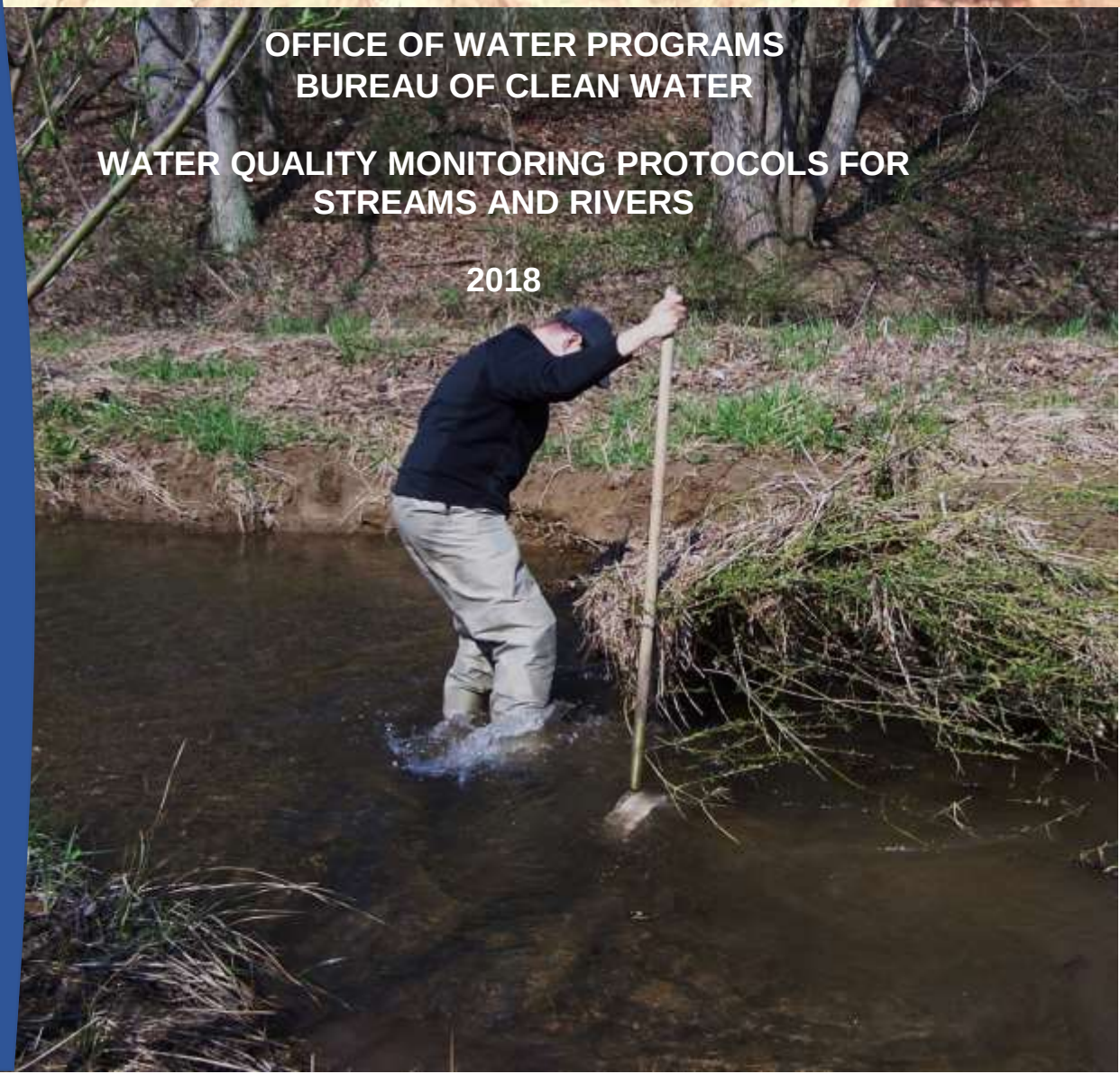
pennsylvania
DEPARTMENT OF ENVIRONMENTAL PROTECTION



OFFICE OF WATER PROGRAMS
BUREAU OF CLEAN WATER

WATER QUALITY MONITORING PROTOCOLS FOR STREAMS AND RIVERS

2018

THIS PAGE INTENTIONALLY LEFT BLANK

pennsylvania
DEPARTMENT OF ENVIRONMENTAL PROTECTION

OFFICE OF WATER PROGRAMS

BUREAU OF CLEAN WATER

WATER QUALITY MONITORING PROTOCOLS FOR STREAMS AND RIVERS

2018

Edited by:

Dustin Shull and Josh Lookenbill
PA Department of Environmental Protection
Office of Water Programs
Bureau of Clean Water
11th Floor: Rachel Carson State Office Building
Harrisburg, PA 17105

2018

TABLE OF CONTENTS

Table of Contents ii
Abbreviations iv
Chapter 1 Introduction 1-1
Purpose 1-3
Collection Framework and Processes 1-4
Chapter 2 Sampling Design and Planning 2-1
Types of Sampling Design 2-3
Sampling Plans 2-4
Cause and Effect Surveys 2-5
Chapter 3 Biological Data Collection Protocols 3-1
Wadeable Riffle-Run Stream Macroinvertebrate Data Collection Protocol 3-2
Wadeable Limestone Stream Macroinvertebrate Data Collection Protocol 3-8
Wadeable Multihabitat Stream Macroinvertebrate Data Collection Protocol 3-13
Semi-Wadeable Large River Macroinvertebrate Data Collection Protocol 3-19
Qualitative Benthic Macroinvertebrate Data Collection Protocol 3-27
Macroinvertebrate Laboratory Subsampling And Identification Protocol 3-31
Fish Data Collection Protocol 3-42
Fish Tissue Data Collection Protocol 3-60
Mussel Data Collection Protocol 3-69
Periphyton Data Collection Protocol 3-83
Bacteriological Data Collection Protocol 3-133
Chapter 4 Chemical Data Collection Protocols 4-1
In-Situ Field Meter and Transect Data Collection Protocol 4-2
Discrete Water Chemistry Data Collection Protocol 4-8
Continuous Physicochemical Data Collection Protocol 4-22
Sediment Chemistry Data Collection Protocol 4-88
Passive Water Chemistry Data Collection Protocol 4-105
Sample Information System (SIS) Data Entry Protocol 4-114
Chapter 5 Physical Data Collection Protocols 5-1
Stream Habitat Data Collection Protocol 5-2

Pebble Count Data Collection Protocol 5-8
Water Flow Data Collection Protocol 5-14
Appendix A: Biological Data Collection Information and Forms A-1
A-1 Macroinvertebrate Field Data Sheet A-2
A-2 Macroinvertebrate Enumeration Bench Sheet A-5
A-3 Fish Field Data Collection Sheet A-7
A-3 Fish Tissue Field Data Collection Sheet A-15
A-5 Mussel Field Data Collection Sheets A-18
A-6 QBE Periphyton Field Data Collection Sheet A-21
A-7 QMH Periphyton Field Data Collection Sheet A-24
A-8 Algae Identification and Enumeration Laboratory Submission Data Sheet A-27
A-9 Microbiology Sample Submission Sheet for Acidified Chlorophyll-A, Phycocyanin, and AFDM A-29
A-10 Bacteria Monitoring Field Data Sheet A-31
Appendix B: Chemical Data Collection Information and Forms B-1
B-1 Field Meter Calibration Form B-2
B-2 Transect Data Collection Form B-4
B-3 Sample Information System User's Guide B-7
B-4 Sediment Field Data Collection Sheet B-43
B-5 CIM Deployment Form B-46
B-6 CIM Maintenance Visit Field Form B-49
B-7 Temperature Adjustments for pH Buffers B-54
B-8 DO 100% Saturation Chart for Fresh Water B-57
Appendix C: Physical Data Collection Information and Forms C-1
C-1 Riffle/Run Prevalence Habitat Data Collection Form C-2
C-2 Riffle/Run Prevalence Habitat Data Collection Abbreviated (One-Page) Form . C-5
C-3 Low Gradient Habitat Data Collection Form C-7
C-4 Pebble Count Data Collection Form C-10
C-5 Alternative Pebble Count Data Collection Form C-12

ABBREVIATIONS

AFDM	Ash Free Dry Mass
AIS	Aquatic Invasive Species
AL	Auditing Laboratory
ALU	Aquatic Life Use
ANS	Academy of Natural Sciences
AWS	Wildlife Water Supply
BAH	Best Available Habitat
BAT	Best Available Technology
BCD	Buoyancy Control Device
BOD	Biological Oxygen Demand
BOL	Bureau of Laboratories
CBOD	Carbonaceous Biological Oxygen Demand
CIM	Continuous Instream Monitoring
CPOM	Coarse Particulate Organic Matter
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
DES	PFBC Division of Environmental Services
DO	Dissolved Oxygen
DOH	Pennsylvania Department of Health
DTH	Depositional Targeted Habitat
DWM	Diving Safety Manual
DWQ	Division of Water Quality
EDC	Endocrine Disrupting Compounds
EPT	Ephemeroptera, Plecoptera, and Tricoptera
EST	Environmental Sampling Technologies
EV	Exceptional Value Waters
FNU	Formazin Nephelometric Units
GPS	Global Positioning System
GRTS	Generalized Random Tessellation Stratified
HDPE	High-Density Polyethylene
HQ	High Quality Waters
HUC	Hydrologic Unit Code
IBI	Index of Biotic Integrity
ICE	Instream Comprehensive Evaluation
IRS	Irrigation Water Supply
IWS	Industrial Water Supply
LDB	Left Descending Bank
LIS	Line-Intercept Sampling

LWS	Livestock Water Supply
MF	Migratory Fishes
MMI	Multi-Metric Index
MSDS	Material Safety Data Sheets
NAWQA	National Water-Quality Assessment
NELAP	National Environmental Laboratory Accreditation Program
NOAA	National Oceanic and Atmospheric Administration
NPDES	National Pollutant Discharge Elimination System
NHD	National Hydrologic Dataset
NTU	Nephelometric Turbidity Units
PAH	Polycyclic Aromatic Hydrocarbons
PCB	Polychlorinated Biphenyls
PEP	Pennsylvania Epilithic Periphyton Sampler
PFBC	Pennsylvania Fish and Boat Commission
PFD	Personal Floatation Device
PHY	Phytoplankton
PL	Participating Laboratory
POCIS	Polar Organic Chemical Integrative Sampler
PPE	Personal Protective Equipment
PRC	Performance Reference Compounds
PWS	Public Water Supply
QA	Quality Assurance
QBE	Quantitative Benthic Epilithic
QC	Quality Control
QMH	Qualitative Multi-Habitat
qPCR	Quantitative Polymerase Chain Reaction
RBP	Rapid Bioassessment Protocol
RDB	Right Descending Bank
RTH	Richest Targeted Habitat
USGS	United States Geological Survey
SAC	Standard Analysis Code
SAV	Submerged Aquatic Vegetation
SCP	Scientific Collectors Permit
SIS	Sample Information System
SPMD	Semi-Permeable Membrane Device
SRBC	Susquehanna River Basin Commission
TDS	Total Dissolved Solids
TE	Threatened and Endangered Species
TL	Total Length
TMDL	Total Maximum Daily Loads
TSF	Trout Stocking
UDO	Unit Diving Officer
USEPA	United States Environmental Protection Agency

USFWS	United States Fish and Wildlife Service
USGS	United States Geological Survey
VOA	Volatile Organic Analysis
WQN	Water Quality Network
WQS	Water Quality Standards
WWF	Warm Water Fishes

CHAPTER 1 INTRODUCTION

Prepared by:

Josh Lookenbill and Dustin Shull
PA Department of Environmental Protection
Office of Water Programs
Bureau of Clean Water
11th Floor: Rachel Carson State Office Building
Harrisburg, PA 17105

2017

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 in flowing waterbodies. 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 Streams and Rivers' (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. At this time, lake and reservoir data collection protocols are not included in this document, but DEP intends on updating and adding them in the future.

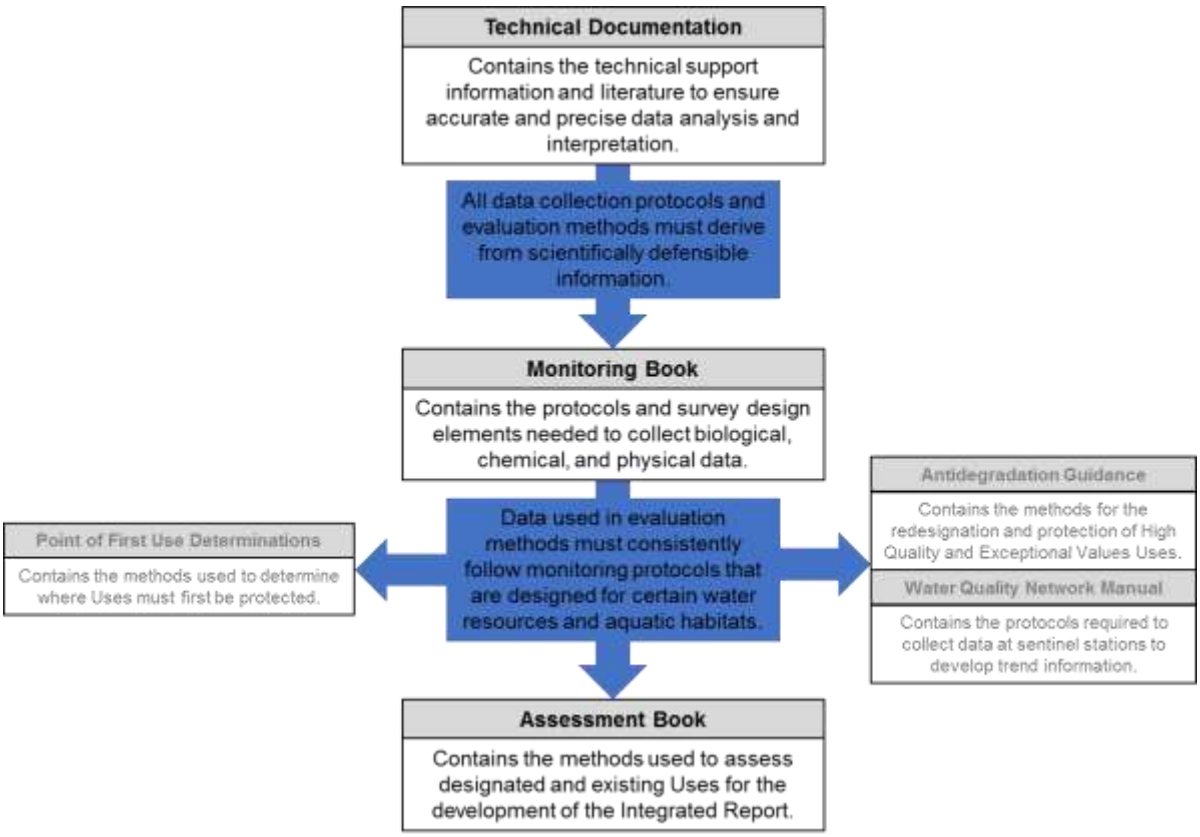


Figure 1. Conceptual framework that DEP uses to make scientifically defensible data collections and decisions.

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. All data collection should 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. The following is a list of monitoring objectives:

    - Assessment of Protected Water Uses
    - Stream Redesignation Evaluation
    - Point of First Use
    - Cause and Effect
    - Water Quality Network Monitoring
    - Protocol, Method and Water Quality Standards Development

- RECONNAISANCE 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.

- SELECT A SAMPLING DESIGN – Select a sampling design that will meet the monitoring objective(s).

    - Probabilistic
    - Targeted

- DEVELOP A SAMPLING PLAN – A sampling plan should be developed that outlines objectives, protocols, methods, standards, and 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 criteria. A review of the quality assurance (QA) criteria

for specific objectives will need to be performed before any collection occurs. Water quality data that are used by DEP need to be collected by individuals trained and subsequently audited by DEP staff. Documentation of trainings and audits will need to be provided upon request. DEP maintains documentation of trainings and audits as part of the QA requirements.

Monitoring for The Assessment of Protected Water Uses

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 the appropriate data collection protocols as wells as requirements described in the appropriate assessment methods (see the Assessment Book, Shull and Pulket 2018). The protected water uses include Aquatic Life, Water Supply, Recreation and Fish Consumption, and Special Protection.

Aquatic Life Use Monitoring

Aquatic Life Uses (ALU) include Cold Water Fishes (CWF), Warm Water Fishes (WWF), Trout Stocking (TSF), and Migratory Fishes (MF). See 25 Pa. Code § 93.3 for definitions and standards.

There are multiple approaches that can be considered in developing a sampling collection plan with the objective of assessing an ALU. The first includes employing a biological data collection protocol (Chapter 3) with an assessment method (Shull and Pulket 2018). DEP currently has four benthic macroinvertebrate data collection protocols with assessment methods. 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. Each assessment method establishes impairment thresholds and/or decision-making matrices to determine impairment. Progressively, alternative approaches include employing a biological data collection protocol or protocols with additional physical, chemical and/or other biological data collections. These additional data collections, often determined during the reconnaissance process, can be used to further assess a waterbody and used to identify potential sources and causes of impairment.

Physical habitat data collection will be completed when employing all biological data collection protocols. Physical habitat data can be used in conjunction with biological assessment data or can be used independently to make ALU assessments. Guidance on physical habitat data collection can be found in Chapter 5 of this book, or in the specific biological data collection protocols in Chapter 3. Specific ALU physical assessment methodology can be found in Chapter 4 of Assessment Book (Shull and Pulket 2018). Collectors should be familiar with physical habitat assessments, and should consult associated habitat data sheets during field reconnaissance to aid in properly identifying and locating sampling locations.

Chemical data can also be collected to characterize water quality during biological data collection or the field reconnaissance process. Chemical data collection protocols can be found in Chapter 4, and are required for many of the data collection protocols found in this book. In-situ field meter data, including cross-section surveys, are highly recommended as a screening process to determine changing water quality that may drive differences in aquatic life. For surface waters with perceived or documented water quality complexities (incomplete mixing, dynamic seasonal/temporal changes, etc.), and especially on larger waterbodies (> 1000 mi2), multiple field meter data collections, cross-section surveys, and/or discrete water chemistry data collections may be completed multiple times targeting changing conditions throughout a period leading up to biological data collection. A "period" is delineated by seasonality or index periods described in the biological collection protocols and assessment methods. For example, instream macroinvertebrate communities significantly change throughout late-May and into June and again typically in October, depending on climatic conditions. Consequently, collectors will usually employ macroinvertebrate collection in November or in April. If collection is scheduled for April, multiple chemical data collection efforts that target changing conditions beginning November and continuing through April would provide data as to the source and/or cause of differences in biological sample results. This information could be collected after biological data collection and after realization of aquatic life use impairment. However, subsequent data collections may not be directly representative of water quality conditions that caused the impairment, especially with highly variable surface waters.

DEP also employs Fish Data Collection, Mussel Data Collection and Periphyton (Algal) Data Collection Protocols that can be found in Chapter 3. While assessment method development is actively being pursued, currently there are no assessment methods for these biological data collection protocols, however they are employed to assess the narrative criteria and measure changes in water quality.

<u>Water Supply Use Monitoring</u>
Water Supply uses include Potable Water Supply (PWS), Industrial Water Supply (IWS), Livestock Water Supply (LWS), Wildlife Water Supply (AWS), and Irrigation (IRS). See 25 Pa. Code § 93.3 for definitions and standards.

Water supply use employs chemical data collection protocols including the 'Field Meter and Transect Data Collection Protocol' and the 'Discrete Water Chemistry Data Collection Protocol', found in Chapter 4. While 25 Pa. Code § 93.4 and § 96.3(c) identifies PWS as applying to all surfaces waters, § 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"*

Therefore, reconnaissance will need to identify surface potable water supply withdrawals and sampling plans will need to target these locations, especially when chemistry data collected involves the parameters listed in § 96.3(d).

Recreational Use and Fish Consumption Monitoring

Recreation and Fish Consumption uses include Boating (B), Fishing (F), Water Contact (WC), and Esthetics (E). Primary objectives for recreational use and fish consumption are Fishing (F) and Water Contact (WC). See 25 Pa. Code § 93.3 for definitions and standards.

A sample collection plan with the objective of assessing the water contact (WC) Use will employ Chapter 3, 'Bacteriological Data Collection Protocol' and Chapter 2 of the Assessment Book (Shull and Pulket 2018), 'Bacteriological Assessment Method for Recreational Use'. Chemical data collection protocols found in Chapter 4 of this book, including the 'In-situ Field Meter and Transect Data Collection Protocol' and the 'Discrete Water Chemistry Data Collection Protocol', should also be considered as part of the field reconnaissance effort.

Collecting fish consumption/fish tissue samples is typically accomplished by employing the 'Fish Data Collection Protocol', Chapter 3, and samples are often collected as a secondary objective during community surveys. Other acceptable collection methods include seine, gill net, rotenone, angling, etc. Refer to Chapter 2 of the Assessment Book (Shull and Pulket 2018), 'Fish Tissue Consumption Assessment Method' for additional requirements that will need to be addressed in the sample collection plan.

Special Protection Monitoring

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 and 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

Stream 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 is 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 (DEP 2013). 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’ 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 sewer. The point of first surface water use establishes where Chapter 93 Water Quality Standards (WQS) must be attained. 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 2000). 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. The ‘Cause and Effect Sampling Design and Planning Protocol’ found in Chapter 2 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 United States 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, Chapter 3), chemical/physical data collection (Chapters 4 and 5), and stream discharge data collection (Chapter 5). In addition to DEP data collection protocols, collectors will refer to the 'Pennsylvania Surface Water Quality Monitoring Network Manual' (DEP 2017), which also includes references to applicable USGS data collection protocols.

Protocol, Method, and Standards Development

DEP Division of Water Quality (DWQ) is responsible for developing surface water data collection protocols, water quality criteria and protected water uses, assessment methods, Pennsylvania Integrated Water Quality 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, at a statewide level, WQS are developed to establish the criteria and protected uses that surface waters must meet. Data collection protocols and assessment methods are developed, at a statewide level, to collect consistent and data that will to determine if surface waters meet WQS. In addition, data collection protocols also need to be applicable to other data collection objectives.

Sampling design for a development objective will pursue the range of possible water quality conditions across the State. Reconnaissance will focus on a range of conditions and attributes that describe this range and not necessarily the potential to delineate a water quality condition or the source or cause on a waterbody. A range of conditions will be qualified by identifying classification of waterbodies. Attributes that are selected to describe a range of water quality conditions will rely on published literature. Development objectives, particularly the development of standards, may also rely on laboratory investigations.

LITERATURE CITED

DEP. 2017. Pennsylvania's Surface Water Quality Monitoring Network Manual. (Available from: http://www.depgreenport.state.pa.us/elibrary/GetDocument?docId=4860&DocName=3800-BK-DEP0636.pdf)

DEP. 2013. Water Quality Antidegradation Implementation Guidance. (Available from: http://www.depgreenport.state.pa.us/elibrary/GetDocument?docId=7842&DocName=391-0300-002.pdf).

DEP. 2000. Policy and Procedures for Evaluating Wastewater Discharges to Intermittent and Ephemeral Streams, Drainage Channels and Swales, and Storm Sewers. (Available from:

http://www.depgreenport.state.pa.us/elibrary/GetDocument?docId=7790&DocName=391-2000-014.pdf)

Shull, D. R., and M. Pulket (editors). 2018. Assessment Methodology for Rivers and Streams. Pennsylvania Department of Environmental Protection, Harrisburg, Pennsylvania.

CHAPTER 2 SAMPLING DESIGN AND PLANNING

Prepared by:

Josh Lookenbill
PA Department of Environmental Protection
Office of Water Programs
Bureau of Clean Water
11th Floor: Rachel Carson State Office Building
Harrisburg, PA 17105

2017

TYPES OF SAMPLING DESIGN

A sampling design must be chosen in order to form a data set that is appropriate to describe the desired objective. 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 must consider population characteristics (Strahler stream order, landuse, number and location of point source discharges, etc.) and must 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 method. This method 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 methods 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 method 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 judgement, 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 landuse 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 DEP Water Quality Network (WQN) and U.S. Geological Survey (USGS) stream gaging network.

Sampling sites and locations are positioned to account for changes in water quality due to influences such as major tributaries, point and nonpoint source impacts, landuse changes, soil characteristics, and geology. Additional samples are collected at the limits of these changes to effectively "bracket" potential sources of water quality differences. Initial sampling site placement should target 3rd order or higher waterbodies. Sampling sites located on 1st and 2nd order waterbodies are chosen based on the variability of potential changes to water quality, but not all 1st and 2nd order waterbodies will have sampling sites located on them. It is important to identify 1st and 2nd order tributaries that may be ephemeral or intermittent, as assessment methods are typically not developed to account for this variable. Also, the minimum length of any use assessment unit is ½ mile. Any impact delineated in length that is less than ½ mile is considered a localized impact, potentially a compliance issue, but not a nonattainment of use. Additional field reconnaissance is highly recommended to verify potential sources of water quality impacts. Field reconnaissance will include visual observations and physical habitat evaluations (see Chapter 5), and can also include chemical data collection and/or field meter and cross-section survey data collection (see Chapter 4). 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.

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. More information on developing Project Plans can be found in United States Environmental Protection Agency (USEPA) resources (USEPA 2002a, USEPA 2002b, and USEPA 2006).

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 legal or regulatory actions. Also, consider what criteria may be used during its interpretation. DEP has numeric and narrative water quality criteria documented in The Pennsylvania 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 narrative or general sediment quality criteria at this time. However, in order to apply these or other results that do not have numeric criteria, one can refer to the Pennsylvania Code, Chapter 93, Water Quality Standards: § 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.

CAUSE AND EFFECT SURVEYS

Cause and effect surveys are designed 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. 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 stream and river habitats, the sampling design and type of sampling gear used are dependent on stream-type, 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 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 stream 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 be violations of WQS and 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, ‘In-situ Field Meter and Transect Data Collection Protocol’, or equal-width-increment or equal-discharge-increment methods (U.S. Geological Survey, 2006). When collecting biological samples, the equal-width-increment method 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 methodologies.

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 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 river 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, habitat assessment, field measurements or water chemistry, channel cross-section and stream flow (as needed), cross-section surveys (as needed), bacteria (as needed), and fish (as needed).

Data Collection

Benthic Macroinvertebrates (required)

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. Sampling protocols include semi-quantitative (population densities derived by estimation), qualitative (population densities not calculated), and quantitative (population densities derived by measurement) methods. The survey data needs will determine which method is most suitable. In most instances, semi-quantitative sampling methods are preferred and will meet most data requirements. Other optional sampling methods include qualitative and quantitative. Qualitative methods (Chapter 3) are appropriate Cause and Effect Survey methods. Qualitative methods may be more appropriate than quantitative or semi-quantitative methods, and may be used in place of, or in conjunction with semi-quantitative sampling when the survey requires a more immediate result or the targeted surface water is not characteristic of surface waters used to develop a methodology 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 Pulket 2018). 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 IBI describes 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 should 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 Division of Water Quality, Assessment Section. Follow-up surveys may be conducted when small IBI score differences are found between control and impact sites, to confirm, or re-evaluate initial cause and effect survey results.

<u>Fish (optional)</u>
For most cause and effect surveys semi-quantitative fish sampling should be considered. See the DEP Fish Data Collection Protocol in Chapter 3 of this book. The objective is to acquire a representative sample of the fish population by sampling all physical stream habitats in relative proportion to their availability. The collected sample

should contain most of the species in the stream at the time of sampling in numbers proportional to their actual abundances.

In some cases, the cause and effect assessment may allow for a less rigorous qualitative fish sampling to demonstrate the presence or absence of discharge impacts. For small or large wadeable streams, sampling is conducted over a representative 100-meter minimum reach of stream. Sampling of the reach is continued until no new species of fish are found. When possible, the fish are identified in the field and released. Voucher specimens and specimens which cannot be field identified are preserved in a 10% formalin solution for laboratory identification. A fish health evaluation should also be considered.

If fish kills are a component of a cause and effect survey investigation, quantitative methods 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 (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 attainment status of stream segments for potable water supply and recreational uses. The samples are collected using methods outlined in Chapter 3 of this book.

Habitat Evaluation (required)

A habitat assessment is conducted on a measured 100-meter reach of stream at a minimum for wadeable surface waters. The habitat assessment process involves rating twelve parameters as optimal, suboptimal, marginal, or poor by using a numeric value (ranging from 20-0), based on the criteria included in the Riffle/Run Habitat Assessment protocol. The Habitat Assessment protocol is provided in Chapter 5 of this book. The field data sheets are provided in Appendix C of this book.

Field Measurements and Water Chemistry (required)

Detailed field observations on landuse and potential sources of pollution are recorded on field data collection forms or in field books. 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 or DMRs that document the chemical violation would suffice. For nonpoint discharges, grab samples are collected upstream of the impacted segment and from within the impacted segment.

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

LITERATURE CITED

Davis, W. S., and T.P. Simon. 1995. Introduction to Biological assessment and criteria: tools for water resource planning and decision making, W.S. Davis and T.P. Simon, eds. (pp. 3 – 6). CRC Press, Boca Raton.

Kincaid, T.M. & A.R. Olsen. 2011. spsurvey: Spatial Survey Design and Analysis. R package version 2.2.

Kincaid, T.M. 2012a. Analysis of a GRTS Survey Design for a Linear Resource. CRAN – R project.

Kincaid, T.M. 2012b. GRTS Survey Designs for a Linear Resource. CRAN – R project.

Southwick, R. I., and A. J. Loftus. 2017. Investigations and Monetary Values of Fish and Freshwater Mussel Kills. American Fisheries Society. ISBN 978-1-934874-47-9.

Turnipseed, D.P., and V. B. Sauer. 2010. Discharge measurements at gaging stations: United States Geologic Survey. Techniques and Methods book 3, chap A8, 87 p.

USEPA. 2002a. Guidance on choosing a sampling design for environmental data collection for use in developing a quality assurance project plan. EPA/240/R-02/005. EPA QA/G-5S. U.S. Environmental Protection Agency. Washington, DC.

USEPA. 2002b. Guidance for quality assurance project plans. EPA/240/R-02/009. EPA QA/G-5. U.S. Environmental Protection Agency. Washington, DC.

USEPA. 2006. Guidance on systematic planning using the data quality objectives process. EPA/240/B-06/001. EPA QA/G-4. U.S. Environmental Protection Agency. Washington, DC.

USGS. 2006, Collection of water samples (ver. 2.0): U.S. Geological Survey Techniques of Water-Resources Investigations, book 9, chap. A4, September 2006.

CHAPTER 3 BIOLOGICAL DATA COLLECTION PROTOCOLS

WADEABLE RIFFLE-RUN STREAM MACROINVERTEBRATE DATA COLLECTION PROTOCOL

Prepared by:

Brian Chalfant
PA Department of Environmental Protection
Office of Water Programs
Bureau of Clean Water
11th Floor: Rachel Carson State Office Building
Harrisburg, PA 17105

2013

Edited by:

Dustin Shull
PA Department of Environmental Protection
Office of Water Programs
Bureau of Clean Water
11th Floor: Rachel Carson State Office Building
Harrisburg, PA 17105

2017

INTRODUCTION

The USEPA's Rapid Bioassessment Protocols for use in Wadeable Streams and 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 the 'Wadeable Multihabitat Stream Macroinvertebrate Collection Protocol' (McGarrell and Pulket 2007). 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 the wadeable freestone riffle-run stream macroinvertebrate assessment method (Chalfant 2015) for more information on collection results and season.

MACROINVERTEBRATE FIELD 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 immediately upstream of the net to an approximate depth of 10 cm, as substrate allows. During each kick, the net should be held stationary. 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 10 regular samples for QA/QC purposes.

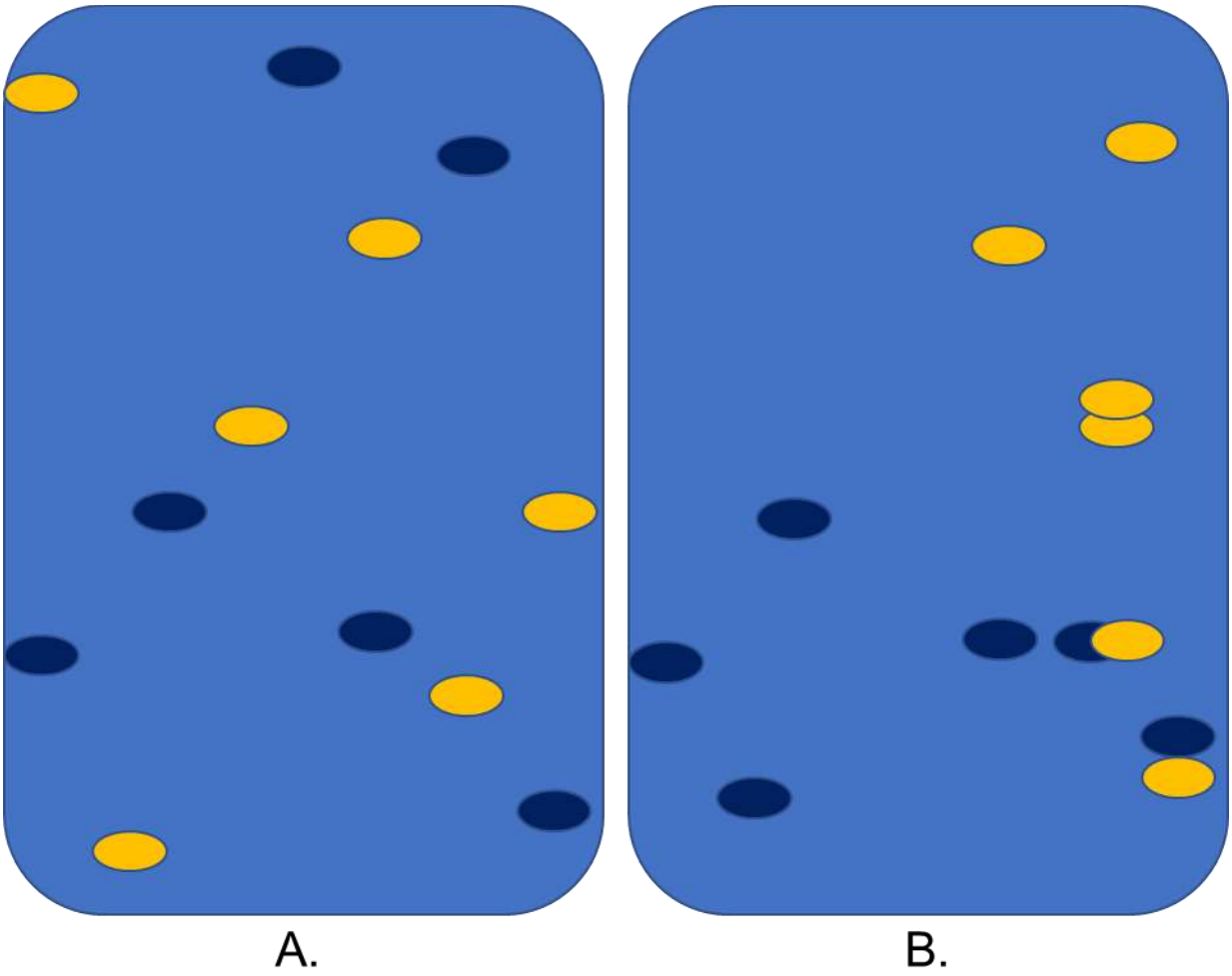


Figure 2. Blue rectangles illustrate a 100-meter reach within a stream, dark blue ellipses show the initial set of kicks during sample collection, and yellow ellipses show the replicate set of kicks during sample collection. (A) Correct sample collection with replicate collection; all kicks are collected 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. 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. 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.

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 or SAC 18 (for special protection). For more information on water chemistry collection and SACs, refer to the Discrete Water Chemistry Data Collection Protocol in Chapter 4. Because riffle-run surveys are designed for high gradient streams, the high gradient habitat assessment form should be used. The DEP high gradient habitat form can be found in Appendix C-1 and C-2.

