equipment be cleaned and checked against calibration standards to describe the accuracy of the continuous measurements every three to six weeks. QC documentation collected during field visits will be used to aid in data management prior to making data final. Methods to perform field visits and correct data are further detailed in DEP's *Continuous Physicochemical Data Collection Protocol* (Hoger and Arnold 2023a).

Laboratory Quality Control Samples

Protocols for biological data validation are found in DEP's Monitoring Book, Chapter 3 (Lookenbill and Arnold 2023). Biologists should submit 10% of each type of biological sample to the Project Manager for taxonomic verification and QA. To accomplish the 10% quality assurance of all samples identified, taxonomists should submit 10% of the samples they have identified in a calendar year. The most common type of certification is the genus level EPT certification provided by the Society of Freshwater Science. DEP may request sample vials for any results entered into internal databases for quality assurance purposes. For periphyton identification, additional QA/QC requirements are described in the *Periphyton Data Collection Protocol* (Butt 2017).

The quality control laboratory protocol for chemical sampling is described in the *Quality Assurance Manual for the PA Department of Environmental Protection Bureau of Laboratories* (DEP 2019) and in test specific Standard Operating Procedures (SOP). The BOL Quality Assurance Manual is provided in Supplemental Document # 1 and SOPs can be made available upon request.

Data Collection Audits

Field investigators will endure quality assurance audits to ensure adherence to protocols. In addition, an auditor shall accompany an individual collector on at least one survey at a minimum of every five years. The auditor shall also randomly select any documentation to verify that data are accurate and complete, and that appropriate analytical techniques are used. The auditor will maintain records for each individual to include: (1) the date of the audit; (2) a list of protocols for which the individual was evaluated; (3) any deficiencies noted; and (4) recommendations offered and corrective actions taken. All data collected in the field need to be recorded for verification purposes in the appropriate field data sheets or field notebooks. In addition, collectors are required to scrutinize their own data as a form of personal auditing.

The field investigator will review chemical and biological sample replicate results and note if target parameters are within range of environmental samples. Similarly, the field investigator will review sample results and note if target parameters are detected in the blank and flag samples accordingly.

Logs and field notes shall be maintained by the collector. Records should be reviewed for completeness periodically throughout the year and compared to data entries in the various entry databases. Calibration logs should be kept with the field meter and archived in-house for reference at the end of each sampling year. Calibration checks should be performed throughout the day if multiple samples will be collected. Results of calibration and preventive maintenance recommended by the manufacturer must be recorded in an equipment logbook maintained for each piece of equipment. Dates of equipment use, calibration results, and operator maintenance activities are recorded. Continuous instream monitoring equipment will have a similar log, requirements are further detailed in *Continuous Physicochemical Data Collection Protocol* (Hoger 2017).

Results QC

Documentation of taxonomic QC results will be stored in WQD, Central Office and samples which were quality assured will be indicated as so in the SLIMS database. Similarly, fish identifications will be documented in the associated Excel and Access databases. Furthermore, all data entries should undergo verification by checking one's work. During results retrieval and data interpretation of results, biological data should again be verified against enumeration forms and database entries.

Subsampling procedures for benthic macroinvertebrate samples and the sequential identifications will undergo QA/QC. Biologists who are involved with macroinvertebrate subsampling will need to be properly trained with documentation that tracks sorting error as is described in the QAPP, Appendix E.1. Macroinvertebrate identifications will undergo QC for each biologist involved in taxonomy. The macroinvertebrate identification QC will be documented according to details provided in Appendix E.1. Fish identification QC is also detailed in Appendix E.1.

The protocol for validation of chemical data is found in the *Quality Assurance Manual for the PA Department of Environmental Protection Bureau of Laboratories* (DEP 2019). Each collector is also responsible for checking replicates and blanks against samples used for final reporting and calculations. If values are erroneously off from replicates and blanks, those data must by flagged and further investigated. If a sample is suspected those data should be removed from the final reporting data set. Similarly if any sample results are flagged by the lab as suspect of contamination, not preserved properly, or outside QC thresholds, these data should also be removed from the final reporting.

The field investigator will also review sample results to determine if the environmental sample and replicate(s) are less than 10% comparatively. For blanks the collector should note if target parameters

are detected in any blank samples. If discrepancies exist, the collector should flag samples accordingly and leave out of any data interpretation analyses.

Continuous data that do not meet the thresholds given in the table Maximum Allowable Limits for Reporting Data (Hoger and Arnold 2023a) are removed from the dataset and will not be used for assessment or other regulatory decisions. Continuous data that are graded “Unverified” due to a lack of quality control checks, will be reported but not used for assessments or regulatory decisions.

B6. Equipment Maintenance and Inspection

Collectors are expected to care for their own equipment. Equipment should be inspected prior to each scheduled field outing. If the equipment malfunctions the collector should troubleshoot or seek assistance from the program specialist. Parts should be replaced, or back-up equipment should be used.

Field meters, continuous water quality monitoring and flow meters should be inspected for malfunctions during each use and/or during routine maintenance visits. Probes should be inspected for damage such as cracks and scrapes. Calibration logs aid in tracking a probe’s life. Temperature probes are checked against another piece of equipment. This should be done periodically to ensure proper readings. DO probe toppers should be replaced every few years as general maintenance; in addition, zero-DO checks should be done periodically to determine how well the probe responds to changing conditions. To make a zero-DO solution dissolve 0.5g of sodium sulfite and 6-10 crystals of cobalt chloride in 500mL of water, place the probe in solution and document response time for DO to reach 0.3mg/L. If the response is slow and readings do not stabilize within 2-3 minutes the probe may need to be replaced. Probes that measure pH have a short life span and should be replaced at least every year or sooner if warranted. DEP records pH millivolts in calibration logs to determine the response life of the pH probe. If pH millivolts drift outside the ranges indicated below, the probe should be replaced. All other parameters follow specifications found in the equipment’s operational manual.