LITERATURE CITED

Barbour, M.T., J. Gerritsen, B.D. Snyder, and J.B. Stribling. 1999. Rapid bioassessment protocols for use in streams and wadeable rivers: periphyton, benthic macroinvertebrates and fish, second edition. EPA 841-B-99-002. United States Environmental Protection Agency; Office of Water. Washington, D.C.

Chalfant, B. A. 2015. Wadeable freestone riffle-run stream macroinvertebrate assessment method. Chapter 2, pages 2-22. *In* Shull, D. R., and M. Pulket. (editors). Assessment Methodology for Streams and Rivers. Pennsylvania Department of Environmental Protection. Harrisburg, Pennsylvania.

McGarrell, C., and M. Pulket. 2007. Wadeable Multihabitat Stream Macroinvertebrate Collection Protocol. Chapter 3: Biological Data Collection Protocols. Pennsylvania Department of Environmental Protection. Harrisburg, Pennsylvania.

WADEABLE LIMESTONE STREAM MACROINVERTEBRATE DATA COLLECTION PROTOCOL

Prepared by:

William Botts
PA Department of Environmental Protection
Office of Water Programs
Bureau of Clean Water
11th Floor: Rachel Carson State Office Building
Harrisburg, PA 17105

2009

Edited by:

Amy Williams
PA Department of Environmental Protection
Office of Water Programs
Bureau of Clean Water
11th Floor: Rachel Carson State Office Building
Harrisburg, PA 17105

2017

INTRODUCTION

Limestone streams are unique aquatic systems that are typically are 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 method 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.

FIELD 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 Method
The DEP Rapid Bioassessment Protocol (RBP) is a modification of the USEPA RBP III (Plafkin et al. 1989). Modifications include: 1) the use of a 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 the 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.

FIELD METHODS

Benthic Macroinvertebrate Sampling
The handheld D-frame sampler consists of a bag 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 $1m^2$ (1 x 1 m) immediately upstream of the net to a depth of 10cm (approximately 4 inches, as the substrate allows) for approximately one minute. 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 method 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 gradient 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. 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). 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.

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 or SAC 18 (for special protection). For more information on water chemistry collection and SACs, refer to the Discrete Water Chemistry Data Collection Protocol in Chapter 4. The Riffle/Run Prevalence Habitat assessment form should be used for limestone macroinvertebrate collections. This form can be found in Appendix C-1 and C-2.

LITERATURE CITED

Plafkin, J.L, M.T. Barbour, K.D. Porter, S.K. Gross, and R.M. Hughes. 1989. Rapid Bioassessment Protocols for Use in Streams and Rivers: Benthic macroinvertebrates and fish. EPA/440/4-89-001. U.S. Environmental Protection Agency, Office of Water, Washington, D.C.

WADEABLE MULTIHABITAT STREAM MACROINVERTEBRATE DATA COLLECTION PROTOCOL

Prepared by:

Charles McGarrell and Molly Pulket
PA Department of Environmental Protection
Office of Water Programs
Bureau of Clean Water
11th Floor: Rachel Carson State Office Building
Harrisburg, PA 17105

2007

Edited by:

Molly Pulket
PA Department of Environmental Protection
Office of Water Programs
Bureau of Clean Water
11th Floor: Rachel Carson State Office Building
Harrisburg, PA 17105

2017

INTRODUCTION

The USEPA's RBP for use in Wadeable Streams and 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 the 'Wadeable Riffle-Run Stream Macroinvertebrate Data Collection Protocol' (Chalfant 2013), 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 protocol identifies appropriate field collection methods 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.

MACROINVERTEBRATE FIELD COLLECTION

Aquatic macroinvertebrate samples are collected using a multihabitat sample collection method 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 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. Each jar is labeled with the date, time, collector (collectively referred to as a station ID (yyyymmdd-HHMM-Collector)), and location. 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.

Table 1. Stream Habitat Types and Field Sampling Techniques

Habitat Type	Description	Sample Technique
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.
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")
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
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.
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

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 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 the Discrete Water Chemistry Data Collection Protocol in Chapter 4. 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-3.

LITERATURE CITED

Barbour, M.T., J. Gerritsen, B.D. Snyder, and J.B. Stribling. 1999. Rapid Bioassessment Protocols for Use in Streams and Wadeable Rivers: Periphyton, Benthic Macroinvertebrates and Fish, Second Edition. EPA 841-B-99-002. U.S. Environmental Protection Agency; Office of Water, Washington, D.C.

Chalfant, B. A. 2013. Wadeable Riffle-Run Stream Macroinvertebrate Data Collection Protocol. Chapter 3, pages 2–7. *In* Shull, D. R., and M. J. Lookenbill. (editors). Water quality monitoring protocols for streams and rivers. Pennsylvania Department of Environmental Protection. Harrisburg, Pennsylvania.

SEMI-WADEABLE LARGE RIVER MACROINVERTEBRATE DATA COLLECTION PROTOCOL

Prepared by:

Dustin Shull
PA Department of Environmental Protection
Office of Water Programs
Bureau of Clean Water
11th Floor: Rachel Carson State Office Building
Harrisburg, PA 17105

2017

INTRODUCTION

Collection of macroinvertebrate data in large semi-wadeable 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, semi-wadeable and non-wadeable. Semi-wadeable 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 non-wadeable macroinvertebrate data collection methodologies. In addition, large semi-wadeable rivers potentially have differences in water quality across their width. Well over half of the large rivers within Pennsylvania are considered semi-wadeable (Figure 1). Therefore, it is important that a standardized protocol be established for the collection of physical, chemical, biological data in these river systems.

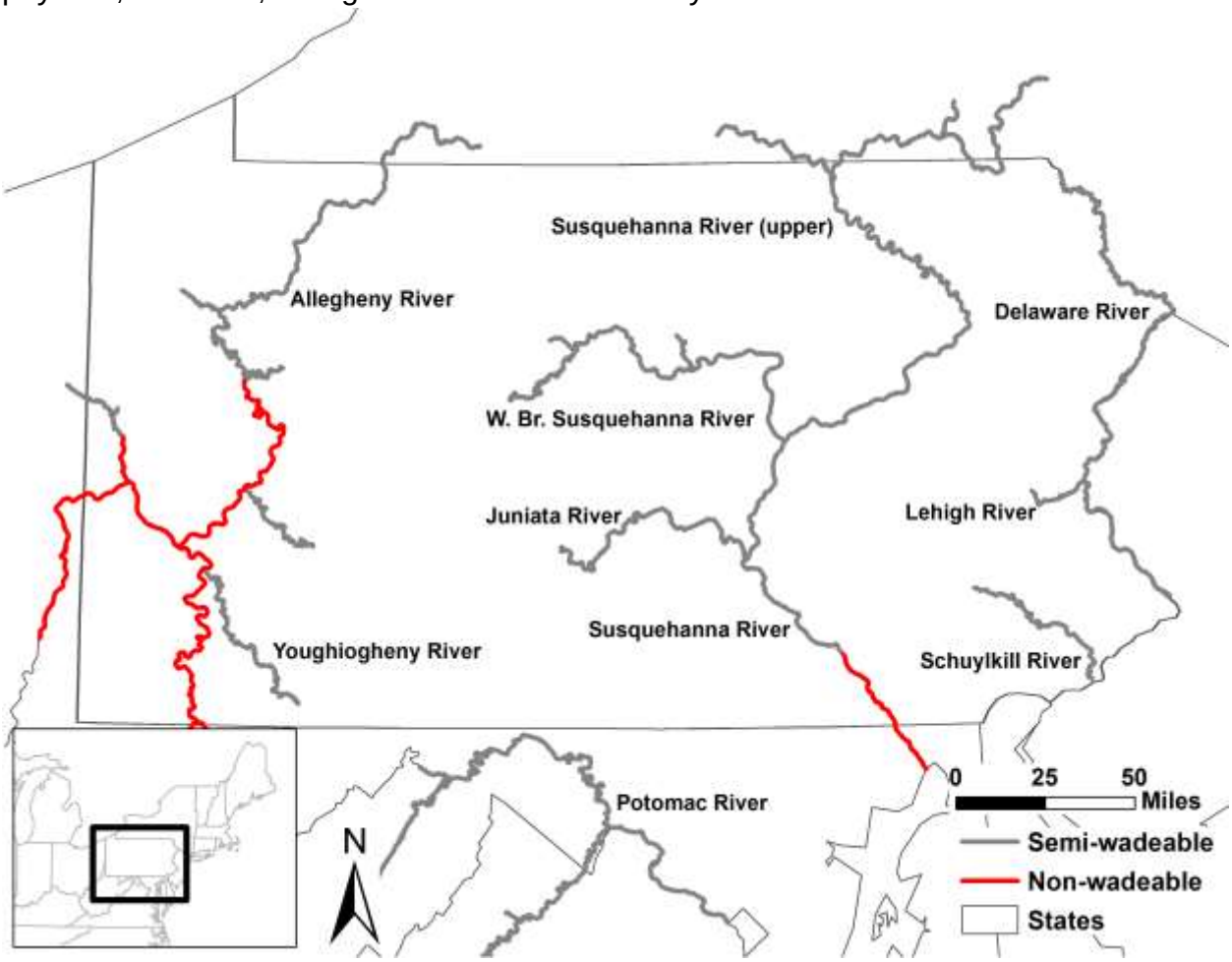


Figure 1. Semi-wadeable and non-wadeable rivers throughout Pennsylvania.

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 semi-wadeable rivers within the boundaries of Pennsylvania.

Working on, in, and around large semi-wadeable 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. 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.

SAFETY PROCEDURES

Appropriate ALU assessment begins with data collection that follows proper guidelines and safety procedures. Safety is of utmost concern when working in and on large semi-wadeable rivers. Individuals that lack experience and education related to navigating these waterbodies put themselves and others at risk of injury or loss of life. In most, if not all cases, some form of watercraft is required for this sampling method. 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 method obtain and keep a boating safety certification on them during semi-wadeable surveys. More information on boating courses and safety certifications are available here:
http://www.fishandboat.com/Boat/BoatingCourses/Pages/default.aspx

Large semi-wadeable rivers present difficulties for both wadeable and non-wadeable water navigation. 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 semi-wadeable 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 method. 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 semi-wadeable rivers is overly risky. In fact, with the proper training and experience this sampling method 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 semi-wadeable rivers, and certainly different when visiting multiple semi-wadeable 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.

WATER QUALITY DATA COLLECTION

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 semi-wadable river.

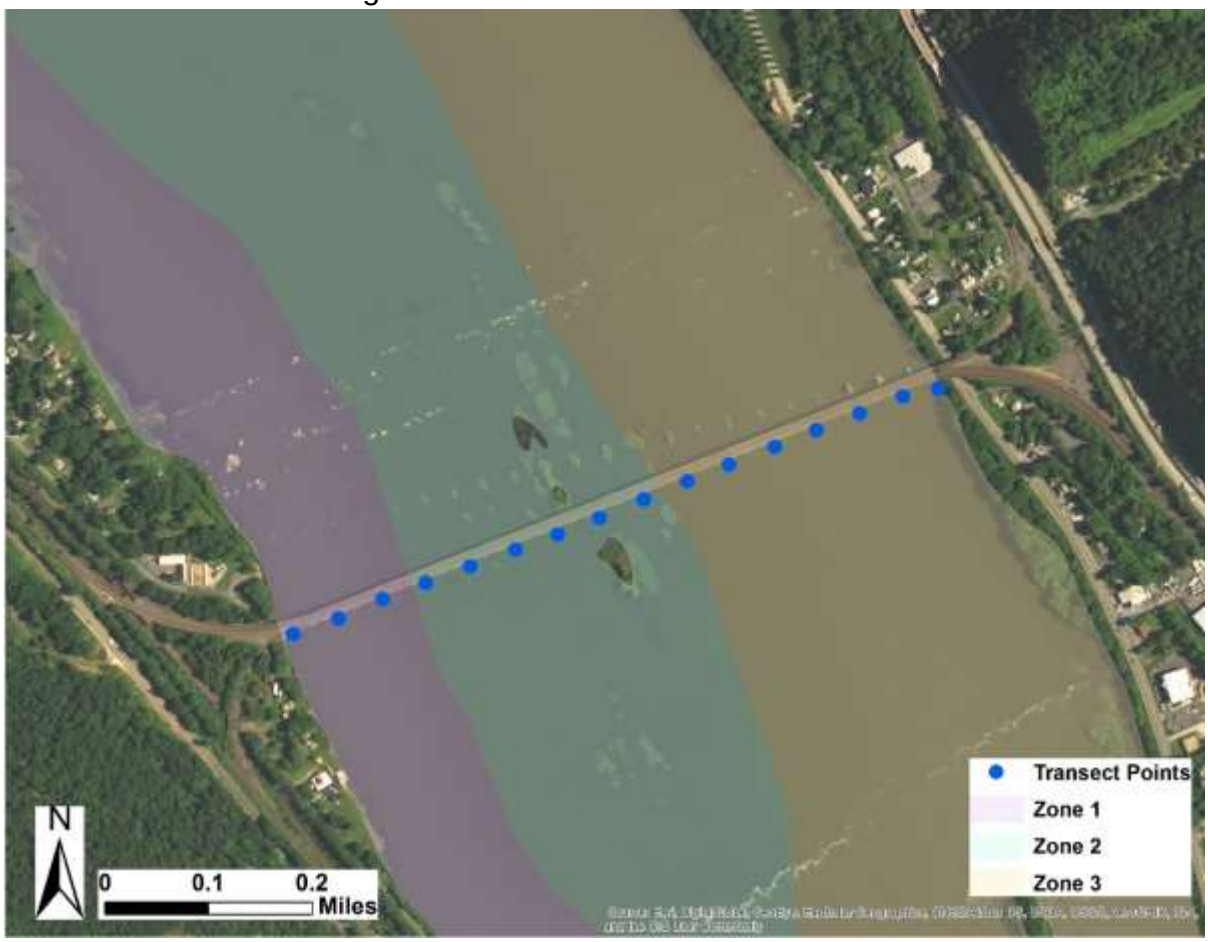


Figure 2. Example of cross-section data collection where data are collected at established transect points over time. In this example, the cross-section survey data identify three unique major water influences that create the three zones where macroinvertebrate, chemical, and physical data should be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

There are three water chemistry collection methods that are acceptable when collecting chemical data on semi-wadeable rivers: fixed-width sampling, isokinetic sampling (USGS 2006), and discrete mid-channel/mid-depth sampling (see the 'Discrete Water Chemistry Data Collection Protocol' in Chapter 4). Fixed width and isokinetic sampling is 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 SAC 612 for semi-wadeable 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 the 'Discrete Water Chemistry Data Collection Protocol' in Chapter 4 (Shull 2013).

MACROINVERTEBRATE COLLECTION

Semi-wadeable large river macroinvertebrate samples are collected using 6D-200 field collection described in the 'Wadeable Riffle-Run Stream Macroinvertebrate Data Collection Protocol' (Chapter 3, Chalfant 2013). 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 method 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. 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). Once field collections are complete, samples are taken to the laboratory for analysis. Macroinvertebrate processing and identification is conducted using standard DEP methods, which are also described in detail later in this Chapter.

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 appropriate habitat type, then samples should not be used for assessments. The responsibility for this is 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 semi-wadeable 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 and C-2.

LITERATURE CITED

Chalfant, B. A. 2013. Wadeable Riffle-Run Stream Macroinvertebrate Data Collection Protocol. Chapter 3, pages 2–7. *In* Shull, D. R., and M. J. Lookenbill. (editors). Water quality monitoring protocols for streams and rivers. Pennsylvania Department of Environmental Protection. Harrisburg, Pennsylvania.

Shull, D. R. 2013. Discrete Water Chemistry Data Collection Protocol. Chapter 4, pages 8–21. *In* Shull, D. R., and M. J. Lookenbill. (editors). Water quality monitoring protocols for streams and rivers. Pennsylvania Department of Environmental Protection. Harrisburg, Pennsylvania.

USGS. 2006. National Field Manual for the Collection of Water Quality Data. Chapter 4.1 - Surface Water Sampling: Collection methods at flowing-water and still-water sites. U.S. Geological Survey, Water Resources Office of Water Quality. Reston, VA. (Available from: https://water.usgs.gov/owq/FieldManual/Chapter4/pdf/Chap4_v2.pdf)

QUALITATIVE BENTHIC MACROINVERTEBRATE DATA COLLECTION PROTOCOL

Prepared by:

Tony Shaw
PA Department of Environmental Protection
Office of Water Programs
Bureau of Clean Water
11th Floor: Rachel Carson State Office Building
Harrisburg, PA 17105

2006

Edited by:

Josh Lookenbill
PA Department of Environmental Protection
Office of Water Programs
Bureau of Clean Water
11th Floor: Rachel Carson State Office Building
Harrisburg, PA 17105

2017

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.

KICK-SCREEN

A common qualitative sampling method 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 1x1meter 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 1x1m area immediately upstream of the net to a depth of 7-10cm, 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, C-2, or C-3) in this book.

D-FRAMED 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 methods 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 the ‘Wadeable Multihabitat Stream Macroinvertebrate Data Collection Protocol’ also found in this chapter. 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 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.

ADDITIONAL DATA

Field meter (temperature, specific conductance, pH, dissolved oxygen, and turbidity if available), water chemistry, and habitat assessment data should also be collected. 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, C-2, or C-3) in this book.

MACROINVERTEBRATE LABORATORY SUBSAMPLING AND IDENTIFICATION PROTOCOL

Prepared by:

Dustin Shull
PA Department of Environmental Protection
Office of Water Programs
Bureau of Clean Water
11th Floor: Rachel Carson State Office Building
Harrisburg, PA 17105

2017

R script provided by:
Zachary Smith
Interstate Commission on the Potomac River Basin
30 West Gude Drive Suite 450
Rockville, Maryland 20850 USA

INTRODUCTION

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

SUBSAMPLING 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
- 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 method 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 method may be more efficient than another. The subsampling method 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 freestone IBI = 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.

<u>Example Using R Script</u>

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 (Table 1). To rarefy the samples, the following 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	Hydropysche	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	Hydropysche	70
B	Paracapnia	14

```
# Set working directory
setwd("C:/Desktop/")
#Load packages
suppressPackageStartupMessages(library(tidyverse))
suppressPackageStartupMessages(library(vegan))
#Import the dataset
df <- read.csv("Example.csv", na.strings = c("NA",""))
#Convert the dataset to wide format
df.wide <- df %>%
  select(Sample, TaxaID, Count) %>%
  group_by(Sample, TaxaID) %>%
  summarize(Count = sum(Count)) %>%
  ungroup() %>%
  spread(TaxaID, Count, fill = 0)
```

The next set of code will loop through each sample and rarefy taxa counts to the specified range. 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. 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 subsample 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.

```
rarefied.df.wide <- purrr::map_df(1:nrow(df.wide), function(row.i) {
  suppressWarnings(
    data.frame(vegan::rrarefy(df.wide[row.i, 2:ncol(df.wide)], sample(180:220, 1)))
  )
}) %>%
  bind_cols(df.wide[, 1]) %>%
  select(Sample, everything())
```

The following code checks that samples were rarefied correctly. Min may be lower than the specified lower bound (i.e., 180), if the sample contained fewer than 180 taxa; however, Max should never be greater than the specified upper bound (i.e., 220).

```
rowSums(rarefied.df.wide[, 2:ncol(rarefied.df.wide)]) %>%
  summary()
```

To complete the rarefaction process, the final table is reformatted and exported in the code below. An example output table is also provided (Table 2). It is 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 <- gather(rarefied.df.wide,  TaxaID, value='Count', gather.cols =
2:ncol(rarefied.df.wide), factor_key=TRUE)
#remove taxa with zeros
rarefied.df <- rarefied.df[apply(rarefied.df!=0, 1, all),]
#arrange based on sample identifier
rarefied.df <- arrange(rarefied.df,Sample)
#Export taxa list
write.table(rarefied.df,
          "C:/Desktop/ExampleRarefied.csv",
          sep=",", row.names = FALSE)
```

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	Hydropysche	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	Hydropysche	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 must be identified to the genus level. 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 should be avoided, because it can 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 the sample and the identifications must be forwarded to a certified taxonomist for further evaluation. In addition, 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 (Turbellaria)
Aquatic Earthworms & Tubificid Worms	Class (Oligochaeta)
Leeches	Class (Hirudinea)
Proboscis worms	Phylum (Nemertea)
Roundworms	Phylum (Nematoda)
Moss Animalcules	Phylum (Bryozoa)
Water mites	Subclass (Acari)

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 Assurance

At least 10% of all samples identified by a biologist are quality assured by a certified taxonomist. The most common type of certification is the genus level EPT certification provided by the Society of Freshwater Science. Identification results between the biologist and certified taxonomist must be 90% or greater. DEP may request sample vials for any results entered into internal databases for quality assurance purposes.

LITERATURE CITED

Barbour, M.T., J. Gerritsen, B.D. Snyder, and J.B. Stribling. 1999. Rapid bioassessment protocols for use in streams and wadeable rivers: periphyton, benthic macroinvertebrates and fish, second edition. EPA 841-B-99-002. United States Environmental Protection Agency; Office of Water. Washington, D.C.

Heck, K. L., Jr., G. Belle, and D. Simberloff. 1975. Explicit Calculation of the Rarefaction Diversity Measurement and the Determination of Sufficient Sample Size. Ecology 56:1459–1461.

Hurlbert, S. H. 1971. The Nonconcept of Species Diversity: A Critique and Alternative Parameters. Ecology 52:577–586.

Sanders, H. L. 1968. Marine Benthic Diversity: A Comparative Study. The American Naturalist 102:243–282.

Simberloff, D. 1978. Use of Rarefaction and Related Methods in Ecology. Biological Data in Water Pollution Assessment: Quantitative and Statistical Analyses,

ASTM STP 652, K.L. Dickson, John Cairns, Jr., and R.J. Livingston, Eds., American Society for Testing and Materials.

FISH DATA COLLECTION PROTOCOL

Prepared by:

Timothy Wertz
PA Department of Environmental Protection
Office of Water Programs
Bureau of Clean Water
11th Floor: Rachel Carson State Office Building
Harrisburg, PA 17105

2017

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 methodologies can be applied to all wadeable and non-wadeable streams for developing indices and other assessment methodologies, promoting spatial and temporal trend analyses, and providing the foundation for a robust fish bioassessment program. All DEP fish bioassessments should strictly adhere to the following standardized collection protocol. Other collection protocols may be considered for various assessment purposes only after rigorous analysis for similarity.

BASIC REQUIREMENTS

Field sampling methods, 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 PFBC, and has met the quality control requirements of DEP (described below). 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 methodology adopted by DEP. Other fish collection methods 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).

REPRESENTATIVE SAMPLING

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

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 6mm (1/4in). 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 methods 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 methods, wadeable and non-wadeable, are described below. Strict *a priori* designations of wadeable or non-wadeable 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 non-wadeable not by size, but in its ability to be efficiently surveyed during the June 1st - September 30th sampling season utilizing the following collection methods (a stream reach may be considered for both methods throughout the same period if both methods can efficiently be implemented). Sites that can have both methods efficiently implemented are referred to as “transitional zones” and are typically large wadeable sites and small non-wadeable sites (Flotemersch et al., 2006). If a site is considered within a transitional zone both methods should be applied within a short timeframe (days-week), as feasible, to facilitate direct comparisons between collection methods. 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 methods 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, watershed 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 represent modifications from literature and preliminary data exploration (Gorman 1986, Schaefer and Kerfoot 2004, Hitt and Angermeier 2008, DEP unpublished data) 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 three stream orders smaller (adventitious). Should be avoided within the reach, when feasible.
Proximity to tributary	Major	1.5	- Defined as tributaries within three stream orders of receiving stream.

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 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 in an 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 zig-zags as they proceed upstream for adequate coverage. Similar collection methods that may employ transect zig-zags 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. 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 and is described in detail below.

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

NON-WADEABLE PROTOCOL

For semi-quantitative fish sampling in non-wadeable 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 non-wadeable sites, two reaches representing the right and left descending banks (RDB and LDB) are applied. For large non-wadeable 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 non-wadeable 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 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 non-wadeable 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 and is 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. This 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 shorter life-spans (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 5mm 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 is 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 the In-Situ Field Meter and Transect Data Collection Protocol (Chapter 4). 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. Final habitat measures are modified from the; Riffle/Run Prevalence Habitat Data Collection Form (Appendix C). These modifications reflect the measurements appropriate for the wadeable and non-wadeable methods. The non-wadeable habitat section does not include bank or riparian zone measures nine through twelve.

LABORATORY METHODS

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 sequential levels of ethyl alcohol washes from 35% to 50% to 70%. The ideal concentration for long term storage is a 70% ethyl 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 (AFS; 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.

- **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-TIME-NAME (Name = 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-TIME-NAME (Name = 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).

QUALITY CONTROL

The QA/QC of data collection is needed to maintain consistent application of these methods. 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 method (e.g., wadeable and non-wadeable) 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 SAFETY

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 United States Fish and Wildlife Service (USFWS) offers educational guidance and certification in electrofishing principles and safety and is considered the standard training source for electrofishing. Currently, USFWS 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 methods 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 non-wadeable 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.

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.*

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.

BIOSECURITY - AQUATIC INVASIVE SPECIES

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. 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 within Pennsylvania have, or have had documentation of AIS, it is best to consider all streams 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 PFBC's Biosecurity Protocol (available upon request).

LITERATURE CITED

Baumann, P., Cairns, V., Kurey, B., Lambert, L., Smith, I. and Thoma, R., 2000. Lake Erie Lakewide Management Plan (LaMP) Technical Report Series. *LaMP)*.

Flotemersch, J.E., Stribling, J.B. and Paul, M.J., 2006. *Concepts and Approaches for the Bioassessment of Non-wadeable Streams and Rivers*. US Environmental Protection Agency, Office of Research and Development.

Gorman, O.T., 1986. Assemblage organization of stream fishes: the effect of rivers on adventitious streams. *The American Naturalist*, *128*(4), pp.611-616.

Hitt, N.P. and Angermeier, P.L., 2008. Evidence for fish dispersal from spatial analysis of stream network topology. *Journal of the North American Benthological Society*, *27*(2), pp.304-320.

Lazorchak, J.M., Klemm, D.J. and Peck, D.V. eds., 1998. Environmental monitoring and assessment program surface waters: field operations and methods for measuring the ecological condition of wadeable streams.

Page, L.M., Espinosa-Pérez, H., Findley, L.T., Gilbert, C.R., Lea, R.N., Mandrak, N.E., Mayden, R.L. and Nelson, J.S., 2013. *Common and scientific names of fishes from the United States, Canada, and Mexico* (p. 243). Bethesda, Maryland: American Fisheries Society.

Pusey, B.J., Kennard, M.J., Arthur, J.M. and Arthington, A.H., 1998. Quantitative sampling of stream fish assemblages: single-vs multiple-pass electrofishing. *Australian Journal of Ecology*, *23*(4), pp.365-374.

Ruaro, R. and Gubiani, É.A., 2013. A scientometric assessment of 30 years of the Index of Biotic Integrity in aquatic ecosystems: applications and main flaws. *Ecological Indicators*, *29*, pp.105-110.

Schaefer, J.F. and Kerfoot, J.R., 2004. Fish assemblage dynamics in an adventitious stream: a landscape perspective. *The American Midland Naturalist*, *151*(1), pp.134-145.

Shank, M.K., Henning, A.M. and Leakey, A.S., 2016. Examination of a Single-Unit, Multiple-Pass Electrofishing Protocol to Reliably Estimate Fish Assemblage Composition in Wadeable Streams of the Mid-Atlantic Region of the USA. *North American Journal of Fisheries Management*, *36*(3), pp.497-505.

Walsh, S.J. and Meador, M.R., 1998. *Guidelines for quality assurance and quality control of fish taxonomic data collected as part of the National Water-Quality Assessment Program*. US Department of the Interior, US Geological Survey.

FISH TISSUE DATA COLLECTION PROTOCOL

Prepared by:

Timothy Wertz and Josh Lookenbill
PA Department of Environmental Protection
Office of Water Programs
Bureau of Clean Water
11th Floor: Rachel Carson State Office Building
Harrisburg, PA 17105

2017

INTRODUCTION

Routine monitoring for contaminants in fish tissue was initiated in 1979 as part of a nationwide network of stations encouraged by the 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 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.

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 (see Chapter 2 of the 'Assessment Book', Shull and Pulket 2018). 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.

SAMPLE COLLECTION

Once the site and representative sample location are determined, the next step is to determine what gear is needed survey the area. The most common method 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 at or exceed 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 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.

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 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 SIS database as soon as possible after the sample has been collected. General SIS data entry methods are described in Chapter 4 of this book entitled 'Sample Information System (SIS) Data Entry Protocol'. Fish tissue data entry is similar to most SIS data entry, with only a few unique differences described in Figures 1-3.

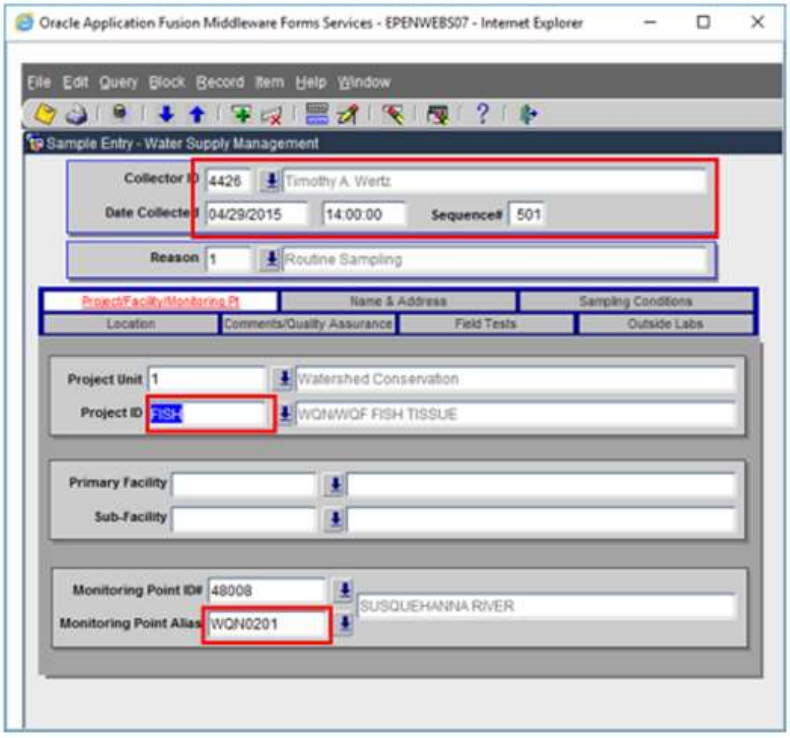


Figure 1. Important SIS data entry blocks for Project/Facility data.

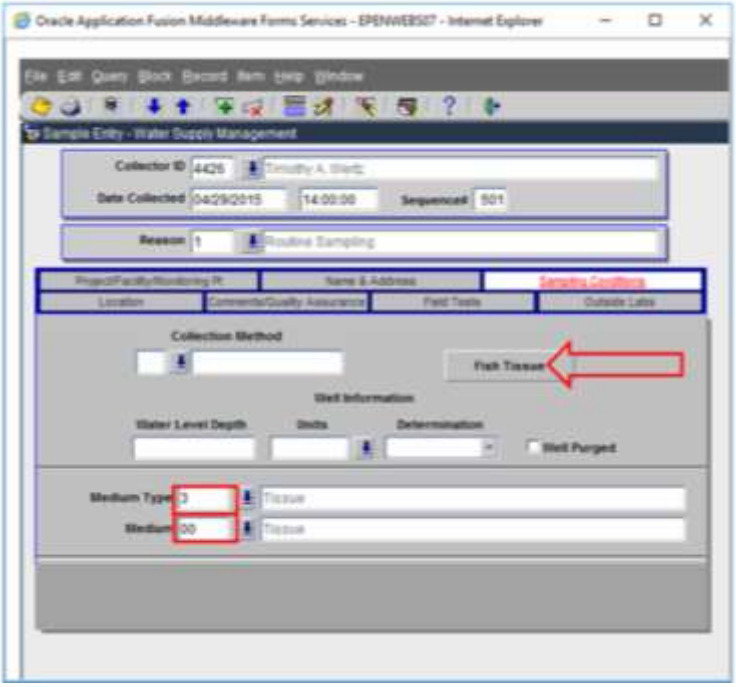


Figure 2. Important SIS data entry blocks and fish tissue button (red arrow), for Sampling Conditions data.

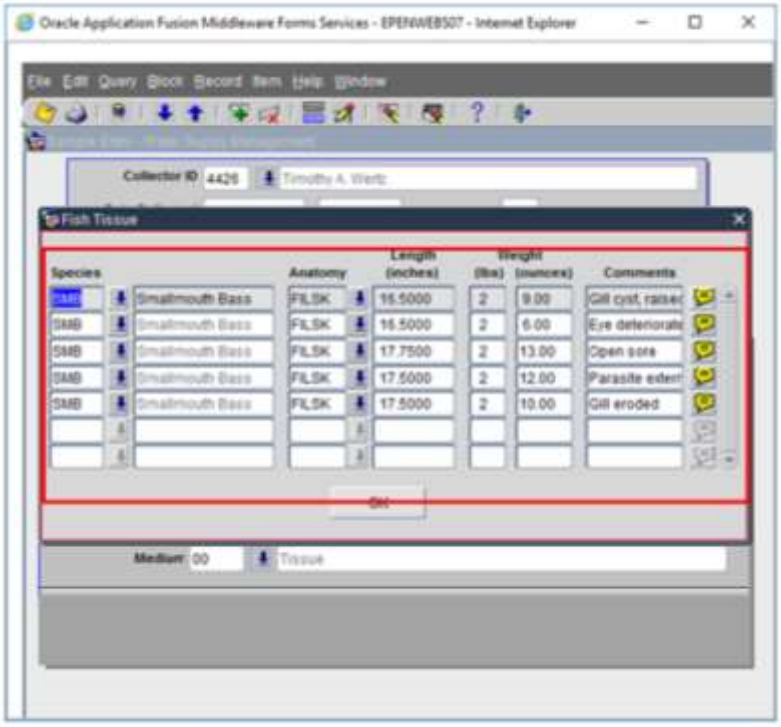


Figure 3. Important SIS data entry blocks for Fish Tissue data.

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.

SHIPPING SAMPLES

Before samples can be delivered to the BOL for analysis, a laboratory sample submission sheet (Submission Sheet) must be completed for each sample with the collection information and SAC needed. 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 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
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 sample submission sheet 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 can be placed in a cooler without additional ice and couriered to the BOL. 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.

LAB SAMPLE PREPARATION

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 if needed. 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.

MUSSEL DATA COLLECTION PROTOCOL

Prepared by:

Richard Spear, Daniel Counahan, and Dustin Shull
PA Department of Environmental Protection
Southwest Regional Office
Office of Water Programs
Clean Water Program
400 Waterfront Street
Pittsburgh, PA 15222

2017

INTRODUCTION

Larger rivers and streams are highly complex ecosystems with a great deal of interdependence between biological communities. As a result, biological components of this assessment must include a diversity of sampling including, but not limited to: fish, benthic macroinvertebrates and mollusk sampling. Current mussel sampling methodologies are not suited for a rapid and cost-effective means of assessing a representative portion of the mussel community. Therefore, DEP developed a mussel sampling protocol that incorporates random sampling to allow for a semi-quantitative assessment of mussel communities. The DEP freshwater mussel sampling protocol enables sampling in reaches where fishes and benthic macroinvertebrates were sampled to provide comprehensive sampling of aquatic biological communities. This protocol can also be described as a cursory evaluation of the mussel community so that future/more intensive surveys can be conducted.

The DEP freshwater mussel sampling protocol is designed to 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. Additionally, this survey can be used as a rapid and cost-effective way of searching for rare, threatened, endangered, and invasive mussels. Protocols such as the ones described by Smith, 2006 and Clayton et al., 2013 are also recommended for this purpose.

This protocol is composed of 12 five-minute dives along linear transects that are randomly spaced from both the downstream sampling point and the shoreline. The objective of this survey method is to build Pennsylvania's capacity to assess conditions of large rivers by providing a foundation for the development of large river assessment protocols for non-wadeable streams. These large river protocols will provide the capability to conduct bioassessments and monitor environmental conditions with a scientifically defensible method applicable to Pennsylvania's rivers and streams.

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.

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, September 2010.

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 Department's Policy for Scientific Diving Personnel and Diving Operations (available upon request). This plan is comparable to and was derived from USEPA's Diving Safety Manual (DSM), and specifically, Appendix A of the DSM USEPA Diving Safety Rules. 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. 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.
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 when their tank pressure decreases to 500 psi. A diver must begin an ascent with at least 500 psi in his/her cylinder if they must make a safety stop at 20 feet or if required by 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 exceed 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. Responsible for preparing for the diving operation including checking equipment such as the pressure in the O_2 cylinders
2. Insures that all divers are physically fit to dive
   Assists the Tender and be in complete charge of the diving operation,
3. Insures that all diving operations are conducted safely in accordance with USEPA's DSM and standards
4. Promptly prepares a Dive Report upon completion of the diving activities
5. Gives 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," and
   h. Reviewing emergency and evacuation procedures.
6. 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, and
   c. Review CPR and the use of medical O_2, if necessary.
7. 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. Insure 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 violates the dictates of USEPA's safety policies or standards.

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 and copies of the log 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 to the UDO within 10 Working days of completion of the operation.

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 (above). Equipment needs will greatly depend on weather conditions and 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, bucket, camera, calipers, and scale. A checklist of required and suggested equipment is provided below:

Dive Equipment

- ☐ Masks
- ☐ Snorkels
- ☐ Wetsuits
- ☐ Gloves
- ☐ Boots
- ☐ Hoods (If Applicable)
- ☐ Fins (If Applicable)
- ☐ Buoyance 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: _____

Survey Location and Site Selection

Survey locations are typically situated at or near large river 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 500m by 50m 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 or deep mussel communities. A minimum of 4 shallow water sites will be selected at each sample site to ensure adequate coverage of this habitat type. 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 50m buffer. No transects will be performed between any lock chamber mooring points and the lock and dam structure. If a survey is located near a United States Army Corp of Engineer (USAOE) lock and dam, then the USACOE 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.

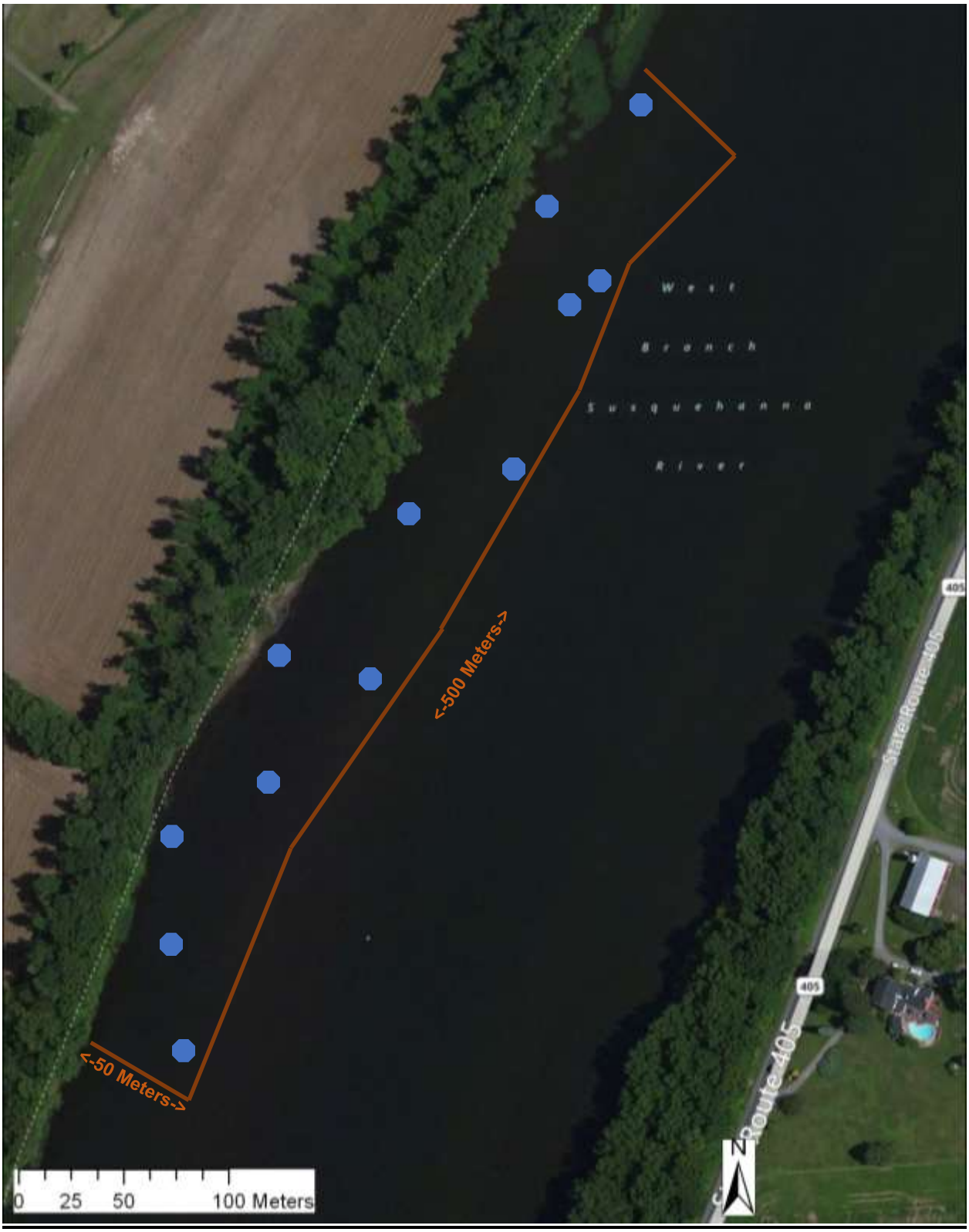


Figure 1. Twelve randomly selected sites (blue dots) within a 500x50m sampling location.

Mussel Data Collection Procedure

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’ dive reel that is marked in 1 m 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. All shell material, live or dead, will be placed in a numbered bag. On a wrist dive slate or verbally, the diver will record or communicate the substrate composition percentages for fines, sand, gravel, cobble, boulder and bedrock. Invasive mollusks (Zebra mussels and Corbicula) 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. While returning to the boat, 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.

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. A Secchi disk is used to measure the depth of the photic zone. Water chemistry measurements will be recorded at one of the 12 sites. All live mussels are identified to species level on-site. However, 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), measured (length, width and depth in millimeters), photographed and returned to the river. Measurements of length, width and depth are described in Figure 2. 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.”

A. B.

C.

Figure 2. 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).

LITERATURE CITED

Clayton, Janet., B. Douglas, P. Morrison, and R. F. Villella, 2013. West Virginia Mussel Survey Protocols.

Smith, D.R. 2006. Survey design for detecting rare freshwater mussels. Journal of the North American Benthological Society 25(3): 701-711.

PERIPHYTON DATA COLLECTION PROTOCOL

Prepared by:

Jeffery Butt
PA Department of Environmental Protection
Office of Water Programs
Bureau of Clean Water
11th Floor: Rachel Carson State Office Building
Harrisburg, PA 17105

2017

Disclaimer:
The mention of specific trade names or commercial products does not constitute endorsement or recommendation for use.

PURPOSE

The purpose of this document is to provide a standard protocol for DEP's collection of periphyton in lotic systems of Pennsylvania. The following sample and subsampling types and methods, laboratory methods, as well as quality issues, will be discussed:

- Collection of Qualitative Multi-Habitat Periphyton samples
- Collection of Quantitative Benthic Epilithic Periphyton samples
- Subsample for Identification and Enumeration of Algal Taxa
- Subsample for the quantitative determination of Chlorophyll-a and Phycocyanin Photopigments
- Subsample for the quantitative determination of Ash Free Dry Mass (AFDM)
- Subsample for the characterization of Cyanotoxins as part of qualitative multi-habitat periphyton and benthic epilithic periphyton quantitative samples
- General laboratory considerations and methods required to process the above sample types
- Quality and harmonization issues concerning the identification and counting of algae taxa.

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 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 methods to collect periphyton from lotic systems. These methods are:

- Qualitative Multi-Habitat (QMH) Periphyton Sampling Method
- Quantitative Benthic Epilithic (QBE) Periphyton Sampling Method