- Buffer 7: readings should be -50 to 50 mV, 0 mV is ideal
- Buffer 4: readings should be +165 to +180 mV from the buffer 7 reading
- Buffer 10: readings should be -165 to -180 mV from the buffer 7 reading.

B7. Instrument Calibration

Field meters used to collect in-situ discrete readings, transects, and vertical profiles need to be checked for calibration issues and if found will need to be calibrated at the start of each sampling day. Continuous water quality monitors are calibrated and maintained prior to deployment and during routine maintenance visits. Flow meters are calibrated by the manufacturer every three years. Calibration logs are kept separate with each unique piece of equipment. See Table 5 for calibration recommendations. Calibration logs should contain ‘Before’ calibration and ‘After’ calibration readings. Collectors can find calibration log forms in Appendix B of DEP’s Monitoring Book (Lookenbill and

Arnold 2023). It is recommended that collectors perform calibration checks on field meters at the end of day that included collecting transect and/or vertical profile data. Performing end of day checks aid in verifying data are accurate according to criteria. These checks can be logged in the same logbook using the same form as the calibration log, where 'After' calibration readings are rendered 'Not Applicable (NA).' More detail on field meter use and maintenance can be found in the Monitoring Book *Chapter 4.1, In-Situ Field Meter, Transect, and Vertical Profile Data Collection Protocol* (Hoger and Arnold 2023b).

B8. Inspection/Acceptance of Supplies and Consumables

All supplies used in data collection should be inspected by the collecting biologist prior to initial use. Equipment should be checked over at the time of purchase and/or receiving. Equipment should be returned to the manufacturer if defects are found. In addition, equipment quality should be inspected prior to and during each use. Standards used for calibration and sample preservation should be used within expiration dates and a log of standards used should be maintained. Expired solutions should be disposed of or recycled properly.

B9. Non-Direct Measurements

Non-direct measurements associated with projects are tools to help define watershed and land use characteristics. These characteristics are provided in the final report. To obtain land use descriptions, DEP often uses USGS Streamstats or ArcGIS software. These are tools commonly used in the field of hydrology and are acceptable tools to obtain watershed and land characteristics.

B10. Data Management

Data management should generally follow that which is described herein and DEP data collection protocols and assessment methods. Data management starts in the field through the use of field forms and field notebooks. Data contained in field documentation are entered or transferred into DEP's SIS, SLIMS, AQUARIUS, Excel or Access databases as soon as possible. The SIS database is used to store in-situ discrete field readings and laboratory results. This database organizes, stores and geo-references data for multiple end uses within DEP. SIS will generate reports which are emailed to the collector once all chemistry results have been completed by BOL. The collector is responsible for tracking and filing these BOL auto-generated reports at their discretion. The final BOL report is used as documentation that samples have been marked complete by BOL. SIS will also be used by the reporting biologist to obtain results for organization and interpretation in a final report. The SLIMS database will generate IBI metric reports for benthic macroinvertebrate samples. In addition, SLIMS will assist in documenting use assessment determinations for stream segments and waterbodies.

Data should further be organized into templated spreadsheets for calculations of indices, models and Chapter 93 WQS criteria violations. These spreadsheets should be maintained by the biologist responsible for the data. It is recommended that each lake, stream, river, or basin have its own

working folder on the responsible biologist's computer, which is separate from the final "Stream File," where spreadsheets, tables, charts and draft reports are created. The information in these files should be organized into a final report, which is placed in the final "Stream File" folder which was mentioned in the Final Filling Section of A9 of this QAPP.

SECTION C: ASSESSMENT AND OVERSIGHT

C1. Assessments and Response Actions

Assessments to be made include progress reports which will be provided to EPA mid-cycle and annually as is required. Additionally, performance assessments will be provided to collectors and report writers at the time of auditing; and project assessments will be made on an as needed basis.

On-going QC by all participating parties should have a line of communication open between the field biologist, the data assessor and the DEP Project Manager. If at any point during data collection, processing, interpreting and reporting an issue arises, the responsible party should reach out to resources by providing a summary of the issue to include the problem description and the corrective action needed. The responsible party needs to document steps taken until the issue has been resolved; documentation can include retaining email chains or creating a timeline spreadsheet.

C2. Reports to Management

Reports regarding ongoing performance and system audits, data quality evaluations, and significant quality assurance problems will be included in the mid-cycle and annual grant status report to USEPA and will be provided to the DEP's Quality Systems Program Manager and the WQD, Monitoring Section.

Based on priorities, the field biologist should complete a final report to the DEP Project Manager in a timely fashion. It is understood that project priorities may change within the DEP due to time sensitive deadlines; if an extension or assistance in data management is needed, biologists can request assistance from WQD, Central Office staff.

SECTION D: DATA VALIDATION AND USABILITY

D1. Data Review, Verification and Validation

The protocol for validation of biological data is found in DEP's Monitoring Book, Chapter 3 (Lookenbill and Arnold 2023). The protocol for validation of chemical data is found in the *Bureau of Laboratories, Quality Assurance Manual for the PA Department of Environmental Protection Bureau of Laboratories* (DEP 2019) and BOL's SOPs. The protocol for validation of physicochemical data is found in DEP's *Continuous Physicochemical Data Collection Protocol* (Hoger and Arnold 2023a).

A log is maintained of field instrumentation calibrations, performance, and repairs. Additionally, the biologist responsible for interpreting data will provide a review of results to include duplicate and blank comparison and best professional judgement determinations based on knowledge of the site and equipment used. If detected, erroneous data will be flagged, described, and ultimately removed from the final dataset. Calculations using indices, models and Chapter 93 WQS criteria are checked during internal review. All continuous data undergoes final review approval according to DEP data collection protocols.