The field procedures employed to collect both the QMH Periphyton and the QBE Periphyton samples are appreciably derived from those described in the USGS document "Revised Protocols for Sampling Algal, Invertebrate, and Fish Communities as Part of the National Water-Quality Assessment Program, Open-file Report 02-150" (Hereafter this USGS document will be referred to as the USGS OFR 02-150 and is found in the Moulton, et al. 2002 reference). 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, 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 Temperature, Specific Conductivity, pH, and Dissolved Oxygen (both mg/l and % saturation).

This document doesn't 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, Chemical Data Collection Protocols.

HISTORY OF LOTIC PERIPHYTON SAMPLING BY DEP

DEP began actively collecting lotic periphyton samples in 2005 at locations associated with its Water Quality Network (WQN) sites. In 2012, Biologists began collecting samples for use in the development of a nutrient trophic index and concluded this sampling in 2014. The samples collected for the nutrient trophic index have also been applied to the development of an algae based Multi-Metric Index (MMI) for assessing wadable and semi-wadeable Pennsylvania rivers and streams. DEP Periphyton

sampling continues to this day for the purposes of developing this MMI as well as for routine monitoring to document existing or changing biological conditions in rivers and streams.

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 collection protocols include (Moulton 2002):

- 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 methods defined above, in general, targets a single habitat type, this sampling method 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 algae. These two methods include:

- QMH Periphyton Sampling Method (Similar to the USGS QMH method)
- QBE Periphyton Sampling Method (Similar to the USGS RTH method).

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.

Large semi-wadable rivers are oftentimes sampled only in the mid-July to August time-period. This selection of sampling period is usually driven by river flows and the ability of the biologist to access the benthos by wading. Flows in Pennsylvanian larger rivers and streams during the spring and early summer, as well as later in the fall, often make wading, and consequentially benthic algae collection, impractical, if not dangerous, for the field biologist. Thus, due to practicality and safety, DEP biologists should begin their algae sampling each year in the spring or early summer and work in watershed head waters. Thereafter, these same biologists should progress downstream into the larger streams and rivers as the season progresses into mid to late summer and flows in those locations characteristically diminish.

QMH PERIPHYTON SAMPLING METHOD

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 Method discussed below) community data, photopigment, or 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 method 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 method.

With this method, 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 method is intended for use in Pennsylvania wadeable riffle-run streams and semi-wadeable large rivers. Refer also to the Wadeable Riffle-Run Stream Macroinvertebrate Data Collection Protocol and the Semi-Wadeable Large River Macroinvertebrate Data Collection Protocol sections in this Chapter. These other protocol sections provide terms and definitions that also apply to this section, as well as provide important safety information for field staff to consider.

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
- Field Meter Discrete Values – refer to the Field Meter and Transect Data Collection Protocol Section in Chapter 4 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 methods 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 method).

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 method 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 methods, by type, will be performed as follows:

- For each sampling method 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) doesn't exceed 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, 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) doesn't exceed 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) doesn't exceed 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) doesn't exceed 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 method 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	120ml Plastic	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 2000 ml 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 method 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 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 shouldn't 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 insure 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.

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 name), 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 ID Lab 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 ID Lab in providing more accurate algae taxa identifications, especially as they may pertain to species or variation level identifications of diatoms.

QBE PERIPHYTON SAMPLING METHOD

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 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 methods 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 U.S. Geological Survey National Water-Quality Assessment Program (USGS NAWQA) 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 method.

This method is intended for use in Pennsylvania wadeable riffle-run streams and semi-wadeable large rivers. Refer also to the Wadeable Riffle-Run Stream Macroinvertebrate Data Collection Protocol and the Semi-Wadeable Large River Macroinvertebrate Data Collection Protocol sections in this Chapter. These sections provide terms and definitions that also apply to this section, as well as provide important safety information for field staff to consider.

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 the Field Meter and Transect Data Collection Protocol section of Chapter 4. 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 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 semi-wadeable 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 semi-wadeable large river requiring a 27-rock sample. Refer to the Wadeable Riffle-Run and Semi-Wadeable Large River Macroinvertebrate Data Collection Protocol sections of this Chapter for a more complete definition of these type of river 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 RDB (Right Descending Bank) and the LDB (Left Descending Bank) locations on the QBE Periphyton Field Data Sheet. In streams to 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 semi-wadeable 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 dice (the first dice roll represents the tens place of a percentage use in the location calculation – for example a roll of 1 represents 10. The second dice roll represents the units place – for example a roll of 5, when combined with the previous dice 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.

<u>Setting up Transects and Making the Sample Rock Selections in Semi-Wadeable Large Rivers</u>

To facilitate the random rock selection in semi-wadeable 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 semi-wadeable 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 (refer also to the In-Situ Field Meter and Transect Data Collection Protocol section in Chapter 4 for detailed field meter and transect methods and also to the Semi-Wadeable Large River Macroinvertebrate Data Collection Protocol section in this Chapter for additional discussion pertaining to delineating tributary water influence transitions and defining multiple river zones). When such water quality heterogeneity is observed, a semi-wadeable 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 (refer to Figure 2 in the Semi-Wadeable Large River Macroinvertebrate Data Collection Protocol section of this chapter. 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 a 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 Pennsylvania Epilithic Periphyton (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.

   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.

3. 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.

4. 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.

5. 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. Through cleaning is required to prevent the contamination of samples with residue from other sites.

6. 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 Discrete Values – refer to the Field Meter and Transect Data Collection Protocol Section in Chapter 4 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 Method 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 or filter requirements, preservation method, as well as transportation and storage needs for the QBE subsamples.

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 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				

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 held-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 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.

One 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 insure 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 already exceeds 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 insure accuracy of volume measurement in a 1000ml graduated cylinder which are often incremented in units of 100ml.

<u>The total algae slurry volume must be recorded on the field data sheet and, eventually, reported to the laboratory performing the identifications/enumerations.</u> 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 µm^3/cm^2) estimates, accurate reporting of algae slurry volume and surface area scrubbed is essential.

Also note that 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. This is why multiple ID/Enumeration bottles are collected from each sample. This QA process is discussed in detail later in this protocol.

<u>Subsampling for Algae Identification/Enumeration</u>
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 insure accuracy of volume measurement. Thereafter, pour this volume into a labeled 120ml algae ID/enumeration 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 ID/enumeration 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 insure 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 ID/enumeration bottle,

return the slurry beaker to the magnetic stirrer to re-establish homogenization prior to pouring the second 100ml algae ID/enumeration sample and later, the third 100ml algae ID/enumeration subsample.

Preserve each 100ml ID/enumeration subsample with 7ml of formaldehyde (volume of formaldehyde preservative must also be reported to the ID/enumeration laboratory). Refer also to Table2 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 made 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 ID Lab 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 ID Lab 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 an identification lab. Often, DEP will retain these samples until the end of a collection season prior to shipping all samples together to the identification/enumeration laboratory.

When ID/Enumeration subsampling is complete, the biologist may then proceed to subsampling for Phycocyanin, Acidified Chlorophyll-a, AFDM, and Cyanotoxins.

<u>Subsampling for Phycocyanin, Acidified Chlorophyll-a, and AFDM</u>

After the Algae Identification/Enumeration subsampling has been completed, 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.

At present, all phycocyanin, acidified chlorophyll-a and AFDM subsamples are delivered to DEP Bureau of Laboratories (BOL) for further processing. These samples may either be hand delivered to BOL or may be shipped to BOL by courier. Refer to Table 2 for

methods of transportation and storage. All necessary laboratory submission sheets must be completed and delivered to BOL along with the sample delivery. Phycocyanin has a very short holding time for pre-processing at BOL and should either be hand-delivered to BOL at the end of the field day or delivered overnight by courier.

Replicate samples for Phycocyanin, Acidified Chlorophyll-a, and AFDM

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.

Phycocyanin and acidified chlorophyll-a photopigments and AFDM are used by DEP to estimate the algae biomass in a stream. This algae biomass, in turn, may be used to infer characteristics regarding the nutrient status of a stream and also provide information used to judge the biological condition status of that stream. Phycocyanin is a photopigment that, in Pennsylvania, is nearly exclusively descriptive of cyanobacteria. Chlorophyll-a is a photopigment that is present in all major algal groups.

Subsampling for Phycocyanin

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. As with the algae identification/enumeration subsampling procedure, don’t 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 insure 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.

As indicated in Table 2, phycocyanin subsamples require no preservation additives but does require appropriate low temperature transportation and storage.

The phycocyanin bottle should be labeled with the same GIS Key as was used for the Algae ID/Enumeration sample, the word “Phycocyanin,” the stream name, and the three collector-sequence numbers 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 30ml submission – BOL typically does this by re-homogenizing the 30ml sample and then extracting three ~5ml subsamples. Thus, the three-subsample replicate requirement is achieved through this single 30ml submission to BOL.

On the field data sheet, record the three collector-sequence numbers that are to be used for phycocyanin submissions to 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. Indicate the BOL SAC Code of B031 for the phycocyanin test on the Sample Submission Sheet. Also, indicate on the BOL submission sheet that 30ml of sample is supplied and that three 5ml subsamples are drawn from this single submission (Refer to Appendix A-9 for BOL Microbiology Sample Submission Sheet).

After subsampling for phycocyanin, place the algae slurry beaker back on the magnetic stirrer to re-homogenize the sample prior to proceeding to the AFDM subsampling.

Prepare the bottled phycocyanin sample for transportation as defined in Table 2. If the biologist hand delivers this sample to BOL, it must be placed into the appropriate refrigerator in the in the Bacteriological Services Section (consult with lab staff to confirm the location of this refrigerator). In this case, sample submission sheets should be hand-delivered to a member of the Bacteriological Services Section so that they are made aware that a phycocyanin sample has been dropped off – 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. When phycocyanin samples are delivered by overnight courier, the Bacteriological Services section is made aware of the arrival of the sample as part of BOL's normal morning sample check-in process. 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 by overnight courier at the end of each field day. In either case, phycocyanin samples should be transported on wet-ice.

Subsampling for AFDM
AFDM is subsampled in a manner very much like that used for acidified chlorophyll-a (see below). However, preservation methods differ between the AFDM and acidified chlorophyll-a subsampling procedures (see Table 2).

AFDM is subsampled by extracting three 2ml replicates of homogenized algae slurry. This is performed using a 2ml spring loaded Hensen-Stemple Pipette (Wilco 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 5) 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. 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 that doesn't exceed 3psi (exceeding this pressure may damage the GF/F filter, resulting in the loss of periphyton slurry material).

With the vacuum still applied, rinse the side walls of the filter apparatus cup with deionized 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 Whatman™ 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. 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 6). 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 zip-lock bag. Each AFDM filter is to be placed into its own small plastic zip-lock bag. Place the three plastic bags into a small paper envelop and seal this paper envelope. This paper envelope should be labeled with the same GIS Key as was used for the Algae ID/Enumeration sample, the word "AFDM," the stream name, and the three collector-sequence numbers assigned to the subsamples. This envelope is then sealed inside a zip-lock plastic bag - multiple paper envelopes may be sealed into a single zip-lock plastic bag. Record the three collector-sequence numbers for this sub 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 BOL SAC Code for AFDM-a is B027.

Prepare the AFDM sample for transportation as defined in Table 3. When delivering this sample to BOL, it must be placed into the appropriate -80°C freezer in the Bacteriological Services Section (consult with lab staff to understand the location of this freezer). Sample submission sheets should be hand-delivered to a member of the Bacteriological Services Section so that they are made aware that AFDM samples have been delivered. AFDM samples may also be delivered to BOL via courier on dry-ice. However, 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 5. Vacuum-draw filtering devices on left and algal slurry beaker on a magnetic stirrer on the right.

Figure 6. The proper method for folding the chlorophyll-a and AFDM filters.

Subsampling for Acidified Chlorophyll-a

Prior to proceeding with the acidified chlorophyll-a subsampling, thoroughly clean the vacuum-draw filtering cups then rinse with de-ionized water. Acidified Chlorophyll-a is subsampled in a manner very much like that used for AFDM (see above). However, preservation methods differ between the AFDM and acidified chlorophyll-a subsampling procedures (see Table 2).

Chlorophyll-a 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 5) 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. 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 that doesn't exceed 3psi or persists for longer than 10 minutes (exceeding this pressure and/or duration may damage cells resulting the loss of photopigments) (Arar & Collins, 1997).

With the vacuum still applied, rinse the side walls of the filter apparatus cup with deionized 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.

After the algae slurry water is drawn through the filter, and the algae is deposited on the GF/F filter, release the vacuum on the filtering device. Fill the filter cup with a small quantity of de-ionized water so that a few milliliters of water pools on top of the filter. Into this maintained pool of water, add drop-wise 1ml of saturated $MgCO_3$ solution preservative. This 1ml of $MgCO_3$ should be uniformly distributed around the pool of de-ionized water so that it distributes evenly across the entire filter surface. Then re-draw the vacuum on the filtering device (again, don't exceed 3psi) and allow the water and $MgCO_3$ to drain. New saturated $MgCO_3$ preservative may be obtained from BOL Microbiological Services at the beginning of the sampling season. This saturated $MgCO_3$ has a long shelf-life and will remain viable through the entire sampling season but should be stored in a cool and dry place in the sampling kit.

With the vacuum still applied, rinse the side walls of the filter apparatus cup with enough de-ionized water to make sure that all MgCO3 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 Whatman™ 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. Using two forceps, one in each hand, carefully fold the circular GF/F filter in half so that the algae material is "sandwiched" inside the now folded half-circle. Continuing to use forceps, perform one more fold, creating a quartered circle (see Figure 6). 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 foil to overlap the filter. 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. Each acidified chlorophyll-a filter is to be placed into its own small plastic zip-lock bag. Place these plastic bags into a single

small paper envelop and seal this paper envelope. This paper envelope should be labeled with the same GIS Key as was used for the Algae ID/Enumeration sample, the words “Chl-a Acidified,” the stream name, and the three assigned collector-sequence numbers. This paper envelope is then sealed inside a zip-lock plastic bag – multiple paper envelopes may be sealed into a single zip-lock plastic bag. Record the three collector-sequence numbers for this subsample on the field data sheet.

Transfer the three collector-sequence numbers, along with an accurate date and time, the GIS Key, and stream name to the BOL Microbiology Sample Submission Sheet. The BOL SAC Code for Chlorophyll-a is B019 – additionally state on the Sample Submission Sheet that the test is for “Acidified Chlorophyll-a with Pheophytin.” SAC code B019 is also used for Non-acidified Chlorophyll-a, so care must be taken to clearly communicate the need for the acidified chlorophyll-a with pheophytin test.

Prepare the acidified chlorophyll-a samples for transportation as defined in Table 3. When hand-delivering this sample to BOL, it must be placed into the appropriate -80°C freezer in the Bacteriological Services Section (consult with lab staff to understand the location of this freezer). Sample submission sheets should be hand-delivered to a member of the Bacteriological Services Section so that they are made aware that chlorophyll-a samples have been delivered. Acidified chlorophyll-a samples may be delivered to BOL via courier on dry-ice. However, because of the holding times involved (30 days), acidified chlorophyll-a samples may be held for a few days on dry-ice and delivered to BOL at the end of the field week.

The 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).

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.

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 the Appendix), 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). Methods for estimating canopy closure follow Platts et al (1987). Measuring canopy closure is suggested over measurement of 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 (four 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 (eight x 17) to calculate a percent closure.