D2. Verification and Validation of Protocols

Errors are detected through verification of data by the biologist in charge of the survey, in-house review of reports, and supporting calculations and field audits. When problems are noted, the responsible individual is notified, provided with the appropriate protocol and training, and reevaluated before performing the task again. The auditor shall maintain the records of any corrective actions on additional auditing forms.

With any data management, integrity is of utmost importance to provide quality results and analyses of the data. It is necessary that collectors cross-validate all field notes with data entries into DEP's data management databases. DEP currently maintains a biological (SLIMS, Excel, Access), discrete physicochemical (SIS) and continuous physicochemical (AQUARIUS) databases. The collector should quality assure their data at the end of the sampling event and again prior to the development of any reports. In addition, all reports will need to be reviewed internally and by the DEP Project Manager prior to submittal to USEPA and prior to distribution to the public to ensure accuracy of the reporting. In addition, all calculations used to generate index scores, models, and water quality criteria exceedances will be reviewed for integrity prior to distributing for use by biologists responsible for interpreting the data. If any problems arise with calculations, formulas, spreadsheets, databases, etc., which are provided to facilitate data interpretation, the user should note issues and inform the DEP Project Manager of discrepancies and problems, at which time the appropriate corrective action will be determined, and updated materials will be distributed.

D3. Reconciliation with User Requirements

The data generated from the surveys and reports will be analyzed and reconciled by cross referencing DEP assessment methods (Shull and Whiteash 2023); assessments are made in accordance with Section 96.3 and 96.5(b) of DEP's regulations. The end product should reconcile with requirements documented in DEP's Monitoring Book (Lookenbill and Arnold 2023), DEP's *Water Quality Assessment Methodology for Surface Waters* (Shull and Whiteash 2023), and DEP's *Water Quality Antidegradation Implementation Guidance, Document Number 391-0300-002* (DEP 2003).

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E.1 BENTHIC MACROINVERTEBRATE SUBSAMPLING AND TAXONOMIC IDENTIFICATION QUALITY CONTROL

INTRODUCTION

The Department of Environmental Protection, Bureau of Clean Water, Water Quality Division (WQD) maintains that all biologist involved in the processing and identification of macroinvertebrate samples be properly trained and audited by WQD staff according to protocols found in Chapter 3 of DEP's *Water Quality Monitoring Protocols for Surface Waters* (Lookenbill and Arnold 2023).

Steps to ensure the proper identification of benthic macroinvertebrate and fish taxonomy are summarized as follows:

1) At least 10% of all samples identified by a biologist are to be quality assured by a certified taxonomist. Identification results between the biologist and certified taxonomist must be 90% or greater.

2) DEP, at its discretion, may request sample vials for any results entered into internal databases for QA purposes.

As part of the taxonomic quality control (QC), documentation of quality assurance of subsampling and identification procedures shall follow that described below.

QC – BENTHIC MACROINVERTEBRATE SUBSAMPLING

Subsampling macroinvertebrates in the laboratory should follow a training, audit and QA schedule, as described herein. Each new sorter will go through a period of training. This training requires samples be sorted and checked by supervising staff. A sample here is defined as being a sample that has been picked to completeness, following the procedures outlined in the *Macroinvertebrate Laboratory Subsampling and Identification Protocol* (Brickner 2020). The individual must follow protocol sorting grid by grid by taking contents out of the randomly selected grid and placing the contents into a viewing pan. The sorter will then pick out all organisms one by one and place them in a petri dish. After each grid is sorted and no remaining organisms can be found, the viewing pan should be checked by supervising staff. In addition, the supervising staff should check the petri dish for any specimens that do not belong; specimens that do not belong include pupae, half body specimens, adult specimens (except aquatic adult Coleopterans), exoskeletons (exuviae), and specimens degraded beyond identification. The supervising staff and sorter will track the number of misses per viewing pan and number of specimens that do not belong after each petri dish check. If the supervising staff finds the sorter missing more than 10 specimens on their first scan check, the sorter will be given a second opportunity to re-look with instruction given to pay better attention to commonly missed specimens. A re-look does not count against the sorter and their training; however, the supervising staff should document a re-look was required and should document the instruction given. During the initial training, if grid error (Equation 1) calculated across all grids sorted during the full sample sort is greater than 10% the individual fails their training and will need to go through a subsequent training period until the individual passes; it is recommended that a minimum of three full

samples be checked prior to completing an audit for sign-off of independent sorting, but this is left up to the discretion of supervising staff.

Once training is passed the individual will complete one more 'full' sample sort for the final audit. If the four or more grids sorted out of Pan 1 results in a GE of greater than 5%, the audit fails, otherwise the sorter passes and can be cleared to pick without any oversite. At the discretion of supervising staff, seasonal staff will be audited as needed; and, agency wide, permeant staff will be audited every three years. An audit will consist of one sample that is picked to completeness and checked by supervising staff. To pass the audit, the one sample QA check should yield no greater than 5% grid error (Equation 1). If the audit fails, the individual will need monitored until a passing grade is achieved. With two subsequent fails the individual will have to be re-trained. If the audit passes the individual may continue to sort independently, until the next auditing event.

Equation 1: Grid Error (GE), expressed as a percentage

Described as the mean weighted grid error for the full sample sort. Calculated according to percent error documented in each pan and weighted by the ratio of organisms per viewing pan divided by the total number of organisms sorted.

GE = $(\sum_{i}^{\square} sortE * W)$/P

Where: sortE = sorting error calculated by number of misses divided by the total number of organisms sorted out of the viewing pan; W = weight given to each viewing pan based on the total population of the full sample sort; P= number of pans sorted

Example:
Pan 1: Number sorted 33, misses 5, petri remove 0, total 38
Pan 2: Number sorted 41, misses 5, petri remove 1, total 45
Pan 3: Number sorted 79, misses 11, petri remove 0, total 90
Pan 4: Number sorted 62, misses 5, petri remove 0, total 67
Total number after four girds 240

Sorting error calculation (sortE):
Pan 1 = (1-(33/38))*100 = 13.16%
Pan 2 = (1-(41/45))*100 = 8.88%
Pan 3 = (1-(79/90))*100 = 12.22%
Pan 4 = (1-(62/67))*100 = 7.46%

Weight calculation (W):
Pan 1 = 38/240 = 0.158
Pan 2 = 45/240 = 0.188
Pan 3 = 90/240 = 0.375

Pan 4 = 67/240 = 0.279

GE = ((13.16*0.158)+(8.88*0.188)+(12.22*0.375)+(7.46+0.279))/4 = 2.6%

QC – BENTHIC MACROINVERTEBRATE IDENTIFICATION

As described above, biologists are to uphold standards of identifying organisms to specific taxonomic levels. Ephemeroptera, Plecoptera, and Trichoptera (EPT) taxa are to be identified to Genus; midges, snails, clams and mussels are to be identified to Family level; flatworms, proboscis worms, roundworms and moss animalcules are to be identified to Phylum level; aquatic earthworms and tubificid worms are to be identified to Class; and water mites are to be identified to Subclass. If for any reason the highest taxonomic identification requirements cannot be made, the Monitoring Book states that "…the biologist must forward identifications to a certified taxonomist for further evaluation…(Lookenbill and Arnold 2023)." It was noted that 10% of all samples identified by a biologist are to be QAed by a certified taxonomist. To accomplish the 10% quality assurance of all samples identified, taxonomists should submit 10% of the samples they have identified for a calendar year. The easiest way to accomplish this is to flag every tenth sample identified to be submitted for QA. This subset should represent an even distribution of all samples collected and/or identified for a calendar year. Samples should be delivered to the DEP staff responsible for sample QA beginning January 1st of the succeeding year and no later than February first. DEP staff, Regional and Central Office, have the option to request the QA of samples that were not collected or identified by them if they have a specific interest in it (i.e. permitting).

Samples submitted for taxonomic verification will undergo calculations to determine percent disagreements in taxonomy, enumeration and Index of Biotic Integrity (IBI) metrics between the biologist and quality assurer results. Errors documented by the taxonomic verification QA procedure were developed similarly to that described in Stribling et al. 2008. Of interest is Percent Taxonomic Disagreement (PTD), Percent Enumeration Disagreement (PED), Percent IBI Disagreement (PID). Percent taxonomic disagreement takes into account differences in specimen identifications between the biologist and the quality assurer. Individual taxon agreements are determined by comparing lists, and a percent difference is calculated according to Equation 2; calculated PTD error should be no greater than 10%. Percent enumeration disagreement is a calculation that determines the counting error. PED is calculated according to Equation 3 and should be no greater than 5%. Percent IBI disagreement considers differences in the IBI scores generated by the two taxa lists (Equation 4); calculated PID error should be no greater than 10%. If any calculated error, (PTD, PED or PID) is greater than the 10% or 5% criteria, corrective action be taken. Corrective action could include an opportunity for the biologist to re-look at the samples, a conversation between the biologist and quality assurer or the recommendation to seek further training in the identification of problem taxa.

Equation 2 – Percent Taxonomic Disagreement (PTD), expressed as a percentage

$$PTD = \left(1 - \left[\frac{a}{Nmax}\right]\right) x\ 100$$

Where: a = total number of agreements (summed across all individuals and taxa); Nmax = total number of individuals identified (the greater of the two totals)

Equation 3 – Percent Enumeration Disagreement (PED), expressed as a percentage

$$PED = \left(\left[\frac{(ni-nq)}{(ni+nq)}\right]\right) \times 100$$

Where: ni = number of individuals counted by the biologist; nq = number of individuals counted by the quality assurer

Equation 4 – Percent IBI Disagreement (PID), expressed as a percentage

$$PID = \left(\left[\frac{(Si-Sq)}{Sq}\right]\right) \times 100$$

Where: Si = the IBI score generated by the taxa list provided by the biologist; Sq = the IBI score generated by the taxa list provided by the quality assurer

QC – FISH IDENTIFICATION

Fish samples identified by DEP biologists should undergo QA/QC similarly to benthic macroinvertebrate samples. 10% of all samples identified by a biologist are to be QAed by a certified taxonomist. To accomplish the 10% quality assurance of all samples identified, taxonomists should submit 10% of the samples they have identified for a calendar year. The easiest way to accomplish this is to flag every tenth sample identified to be submitted for QA. This subset should represent an even distribution of all samples collected and/or identified for a calendar year. Samples should be delivered to the DEP staff responsible for sample QA beginning January first of the succeeding year and no later than February first. DEP staff, regional and central office, have the option to request the QA of samples that were not collected or identified by them if they have a specific interest in it (i.e. permitting).

Samples submitted for taxonomic verification will undergo calculations to determine percent disagreements in taxonomy and enumeration between the biologist and quality assurer results. Errors documented by the taxonomic verification QA procedure were developed similarly to that described in Stribling et al. 2008. Of interest is Percent Taxonomic Disagreement (PTD) and Percent Enumeration Disagreement (PED). Percent taxonomic disagreement takes into account differences in specimen identifications between the biologist and the quality assurer. Individual taxon agreements are determined by comparing lists, and a percent difference is calculated according to Equation 2; calculated PTD error should be no greater than 10%. Percent enumeration disagreement is a calculation that determines the counting error. PED is calculated according to Equation 3 and should be no greater than 5%. If any calculated error, (PTD or PED) is greater than the 10% or 5% criteria, corrective action be taken. Corrective action could include an opportunity for the biologist to re-look at