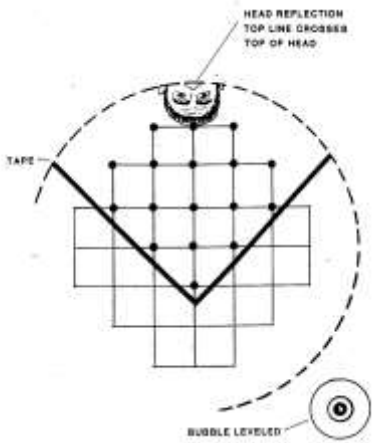


Figure 6. Concave spherical densiometer with placement of head reflection, and 17 observation points. From Platts et al. (1987).

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

LABORATORY METHODS

Although strict adherence to the field data collection protocols is necessary to achieve comparability between samples, field data collection represents only a portion of that which is needed to insure 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 methods needed to process periphyton related samples. These laboratory methods include those applied by BOL as well as those that must be applied by contracted labs. These methods 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 Ash-Free Dry Mass samples at its BOL facility. The laboratory methods used to process these samples are extensively defined in BOL documentation and may be obtained from that DEP bureau. BOL methods currently used include:

- Phycocyanin by Fluorometry Rev 001, effective November 2017
- [Acidified] Chlorophyll-a in NPW by SM10200H 1 & 3 (Fluorometric Method Rev 002, effective 3/27/2017
- Ash-Free Dry Mass by SM10200-I Rev 001, effective 2/26/2016
- Plankton Identification and Enumeration Rev 002, effective 5/7/2015.

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 Method description provided below. Further, the use of multiple identification and enumeration processing facilities obligates DEP to employ a rigorous QA Audit process designed to insure 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 Method

The identification and enumeration of periphyton samples collected by either the QMH or the QBE periphyton field methods 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 method 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™ 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 method, 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 method 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 Methods 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 U.S. Geological Survey National Water-Quality Assessment Program, Report No. 02-06, as prepared by Donald F. Charles, Candia Knowles, and Robin S. Davis of the Patrick Center for Environmental Research, The Academy of Natural Sciences, Philadelphia, PA (hereafter referred to as the NAWQA Protocol) (available at https://water.usgs.gov/nawqa/protocols/algprotocol/algprotocol.pdf), and as outlined above.

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 Academy of Natural Sciences. Consequentially, there may be a limited opportunity for flexibility regarding the exact methods by which the identification and enumeration laboratory results are produced. This creates the possibility for the processing laboratory to slightly modify the methods of performing identification and enumeration as detailed by the 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 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 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 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 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 PADEP 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 insure 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, M.T., et al. 1999
- Kahlert, M., et al. 2009
- Kelly, M.G., et al. 2001
- Whittaker, R.H., et al. 1958
- Wolda, H. 1981
- The Academy of Natural Sciences diatom and non-diatom taxa lists.

The body of scientific literature consulted suggested that the appropriate method 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 PO), regardless of how many times the taxa is re-identified throughout the sample set.

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

- *Percent Community Similarity of Diatoms* (PS) – this is also referred to as the Whittaker and Fairbanks (1958) similarity and is recommended by the 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:

$$D = \Sigma \min (a_i , b_i)$$

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 the 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 methods involved with the processes of subsampling, and identifying/enumerating algae samples
- Possible use of taxonomic intercalibration exercises and other harmonization methods (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 is 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). (Duplication from the DEP Algae Database Samples Table). Typically 100ml.
- 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 identification/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 ID/enumeration subsample (ml) + Volume of Formaldehyde added (ml)) / Volume of ID/enumeration subsample (ml). For example, if volume of ID/enumeration 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 subsample ID/enumeration bottle (ml) + Volume of distilled water added (ml)) / Volume of original subsample ID/enumeration bottle(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 exceed, 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 Slurry 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 identification/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 method 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 exceeded.
- 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 methods. One method 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 taxa. The method 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 method 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 method is to use archived taxa-level biovolume data from the Academy of Natural Sciences. This archived data is available from https://diatom.ansp.org/autecology/download.asp?file_id=4 and provides various biovolume statistics for each taxon. These biovolume statistics are compiled from the data the Academy has accumulated over many years of research and would provide a reliable representation of the sampled taxa population. When relying upon this later method, 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 method are that it is not sample specific and the data offered on the Academy website was last updated in 2001. This Academy provided biovolume data is 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 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 in 2013, 2014, 2015, and 2016. At the beginning of each project, DEP will stipulate to the PL which of these two methods 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

LITERATURE CITED

Arar, E.J., G.B. Collins. 1997. Method 445.0: In Vitro determination of Chlorophyll a and Pheophytin a in Marine and Freshwater Algae by Fluorescence. EPA method 445.0. Available from: https://cfpub.epa.gov/si/si_public_record_report.cfm?dirEntryId=309417).

Barbour, M.T., J. Gerritsen, B.D. Snyder, and J.B. Stribling. 1999. Rapid bioassessment protocols for use in streams and wadeable Rivers: periphyton, benthic macroinvertebrates and fish, second edition. EPA 841-B-99-002. (Available from: https://www3.epa.gov/region1/npdes/merrimackstation/pdfs/ar/AR-1164.pdf).

Charles, D.F., C. Knowles, and R.S. Davis. 2002. 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. (Available from: https://water.usgs.gov/nawqa/protocols/algprotocol/algprotocol.pdf).

Hausmann, S., D.F. Charles, J. Gerritsen, and T.J. Belton. 2016. A diatom-based biological condition gradient (BCG) approach for assessing impairment and developing nutrient criteria for streams. Science of the Total Environment 562: 914-927.

Hill, W.R. 1996. Effects of Light, In Algal Ecology, Freshwater Benthic Ecosystems (Edited by Stevenson P.J., Bothwell M.L., Lowe R.L.). pp 121-144. Academic Press, San Diego.

Hill, W.R., and B.J. Roberts. 2009. Quantifying phosphorus and light effects in stream algae. Limnology and Oceanography, 54(1): 368-380.

Kahlert, M., R.-L. Albert, E.-L. Anttila, R. Bengtsson, C. Bigler, T. Eskola, V. Gälman, S. Gottschalk, E. Herlitz, A. Jarlman, J. Kasperoviciene, M. Kokociński, H. Luup, J. Miettinen, I. Paunksnyte, K. Piirsoo, I. Quintana, J. Raunio, B. Sandell, H. Simola, I. Sundberg, S. Vilbaste, J. Weckström. 2009. Harmonization is more important than experience – results of the first Nordic-Baltic diatom intercalibration exercise 2007 (stream monitoring). J. Appl. Phycology 21:471-482.

Kahlert, M., É. Ács, A. S.F. Almeida, S. Blanco, M. Dreβler L. Ector, S.M. Karjalainen, A. Liess, A. Mertens, J.vd Wall, and S. Vilbaste.. 2016. Quality assurance of diatom counts in Europe: towards harmonized datasets. Hydrobiologia 772(1): 1-14.

Kelly, M.G, A. Cazaubon, E. Coring, A. Dell'Uomo, L. Ector, B. Goldsmith, H. Guasch, J. Hürlimann, A Jarlman, B. Kawecka, J. Kwandrans, R. Laugaste, E.-A. Lindstrøm, M. Leitao, P. Marvan, J. Padisák, E. Pipp, J. Prygiel, E. Rott, S. Sabater, H. van Dam, and J. Vizinet. 1998. Recommendations for the routine sampling of diatoms for water quality assessments in Europe. Journal of Applied Phycology 10: 215-224.

Kelly, M. G. 2001. Use of Similarity Measures for Quality Control of Benthic Diatom Samples. Wat. Res. 35(11): 2784-2788.

Lee, S., I Bishop, and S. Spaulding. 2017. Improving diatom taxonomic consistency and reference site detection in NRSA. U.S. EPA Webinar PowerPoint presentation given in March 2017.

Moulton II, S.R., J.G. Kennen, R.M. Goldstein, and J.A. Hambrook. 2002. Revised protocols for sampling algal, invertebrate, and fish communities as part of the National Water-Quality Assessment Program. USGS Open file Report 02-150. (Available from: https://pubs.usgs.gov/of/2002/ofr-02-150/pdf/ofr02-150.pdf**).**

Peterson, C.G., and R.J. Stevenson. 1990. Post-spate development of epilithic communities in different current environments. Canadian Journal of Botany, 68: 2092-2102.

Platts, W.S., E. Armour, G.D. Booth, M. Bryant, J.L. Bufford, P. Cuplin, S. Jensen, G.W. Lienkaemper, G. W. Minshall, S.B. Monsen, R.L. Nelson, J.R. Sedell, and J.S. Tuhy. 1987. Methods for Evaluating Riparian Habitats with Applications to Management. General Technical Report INT-GRT-221. USDA.

Porter, S.D., D.K. Mueller, N.E. Spahr, M.D. Munn, and N.M. Dubrovsky. 2008. Efficacy of algal metrics for assessing nutrient and organic enrichment in flowing waters. Freshwater Biology 53: 1036-1054.

Potapova, M., and D.F. Charles. 2005. Choice of substrate in algae-based water-quality assessment. Journal of the North American Benthological Society 24(2): 415-427.

Potapova, M., and D.F. Charles. 2008. Diatom metrics for monitoring eutrophication in rivers of the United States. Ecological Indicators 7: 48-70.

The Academy of Natural Sciences Diatom and Non-Diatom Taxa lists. (Available from: https://diatom.ansp.org/Taxa.aspx).

USEPA. 2013. Standard operating procedure for In Vitro determination of chlorophyll a in freshwater Phytoplankton by Fluorescence. (Available from https://www.epa.gov/sites/production/files/2017-01/documents/sop-for-in-vitro-determination-of-chlorophyll-a-in-freshwater-201303-11pp.pdf).

Wehr, J.D., and R.G. Sheath. 2003. Introduction to freshwater algae in Freshwater Algae of North America: Ecology and Classification. pp 1-5. Academic Press, New York NY.

Whittaker, R. H. and C. W. Fairbanks. 1958. A Study of Plankton Copepod Communities in the Columbia Basin, Southeastern Washington. Ecology, 39(1): 46-65.

Wolda, Henk. 1981. Similarity Indices, Sample Size and Diversity. Oecologia 50: 296-302.

BACTERIOLOGICAL DATA COLLECTION PROTOCOL

Prepared by:

Megan Bradburn and Shawn Miller
PA Department of Environmental Protection
Office of Water Programs
Bureau of Clean Water
11th Floor: Rachel Carson State Office Building
Harrisburg, PA 17105

2015

Edited by:

Josh Lookenbill
PA Department of Environmental Protection
Office of Water Programs
Bureau of Clean Water
11th Floor: Rachel Carson State Office Building
Harrisburg, PA 17105

2017

INTRODUCTION

All waters of Pennsylvania with the exception of Lake Erie Coastal Beaches and waters specified with exceptions to the criteria in §93.9 a-z of 25 Pa. Code Chapter 93, are evaluated for water contact recreational use attainment according to the criterion for fecal coliform bacteria in §93.7 of Chapter 93. This protocol identifies appropriate sample collection procedures needed to assess for water contact recreational use for applicable waters.

SAMPLING DESIGN CONSIDERATIONS SPECIFIC TO BACTERIA MONITORING

Probability-based or targeted (judgmental) sampling designs can both be used for bacteria monitoring; however, DEP prefers a targeted sampling design. Both sampling design methods are described in more detail in Chapter 2, Sampling Design and Planning. 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 and specifically targeted for recreational use monitoring. Additional sampling locations are chosen throughout a watershed based on changes in landuse, location of major tributaries, and potential sources of impairment. Impairment sources can include municipal point sources, combined sewer overflows (CSOs), and agricultural sources relating to manure application, livestock grazing, and animal feeding. In most cases multiple sampling locations will be needed to bracket potential sources of impairment. Smaller headwater streams (Strahler 2nd order or smaller), especially those with no potential sources of impairment are not prioritized. For larger rivers, streams, or lakes 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 (see Chapter 4, Field Meter and Transect Data Collection). The number and distribution of docks, boating areas, residences, cabins, and access areas should be considered in locating sample sites. Lakes and reservoirs with beaches or swimming areas must include sampling locations from the left perimeter, center, and right perimeter of the swimming area, unless the swimming area is less than 100 feet. If less than 100 feet, sampling locations from the left perimeter and right perimeter are sufficient.

During the swimming season, the DOH and DCNR collect weekly samples for *Escherichia coli (E.coli)* at public beaches for monitoring purposes. *See* 28 Pa. Code §18.30. When violations of criteria occur at public beaches closure notices are issued by DOH. In cooperation with DEP, DOH, and DCNR compiles a list of closures that DEP

will utilize to identify possible recreational use impairment and focus future fecal coliform assessment sampling.

SAFETY PROCEDURES

Appropriate recreational use assessment begins with data collection that follows proper guidelines and 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 can be required for this sampling method. 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 method 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 present difficulties for both wadeable and non-wadeable water navigation. Certain locations, especially on large rivers, may be fully or partially wadeable during certain times of the targeted recreation season, but there are also highly variable currents and drop-offs that are dangerous to wading collectors.

Inherent in these recommendations is the understanding that collectors must be well informed of the unique characteristics of each surface water they intend to sample and be aware of current flow and weather conditions. Equipment considerations may be different between similar sized surface waters, and certainly different when visiting multiple surface waters of different size and type. For instance, boat selection and safety equipment lists would be different depending on whether the collector is visiting a small 3rd order stream or a large 7th order 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 surface water 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/?type=flow

DATA COLLECTION

Bacteriological sampling for determining primary water contact recreational use attainment is conducted during the swimming season (May 1st through September 30th). This is the timeframe the public is likely to be engaging in primary water contact recreational activities. Primary water contact recreational activities are defined 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, water play by children, or similar water-contact activities (USEPA 2012). As a general rule, bacteriological sampling should not take place during those times when physical conditions, such as stream discharge, render primary contact recreation hazardous to the public. Samples collected for assessment purposes should provide the ability to identify an impaired recreational use due to chronic long-term impacts and not acute problems that are transitory. In the past, there was guidance that sampling should not take place immediately following 0.25 inch of rain or more to avoid collecting samples during elevated discharge when fecal levels are elevated, but not necessarily characteristic of a chronic condition. This guidance is usually applicable to smaller waterbodies where discharge responds quickly to precipitation events, but becomes more difficult to apply to larger waterbodies and to some lakes where increased discharge and water quality changes can occur days following 0.25 inch of rain.

The water contact use criterion at 25 Pa. Code §93.7:

(Fecal coliforms/ 100 ml)—During the swimming season (May 1 through September 30), the maximum fecal coliform level shall be a geometric mean of 200 per 100 milliliters (ml) based on a minimum of five consecutive samples each sample collected on different days during a 30-day period. No more than 10% of the total samples taken during a 30-day period may exceed 400 per 100 ml. For the remainder of the year, the maximum fecal coliform level shall be a geometric mean of 2,000 per 100 milliliters (ml) based on a minimum of five consecutive samples collected on different days during a 30-day period.

The criterion specifically defines magnitude, frequency, and duration; and subsequently prescribes the absolute minimum number of samples required in determining impairment for water contact recreational use. At least 5 samples, each separated by at least 24 hours, spanning a maximum of 30 days and a minimum of 14 days are required. The frequency and duration of 5 samples over 30 days were included in USEPA's Recreational Water Quality Criteria Recommendations (2012):

"EPA believes that a shorter duration (i.e., 30 days), used in a static or rolling manner, coupled with limited excursions above the [statistical threshold value] *STV, allows for the detection of transient fluctuations in water quality in a timely manner. In the development of their monitoring program, EPA recommends that states consider the*

number of samples evaluated in order to minimize the possibility of incorrect use attainment decisions…"

Collecting data for water assessments in general will include samples collected frequently enough and over a sufficient period of time to account for sampling error or variations driven by confounding variables (Chalfant 2017). Water contact recreational use samples will include at least 5 bacteriological samples collected within a 30-day period. Collecting samples throughout the entire May 1st through September 30th for surface water assessment is not sustainable. However, it is recommended that additional samples are collected to better understand the variability in the data and target conditions that are likely to characterize impairment. This results in sampling error decreases and a more confident assessment (Chalfant 2017). If information is available that indicates more than 5 samples over a 30-day period are required to confidently assess, then additional samples will be collected.

DEP collects and analyzes DNA for *Bacteroides dorei* to identify the host animal of the bacteria present in surface waters*. B. dorei* is a common bacterium found in the environment and has a known genetic marker for the HF183 human genetic marker. The process to analyze the DNA is known as quantitative Polymerase Chain Reaction (qPCR) which results in genes being copied. qPCR is collected at sampling locations that are suspected to have or have documented elevated levels of bacteria. DNA target sequences are amplified in the presence of primers (short genetic sequences) that are specific to various hosts (humans, cows, birds, pigs, etc). Results from qPCR analyses are reported in units that are calculated based on the target DNA sequences from test samples relative to those in calibrator samples that contain a known quantity of target organisms (Haugland et al., 2005; Wade et al., 2010), which can aide in determining the source of bacterial contamination.

In addition to collecting discrete bacteriological samples, continuous instream monitoring (CIM) data can also be used to make assessment decisions. Water quality data sondes record instream parameters at defined intervals that are sufficiently short to be considered continuous. Bacteriological parameters are not capable of being directly measured on a continuous basis, but models have been developed 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 water contact recreational use data for assessment purposes, provide that CIM data collection protocols are followed (Hoger et al. 2017).

COLLECTION REQUIREMENTS

Collector Identification Number

Collectors 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 can 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

Collectors must submit samples to the DEP Bureau of Labs (BOL) using the “Sample Submission Sheet” (Submission Sheet). 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, collector name, date, phone number, and any additional comments that lab analysts will use to properly handle samples.

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 time or UTC-5 throughout the year.

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 B021 is used by “Water Management” when submitting bacteriological samples for bot Fecal and E.coli with a 24-hour hold time. Unique SACs are created for specific purposes. Table 1 lists other bacteriological test descriptions and SACs.

Table 1. 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 + E.coli	Membrane Filtration (24-hr hold time)	B021
E. coli	Membrane Filtration	B022
Enterococci	Membrane Filtration	B015
Fecal coliforms + Total/Human/Avian/Bovine/Swine Bacteroides	Membrane Filtration + qPCR	B035
E. coli + Total/Human/Avian/Bovine/Swine Bacteroides	Membrane Filtration + qPCR	B037
Fecal + E. coli + Total/Human/Avian/Bovine/Swine Bacteroides	Membrane Filtration + qPCR	B036