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APPENDIX F: BIOSECURITY AND SAFETY PROCEDURES

Appendix F Biosecurity and Safety Procedures

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BIOSECURITY PROCEDURES

The spread of aquatic invasive species (AIS) is always a potential concern when conducting stream surveys. Biosecurity measures should be considered and implemented for all fish collections as well as for all other water quality work. AIS covers a wide range of taxa that include but are not limited to fish, reptiles, mussels, invertebrates, aquatic plants, algae, pathogens, and parasites. While many of the streams and lakes within Pennsylvania have, or have had documentation of AIS, it is best to consider all waterbodies as potential candidates for AIS transfer. To this end, sampling design should be adjusted accordingly. Whenever possible, site selection priority should be placed on sites within the same watershed and ordered upstream to downstream. All gear that may come in contact with the water should be decontaminated prior to leaving the site if they are to be used at another site within the same day, this includes but is not limited to boats, trailers, live-wells, buckets, nets, and waders. Various decontamination products are commercially available and include but are not limited to chlorine solutions or peroxygen solutions. Whenever gear must be used in multiple watersheds adequate time must be allotted for additional decontamination practices that include but are not limited to high-temperature pressure washing, freezing, or drying. Additional information regarding AIS can be found in the Pennsylvania Fish and Boat Commission's website (Aquatic Invasive Species (fishandboat.com)).

GENERAL SAFETY PROCEDURES

Safety is of utmost concern when working in and on any waterbody. Individuals that lack experience and education related to navigating these waterbodies put themselves and others at risk of injury or loss of life. On large waterbodies some form of watercraft will be required for data collection protocols. Whether a kayak, canoe, or powered boat is used, DEP recommends – and in most cases PFBC requires by law – that individuals using watercrafts take boating courses and obtain a boating safety certificate. However, whether laws apply to certain individuals or watercraft or not, DEP strongly recommends that all individuals regardless of age, experience, or navigation procedure obtain and keep a boating safety certification on them during surveys that require watercraft. More information on boating courses and safety certifications are available here: http://www.fishandboat.com/Boat/BoatingCourses/Pages/default.aspx

Surface waters, including large semiwadeable rivers, present difficulties for both wadeable and nonwadeable water navigation. For example, certain locations on rivers such as the Susquehanna River around Harrisburg, PA may be fully or partially wadeable during certain times of year, but there are also highly variable currents and drop-offs that are dangerous to wading collectors. Therefore, personal floatation devices (PFD) should always be worn. However, PFDs should not be used to facilitate wading or navigating the waterbody. Navigation using a watercraft can be equally if not more challenging than wading. Many boats that frequent the large semiwadeable rivers such as the Susquehanna River are outfitted with modified jet propulsion to reduce the risk of serious damage associated with impacting the streambed with the lower unit. Therefore, consideration for modified equipment may be needed to conduct this protocol. In addition, riffles and bedrock substrate often

dominate these rivers regardless of size. Consequently, DEP recommends that at least two individuals work together during these surveys. This is critical for many safety reasons, particularly important when a powered watercraft is underway. While in motion, DEP recommends that the individual not operating the powered watercraft is actively looking for obstacles on and just under the surface. This individual should be actively and clearly communicating obstacles to the operator. This is best done with the use of hand signals that are agreed upon between collectors prior to navigating the water. All of this is not to say that macroinvertebrate sampling in large semiwadeable rivers is overly risky. In fact, with the proper training and experience this sampling protocol can be conducted efficiently and safely using limited resources, and without restrictions based on the collector's physical stature.

Inherent in these recommendations is the understanding that collectors must be well informed of the unique characteristics of each river they intend to sample and be aware of current flow and weather conditions. Equipment considerations may be different between similar sized large semiwadeable rivers, and certainly different when visiting multiple semiwadeable rivers of different sizes. For instance, boat selection and safety equipment lists would be different depending on whether the collector is visiting a 1,000 mi^2 river or 10,000 mi^2 river. These differing characteristics require thorough planning before going into the field, so resources are not wasted due to a lack of preparation. It is also imperative that collectors have access to and continually check river flow conditions. There will be situations when weather in one part of Pennsylvania may be optimal for sampling a site, but weather in another part of Pennsylvania – two or three days before the sampling period – created conditions that reduces the ability to collect. Analysis of flow conditions at all available points upstream of the intended sampling locations are highly recommended. Commonly used discharge data are available here: https://waterdata.usgs.gov/pa/nwis/current.

ELECTROFISHING SAFETY PROCEDURES

DEP recognizes that electrofishing is an inherently hazardous activity for which safety is a primary concern. The environmental conditions under which these operations are conducted further increase the risks. Due to these important concerns for electrofishing safety, all field team members should be familiar with electrofishing techniques, safety precautions, and equipment manufacturer's operating procedures. It is the responsibility of the project coordinator (or crew leader) to verify that crewmembers have a basic understanding of these principles prior to sampling. The safety of all personnel and the quality of the data are assured through the adequate education, training, and experience of all members of the fish collection crew. To this end, the crew leader must have completed electrofishing training and safety courses, be certified in CPR, and should have a minimum of five years of electrofishing experience. The U.S. Fish and Wildlife Service offers educational guidance and certification in electrofishing principles and safety and is considered the standard training source for electrofishing. Currently, U.S. Fish and Wildlife Service offers two courses that are available online. The Electrofishing Principles and Techniques course is required for crew leaders and the Electrofishing Safety course should be completed by every crewmember prior to sampling.

Principles and Techniques of Electrofishing (Online) - CSP2C01
https://nctc.fws.gov/courses/CSP/CSP2C01/resources/
Electrofishing Safety (Online) - CSP2202
https://training.fws.gov/courses/csp/csp2202/resources/index.html

Electrofishing protocols rely on one of three main types of gear platforms: backpacks and towboats with hand-held probes for wadeable streams and standard boats with fixed-boom probes for nonwadeable streams and rivers. Collection gear consists of a wide variety of net sizes, shapes, and configurations. Each team member must be insulated from the water and the electrodes; therefore, non-breathable waders are necessary as they are considered personal protective equipment (PPE), and rubber (linesman) gloves are recommended. Nonbreathable waders or rubber boots are also considered PPE and may be worn during boat electrofishing at the discretion of the crew leader. All waders should have non-slip, rubber soles (no felt soles). Electrode and dip net handles must be constructed of non-conductive materials (fiberglass or wood). Additional equipment used during the survey should also be constructed of non-conductive materials to reduce inadvertent shock potential (e.g., buckets, live-well frames). Electrofishing units must be equipped with functional safety switches and represent best available technology (BAT). Field crew members must remain cognizant of their surroundings and not reach into the water unless the electrodes have been removed from the water or the electrofishing unit has been disengaged. A good anacronym to remember for safety is **S-H-O-C-K-E-D**.

Safety Brief- Crew leaders brief the crew on safety and strategy prior to each collection.
Handle- Be aware of what is touched, stepped on, or ducked under.
Operate- Be aware of probes and the electrical field at all times.
Communicate- Let others know of any potential hazards that may become apparent.
Key Personnel- Know which crew members are trained in case of emergency.
Equipment- Inspect equipment for damage before and after each sampling event.
Debrief- Discuss and critique the survey for improvement.

Each crewmember should also wear polarized glasses to reduce surface glare which enhances their ability to safely negotiate the stream bottom and to see and net fish.

DIVING SAFETY PROCEDURES

DEP recognizes the need to structure and oversee this activity due to the inherent hazards involved in underwater diving. Due to these important concerns for diving safety, all dive team members must be trained in mussel sampling techniques, safety precautions, and equipment manufacturers' operating procedures. In addition, all scientific divers must have completed, at minimum, an approved SCUBA Certification Course, and have adequate training to conduct mussel surveys. It is the responsibility of the Unit Diving Officer (UDO) to verify that the team members have the appropriate training. Specific team member duties should be reviewed during each sampling event.

The safety of all personnel and the quality of the data are assured through the adequate education, training, and experience of all members of the scientific diving team. At least one UDO must be involved in each sampling event. For some survey locations, mid-channel sites may be limited by commercial or recreational traffic that could pose unsafe diving conditions. Any intake structures or other potential hazards will be avoided by an approximate 50-meter buffer. No dives will be performed between any lock chamber mooring points and the lock and dam structure. If a survey is located near a US Army Corp of Engineer lock and dam, then the US Army Corp of Engineer must be notified prior to the survey date. When on site, the lockmaster must be notified via marine radio that the dive team is ready to commence operations.

The safety standards which will apply and will be followed for all DEP operations are derived from USEPA's *Diving Safety Manual Revision 1.2*, (DSM, USEPA 2010b).

Safety Plan

This safety plan establishes general guidelines and procedures for safe and efficient scientific diving. The intent is to provide written guidance for quick reference in the field by the Unit Dive Officer (UDO) and scientific divers and is based on the DEP's Policy for Scientific Diving Personnel and Diving Operations (DEP 2012). This plan is comparable to and was derived from USEPA's DSM (USEPA 2010b, and specifically, Appendix A of the DSM, *USEPA Diving Safety Rules* (USEPA 2010a). A copy of this safety plan is required to be available in the field for all diving operations.

<u>Dive Operations and Rules</u>

1. A Dive Plan for each proposed diving operation will be approved by the UDO.
2. Diving **with air** will be conducted in accordance with the U.S. Navy No-Decompression Limits and Repetitive Group Designation Table for No-Decompression Air Dives, located in Appendix H-10 of the DSM (USEPA 2010b). Diving **with Nitrox** will be conducted in accordance with the National Oceanic and Atmospheric Administration (NOAA) Nitrox Tables for No-Decompression Limits and Repetitive Group Designation Table for No-Decompression Dives (NOAA 2016).
3. During "live-boat" diving, the tender or UDO will maintain continuous visual contact with the divers, or their bubbles after they descend, and keep the boat operator informed of their position. The boat should always be positioned to render immediate assistance to the divers.
4. The dive plan should require that the deepest dive be scheduled first and be followed by shallower dives. If this is not possible, the divers must comply with rule Nos. 5 and 6, below.
5. Generally, all dives should be terminated 5 minutes before the no-decompression limit.
6. During repetitive diving at water depths greater than 50 feet, on ascent the diver should stop at approximately 20 feet for 2 minutes for a safety decompression stop on the second and succeeding dives. If a safety decompression stop is taken, the time spent at the stop must be added to the bottom time for that dive.
7. While diving, divers will remain in contact (visual, auditory, tactile, or through communications from the tender) with at least one other diver. All divers must surface if contact or communication is lost. No one may dive unattended.

8. A diver should start their ascent from the bottom prior to their tank pressure decreasing to 500 psi, so that the tank pressure does not decrease to or below 500 psi at any time. A diver must begin an ascent with enough air in his/her cylinder while taking into consideration if it is necessary to make a safety stop at 20 feet due to bottom time or other environmental conditions.
9. When diving in extremely limited visibility and overhead environments such as piers or other situations presenting entrapment or entanglement hazards, the rule of thirds should be followed: one third of the tank pressure may be used to get to the dive objective, the second third may be used to complete the work assignment and return to the surface, and the final third of the tank pressure is for a safety reserve. This rule does not supersede the 500-psi rule (Rule No. 8).
10. When diving in areas with possibilities of entrapment or entanglement such as described above in Rule No. 9, an extra open-circuit SCUBA regulator will be at the dive site and attached to a standby full SCUBA cylinder complete with backpack.
11. During dives always ascend at a rate of 30 fpm (minor variations in ascent rate between 20 and 40 fpm are acceptable).
12. No dive will go beyond 130 feet of depth.
13. Diving operations must be conducted in accordance with all appropriate DEP policies and standards (e.g., for Nitrox diving or flying after diving).
14. In an emergency, the UDO in charge of the diving operation may have to make field decisions that deviate from the requirements of this safety plan to prevent or minimize a situation which will likely cause death or serious physical harm.
15. Oxygen will be provided on-scene and on boats participating in dive operation.