Total/Human/Avian/Bovine/Swine Bacteroides	qPCR	B038

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. 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. BOL recommends contacting the appropriate BOL staff before submitting samples.

Sampling Supplies and Equipment

- BOL Sample Submission Sheet (Submission Sheet)
- DEP Bacteria Monitoring Field Data Sheet (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 with sodium thiosulfate (qPCR)
- Sample blanks containing sterile (autoclaved) water obtained from BOL

- Disposable, powderless gloves (such as nitrile)
- Coolers and 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)
- Boots and waders (optional)

COLLECTION METHOD

Collectors must ensure that the samples collected will be representative of the aquatic system of interest. 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 location 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 for samples submitted to BOL is the collector number and sequential sample number, collectors may add additional information such as date and time collected, general test(s) description. 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 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 complete a field data sheet (Appendix A-10) for samples collected.

Flowing Waterbodies

For flowing waterbodies, bacteriological samples will be collected by direct surface water sampling. Avoid sampling in eddies, pools, side-channels, or in tributary mixing zones unless necessary due to site-specific or other environmental considerations. 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. 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. In smaller waterbodies that do not require multiple locations across a transect, 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. DEP uses 125-mL or 1-L (qPCR) screw-capped bottles with sodium thiosulfate added to neutralize the effects of residual chlorine. DO NOT RINSE THE BOTTLE BEFORE SAMPLING. Fill the bottle to the shoulder leaving headspace. Once the sample is collected and capped, the collector should rinse any large amount of dirt or debris from the outside of the container.

Lakes and Reservoirs

Bacteriological samples will be collected using disposable, powderless gloves (such as nitrile (elbow length preferred) from lakes. Samples are collected at a depth of one meter when possible and at least a 0.3-meter depth at a minimum. Samples collected at a depth of one meter can be collected using a sampling pole or dispensed from depth specific water sampling equipment (Van Dorn, Kemmerer). Samples from a boat are collected with motor off. 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. DEP uses 125-mL or 1-L (qPCR) screw-capped bottles with sodium thiosulfate added to neutralize the effects of residual chlorine. DO NOT RINSE THE BOTTLE BEFORE SAMPLING. Fill the bottle to the shoulder leaving headspace. Once the sample is collected and capped, the collector should rinse any large amount of dirt or debris from the outside of the container.

Preservation

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. BOL personnel will note whether samples were shipped properly. Improperly shipped samples may be subject to a data release request. The “Sample Submission Sheet” (Submission Sheet) should be filled out, inserted into a Ziploc® 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.

Quality Assurance
For quality assurance purposes, sample blanks containing sterile (autoclaved) water obtained from BOL or the laboratory analyzing samples should be submitted for every 20 samples for each sampling trip/day to determine whether contamination is occurring in any part of the sample collection, handling, or preservation process. Sample blanks are “collected” by transferring sterile water from container received from BOL to a labeled 125-mL. A duplicate grab sample should also be collected every 20 samples or for each sampling trip/day to gauge testing variability and potential sources of contamination due to collection procedures. Duplicate samples are collected simultaneously with the associated environmental sample, using identical sampling and preservation procedures. Both sample blanks and sample duplicates are assigned unique, sequential sample numbers. Label duplicate and blank samples in the same manner as environmental samples. DO NOT IDENTIFY DUPLICATE OR BLANK ON THE SAMPLE BOTTLE. Duplicates and blanks must be documented appropriately in SIS under the ‘Comments/Quality Assurance’ tab.

Field Meters
Field meters provide the ability to collect in-situ data that is not available through grab samples submitted to BOL. Standard field parameters include dissolved oxygen, temperature, specific conductance, pH, and turbidity. Collection of field meter data is recommended for bacteriological sampling and required for rivers and streams greater than 1000 mi^2. More information on the use and proper calibration of field meters is found in the “Field Meter Data Collection Protocol” (Chapter 3).

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. Bacteria samples collected and submitted for compliance purposes have a hold time of 8 hours. A hold time of 24 hours may be applied to samples collected and submitted for assessment purposes.

Shipping Samples
All DEP district and regional offices are designated pick-up locations for water samples; the samples must be dropped off for pick up by 1600 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. Further, the drop-off locations may require a drop-off specific key to open the drop-off entrance lock. Drop-off locations are available on the BOL website.

SAMPLE INFORMATION SYSTEM DOCUMENTATION

General guidelines for data entry are provided in the 'Sample Information System (SIS) Protocol' located in Chapter 4 of this book. 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)

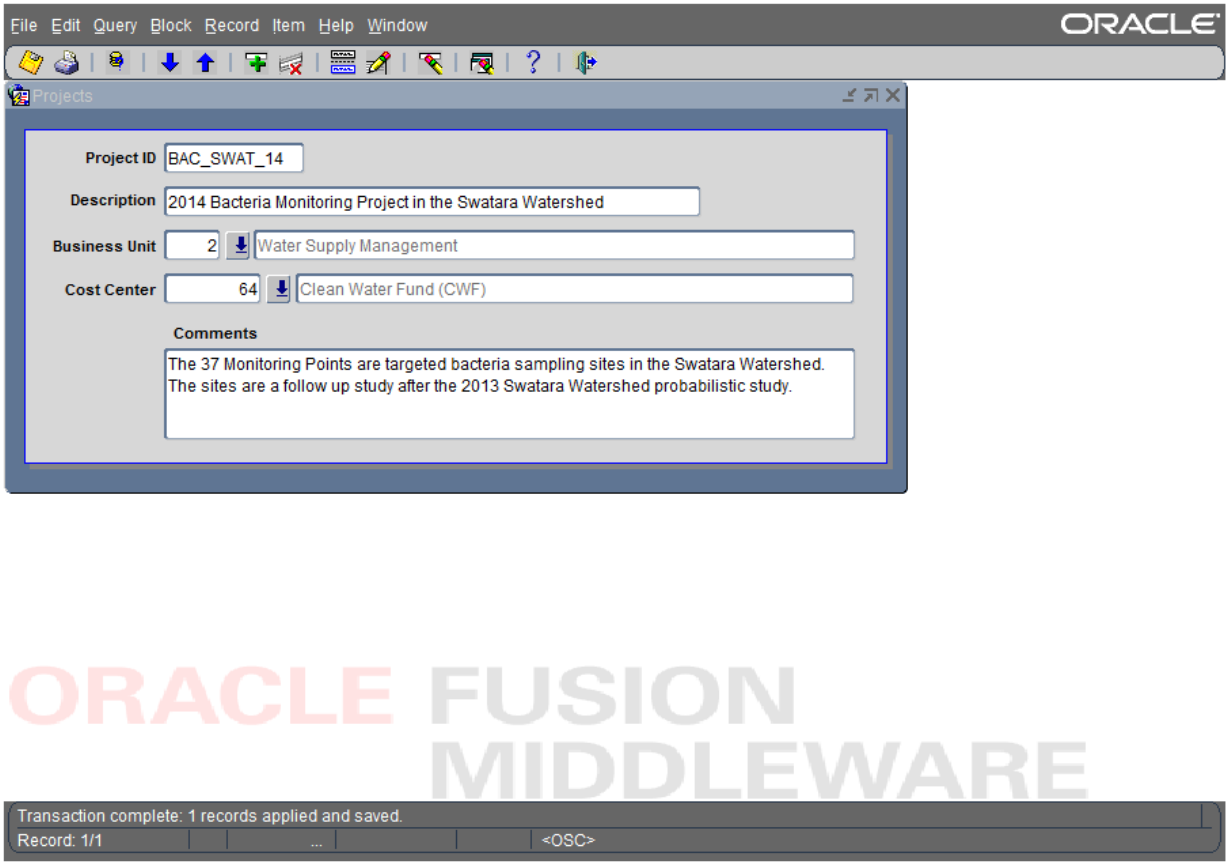


Figure 1. SIS Project entry and edit screen with template information.

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 NHD tab click ‘Launch NHD Locator, snap the monitoring point to a 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’ (Figure 5).

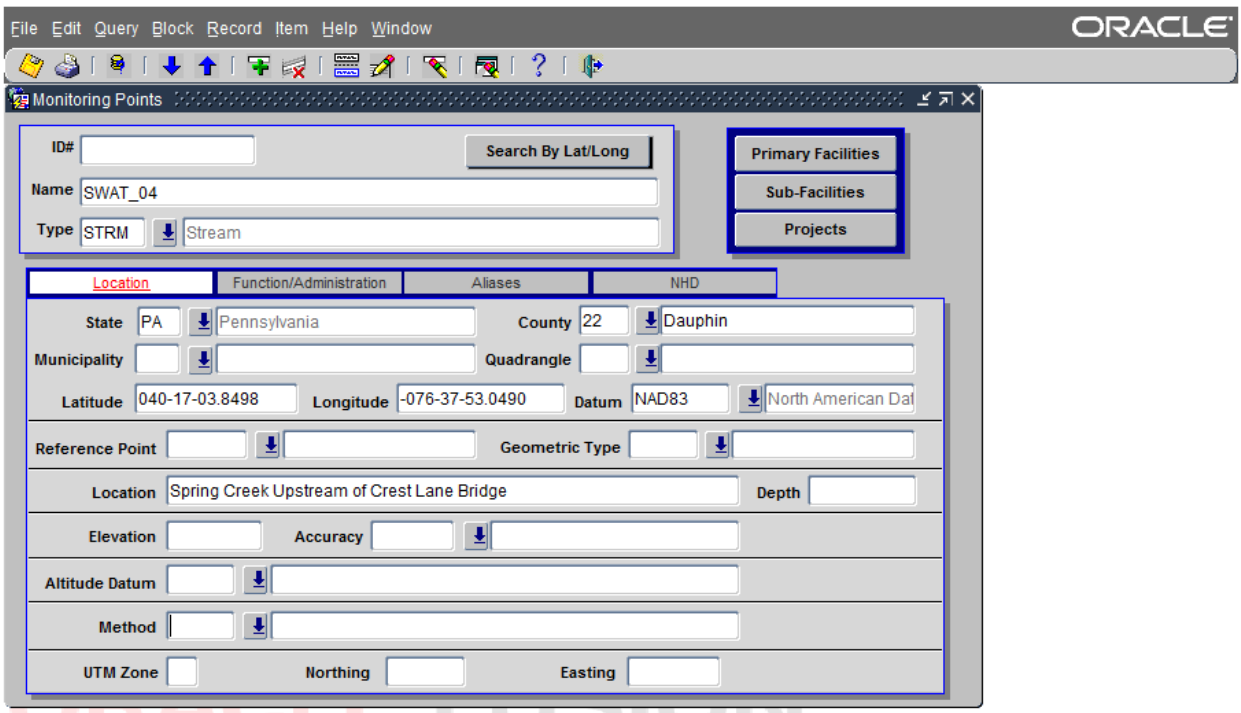


Figure 2. SIS Monitoring Point, Location tab entry and edit screen with template information.

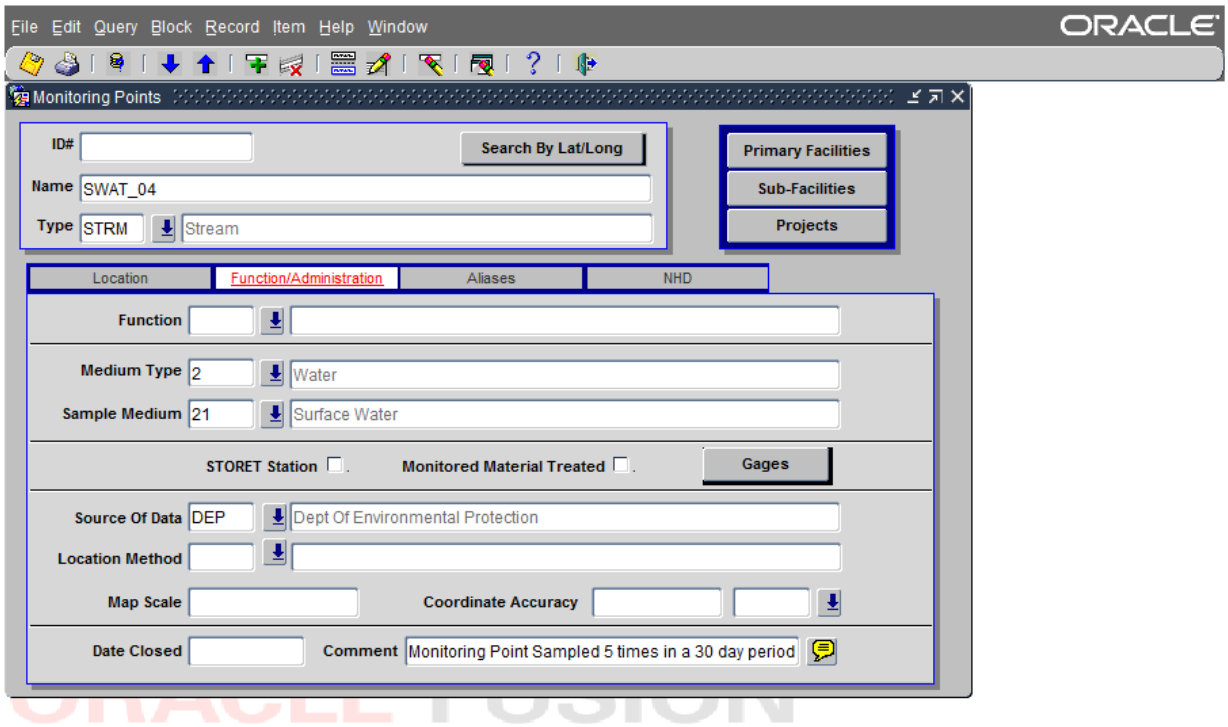


Figure 3. SIS Monitoring Point, Function/Administration tab entry and edit screen with template information.

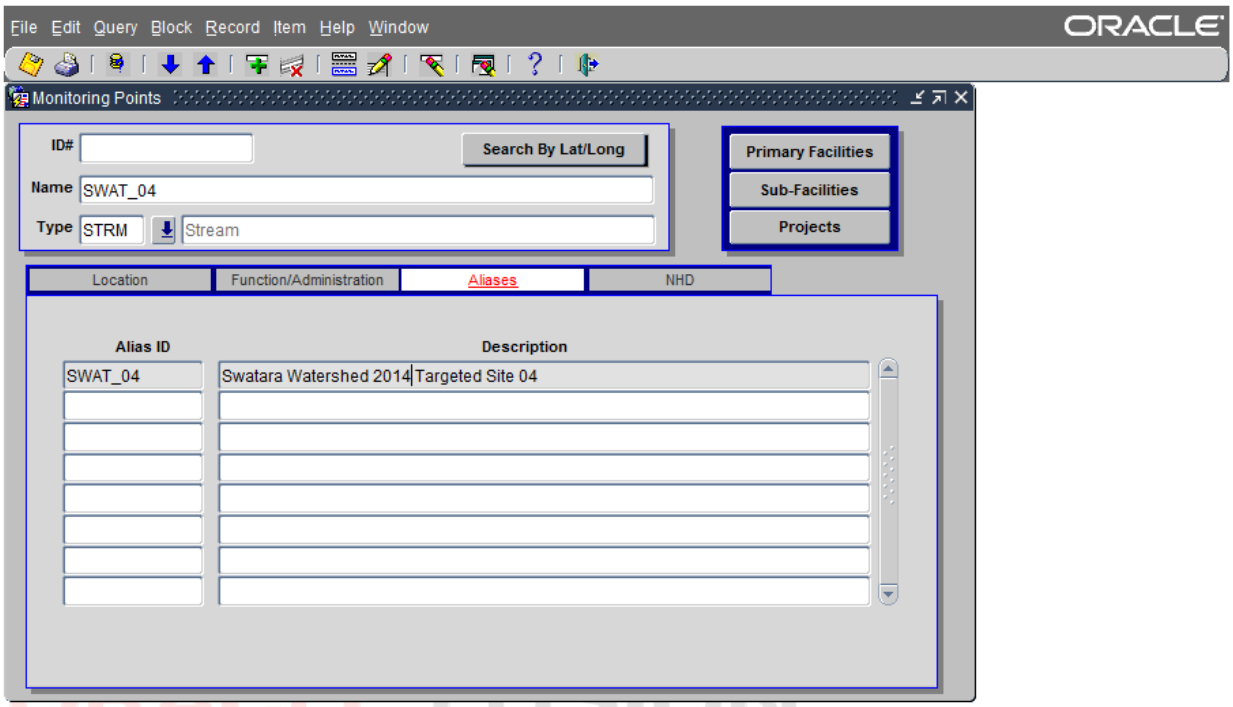


Figure 4. SIS Monitoring Point, Aliases tab entry and edit screen with template information.

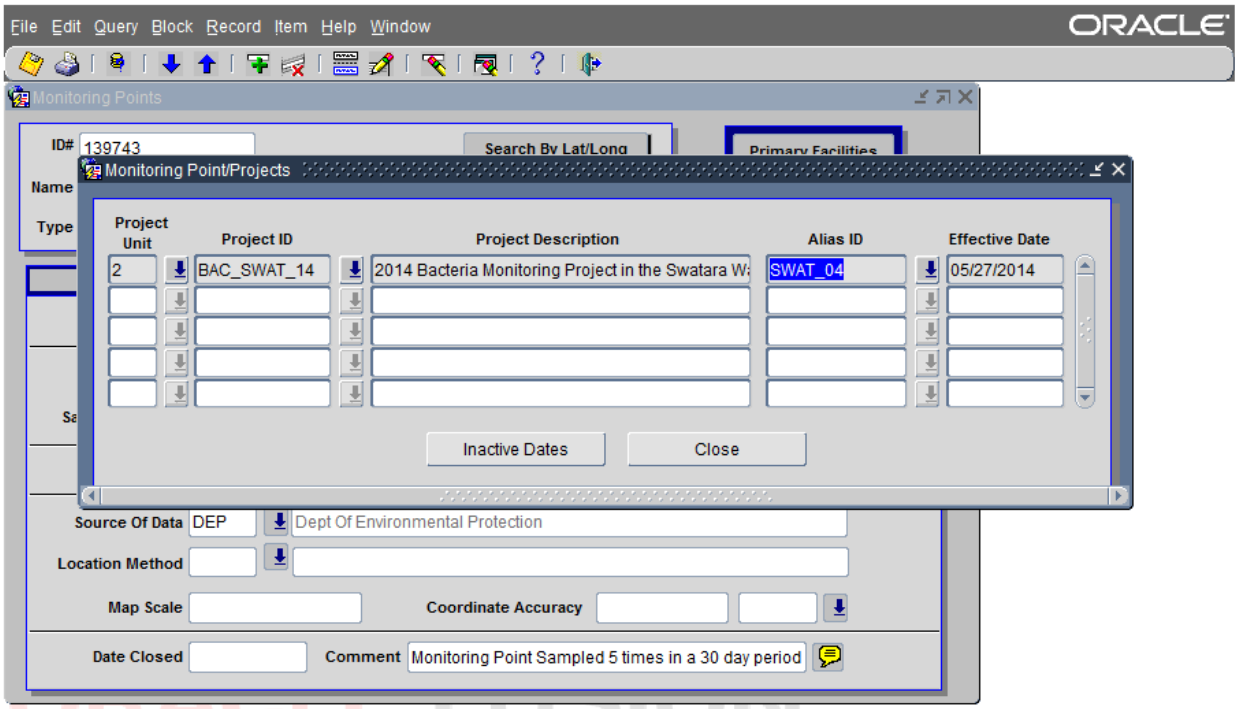


Figure 5. SIS Monitoring Point, Project link and edit screen with template information.

LITERATURE CITED

Haugland, R.A., Siefring, S.C., Wymer, L.J., Brenner, K.P., Durfour, A. 2005. Comparison of Enterococcus measurements in fresh water at two recreational beaches by quantitative polymerase chain reaction and membrane filter culture analysis. Water Research 39: 559-568.

Hoger, M. S., D. R. Shull, M. J. Lookenbill. 2017. Continuous Physicochemical Data Collection Protocol. Chapter 4, pages 22-87. *In* Shull, D. R., and M. J. Lookenbill. (editors). Water quality monitoring protocols for streams and rivers. Pennsylvania Department of Environmental Protection. Harrisburg, Pennsylvania.

Hoger, M.S. 2018. Continuous Physiochemical Assessment Method. Chapter 3, pages 20-35. *In* Shull, D. R., and M. Pulket. (editors). Assessment Methodology for Streams and Rivers. Pennsylvania Department of Environmental Protection. Harrisburg, Pennsylvania.

Rasmussen, T.J., Lee, C.J., and Ziegler, A.C., 2008, Estimation of constituent concentrations, loads, and yields in streams of Johnson County, northeast Kansas, using continuous water-quality monitoring and regression models, October 2002 through December 2006: U.S. Geological Survey Scientific Investigations Report 2008-5014, 103 p.

Stone, M.L., and Graham, J.L., 2014, Model documentation for relations between continuous real-time and discrete water-quality constituents in Indian Creek,

Johnson County, Kansas, June 2004 through May 2013: U.S. Geological Survey Open-File Report 2014–1170, 70 p.

USEPA. 2002. Guidance on Choosing a Sampling Design for Environmental Data Collection for Use in Developing a Quality Assurance Project Plan. EPA QA/G-5S. U.S. Environmental Protection Agency, Office of Environmental Information. Washington, DC.

USEPA. 2012. Recreational Water Quality Criteria. EPA 820-F12-058. Environmental Protection Agency, Office of Environmental Information. Washington, DC.

Wade, T.J., Sams, E., Brenner, K.P., Haugland, R., Chern, E., Beach, M., Wymer, L., Rankin, C.C., Love, D., Li, Q., Noble, R., Dufour, A.P. 2010. Rapidly Measured Indicators or Recreational Water Quality and Swimming-Associated Illness at Marine Beaches: A Prospective Cohort Study. Environmental Health 9: 66.

CHAPTER 4 CHEMICAL DATA COLLECTION PROTOCOLS

IN-SITU FIELD METER AND TRANSECT DATA COLLECTION PROTOCOL

Prepared by:

Mark Hoger
PA Department of Environmental Protection
Office of Water Programs
Bureau of Clean Water
11th Floor: Rachel Carson State Office Building
Harrisburg, PA 17105

2017

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 the Continuous Physiochemical Data Collection Protocol, discussed later in this Chapter.

CALIBRATION

Proper calibration should follow manufacturers' guidelines. In addition, DEP strongly recommends several procedures to further insure reliability and defensibility of data. At a minimum, meters should have calibration checked before each day of use. In most circumstances, it is recommended that the sensors are calibrated at this time. All calibration activity, including checks, need to be logged and records maintained for quality assurance. A calibration form is provided in Appendix B-1.

Calibration for all parameters should bracket any expected values. A summary of calibration recommendations is provided in Table 1. 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 method more closely replicates the deployed environment than calibrating in just the calibration cup, and therefore, can provide more consistent and accurate calibration.

Table 1. 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.

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 life-span 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. Additional information on cleaning and maintenance is provided by Hoger et al. (2017) 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 should 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.

Division of Water Quality 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.

CROSS-SECTION SURVEYS

Waterbodies are dynamic systems changing throughout time and space. Regular cross-section surveys 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 should 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.

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 later in this Chapter.

LITERATURE CITED

Hoger, M. S., D. R. Shull, and M. J. Lookenbill. 2017. Continuous Physicochemical Data Collection Protocol. Chapter 4, pages 22-87. *In* Shull, D. R., and M. J. Lookenbill. (editors). Water quality monitoring protocols for streams and rivers. Pennsylvania Department of Environmental Protection. Harrisburg, Pennsylvania.

DISCRETE WATER CHEMISTRY DATA COLLECTION PROTOCOL

Prepared by:

Dustin Shull
PA Department of Environmental Protection
Office of Water Programs
Bureau of Clean Water
11th Floor: Rachel Carson State Office Building
Harrisburg, PA 17105

2013

Edited by:

Dustin Shull
PA Department of Environmental Protection
Office of Water Programs
Bureau of Clean Water
11th Floor: Rachel Carson State Office Building
Harrisburg, PA 17105

2017

Disclaimer:
The mention of specific trade names or commercial products does not constitute endorsement or recommendation for use.

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 methods described here are adapted from scientific, peer-reviewed methods, 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 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 methods 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" (Submission Sheet). Collectors must submit samples to the DEP Bureau of Labs (BOL) using the laboratory submission sheet". 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, collector name, date, phone number, and any additional comments that lab analysts will use to properly handle samples.

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 collected per SAC 612 during routine semi-wadeable 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
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 HDPE bottle is used for storing an unfiltered, non-chemically preserved sample for inorganic constituent analyses and 125ml High-Density Polyethylene (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)
☐ 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
☐ buckets & rope (applicable length for bridge sampling)
☐ 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:
_____

COLLECTION METHODS

Many surface water samples are manually collected instantaneous “grab” 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 technician 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 exceeded and the sample may be voided.

Field personnel must ensure that the samples collected will be representative of the aquatic system of interest. A grab sample temporally and spatially represents the part of the surface water system being investigated. The method of sampling 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 water body 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 Water Bodies
The most common type of water sampling is conducted in wadeable, flowing water bodies, where water is collected directly into the sample bottle. This method 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.

Field Meters
Field meters provide the ability to collect in-situ data that is not available through grab 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 (e.g., transect data collection for semiwadeable river surveys, in Chapter 2 of this document). Field meter data will be collected per the “In-Situ Field Meter and Transect Data Collection Protocol” in Chapter 3 of this document.

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. 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 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 120ml 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 exceed 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 2 ml 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 ml HDPE	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 water body. 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 (PWS) use, collectors should consider that while 25 Pa. Code § 93.4 and § 96.3(c) identify PWS as applying to all surfaces waters, § 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 upstream of the surface water withdrawal at a minimum of one location or from the facilities raw water faucet that they use for their testing. However, multiple locations may be necessary to identify potential sources of pollution. Analyses are performed for total nitrites, total and dissolved metals, chloride, fluoride, sulfate, color, and dissolved solids using SAC 166. Additional microbiological parameters can be added on a site-specific basis.

Quality Assurance

For quality assurance purposes, sample blanks containing ultrapure water obtained from BOL should be submitted for every 20 samples for each sampling trip/day to determine whether contamination is occurring in any part of the sample collection, handling, or preservation process. A duplicate grab sample should also be collected every 20 samples or for each sampling trip/day to gauge testing variability and potential sources of contamination due to collection procedures. Duplicate samples are collected simultaneously with the associated environmental sample, using identical sampling and preservation procedures. 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.

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 exceeds 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 for pick up by 1600 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 Ziploc® 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 United States Geological Survey (USGS) stream gauging techniques (USGS 2006). Analyses are performed for metals, alkalinity, and acidity using SAC 909.

Acid precipitation sampling should occur in late winter/early spring during heavy snowmelt and/or storm events to capture episodic acidification. Sampling should occur during both base flow and peak flow to characterize worst-case conditions and to document the difference between base flow and worst-case conditions. This protocol includes a filtering method for dissolved aluminum that differs from that prescribed for other dissolved metals. Water for the dissolved aluminum analysis is filtered through a 0.1µm filter rather than through the standard 0.45µm filter. A pump is recommended to aid this filtration process. The result from this alternate dissolved aluminum analysis correlates well with the occurrence of inorganic monomeric aluminum species, which causes lethal responses in fish. Analyses are performed for metals, alkalinity and acidity using SAC 910.

LITERATURE CITED

USGS. 2006. National Field Manual for the Collection of Water Quality Data. U.S. Geological Survey, Water Resources Office of Water Quality. Chapter 4.1 - Surface Water Sampling: Collection methods at flowing-water and still-water sites.

CONTINUOUS PHYSICOCHEMICAL DATA COLLECTION PROTOCOL

Prepared by:

Mark Hoger, Dustin Shull, and Josh Lookenbill
PA Department of Environmental Protection
Office of Water Programs
Bureau of Clean Water
11th Floor: Rachel Carson State Office Building
Harrisburg, PA 17105

2017

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 use attainment. 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 conditions, describing seasonal and diel fluctuations, and documenting potential violations to water quality criteria. These data are also used in conjunction with flow measurements and chemical analyses of grab samples to estimate chemical loads. 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 methods, 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 manual (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 water bodies, 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. 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 water body 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 water body being measured. Thus, an optimal site is one that permits sensors to be located at a point that best represents the section 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 does not exceed 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 DEP's Field Meter and Transect Data Collection Protocol (Hoger 2017).

Some aquatic environments may present unique challenges for optimal site location. 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 water body. 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.

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. Division of Water Quality 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 method 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 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).

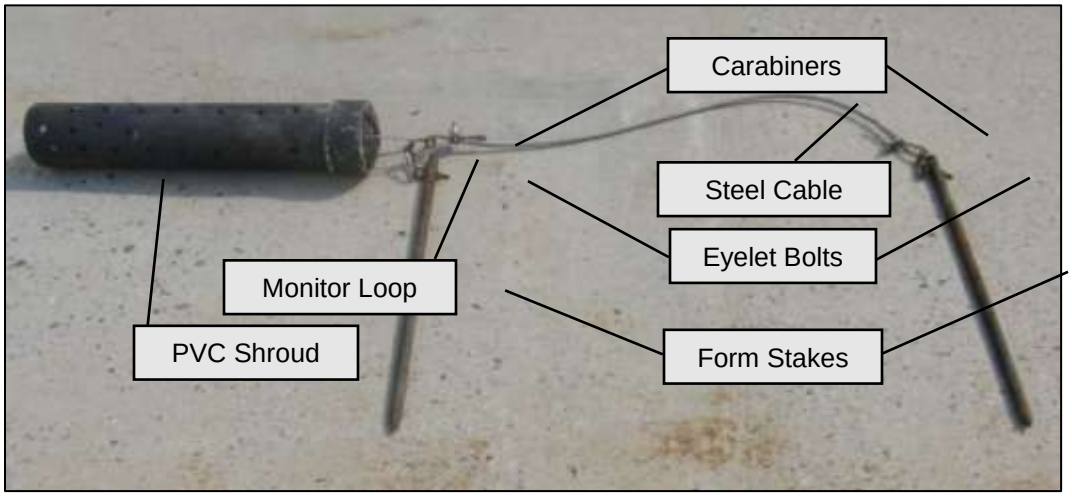


Figure 1. Standard deployment configuration for large, multi-parameter monitors uses two rebar/form stakes used as sediment anchors. Cables and carabiners are attached to stakes using 1/4 in. eyelet bolts. Once attached, the bolt threads are stripped to avoid backing out.

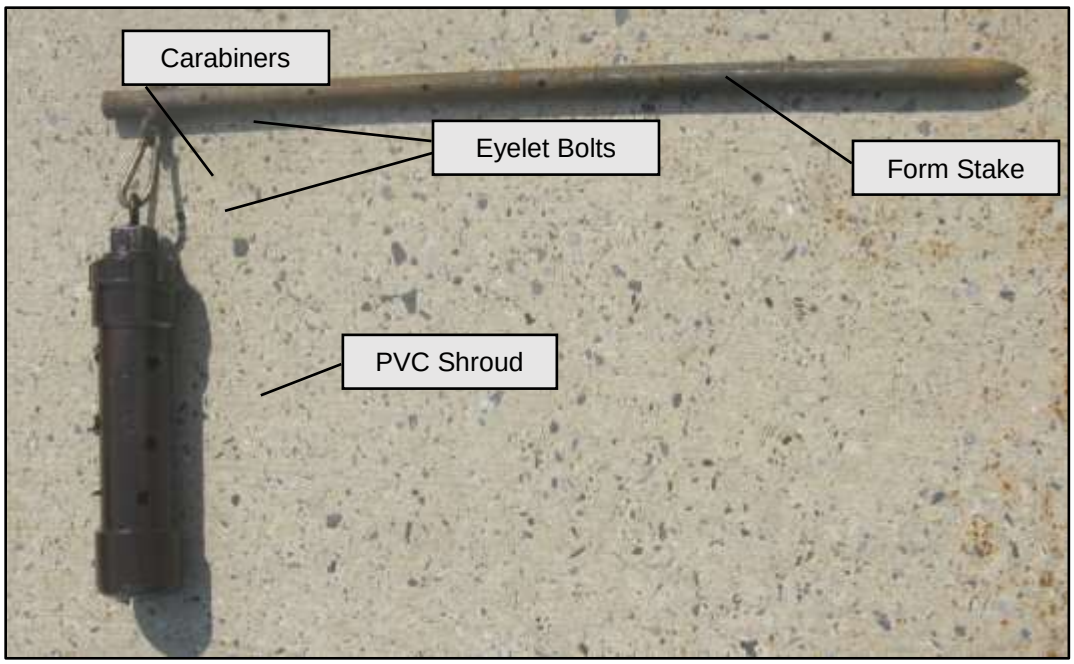


Figure 2. Deployment configuration for smaller monitors typically only includes one form stake.

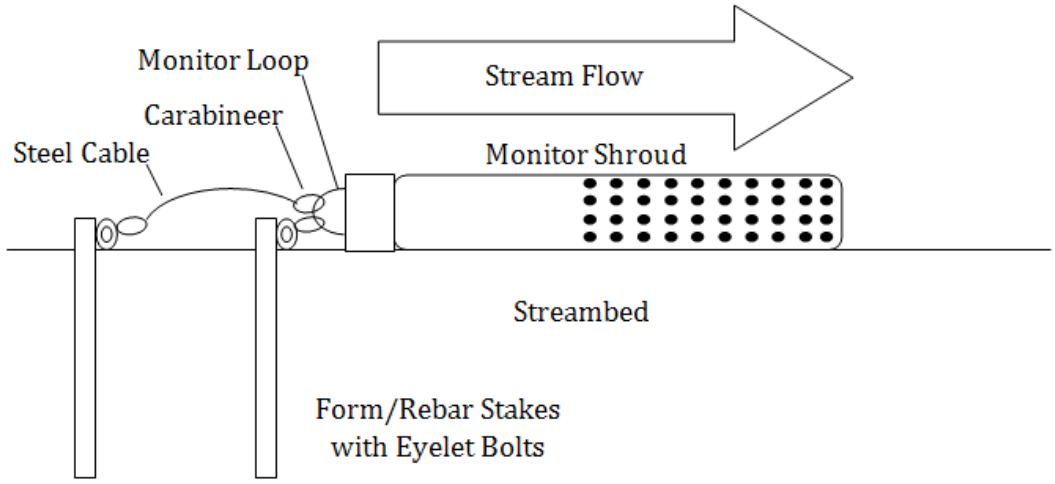


Figure 3. The standard instream configuration for monitors operated by DEP. Large, but manageable, rocks (not shown in figure) are placed around the monitor for stability, concealment, and protection.

<u>Adjusting for Sediment Fouling</u>

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 method is often sufficient.
- *Angle the monitor 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 out 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.

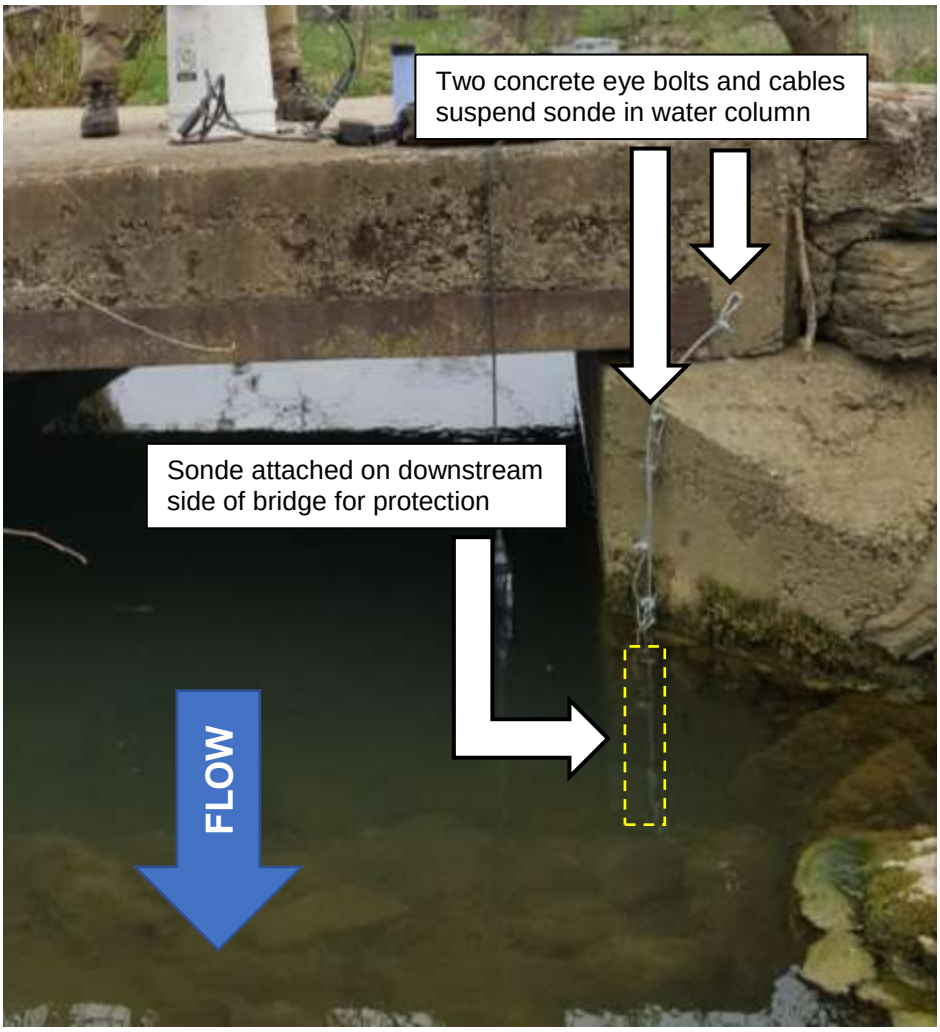


Figure 5. Bridge deployment used at Groff Creek, Lancaster County, PA, to minimize sediment fouling.

<u>Large River Deployment</u>

Accessing sondes at various flows in large rivers can be difficult using the standard deployment method. 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.

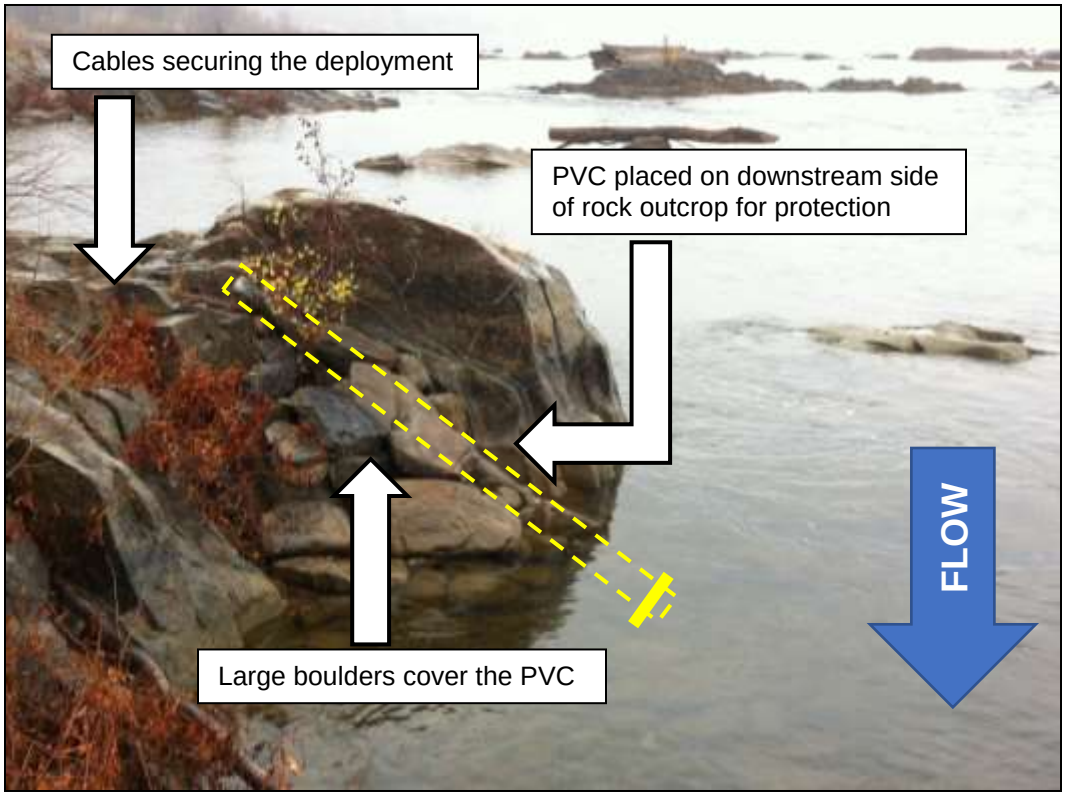


Figure 6. Large river deployment on Susquehanna River at Rockville, PA, employed to facilitate access at various flows.

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 2006).

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). Division of Water Quality 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 method, 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. Division of Water Quality 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).

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).

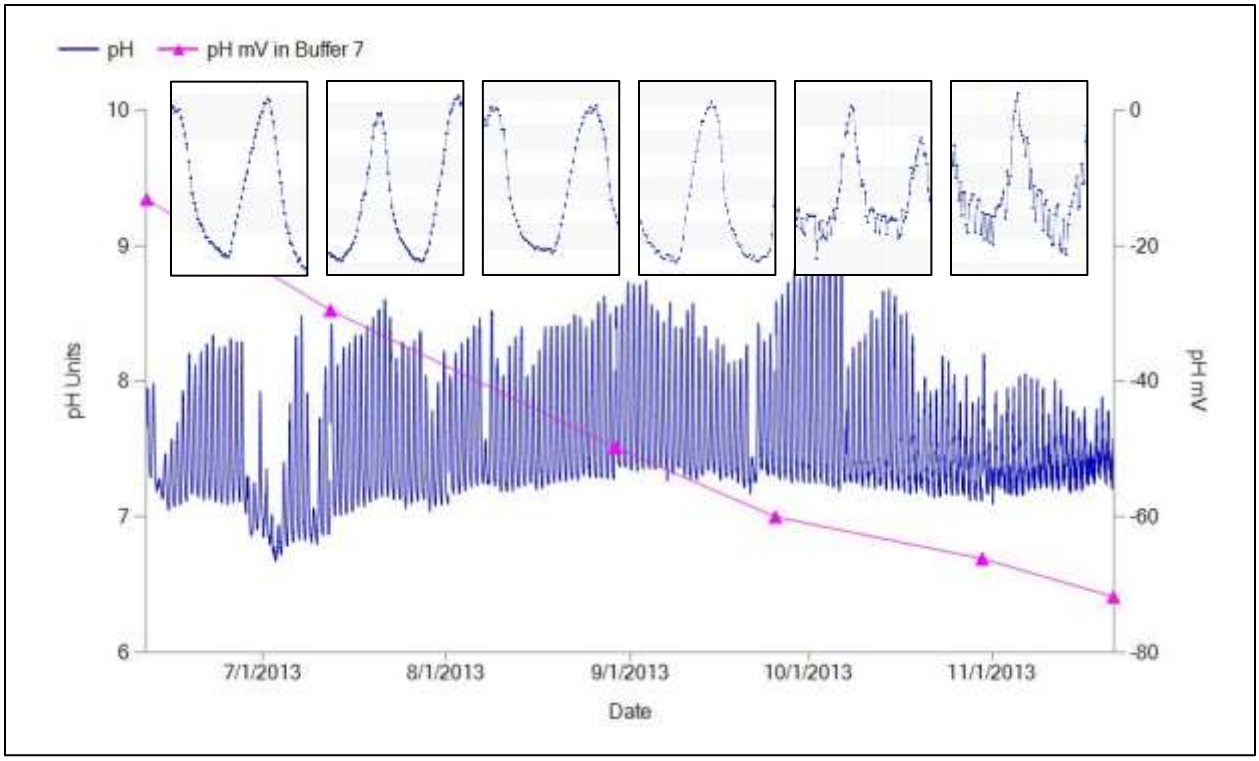


Figure 7. These pH data demonstrate the effects of an aging pH probe, measured by the mV readings departing from 0 mV in buffer 7. The inset boxes are close-ups of the pH at each of the six mV readings during calibration checks. As the mV readings fall outside the optimal range, the pH readings become increasingly erratic.

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. Division of Water Quality 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).

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 method, 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. Division of Water Quality generally recommends that the distance between stream monitor and barometric monitor does not exceed 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 method, 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 Hoger (2017).

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 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. Comments on site conditions, sensor condition, and any other pertinent observations
10. Battery voltage of monitor at departure and if the batteries were replaced, name of new file, and start time of logging
11. Notes on sensor/monitor changes or replacements, and other comments that facilitate processing of the record
12. Cross-section survey data (locations of points, measured values, and corresponding times), and monitor values before and after the cross-section survey (Hoger 2017)
13. 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

Division of Water Quality 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 hand held device)
    a. If monitor is to be submerged during read-out, ensure the cable is designed to operate under water
    b. Stop unattended 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 in 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 2006).

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 insure 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
Division of Water Quality uses three buffers (4, 7, 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. 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.

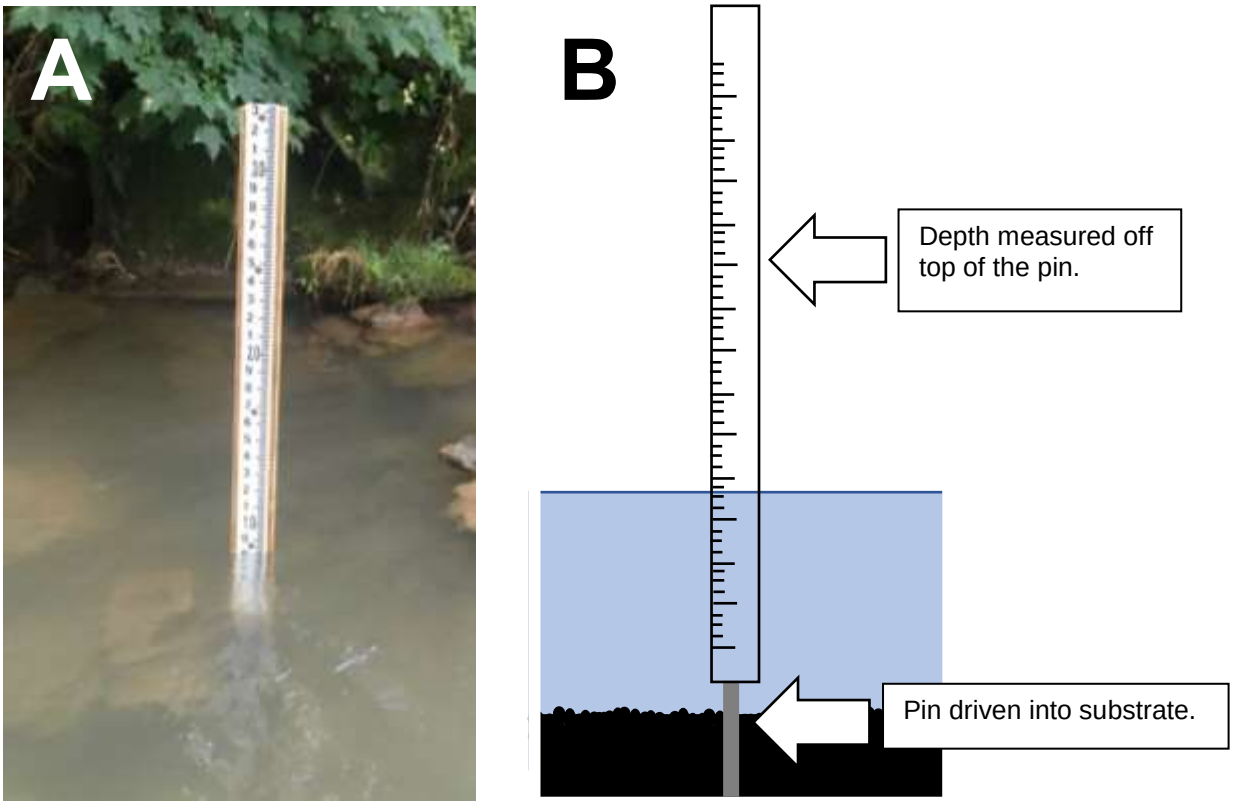


Figure 8. Permanent staff plate deployed at Donegal Creek, near Marietta, PA (A) and depiction of pin used as measuring point (B).

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 is graded and approved, and a final Continuous Instream Monitoring Report (CIMR) is written, reviewed, and approved by DEP staff. DEP uses Aquarius software (current version: 3.10) from Aquatic Informatics for all data management. 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).

<u>Uploading Data</u>

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.

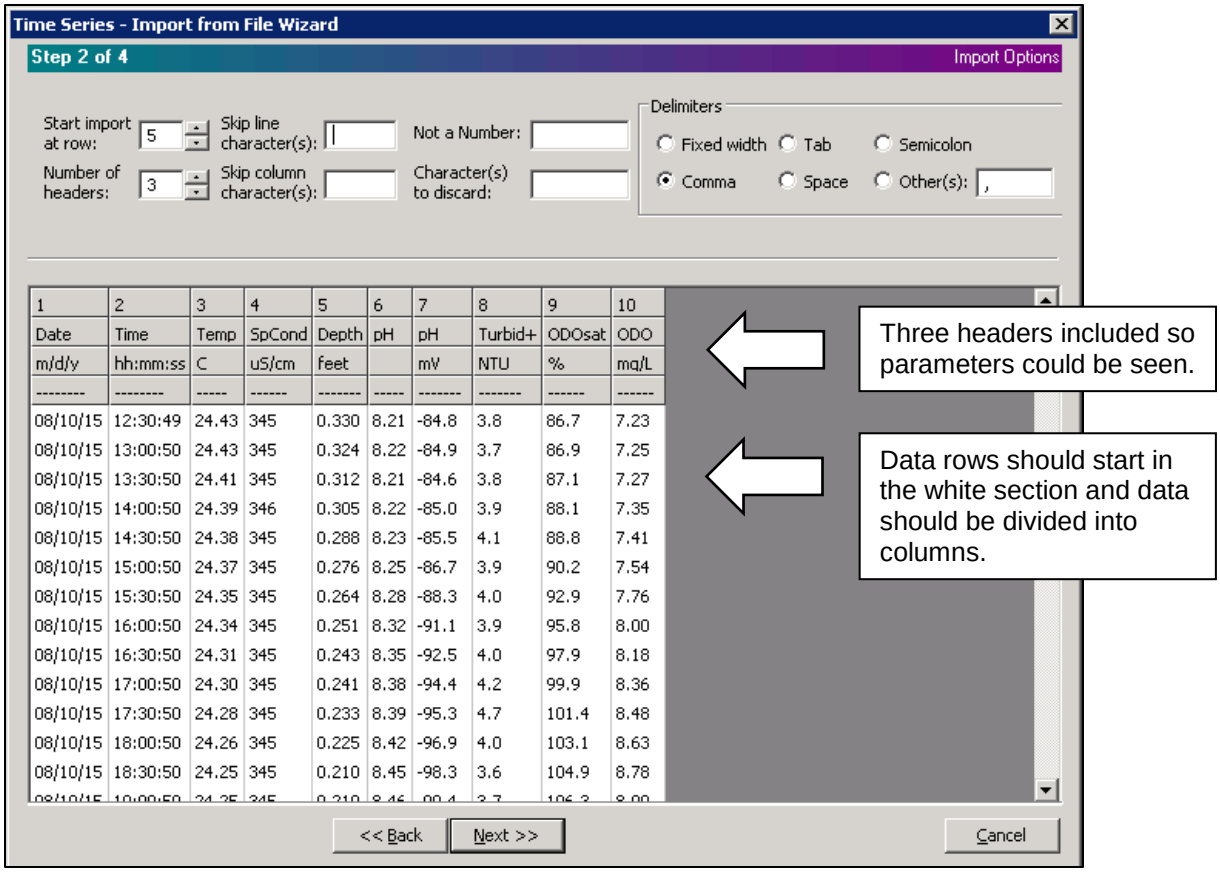


Figure 9. Step 2 of the Config File setup.

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.

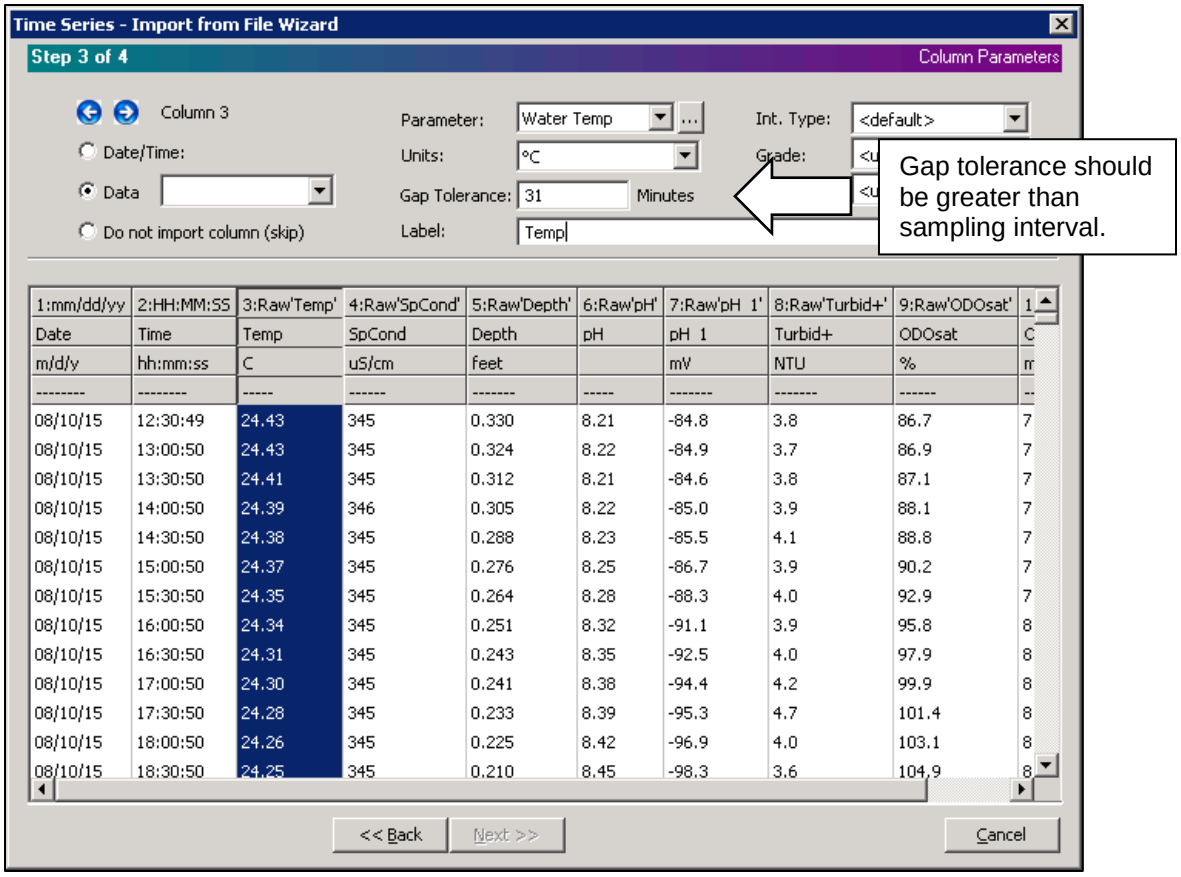


Figure 10. Step 3 of the of Config File setup showing the proper settings for a data column.

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.

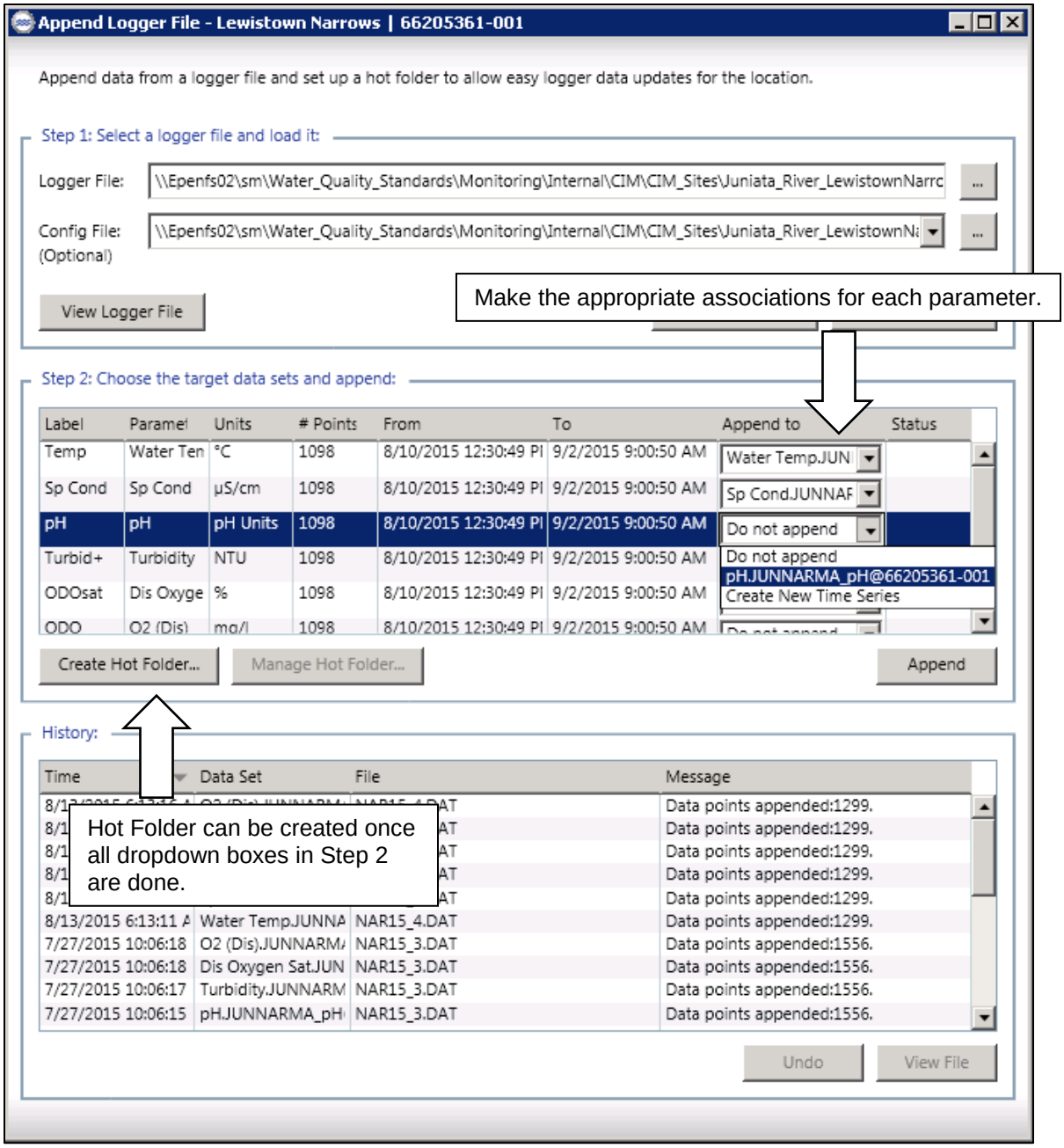


Figure 11. Appending data in the Append Logger File toolbox.

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.

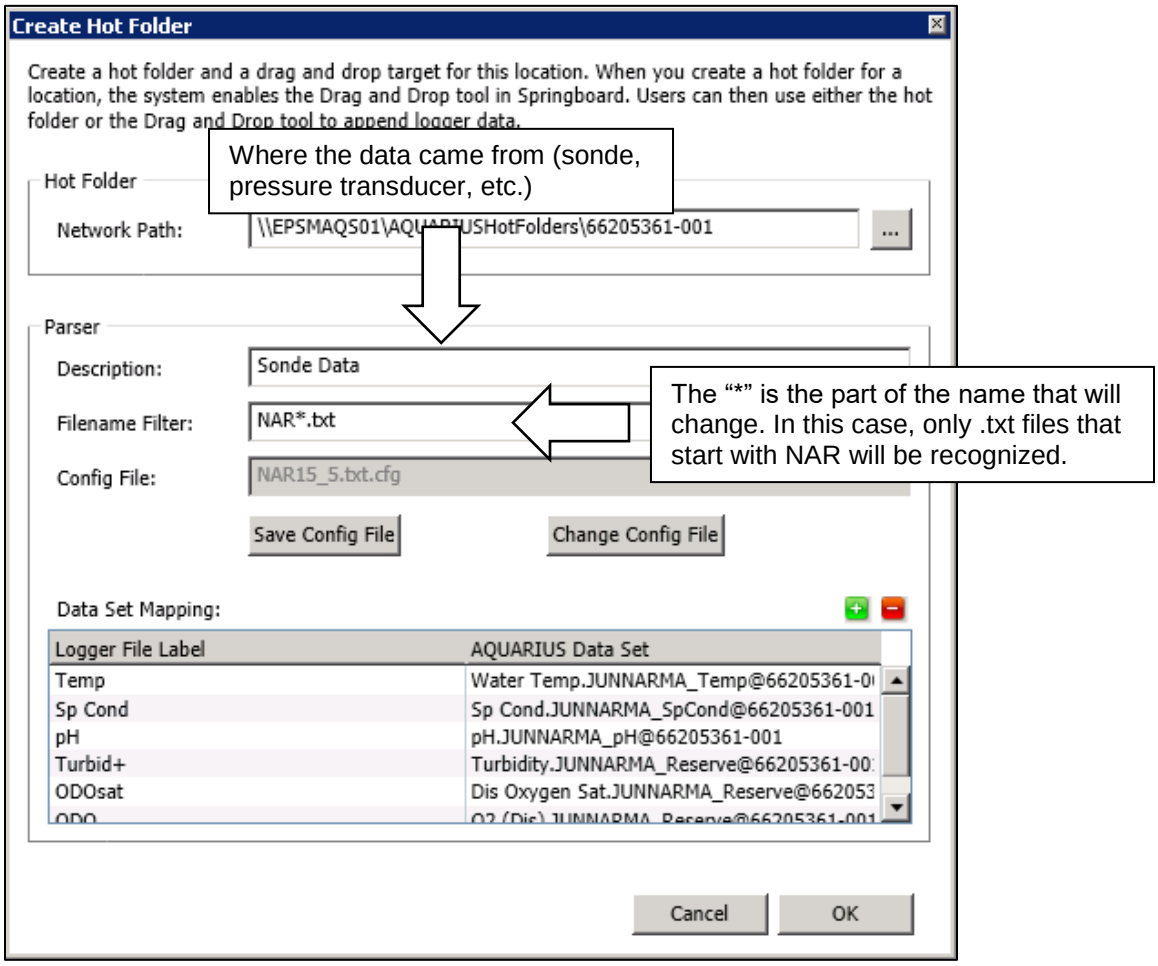


Figure 12. Establishing the settings for a hot folder in the Create Hot Folder toolbox.

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.

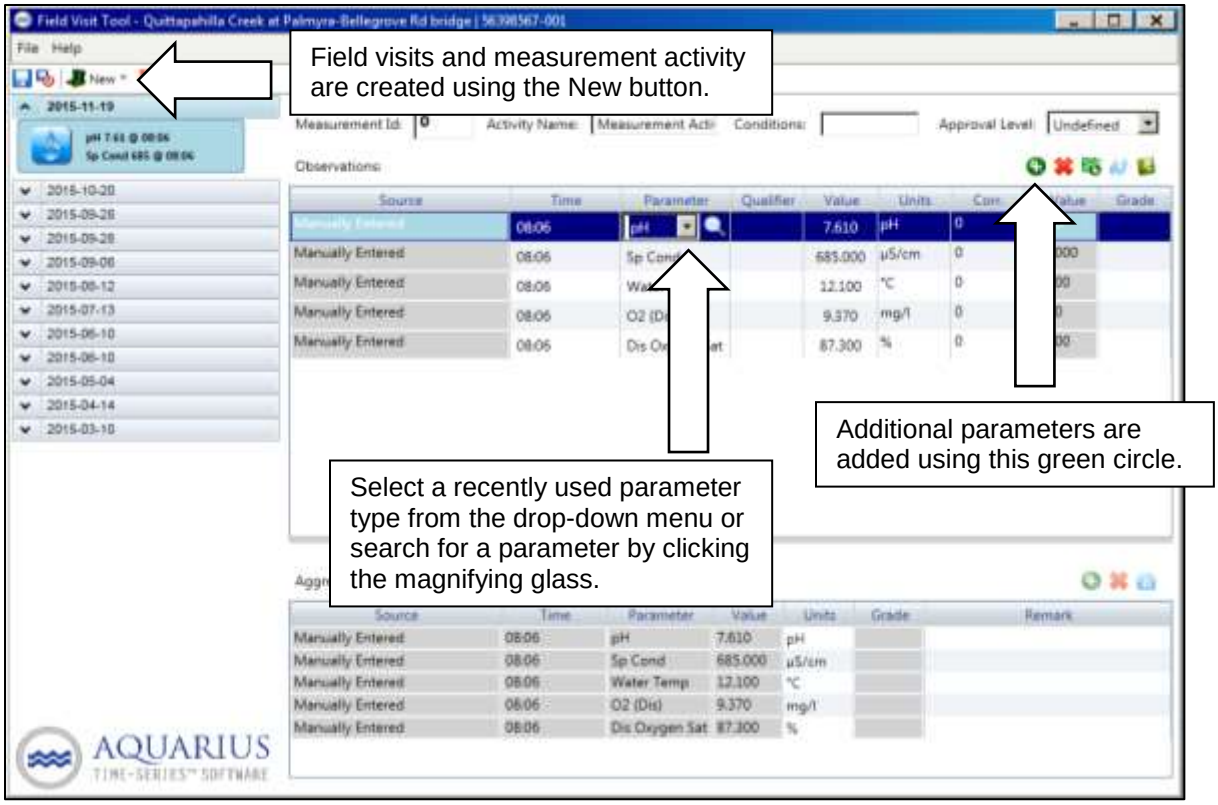


Figure 13. Entering discrete data into the Field Visit toolbox.

<u>Removing Data</u>

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. <u>Removing data cannot be undone.</u> 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.

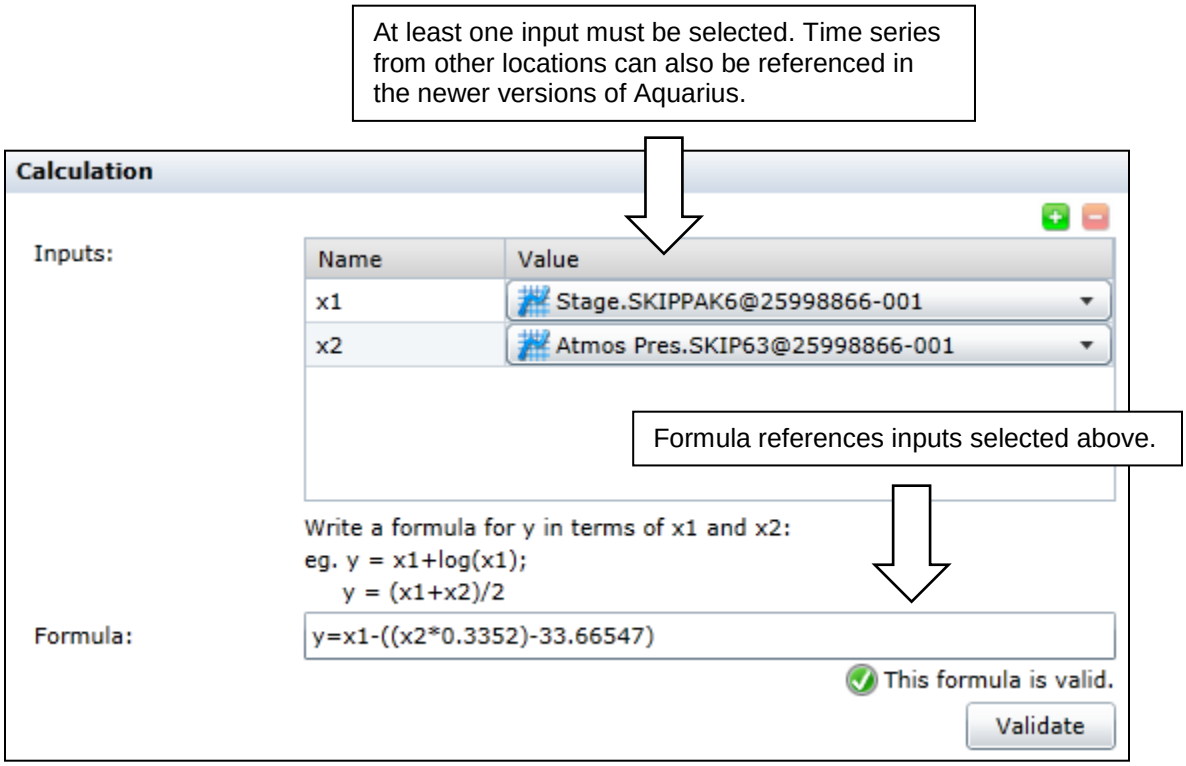


Figure 14. An example of a calculated-derived series in Aquarius that corrects the raw stage for changes in atmospheric pressure for Skippack Creek at Mainland Rd, PA.

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.

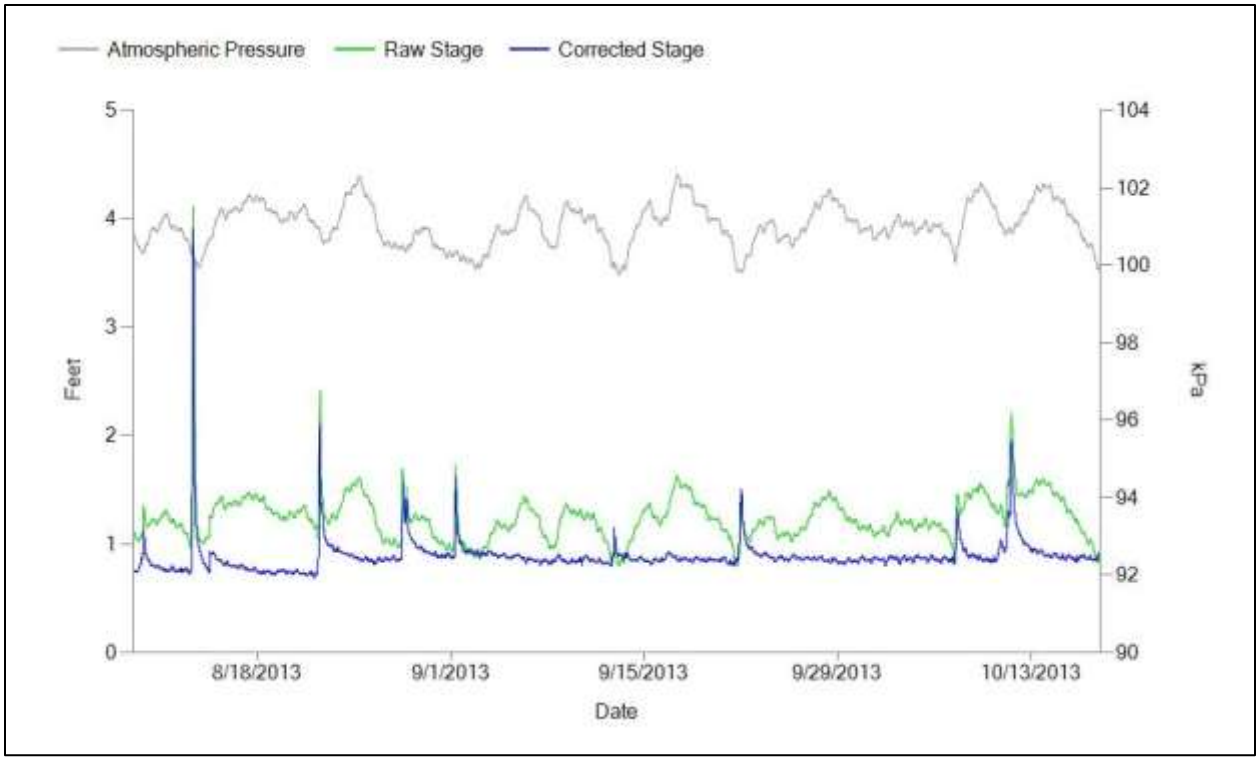


Figure 15. Elimination of changes in atmospheric pressure from non-vented stage data from Skippack Creek at Mainland Rd, PA. Large flow events (e.g., 8/13/13) are apparent in the raw data, but many small flow events are concealed by changes in atmospheric pressure.

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 have included 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. 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)

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 is 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 is 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 is 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.

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.

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

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 methods; 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.

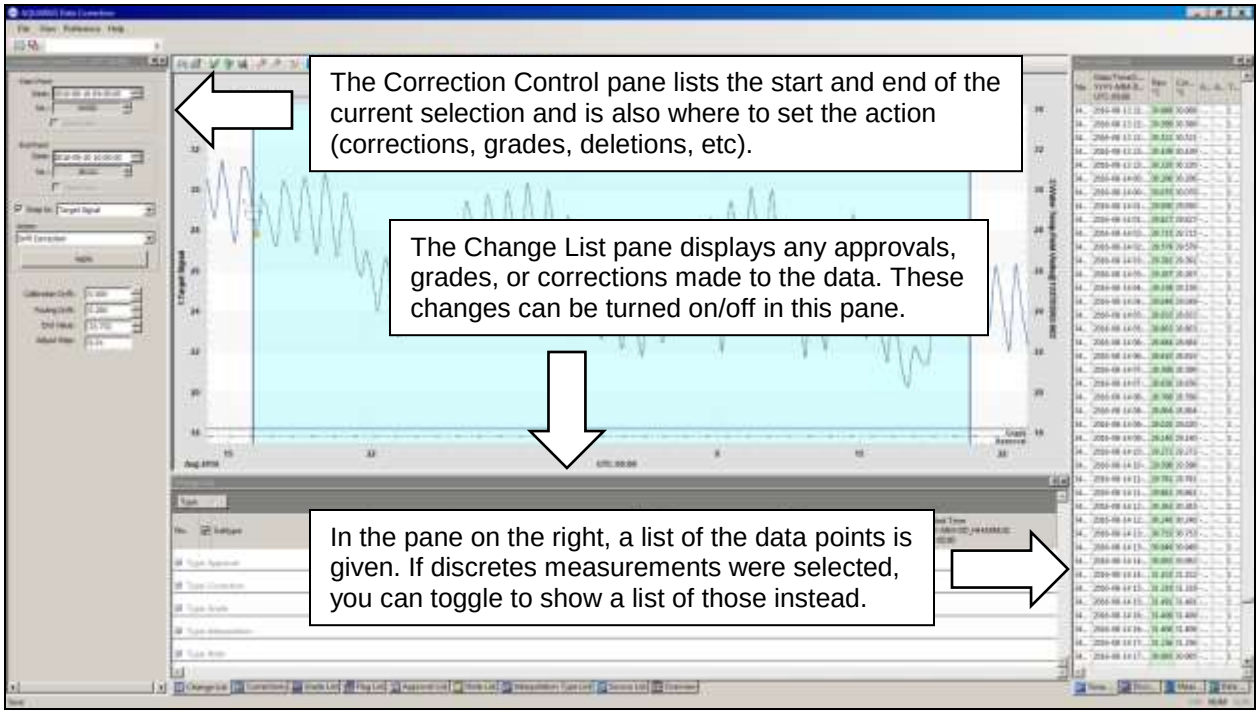


Figure 16. Default layout of the Data Correction window.

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.

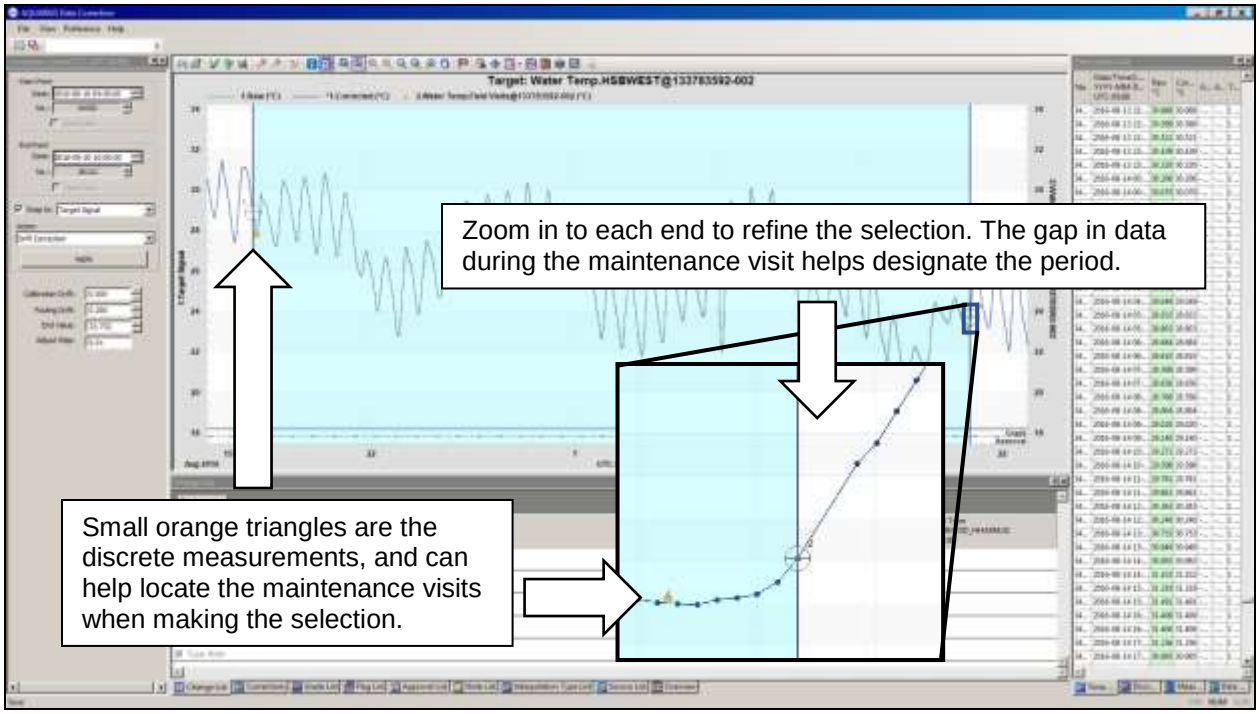


Figure 17. Use the Mark Region tool to select the data to be corrected. Selected region will be highlighted in blue.

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 method 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.

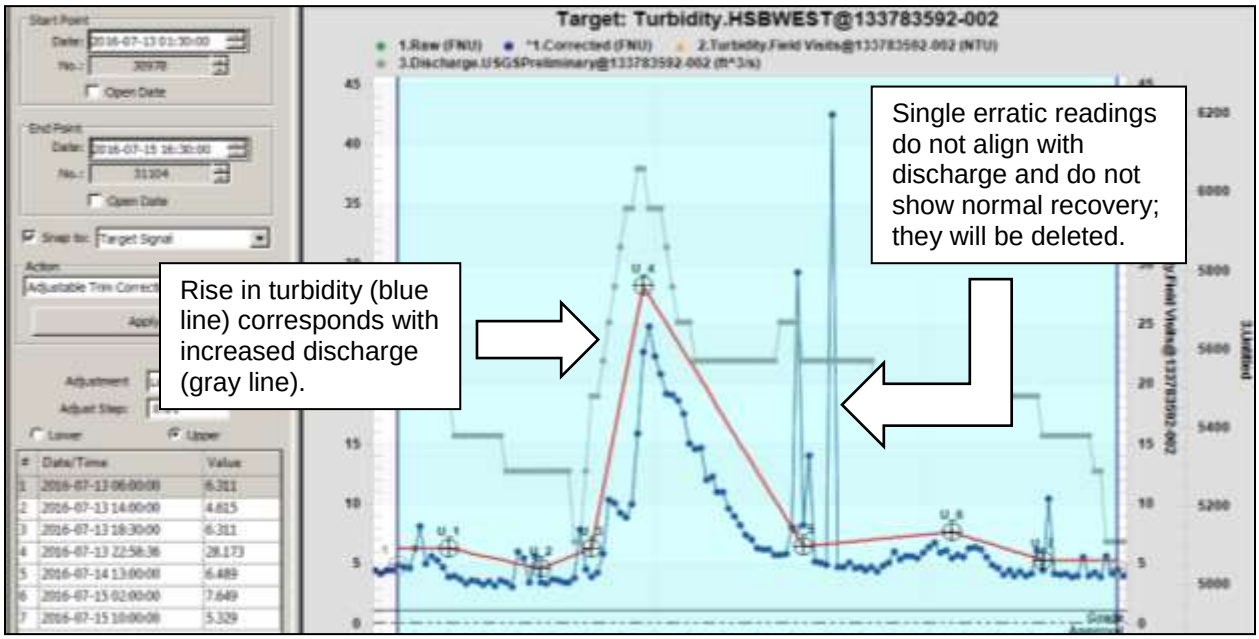


Figure 18. The Adjustable Trim Correction action (red line) allows for a variable threshold to be established. This adjustable threshold can be used to contour data while establishing an upper or lower threshold. This example demonstrates the correction being used to establish an upper threshold to delete 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.

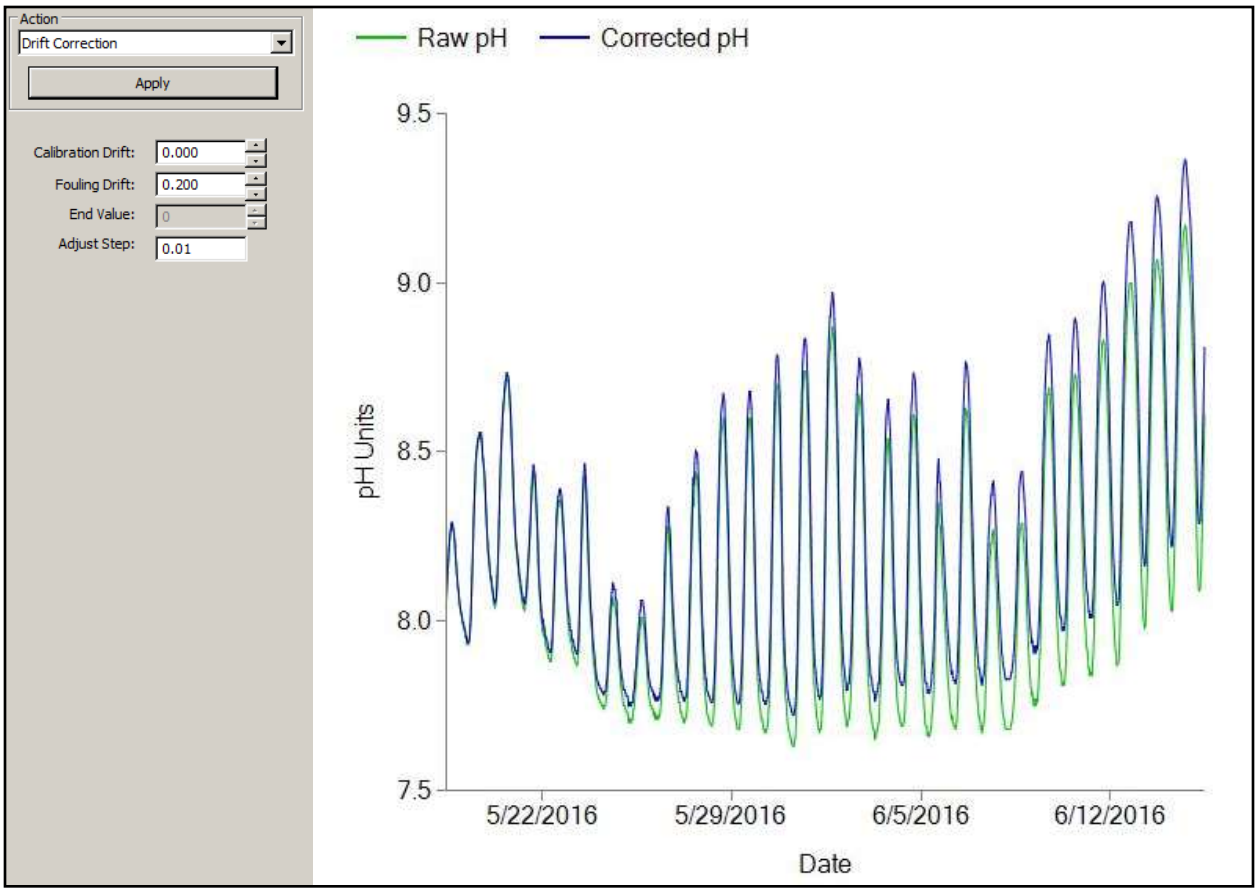


Figure 19. Continuous pH data from the Susquehanna River at Rockville, PA with a drift correction of 0.2 units applied.

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 of the selected region.

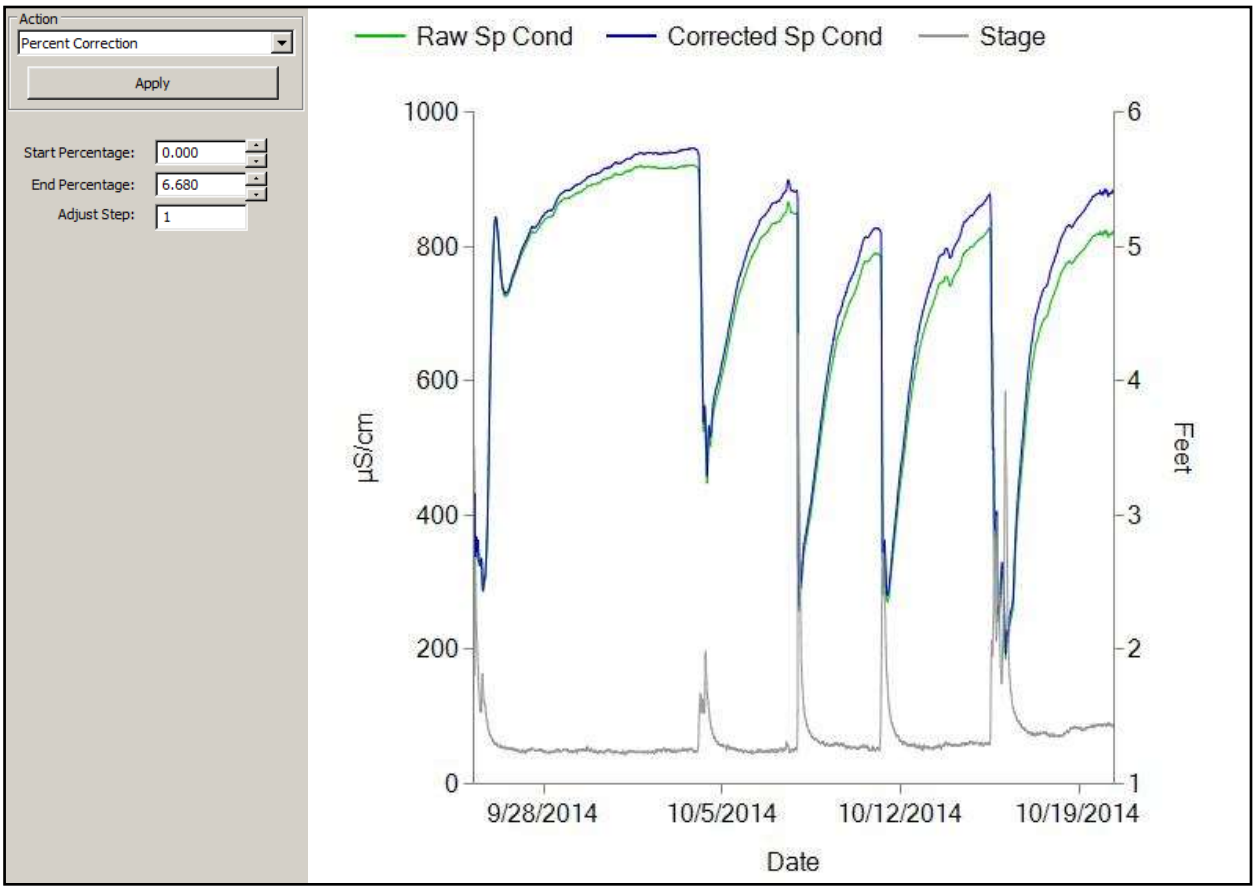


Figure 20. Continuous specific conductance data from Goose Creek at Mosteller Park, PA with a percent correction of 6.68%. Larger raw values are corrected more than the smaller raw values following rain events.

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 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 10 buffer correction and smaller corrections as the values approach the 7 buffer correction. The Correction V Diagram shows the correction applied across the range of values. In this example, the 4 buffer correction entered has no influence on the data because all values are between 7 and 10 pH. The correction is still entered, however, in order to catalog a record of corrections in Aquarius. Additionally, if a subsequent correction were to move any data below 7 pH, the 4 buffer

correction would become applicable. Like the percent correction, both the start and end of the period can be adjusted.

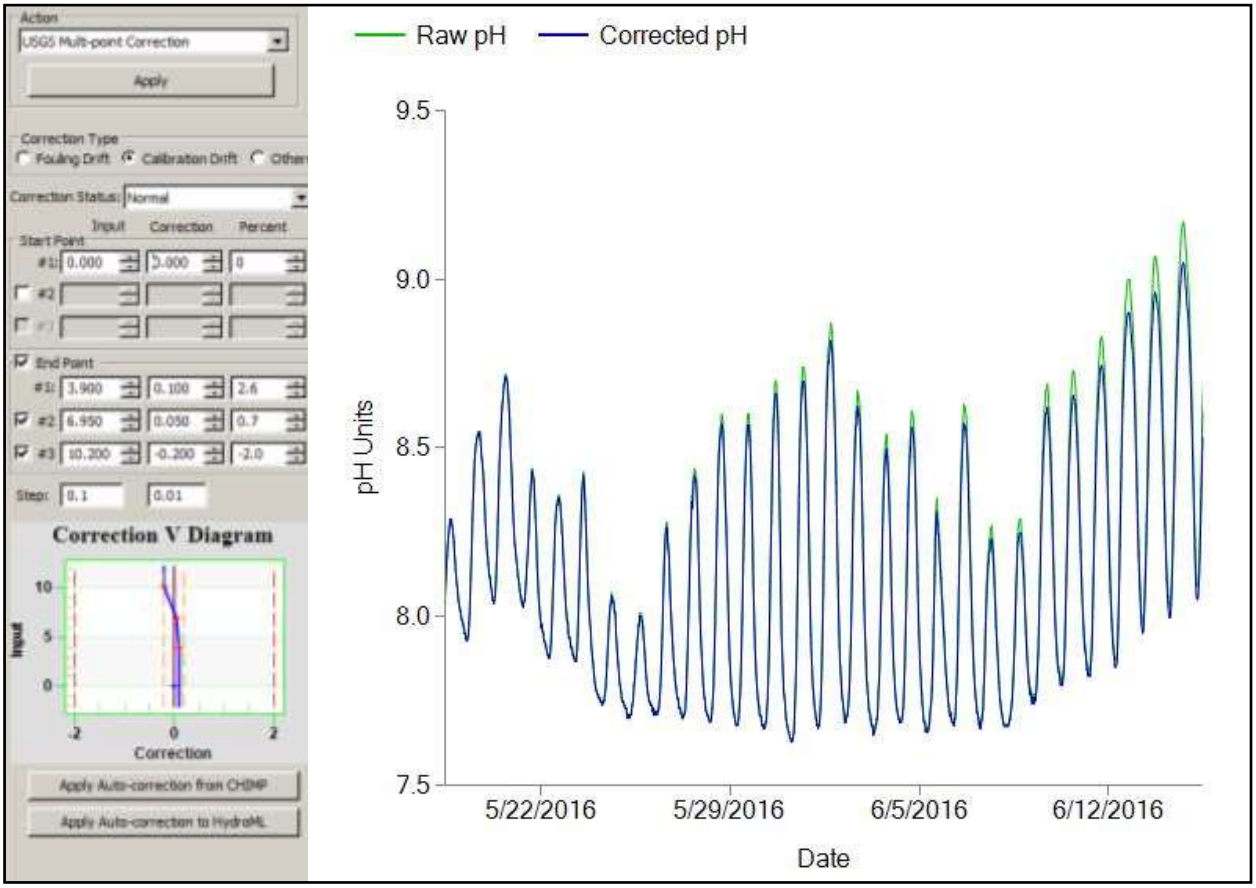


Figure 21. Continuous pH data from the Susquehanna River at Rockville, PA with a USGS multipoint correction applied. Buffers 4, 7, and 10 were used to check pH calibration. Values in buffer are entered under Input (3.90, 6.95, 10.20, respectively). The second column is the correction necessary (0.10, 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 method. 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).

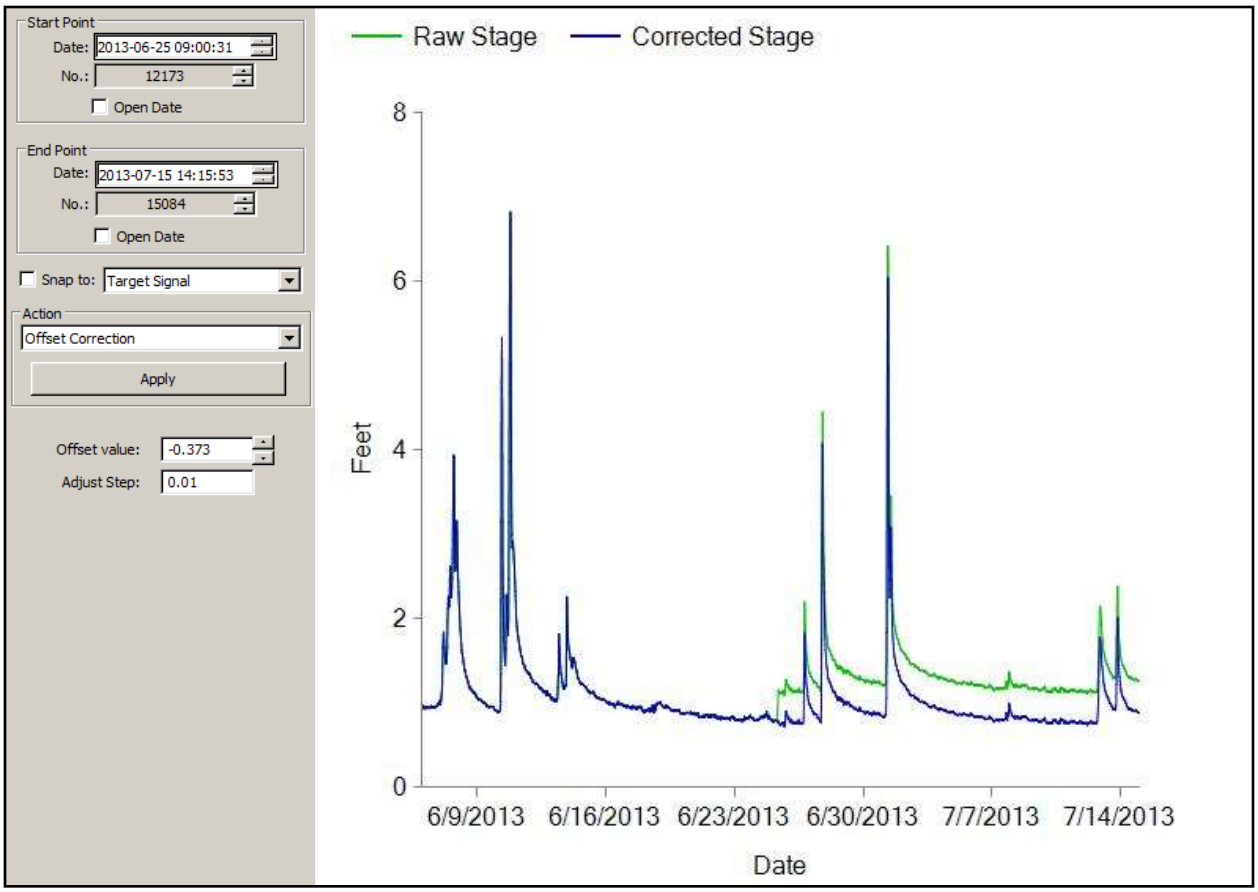


Figure 22. Stage data from Skippack Creek at Mainland Rd, PA with an offset correction of -0.373 feet applied from 6/25/13 to 7/15/13. Data were previously corrected for changes in atmospheric pressure. Only the offset correction is shown here.

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.

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 method 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.

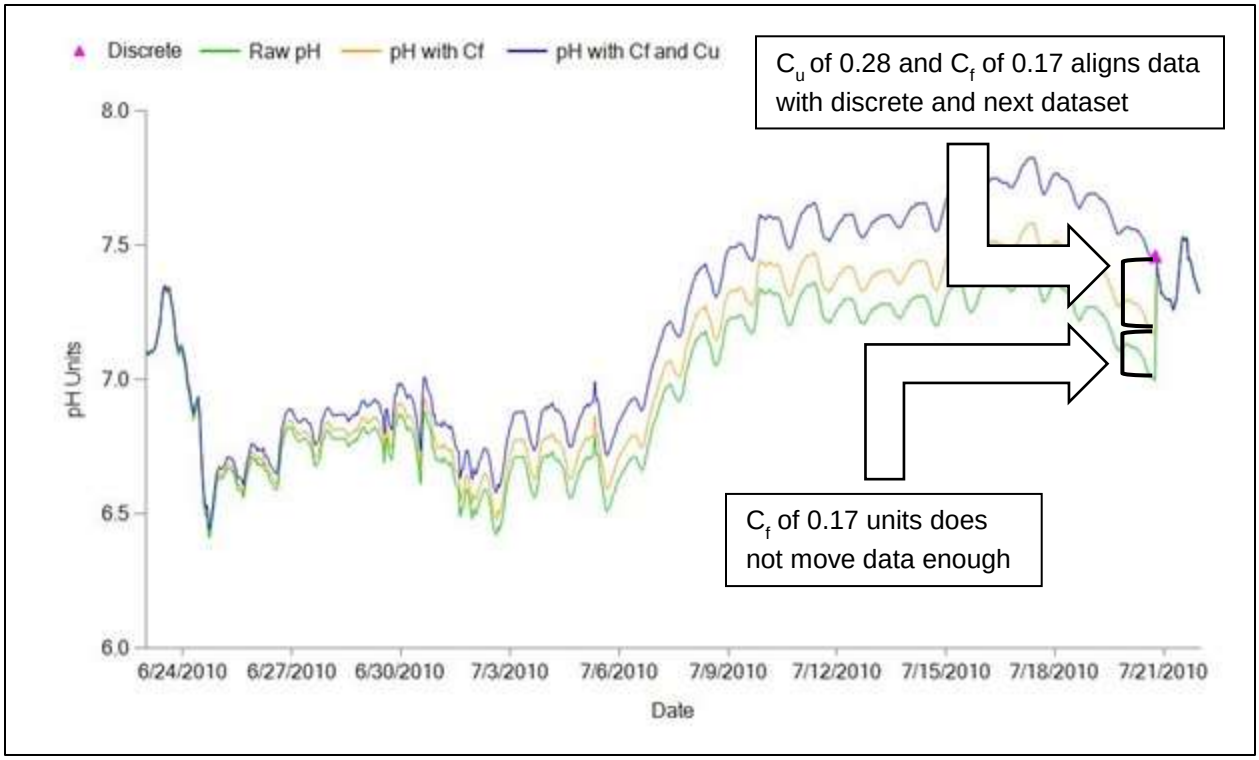


Figure 23. Data correction at Little Kettle Creek, PA. There was no calibration error during this period and fouling error was 0.17 pH units. Correcting for just fouling error (orange line) does not account for the full difference between the continuous data and either the discrete sample point or the 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.

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.

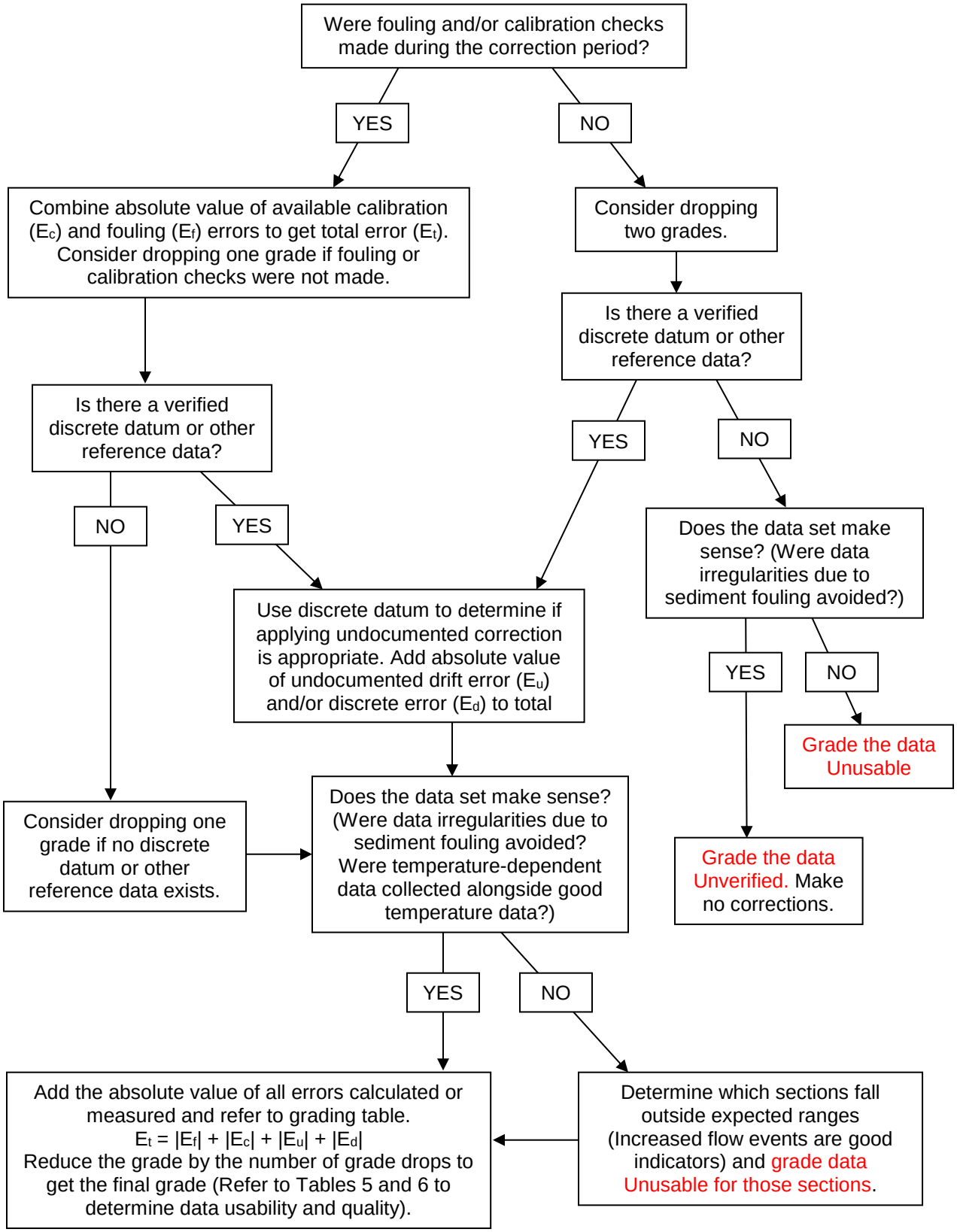


Figure 24. Division of Water Quality standardized grading flow chart

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 method 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) $\%E_m = 100 * [(V_c - V_r) / V_c]$

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 method to be used is determined in the same way as the calibration criteria described earlier—whichever method would result in a better grade is the method 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.

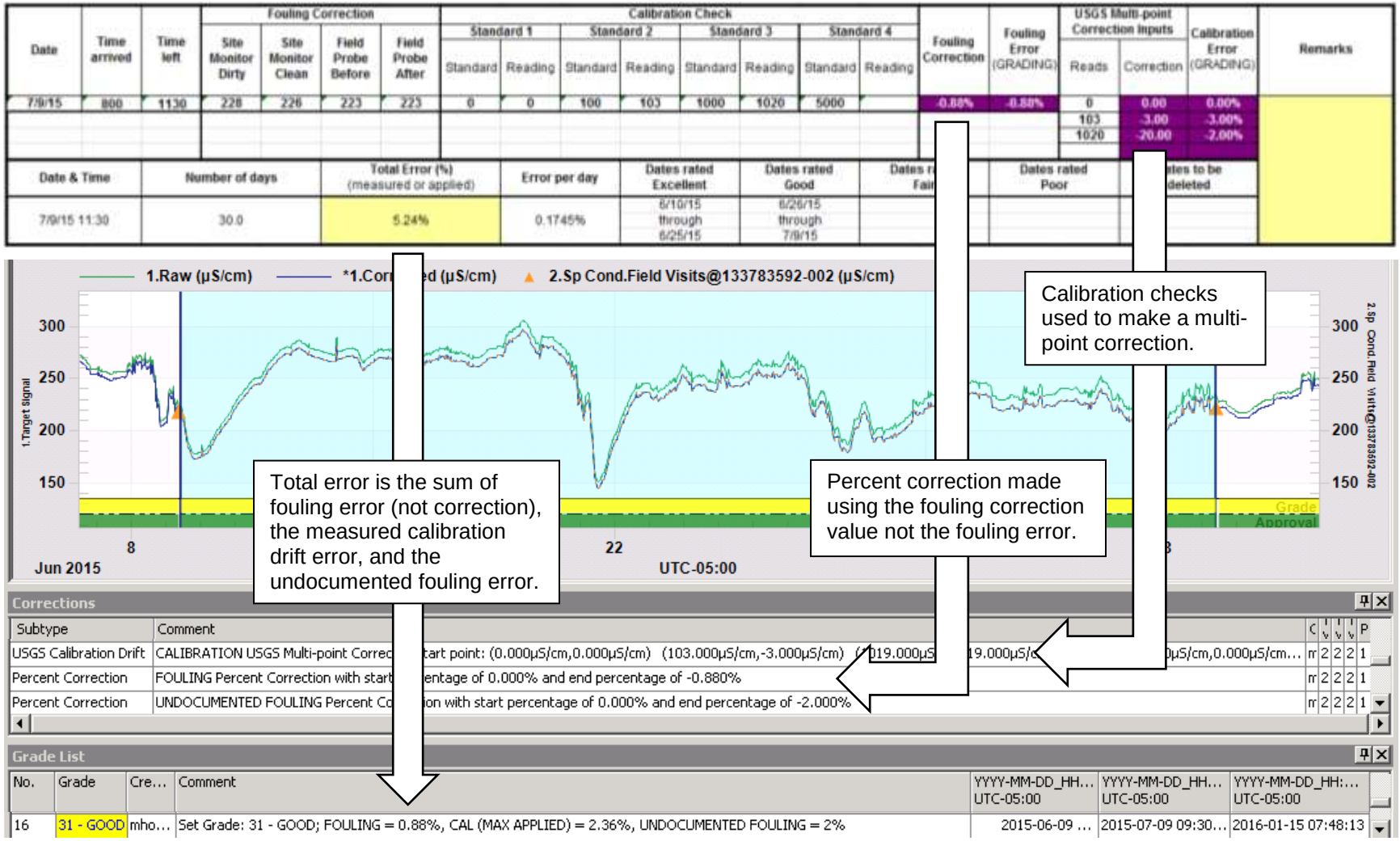


Figure 25. Corrections and grade applied to data from the Susquehanna River at Rockville for the period from 6/9/15 to 7/9/15. The upper section of the figure is taken from the Specific Conductance tab of the Field Form spreadsheet.

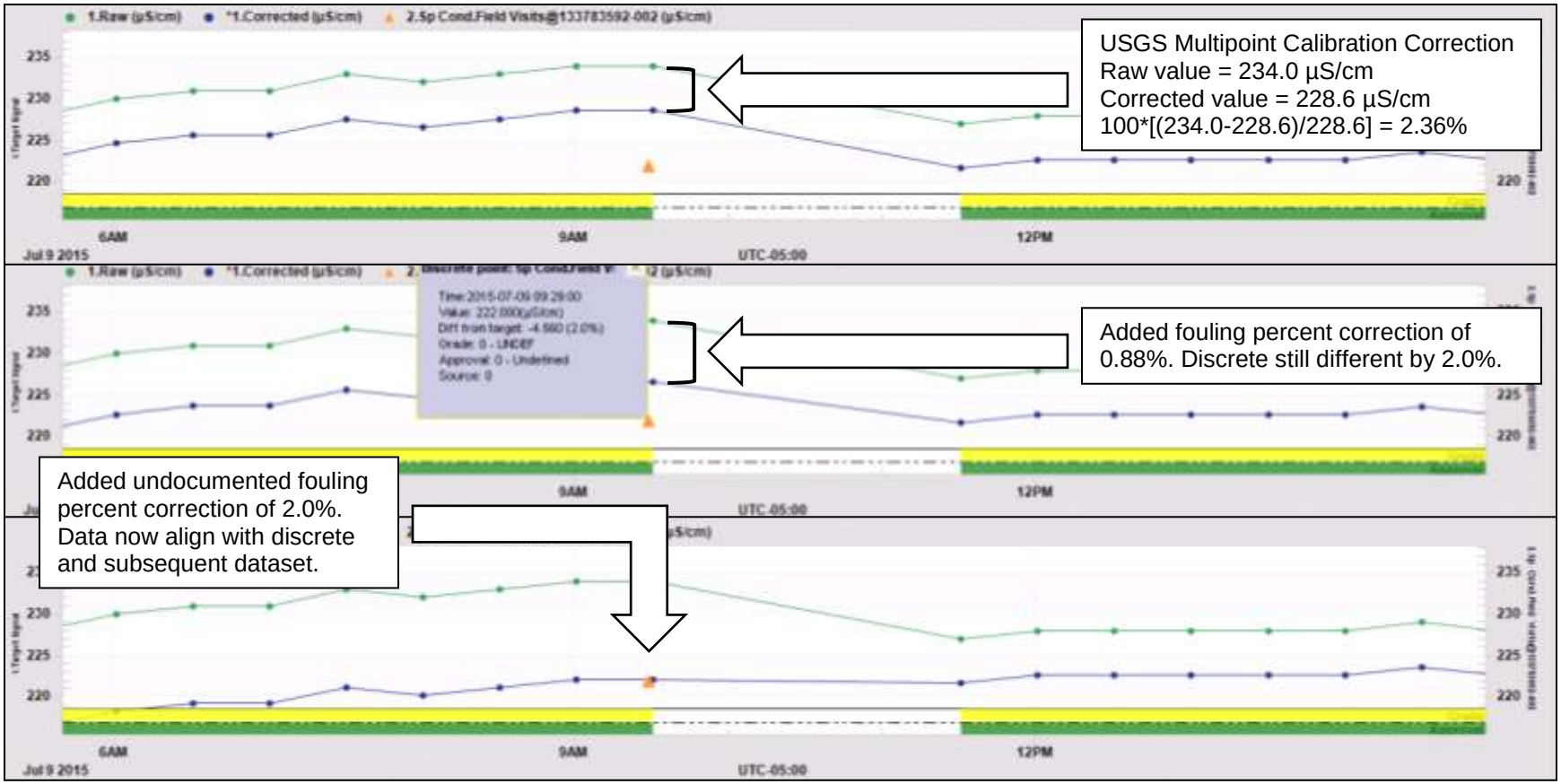


Figure 26. Series of corrections applied in Aquarius to data from the Susquehanna River at Rockville for the period from 6/9/15 to 7/9/15. The errors associated with these corrections are summed to determine the grade.

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.

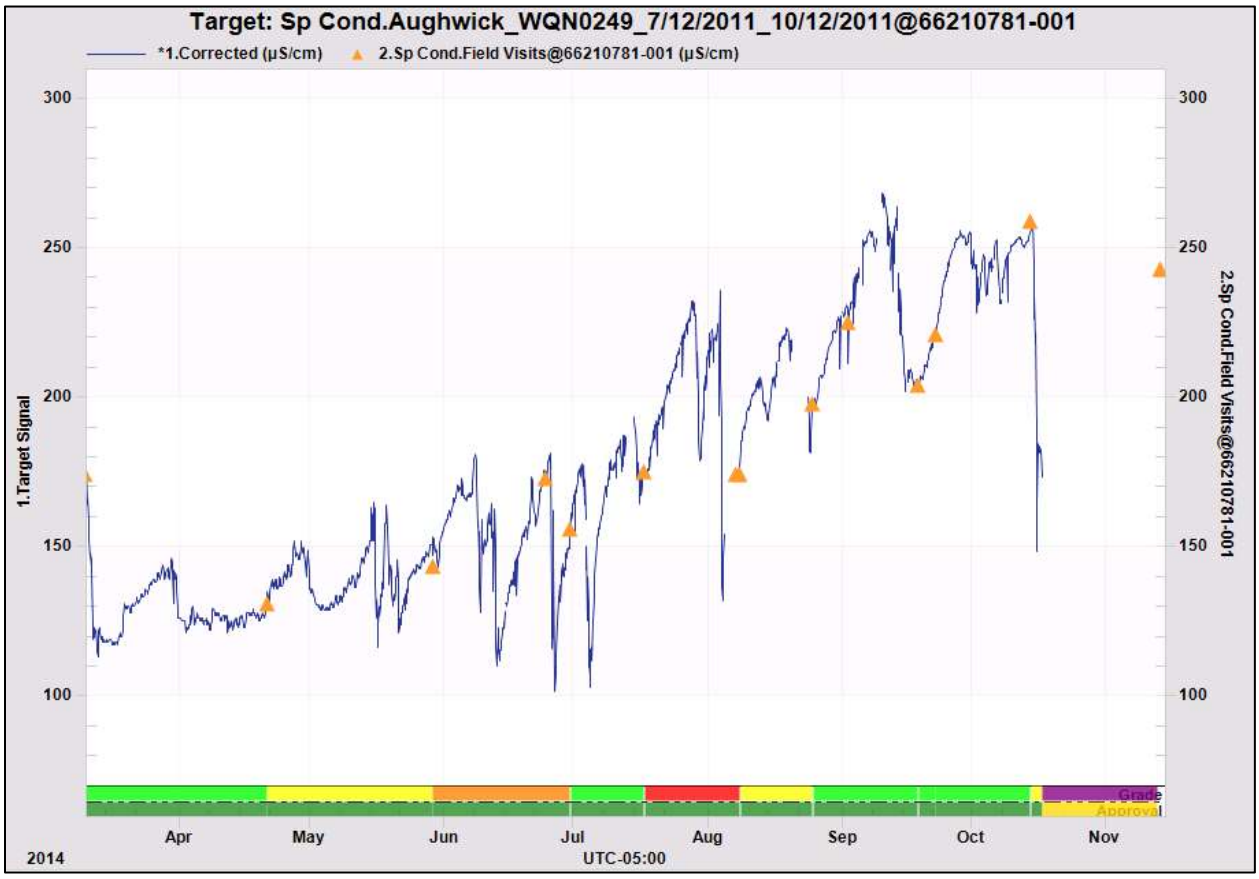


Figure 27. Specific conductance data at Aughwick Creek from 2014 displaying the grades and approval status within the Aquarius data correction tool box. The upper and lower rows of colored bars at the bottom represent grades and approvals, respectively. Because the last period was graded unusable (purple), 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

Division of Water Quality 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 methods 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.

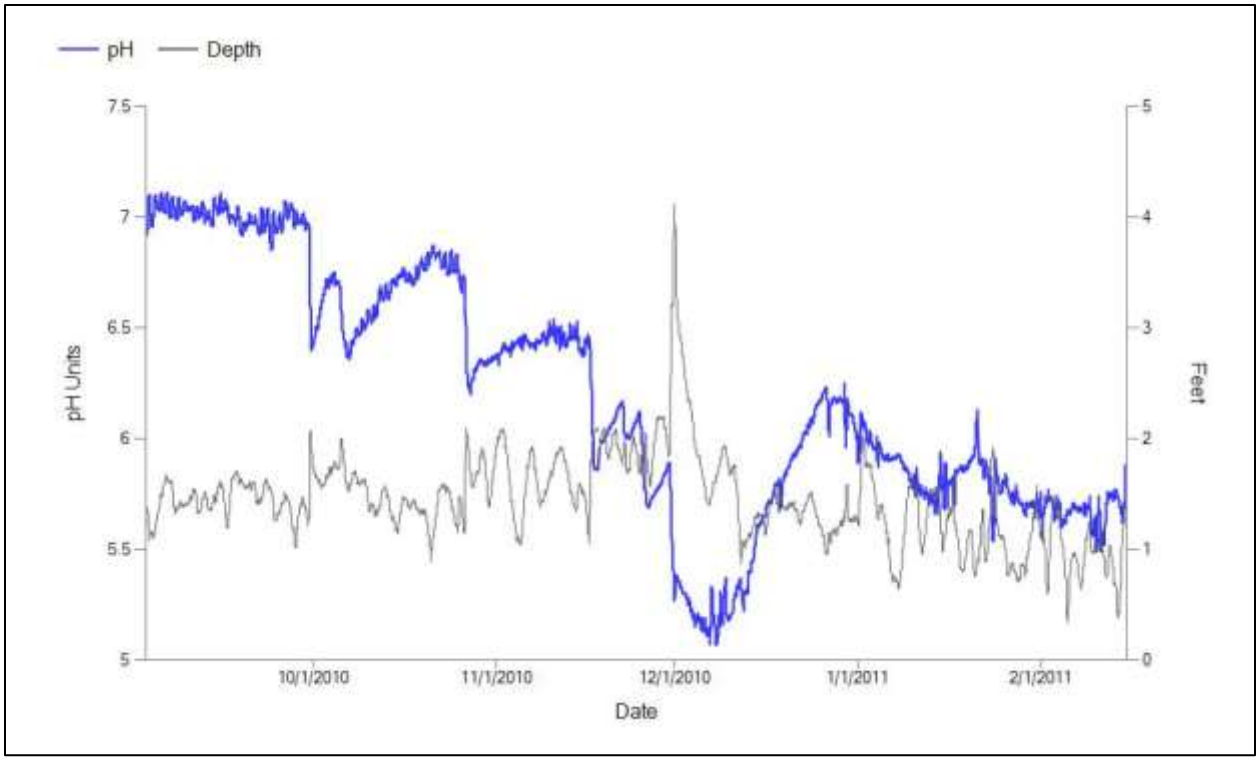


Figure 28. Depth and pH data for the East Branch Clarion River near the mouth of Gum Boot Run from September, 2010 to February, 2011. A critical depression in pH can be seen around the beginning of December, 2010. The depth time series helps identify and explain that the problem is most likely due to acid 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 methods, 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.

LITERATURE CITED

Anderson, C.W., 2005, Turbidity (ver. 2.1): U.S. Geological Survey Techniques of Water-Resources Investigations, book 9, chap. A6., sec. 6.7, September, 2005, accessed September 6, 2011, from http://pubs.water.usgs.gov/twri9A6/.

Hoger, M. S. 2017. In-situ field meter and transect data collection protocol. Chapter 4, pages 2–7. *In* Shull, D. R., and M. J. Lookenbill. (editors). Water quality monitoring protocols for streams and rivers. Pennsylvania Department of Environmental Protection. Harrisburg, Pennsylvania.

Radtke, D.B., J.V. Davis, and F.D. Wilde, 2005, Specific Electrical Conductance (ver. 1.2): U.S. Geological Survey Techniques of Water-Resources Investigations, book 9, chap. A6., sec. 6.3, August 2005, accessed September 6, 2011, from http://pubs.water.usgs.gov/twri9A6/.

Ritz G.F., and J.A. Collins, 2008. pH (ver. 2.0): U.S. Geological Survey Techniques of Water-Resources Investigations, book 9, chap. A6., sec. 6.4, November 2008, accessed September 6, 2011, from http://pubs.water.usgs.gov/twri9A6/.

Rounds, S.A., Wilde, F.D., and Ritz, G.F., 2013, Dissolved Oxygen (ver. 3.0): U.S. Geological Survey Techniques of Water-Resources Investigations, book 9, chap. A6., sec. 6.2, accessed December 22, 2016, from http://pubs.water.usgs.gov/twri9A6/.

Temperature Dependence of pH for BDH Reference Buffers, (n.d.), accessed December 22, 2016, from https://us.vwr.com/assetsvc/asset/en_US/id/12566247/contents.

Wagner, R.J., Boulger, R.W., Jr., Oblinger, C.J., and Smith, B.A., 2006, Guidelines and standard procedures for continuous water-quality monitors—Station operation, record computation, and data reporting: U.S. Geological Survey Techniques and Methods 1–D3,51p.+8attachments; accessed August 30, 2011, at http://pubs.water.usgs.gov/tm1d3

Wilde, F.D. 2006, Temperature (ver. 2.0): U.S. Geological Survey Techniques of Water-Resources Investigations, book 9, chap. A6., sec. 6.1, March 2006, accessed September 6, 2011, from http://pubs.water.usgs.gov/twri9A6/.

SEDIMENT CHEMISTRY DATA COLLECTION PROTOCOL

Prepared by:

Amy Williams
PA Department of Environmental Protection
Office of Water Programs
Bureau of Clean Water
11th Floor: Rachel Carson State Office Building
Harrisburg, PA 17105

2015

Edited by:

Amy Williams
PA Department of Environmental Protection
Office of Water Programs
Bureau of Clean Water
11th Floor: Rachel Carson State Office Building
Harrisburg, PA 17105

2017

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 grab 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 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 impairment or attainment of flowing water bodies. It can be used in conjunction with the Instream Comprehensive Evaluation to assess use attainment.

This document provides guidelines for the standardized collection of streambed sediment samples from flowing waterbody systems. The methods described here are adapted from scientific, peer-reviewed methods, 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 methods described here. The most common sources of error (also known as "interference") are cross-contamination and improper sample collection and 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 can request collector identification numbers for their field staff with the "Collector ID Request Form" found at the eLibrary website (ID Request Form).

DEP Laboratory Sample 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" (Submission Sheet). Collectors must submit samples to the DEP Bureau of Laboratories (BOL) using a "Sample Submission Sheet". Field staff are required to document collector identification number; reason code; cost center; program code; sequence number; date collected; time collected (military time); fixative(s) used; standard analysis code (SAC); under certain circumstances, legal seal numbers for each sample collected; the number of bottles submitted per test suite; collector name; current date; phone number; and any additional comments that lab analysts will use to properly handle samples.

As described previously, collector identification numbers are unique to each field staff member 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. Date and time collected should be accurately documented. If a sample is "fixed" or preserved with acid this must be documented in the appropriate space.

A standard analysis code or SAC is a unique code that details analytical tests to be applied to a specific sample. Suite codes are also available, and are often used for radionuclide and organic samples. Each DEP program uses different SACs or suites for specific projects or purposes. For example, SAC 018 is used by "Water Supply Management" when submitting water chemistry samples for Special Protection Surveys. The analytes/tests listed under SAC 018 are those specifically identified by regulation that surface water must meet and therefore be assessed for if a special protection determination is warranted. Other programs have developed SACs for their unique purposes, and the DEP BOL encourages programs to create SACs tailored to a program's specific needs.

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 needed, and should be used according to 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 for the particular needs.

Collector name, current date, phone number, and number of bottles submitted per test suite were added to the DEP laboratory "Sample Submission Sheet" to meet National Environmental Laboratory Accreditation Program (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, current 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 pieces of information to be documented are any additional comments that lab analysts need 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 need to document turbidity for a particular sample. The sample collector would identify SAC 018 in the appropriate field and indicate in the 'Comment' field to add the turbidity test to the particular sample. If a large number of samples will be submitted with consistent modifications of a particular SAC, the BOL prefers a new SAC be created specifically for those samples. Other important comments to consider include odor, sheen, color, viscosity, foaming, field meter readings, historical knowledge about the site, and any other information that lab analysts may need to safely and correctly handle the samples. The BOL requests collectors contact 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 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.

Parameter-specific equipment requirements, sample collection, labeling, and storage will be covered in subsequent sections below. Recommended sampling equipment and other gear are listed in the below check list:

SEDIMENT SAMPLING

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)
☐ methanol (to rinse organics equipment)
☐ hexane (to rinse organics equipment)
☐ 5% HCl (to rinse trace elements equipment)

FORMS
☐ sediment field forms
☐ laboratory sediment chem. sheets

SHIPPING
☐ courier shipping forms
☐ tape & dispenser
☐ 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 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 method, it can be easy to lose fine sediment, which is desirable for most tests. Additionally, it can be harder 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, hand-held 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 exceeding holding times.

SAMPLING DESIGN CONSIDERATIONS SPECIFIC TO SEDIMENT

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 the section “Types of Sampling Design” in Chapter 2 of this book for suggested sampling plans.

During or after completing a sample collection 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 & 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.

COLLECTION METHODS

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 stream 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 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 & Capel 1994). In general, the way to distinguish between sand, silt, and clay is displayed in Table 1, below:

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

In the event that 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 wash and soak it for 30 minutes in a 0.2% phosphate-free detergent (Shelton & 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.

First, calibrate any field sampling equipment, such as field meters (see the “Discrete Water Chemistry Data Collection Protocol” in Chapter 4 of this book for more details). 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 & 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.

Conductivity measurements may be adequate tools to determine the point of complete mix. Conductivity measurements should be taken at multiple points along a cross-section. The same protocol used to divide a stream for flow measurements may be utilized, as described in DEP's "Discrete Stream Flow Data Collection Protocol". Following the streamflow protocol, each cross-section should be divided into at least 20 sub-sections to ensure that no more than 5% of the total stream discharge flows through any one sub-section. Conductivity measurements are then taken at each sub-section. The point of complete mix occurs when the conductivity measurements across a cross-section are approximately stable. Stability occurs when the relative percent difference between the range of measurements is less than 10% (Colorado Department of Public Health and Environment 2002). Inevitable variability of conductance occurs across a stream channel, but past studies have indicated that a stream fully mixed with a discharge will often have ranges of conductivities across a transect that are >1% different, but are very rarely >10% different (Colorado Department of Public Health and Environment 2002). Transects should be done at regular intervals downstream until the point of complete mix is reached.

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 methods 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 Samples

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 methods 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. USEPA has a detailed description on how to collect volatile organic compounds in USEPA Test Method 5035A (2002c) - 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 Samples

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 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 methods 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 method 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 method 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 (2002c) - 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.

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, SAC code, and any additional analytical tests performed or excluded.

Quality Assurance

A duplicate grab sample should be collected every 20 samples or for each sampling trip/day to gauge testing variability and potential sources of contamination due to collection procedures. Duplicate samples are collected simultaneously or sequentially with the associated environmental sample, using identical sampling and preservation procedures. Sequentially collected duplicates may measure inhomogeneities present in the sediment. Duplicates are assigned unique sequential sample numbers. The collector needs to carefully annotate which sample is a duplicate in their field book. Duplicates must be documented appropriately in SIS under the 'Comments/Quality Assurance' tab.

"Split samples" are collected as one sample and then divided in two for separate analysis (Radtke 2005). These can be sent to different labs for analysis or be tested at the same lab, and can help determine lab analysis variability.

"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 & 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 Holding Times

Samples need to be shipped or delivered to the lab as soon as possible. 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 exceeds 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.

Shipping

All DEP district and regional offices are designated pick-up locations for water and sediment samples. In most cases, samples must be dropped off for pick up by 1600 hours. Other locations exist, such as at some Pennsylvania Department of Transportation facilities and private businesses, but these drop-off locations may require call-ahead notice to the current courier, as they may not be visited daily. Further, the drop-off locations may require a drop-off specific key to open the drop-off entrance lock. Collectors need to coordinate with the current courier for specific drop-off and pick-up requirements.

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 4°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 samples and should never be used for storage or shipping. The “Sample Submission Sheet” should be filled out, inserted into a Ziploc® 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. Be sure that any required legal seals are in place.

LITERATURE CITED

Colorado Department of Public Health and Environment. 2002. Water Quality Control Division. Colorado Mixing Zone Implementation Guidance. (Available from: https://www.colorado.gov/pacific/sites/default/files/WQ_Mixing-Zone-Policy.pdf).