<u>Responsibilities of Dive Personnel</u>

UDO:

1. Preparing for the diving operation including checking equipment such as the pressure in the O_2 cylinders.
2. Ensuring that all divers are physically fit to dive.
3. Assisting the Tender and is completely in-charge of the diving operation.
4. Ensuring that all diving operations are conducted safely in accordance with USEPA's DSM and standards (USEPA 2010b).
5. Promptly prepares a Dive Report upon completion of the diving activities.
6. Giving the pre-dive Briefing which will include:
    a. Designating dive buddy pairs including the alternate UDO.
    b. Providing a brief description of the dive site.
    c. Discussing the objectives of the diving operation.
    d. Reviewing the operation of equipment to be used.
    e. Identifying any potential pollution sources.
    f. Discussing environmental and any hazardous conditions.
    g. Monitoring divers for signs/symptoms of "bubble trouble."
    h. Reviewing emergency and evacuation procedures.

7. For the emergency and evacuation procedures, the following will be identified and discussed in as much detail as is appropriate for conditions at the dive site:
    a. Establish evacuation routes and means of transportation.
    b. Review methods of communication.
    c. Review CPR and the use of medical O_2, if necessary.
8. In the Event of a Diving Accident the following will be conducted:
    a. Maintain heart and breathing functions.
    b. Do NOT remove O_2 from patient unless necessary.
    c. Reconstruct dive profiles while in route to the chamber.
    d. Ensure dive partner accompanies victim or goes to chamber ASAP.
    e. Retain all dive gear for examination.

Scientific Divers:

1. Dive only if they are physically and mentally fit and properly trained for the task to be performed.
2. Keep their diving equipment in safe operating condition.
3. Wear a compass and depth and tank pressure gauges and, when necessary, a dive watch or bottom timing device.
4. Refuse to dive if diving conditions are unsafe or unfavorable, or if the diving operation breaches the dictates of USEPA's safety policies or standards (USEPA 2010a, USEPA 2010b).

Standby Divers:

1. Be fully equipped and ready to give immediate assistance at the dive site.
2. Receive the same briefing and instructions as the working divers.
3. Monitor the progress of the diving operations.

Tenders:

1. Assist divers and track their location in the water.
2. Record each diver's bottom time, tank pressures, and maximum depth.
3. Alert the divers, when necessary, on the status of their bottom time via the Diver Recall Unit.
4. Advise other vessels of diving operations and warn off boat traffic which may pose a hazard to the divers.
5. Perform no other concurrent function which interferes with these duties.

<u>Number of Personnel Per Dive Team</u>

Except under emergency conditions, the minimum number of personnel required per dive team will be as follows (Table 1).

Table 1. Dive team members required.

Water Depth/Situation	Number of Divers	Number of Standby Divers	Number of Tenders	Total Individuals
Under 15ft/diver visible at all times	1	1[1/]	1	3
Between 15 and 60ft/without unusual conditions[3/]	2	-	1[2/]	3
Between 15 and 60ft/with unusual conditions	2	1[1/]	1	4
Between 60 and 130ft/all conditions	2	1[1/]	1	4

[1/]The Standby Diver will be the UDO or Alternate UDO, if the UDO is diving.
[2/]The Tender will also be the UDO or Alternate UDO, if the UDO is diving.
[3/]Unusual conditions as determined by the UDO considering weather, water currents, visibility in the water, potential entanglements, or any other factor that may compromise the safety of the diving operations.

Decompression Diving
No decompression diving will be conducted

Dive Buddy
No one may dive unattended. Divers must have visible, auditory, or tactile, communication with at least one dive buddy or a tender at all times.

Dive Computers
As specified in the Dive Plan, determined by the UDO, and in accordance with the DSM, dive computers may be used to control the dive profile.

Dive Equipment
All dive equipment will conform to USEPA regulations.

Flying after Diving: wait a minimum surface interval of 12 hours prior to flying after diving. When making daily, multiple dives for several days or making a dive requiring an emergency decompression stop, extend the surface interval beyond 12 hours. Whenever possible wait 24 hours before flying.

Record Keeping
All diving operations will be logged in accordance with the USEPA diving directives in Section 3.4 of the DSM (USEPA 2010b) and copies of individual divers' logs will be provided to the UDO on the first working day and no more than five working days after the operations are complete. A complete dive report will be provided by the Divemaster in charge to the UDO within 10 Working days of completion of the operation.

LITERATURE CITED

DEP. 2012. Policy for Scientific Diving Personnel and Diving Operations. Pennsylvania Department of Environmental Protection, Harrisburg, Pennsylvania.

NOAA. 2016. NOAA No-decompression tables for Nitrox Dives. NDP 07 -2015. U.S. Department of Commerce, National Oceanic and Atmospheric Administration, Office of Marine and Aviation Operations, Silver Spring, MD.

USEPA. 2010a. Appendix A EPA diving safety rules. Diving safety manual (Revision 1.2). U.S. Environmental Protection Agency, Office of Administration and Resources Management, Safety, Health and Environmental Management Division, Washington, D.C.

USEPA. 2010b. Diving safety manual (Revision 1.2). U.S. Environmental Protection Agency, Office of Administration and Resources Management, Safety, Health and Environmental Management Division, Washington, D.C.

APPENDIX G: UNDENATURED ETHANOL POLICY

PURPOSE

This policy is established to manage the purchase, storage, access, use and tracking of tax-free undenatured alcohol and to ensure compliance with the following: Pennsylvania Liquor Control Board (PLCB) Code (47 P.S. §§ 1-101 et seq.); Federal Alcohol Administration Act (27 U.S.C. Chapter 8, Subchapter I); the Internal Revenue Code (26 U.S.C. Chapter 51, Subchapter D); and regulations at 40 Pa. Code Chapter 11, Subchapter A (relating to general provisions) and 27 CFR Chapter I, Subchapter A (relating to alcohol). All undenatured ethanol purchased by the DEP must be used for official business only.

SCOPE

This policy applies to DEP staff that use undenatured ethanol for activities associated with water quality data collection in surface waters of Pennsylvania. Each DEP bureau and/or regional office is responsible for naming a designee to be responsible for the direct control of undenatured ethanol storage, access and issue from stock. This designee is also responsible for meeting the recordkeeping requirements required by the PLCB Code 40 Pa. Code § 11.35 (relating to records and reports) and federal 27 CFR § 22.161 (relating to records).

This policy does not apply to other forms of alcohol that are not regulated by the Pennsylvania Liquor Control Board (PLCB) including denatured alcohol and undenatured alcohol that has been used for sterilization, sample preservation, specimen preservation, laboratory analysis, etc. Other forms of alcohol that are otherwise classified as hazardous materials should be managed according to requirements established for hazardous materials or hazardous waste.

Central Office and Regional Office Program Managers are responsible for the implementation of and adherence to this policy within respective programs. All employees using undenatured ethanol are required to be informed of this policy prior to using undenatured ethanol.

DEFINITIONS

Undenatured Ethanol: A flammable, colorless, slightly toxic chemical compound with a distinctive perfume-like odor, with a molecular formula of C_2H_5OH, commercially available as a 95% ethanol, 5% water mixture; is suitable for human consumption.

Proof: The ethyl alcohol content of a liquid at 60 °Fahrenheit, stated as twice the percent of ethyl alcohol by volume.

Proof Gallon: A standard US liquid gallon (128 fluid ounces) containing 50% ethanol by volume of ethyl alcohol having a specific gravity of 0.7939 at 60 °Fahrenheit referred to water at 60 °Fahrenheit as unity, or the alcoholic equivalent thereof. 27 CFR § 22.11 (relating to terms) .

Tax-Free Alcohol: Alcohol mixtures suitable for human consumption obtained without applicable federal and state excise and liquor taxes.

PERMITS

A federal Alcohol and Tobacco Tax and Trade Bureau permit (TTB permit), industrial use of distilled spirits permit, and a Pennsylvania Liquor Control Board Wholesale Alcohol Purchase AE Permit (alcohol tax exempt permit) are required to purchase and use undenatured ethanol. 27 CFR § 22.41 (relating to application for industrial alcohol user permit), 26 USC § 5271 (relating to permits) and 40 Pa. Code §§ 11.21, 11.22 (relating to class of permit and application for permits).

Each DEP region or bureau using undenatured ethanol must obtain its own TTB Permit and PLCB Wholesale Alcohol Purchase AE Permit prior to purchasing tax-free, undenatured alcohol. The permittee must first acquire the TTB permit and then apply for the PLCB AE permit. The designee must maintain a photocopy of the permits in a designated file (however named.)

Permits allow purchase of tax-free, undenatured ethanol. Annual purchases may not exceed the Maximum Quantity to be Withdrawn per Calendar Year listed on the TTB permit.

RECEIPT, STORAGE AND DOCUMENTATION OF UNDENATURED ETHANOL

The designee must document the receipt of tax-free, undenatured ethanol in an "Undenatured Ethanol Logbook" and secure storage of the ethanol. The designee must maintain copies of invoices or packing lists from shipments of undenatured ethanol in the logbook and/or the designated file. The DEP region or bureau and program name must be clearly identified on the records. The logbook must completely and accurately document the purchase and use of the tax-free, undenatured ethanol. All records must include proof, date of transaction and quantity purchased. 27 CFR § 22.161 (relating to records). The permits and other records must be kept current at all times. The records and the alcohol storage premises will be available for inspection, during normal business hours, by an appropriate TTB officer and by authorized representatives of the PLCB and the State Police Bureau of Liquor Control Enforcement. 27 CFR § 22.25 (relating to right of entry and examination), 47 P.S. § 5-513 (relating to premises and records subject to inspection) and 47 P.S. § 2-211 (relating to enforcement) and 40 Pa. Code § 11.35 (relating to records and reports). The logbook and records must be retained by the designee for a minimum of six years.

Undenatured ethanol (95% ethanol, 5% water mixture) is classified as a Globally Harmonized System (GHS) Category 2 Flammable Liquid. Therefore, undenatured ethanol must be stored in a locked storage unit that meets National Fire Protection Association (NFPA) 30 or Occupational Safety and Health Administration (OSHA) Class 2 Certification.

USE

Staff shall use tax-free, undenatured ethanol for authorized purposes only and document the use in the "Undenatured Ethanol Logbook". Authorized uses include, but are not limited to, sterilization, sample preservation, specimen preservation and laboratory analysis.

Staff are prohibited from using tax-free, undenatured ethanol for beverage or food product purposes. 27 CFR § 22.102(a). Employees are prohibited from selling tax-free, undenatured alcohol and from using alcohol in the manufacture of any product for sale or selling any products resulting from the use of tax-free alcohol. 27 CFR § 22.102(b).

TRANSPORATION

Staff may transport small quantities of undenatured ethanol from the controlled, secure storage to remote field locations if such prohibitions are not described in the permits.

SEMI-ANNUAL INVENTORY

Each designee must perform a semi-annual inventory of the tax-free, undenatured ethanol for the periods ending June 30 and December 31 of each year or other inventory periods which are approximately 6 months apart upon filing written notice with the appropriate TTB officer establishing other inventory periods. 27 CFR § 22.162. These inventories must be recorded in the “Undenatured Ethanol Logbook”.

G1. UNDENATURED ETHANOL LEDGER

Federal Alcohol and Tobacco Tax and Trade Bureau Permit Number _____
Pennsylvania Liquor Control Board Wholesale Permit Number _____
Program Name and Address _____
Program Designee _____
Purchase, Issue and Use Record

Date	Action (Purchase, Use, Inventory)	Quantity (gallons)	Comments	Issuer Signature	User Signature