Horowitz, A.J. 1985. A primer on trace metal-sediment chemistry. Water-Supply Paper 2277. United States Geological Survey, Alexandria, Virginia.

Oak Ridge Associated Universities (ORAU) Training. 2012. Environmental Monitoring for Radiation. Oak Ridge, Tennessee.

Ohio Environmental Protection Agency. 2001. Sediment sampling guide and methodologies (2nd Edition). Division of Surface Water, Columbus, Ohio (Available from: http://www.epa.ohio.gov/portals/35/guidance/sedman2001.pdf).

DEP. 2009. Instream Comprehensive Evaluation Surveys. Harrisburg, Pennsylvania.

Radtke, D.B. 2005. National field manual for the collection of water-quality data, Chapter A8: Bottom-material samples. Techniques of Water-Resources Investigations. Book 9: Handbooks for Water-Resources Investigations. United States Geological Survey. Reston, Virginia.

Shelton, L.R. & P.D. Capel. 1994. Guidelines for collecting and processing samples of stream bed sediment for analysis of trace elements and organic contaminants for the national water-quality assessment program. Open-File Report 94-458. United States Geological Survey. Sacramento, California.

USEPA. 2002a. Guidance on choosing a sampling design for environmental data collection for use in developing a quality assurance project plan. U.S. Environmental Protection Agency. Washington, DC. EPA/240/R-02/005. EPA QA/G-5S.

USEPA. 2002b. Guidance for quality assurance project plans. U.S. Environmental Protection Agency. Washington, DC. EPA/240/R-02/009. EPA QA/G-5.

USEPA. 2002c. Method 5035A – Closed-System-Purge-and-Trap and Extraction for Volatile Organics in Soil and Waste Samples. Appendix A: The collection and preservation of aqueous and solid samples for volatile organic compound (VOC) analysis.

USEPA. 2006a. Guidance on systematic planning using the data quality objectives process. U.S. Environmental Protection Agency. Washington, DC. EPA/240/B-06/001. EPA QA/G-4.

USEPA. 2012. Sample collection procedures for radiochemical analytes in environmental matrices. U.S. Environmental Protection Agency. Office of Research and Development. National Homeland Security Research Center. Cincinnati, Ohio.

Water Environment Federation. 2002. Handbook on Sediment Quality: Chapter 4. The Federation. Alexandria, Virginia.

PASSIVE WATER CHEMISTRY DATA COLLECTION PROTOCOL

Prepared by:

Amy Williams
PA Department of Environmental Protection
Office of Water Programs
Bureau of Clean Water
11th Floor: Rachel Carson State Office Building
Harrisburg, PA 17105

2013

Edited by:

Amy Williams
PA Department of Environmental Protection
Office of Water Programs
Bureau of Clean Water
11th Floor: Rachel Carson State Office Building
Harrisburg, PA 17105

2017

Disclaimer:
Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

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 ones 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 water body systems. The methods described here are adapted from scientific, peer-reviewed methods, 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 methods described here. The most common sources of error (also known as “interference”) are cross-contamination and improper sample collection and preservation.

COLLECTION REQUIREMENTS

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 method 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 SAMPLING DESIGN

A sample collection plan is recommended before commencing a sampling project. This 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 water body 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 method is judgmental or targeted sampling – sampling sites are chosen based on professional judgment, *not* scientific theory. This is a good method 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.

COLLECTION METHODS

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. 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. 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 Holding Times
Holding times depend on the compounds analyzed. Discuss with the analysis lab(s) regarding holding times.

Shipping
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.

LITERATURE CITED

Alvarez, D.A. 2010. Guidelines for the use of the semipermeable membrane device (SPMD) & the polar organic chemical integrative sampler (POCIS) in environmental monitoring studies. United States Geologic Survey. Techniques and Methods 1–D4.

Environmental Sampling Technologies. SOP E-13, Cleaning of SPMD/POCIS Deployment Devices.

Horowitz, A.J. 1985. A primer on trace metal-sediment chemistry. United States Geological Survey. Water-Supply Paper 2277.

USEPA. 2002a. Guidance on Choosing a Sampling Design for Environmental Data Collection for Use in Developing a Quality Assurance Project Plan. EPA QA/G-5S. U.S. Environmental Protection Agency, Office of Environmental Information. Washington, DC.

USEPA. 2002b. Guidance for quality assurance project plans. EPA QA/G-5. U.S. Environmental Protection Agency, Office of Environmental Information. Washington, DC.

USEPA. 2006. Guidance on systematic planning using the data quality objectives process. EPA QA/G-4. U.S. Environmental Protection Agency, Office of

Environmental Information. Washington, DC.

SAMPLE INFORMATION SYSTEM (SIS) DATA ENTRY PROTOCOL

Prepared by:

Dustin Shull
PA Department of Environmental Protection
Office of Water Programs
Bureau of Clean Water
11th Floor: Rachel Carson State Office Building
Harrisburg, PA 17105

2017

INTRODUCTION

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 is 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 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 (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

STREAM HABITAT DATA COLLECTION PROTOCOL

Prepared by:

Tony Shaw
PA Department of Environmental Protection
Office of Water Programs
Bureau of Clean Water
11th Floor: Rachel Carson State Office Building
Harrisburg, PA 17105

2002

Edited by:

Josh Lookenbill
PA Department of Environmental Protection
Office of Water Programs
Bureau of Clean Water
11th Floor: Rachel Carson State Office Building
Harrisburg, PA 17105

2017

INTRODUCTION

DEP has adopted a modification of the habitat evaluation methods outlined in USEPA's 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 landuses. 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 and 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.

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 in the immediate vicinity of the benthic macroinvertebrate sampling reach.

- **Instream Fish Cover** (riffle/run & low gradient)
  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.
- **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.
  (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.
- **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 sampled 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. This area is usually defined as the length of stream that was electroshocked for fish (or an approximate 100-meter stream reach when no

fish were sampled). It can also take into consideration upstream land-use activities in the watershed.

- **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. 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. 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 & low gradient)
  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.
- **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. 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 4 (Shull and Pulket 2018) for habitat assessment method.

LITERATURE CITED

Barbour, M.T., J. Gerritsen, B.D. Snyder, and J.B. Stribling. 1999. Rapid bioassessment protocols for use in streams and wadeable rivers: periphyton, benthic macroinvertebrates and fish, second edition. EPA 841-B-99-002. United States Environmental Protection Agency; Office of Water. Washington, D.C.

Shull, D. R., and M. Pulket (editors). 2018. Assessment Methodology for Rivers and Streams. Pennsylvania Department of Environmental Protection, Harrisburg, Pennsylvania.

PEBBLE COUNT DATA COLLECTION PROTOCOL

Prepared by:

Gary Walters
PA Department of Environmental Protection
Office of Water Programs
Bureau of Clean Water
11th Floor: Rachel Carson State Office Building
Harrisburg, PA 17105

2009

Edited by:

Gary Walters
PA Department of Environmental Protection
Office of Water Programs
Bureau of Clean Water
11th Floor: Rachel Carson State Office Building
Harrisburg, PA 17105

2017

INTRODUCTION

This survey 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 method of 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 impacted” 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.

FIELD COLLECTION

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, 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 field 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 field 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.

PARTICLE COUNT PROCEDURE

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 field form attached to this document. 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 form attached to this document. 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 Chapter 5 of the Assessment Book (Shull and Pulket 2018) for habitat assessment method.

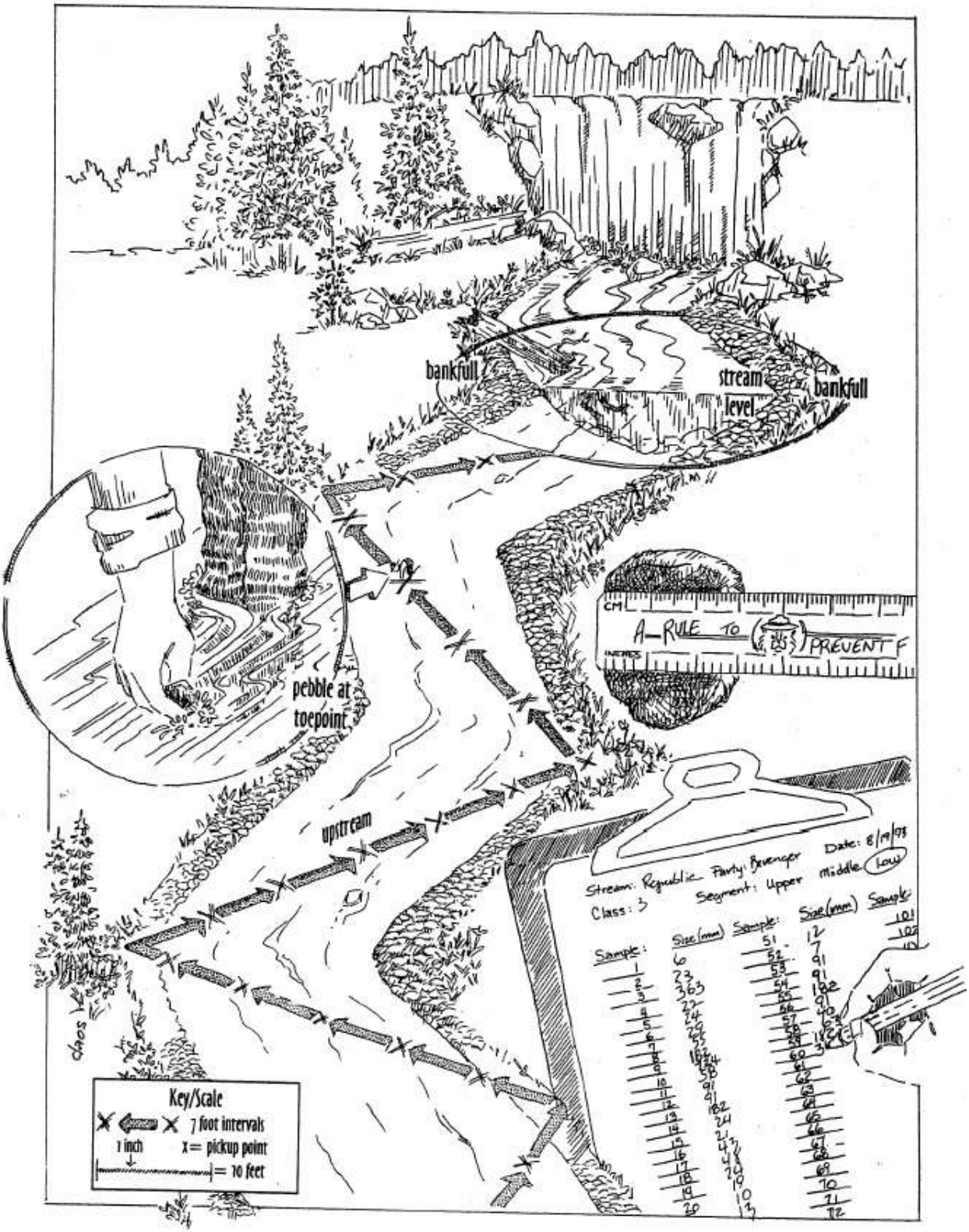


Figure 1. Zig-zag pebble count procedure from Bevenger and King, 1995.

LITERATURE CITED

Rosgen, David L. 1994. A Stream Classification System. Catena. Volume 22. Pp 169-199. Elsevier Science, Amsterdam.

Rosgen, David L. 1996. Applied River Morphology. Wildlands Hydrology Books, Pagosa Springs, Colorado.

Shull, D. R., and M. Pulket (editors). 2018. Assessment Methodology for Rivers and Streams. Pennsylvania Department of Environmental Protection, Harrisburg, Pennsylvania.

USDA Forest Service. 1995. A Pebble Count Procedure for Assessing Watershed Cumulative Effects. Rocky Mountain Forest and Range Experiment Station. RM-RP-319. (Authors: Gregory S. Bevenger and Rudy M. King)

Wolman, M. G. 1954. A Method of Sampling Coarse River-bed Material. Transactions American Geophysical Union. Volume 35. Number 6. Pp 951-956.

WATER FLOW DATA COLLECTION PROTOCOL

Prepared by:

Josh Lookenbill
PA Department of Environmental Protection
Office of Water Programs
Bureau of Clean Water
11th Floor: Rachel Carson State Office Building
Harrisburg, PA 17105

2009

Edited by:

Josh Lookenbill
PA Department of Environmental Protection
Office of Water Programs
Bureau of Clean Water
11th Floor: Rachel Carson State Office Building
Harrisburg, PA 17105

2017

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 is collected along with other water quality data in order to characterize yields or quantities of pollutants that are transported by waterbodies. Chemical data is 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” method 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 method 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 United States 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 uses 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.

LITERATURE CITED

Buchanan, T.J., and W. P. Somers. 1969. Discharge measurements at gaging stations: United States Geologic Survey. Techniques and Methods book 3, chap A8, 65 p.

Mueller, D.S. and C. R. Wagner. 2009. Measuring discharge with acoustic Doppler current profilers from a moving boat: United Stations Geological Survey Techniques and Methods 3–A22, 72 p.

Oberg, K.A., S. E. Morlock, and W. S. Caldwell. 2005. Quality assurance plan for discharge measurements using acoustic Doppler current profilers: United States Geological Survey Scientific Investigations report 2005–5183, 35 p.

Rantz, S.E. and others. 1982. Measurement and computation of streamflow: United States Geological Survey Water-Supply Paper 2175, v. 2, 631 p

Simpson, M.R. 2002. Discharge measurements using a Broad-Band Acoustic Doppler Current Profiler: United States Geological Survey Open-File Report 01–01, 123 p

Turnipseed, D.P., and V. B. Sauer. 2010. Discharge measurements at gaging stations: United States Geologic Survey. Techniques and Methods book 3, chap A8, 87 p.

APPENDIX A: BIOLOGICAL DATA COLLECTION INFORMATION AND FORMS

A-1 MACROINVERTEBRATE FIELD DATA SHEET

3800-FM-WSFR0086 Rev. 12/2008

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-Initials* Example 20040212-0312-XYZ	- - Date Time Initials	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) Basin Survey, (2) Cause / Effect, (3) Fish Tissue, (4) Instream Comprehensive Evaluation [ICE], (5) Point-of-First-Use, (6) SERA, (7) Antidegradation [Special Protection], (8) Toxics, (10) Use Attainability, (11) WQN, (12) Limestone, (13) Low-gradient [Multihabitat]

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 method: Std. kick screen: ☐ D-frame: ☐ Other: ☐ method?: _____
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 warm water, low gradient streams)*	
#9 Condition of Banks + #10 Bank Vegetation = 24 or less *(20 or less for warm water, low gradient streams*	
Total habitat score 140 or less for forested, cold water, high gradient streams *(120 or less for warm water, low gradient streams)*	
Habitat Comments:	

Special Condition
Use this block to describe conditions that justify attainment/impairment 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 Attainability, 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	TAXA	#	QA
1			26		
2			27		
3			28		
4			29		
5			30		
6			31		
7			32		
8			33		
9			34		
10			35		
11			36		
12			37		
13			38		
14			39		
15			40		
16			41		
17			42		
18			43		
19			44		
20			45		
21			46		
22			47		
23			48		
24			49		
25			50		

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 ☐ Non-wadeable (Boatable)

Habitat Parameter	**Riffle/Run – Wadeable & Non-wadeable**			
	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 & Non-wadeable (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-3 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 _____

Method: ☐ 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	Dive
	% Boulder:	% Boulder:
	% Cobble:	% Cobble:
	%Gravel:	%Gravel:
	%Sand:	%Sand:
	%Silt:	%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	Dive
	% Boulder:	% Boulder:
	% Cobble:	% Cobble:
	%Gravel:	%Gravel:
	%Sand:	%Sand:
	%Silt:	%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	Dive
	% Boulder:	% Boulder:
	% Cobble:	% Cobble:
	%Gravel:	%Gravel:
	%Sand:	%Sand:
	%Silt:	%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	**Quantity Rocks Sampled**
1/2 Downstream			All	
3/4 Upstream			> 25 meters	
3/4 Downstream			> 25 meters	
Left Descending Bank			All	
Total / % Closure				

% 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

pennsylvania
DEPARTMENT OF ENVIRONMENTAL PROTECTION

COMMONWEALTH OF PENNSYLVANIA
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 DATA SHEET

pennsylvania
DEPARTMENT OF ENVIRONMENTAL PROTECTION

BACTERIA MONITORING

FIELD DATA SHEET

Project: _____ ***Round for samples:*** ***OF 5***

Weather & Physical Conditions

Weather within past 24 hours? SUNNY - PARTLY CLOUDY - OVERCAST - RAIN - OTHER_____

Precipitation within the past 24 hours? NONE - TRACE - LIGHT - HEAVY

Present weather? SUNNY - PARTLY CLOUDY - OVERCAST - RAIN - OTHER _____

Precipitation currently? NONE - TRACE - LIGHT - HEAVY

Is the water cloudy? NO SOMEWHAT VERY

Sediment odor? NORMAL SEWAGE OIL OTHER_____

Flow of stream/capacity status of impoundment: LESS THAN NORMAL - NORMAL - GREATER THAN NORMAL

Date: _____ Collector: _____

Stream Name	Site ID# OR Coordinates	Time (24 hour clock)	Sample Number Collector ID+Sequence Number	Check if Blank	Check if Dup
				☐	☐
				☐	☐
				☐	☐
				☐	☐
				☐	☐
				☐	☐
				☐	☐
				☐	☐
				☐	☐
				☐	☐
				☐	☐

Stream Name	Site ID# OR Coordinates	Time (24 hour clock)	Sample Number Collector ID+Sequence Number	Check if Blank	Check if Dup
				☐	☐
				☐	☐
				☐	☐
				☐	☐
				☐	☐
				☐	☐
				☐	☐
				☐	☐
				☐	☐
				☐	☐
				☐	☐
				☐	☐
				☐	☐
				☐	☐
				☐	☐
				☐	☐
				☐	☐
				☐	☐
				☐	☐
				☐	☐
				☐	☐

TOTAL NUMBER OF SAMPLES COLLECTED = _____

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

pennsylvania
DEPARTMENT OF ENVIRONMENTAL PROTECTION

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:

1. Open the IntraDEP page (http://intradep/).
2. Click the "Oracle Applications" link.
3. 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.

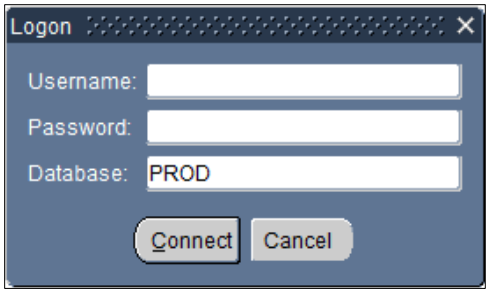


Figure 1: The Logon pop-up window.

4. Click in the *Username* field and enter a user name.
5. Click in the *Password* field and enter the password.
6. Click in the *Database* field and enter "PROD".
7. 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.

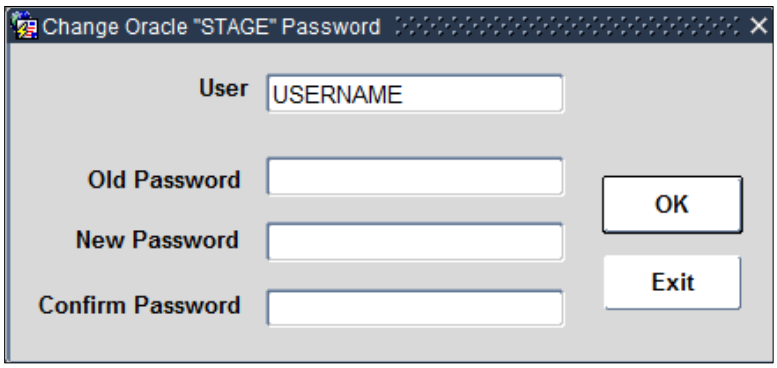


Figure 2: The "Change Oracle Password" pop-up window.

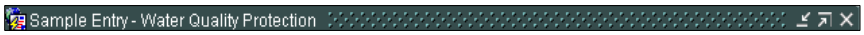
8. Click in the *Old Password* field and enter the current password.
9. Click in the *New Password* field and enter a new password.
10. Click in the *Confirm Password* field and enter the new password a second time.
11. Click the OK button.
12. A message in the hint line at the bottom of the screen will indicate if the password was successfully changed.

<u>SIS OVERVIEW</u>

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

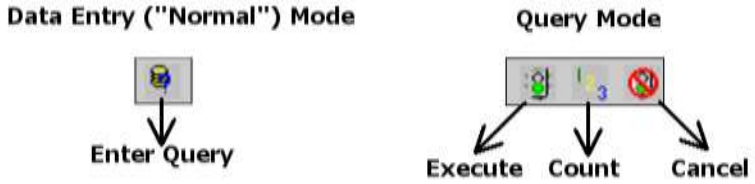
The HELP button provides the technical properties of the field in which the cursor resides.

Exit (CTRL + Q)

The EXIT button closes the current screen.

<u>QUERY MODE</u>

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.

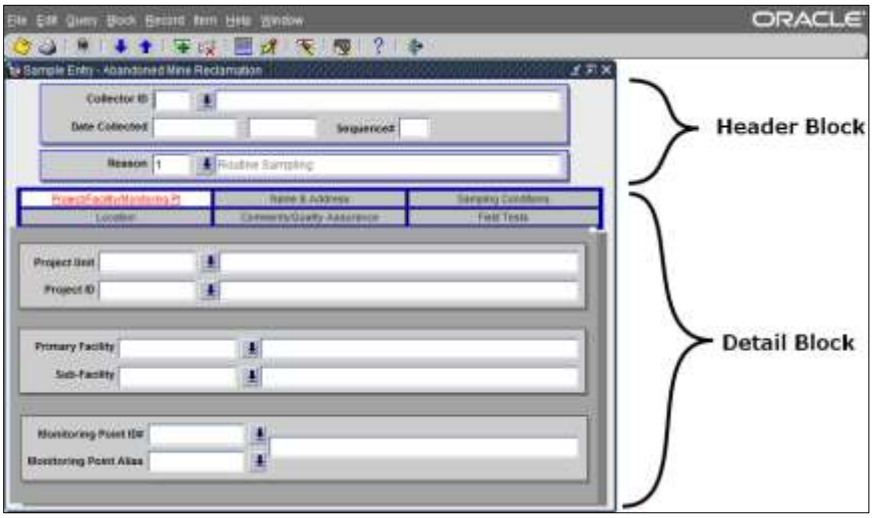


Figure 3: The SAMPLE ENTRY Screen with the header and detail blocks identified.

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.

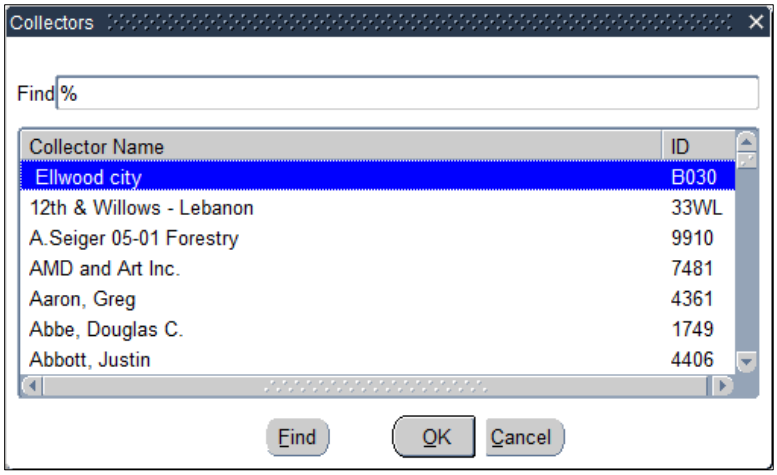


Figure 4: A list of values with the first value selected.

Using the list of values button:

1. Click the list of values button to the right of the field and a list of values pop-up window displays.
2. 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.

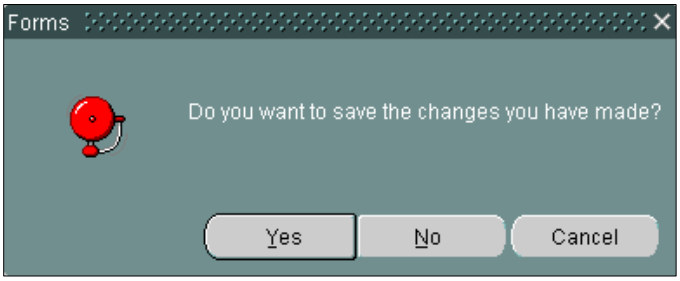


Figure 5: A pop-up message confirming an action.

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.

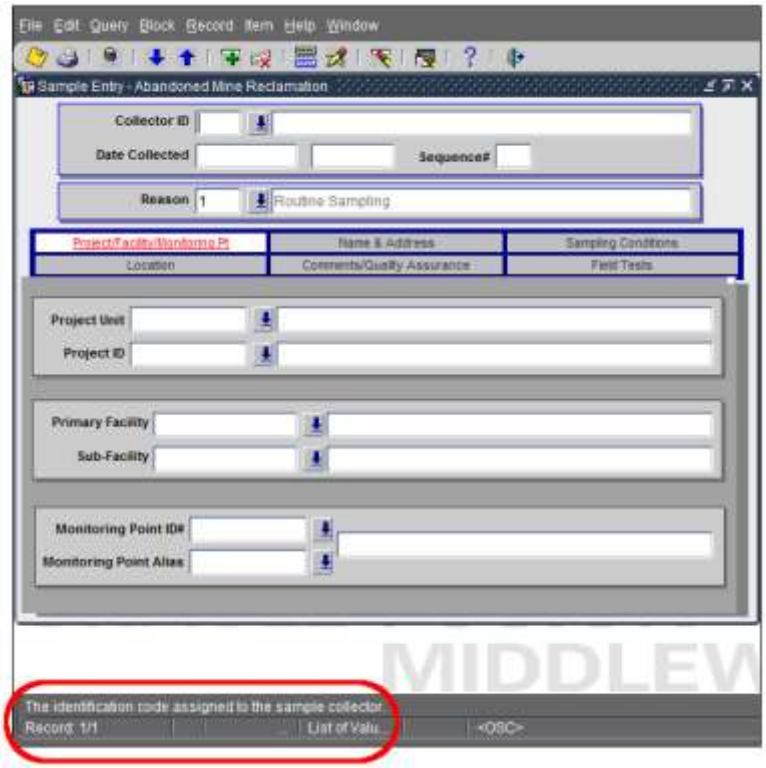


Figure 6: The hint line displaying a description for the *Collector ID* field.

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.

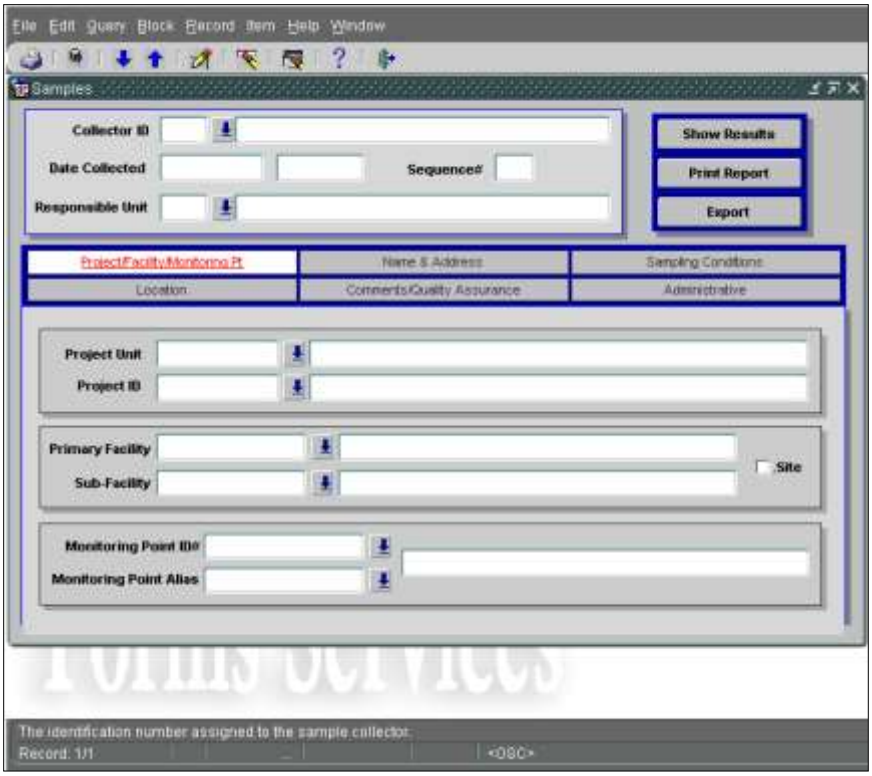


Figure 7: The samples screen.

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.

1. Access the SAMPLES Screen and then click the ENTER QUERY button on the toolbar or press the [F7] key.
2. 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.
3. If known, enter the date that the sample was collected. Press the [TAB] key.
4. If known, enter the time that the sample was collected. Press the [TAB] key.
5. If known, enter the sequence number assigned to the sample.
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.

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.

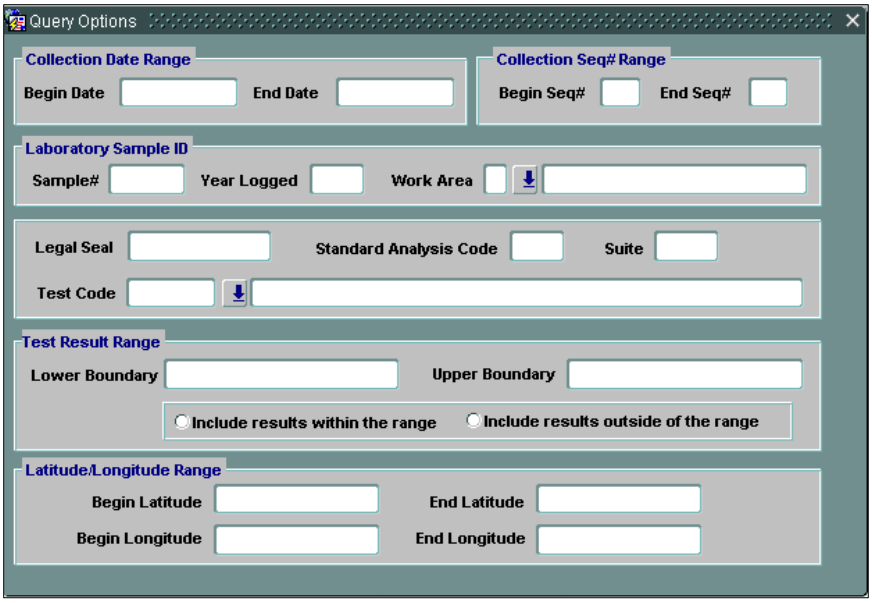


Figure 8: The "Query Options" pop-up window.

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.

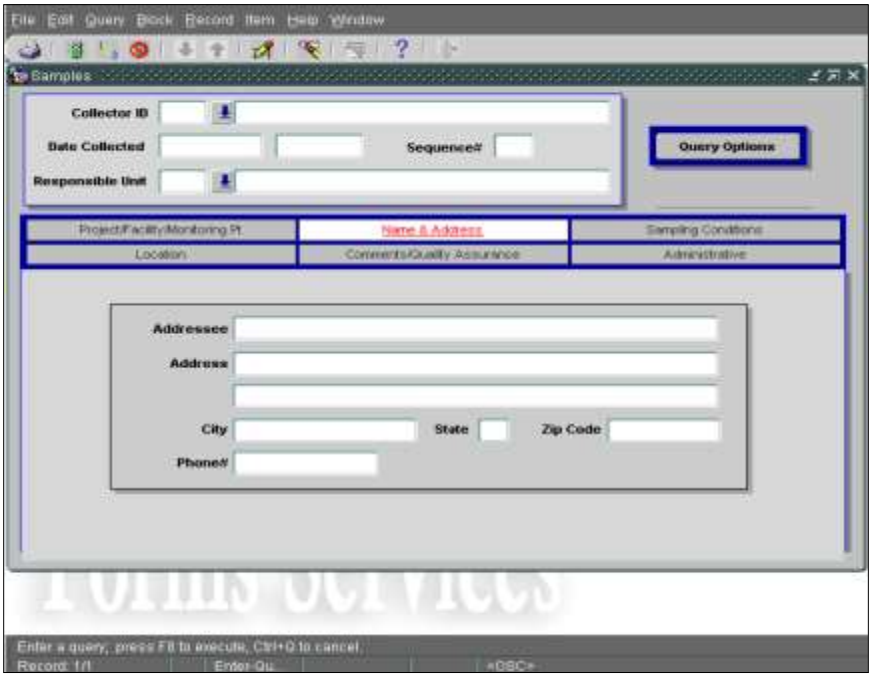


Figure 9: The samples screen with the Name & Address TAB selected.

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:

<u>Sample Query Header Block</u>

- Collector ID*
- Sequence#
- Date Collected
- Responsible Unit*

<u>Project/Facility/Monitoring Pt TAB</u>

- Project Unit*
- Primary Facility*
- Monitoring Point ID#*
- Project ID*
- Sub-Facility*
- Monitoring Point Alias*

<u>Name/Address TAB</u>

- 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 Method*
- Medium*
- Initial Flow
- Units*
- Depth To Water
- Determination*

Location TAB

- State*
- Municipality
- Longitude
- Reference Point*
- Location Method*
- 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.

1. Access the SAMPLES Screen and query a sample record.
2. Click the SHOW RESULTS button to display the SAMPLE ANALYSES/RESULTS Screen, which includes the lab results of sample testing.

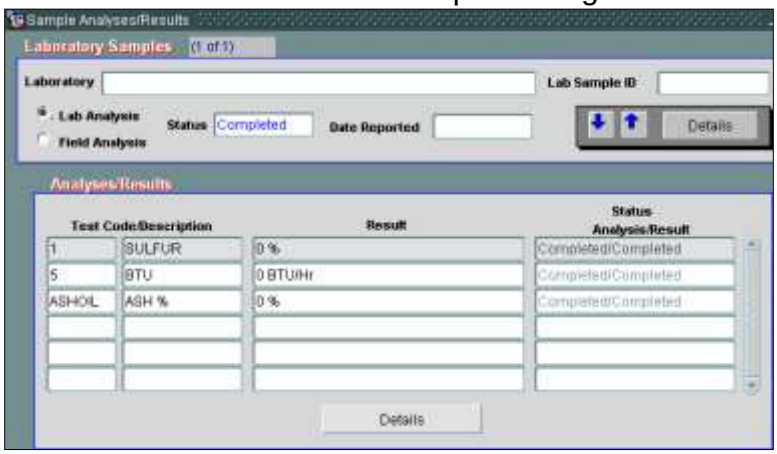


Figure 10: The SAMPLE ANALYSES/RESULTS Screen.

3. 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.
4. 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.

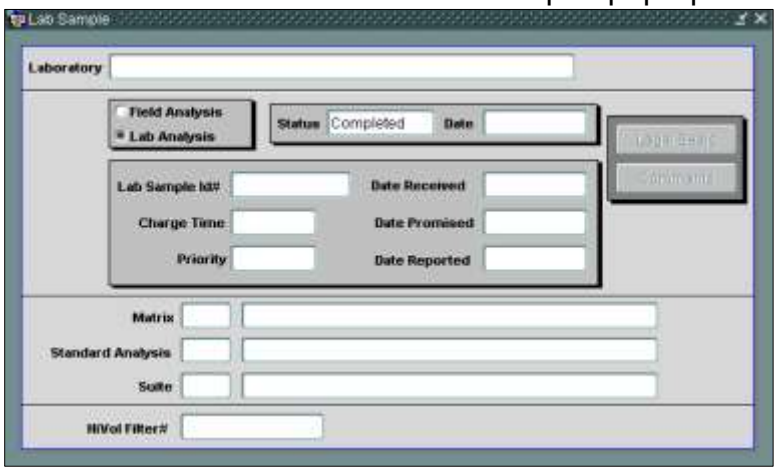


Figure 11: The “Lab Sample” pop-up window.

5. If available, click the LEGAL SEALS or COMMENTS button to view additional details.
6. Click the ☒ to exit the pop-up window and return to the SAMPLE ANALYSES/RESULTS Screen.
7. 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.

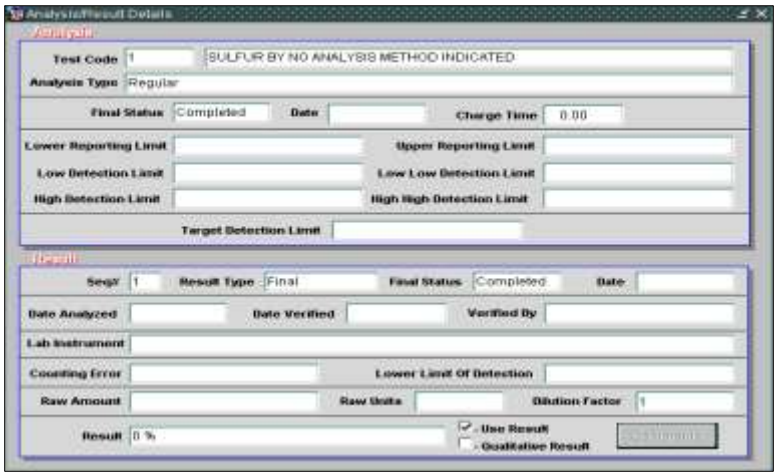


Figure 12: The “Analysis/Result Details” pop-up window.

8. If available, click the COMMENTS button to view additional comments.
9. Click the ☒ to exit the pop-up window and return to the SAMPLE ANALYSES/RESULTS Screen.
10. Click the ☒ again to return to the SAMPLES Screen.
11. 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.

12. 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.
13. 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.

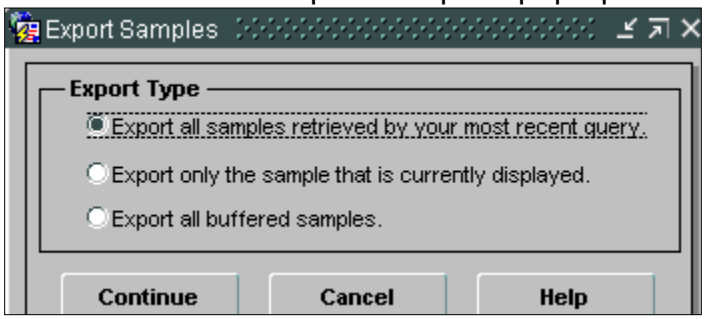


Figure 14: The "Export Samples" pop-up window.

3. Select which samples to include in the report and then click the CONTINUE button. The EXPORT SAMPLES Screen displays.

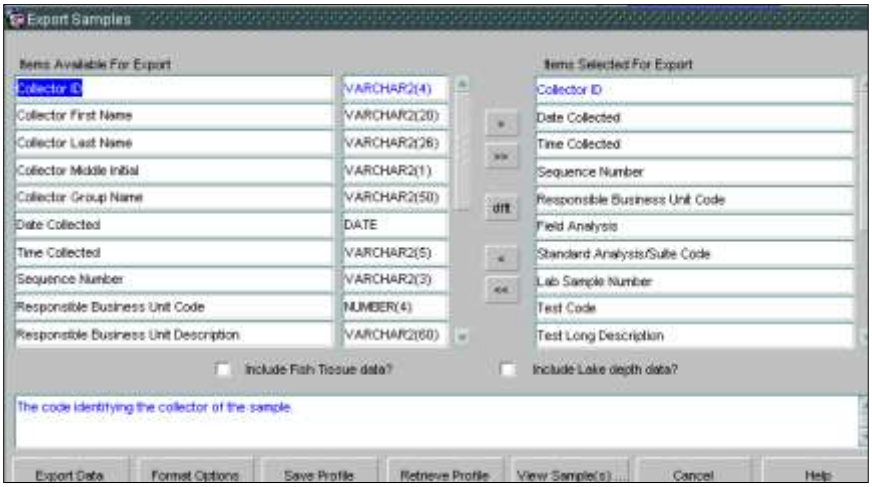


Figure 15: The EXPORT SAMPLES Screen.

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.

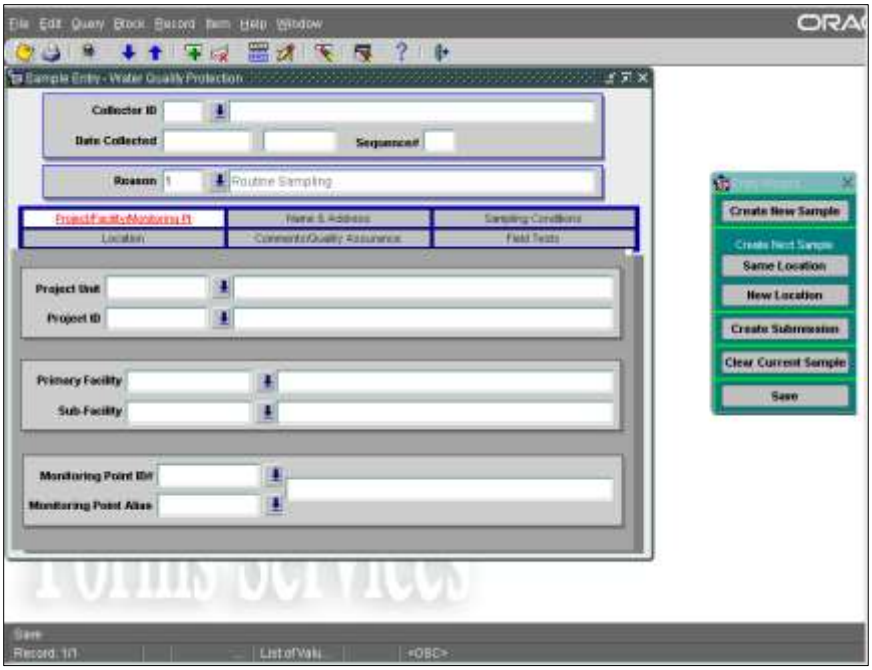


Figure 16: The SAMPLE ENTRY Screen.

> 🕮 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.

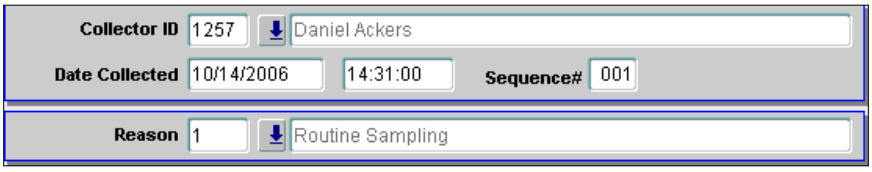


Figure 17: A sample header.

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.

1. Click the Project/Facility/Monitoring Pt TAB.

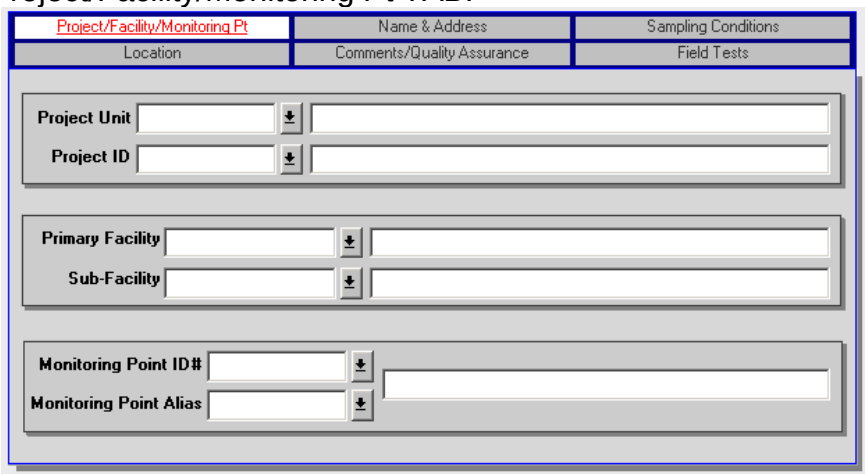


Figure 18: The Project/Facility/Monitoring Pt TAB.

2. 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.
3. If the sample was collected to monitor an existing primary facility and/or sub facility, complete the following steps:
   a. 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.
   b. Optionally, select a sub-facility by clicking the list of values button to the right of the *Sub-Facility* field.
4. If the sample was collected at a particular monitoring point, identify the monitoring point using one of the following methods:
   a. Enter the Monitoring Point ID assigned to the monitoring point and press the [TAB] key.
   b. Enter the Monitoring Point Alias and press the [TAB] key.
   c. 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)

5. 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.
6. 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.

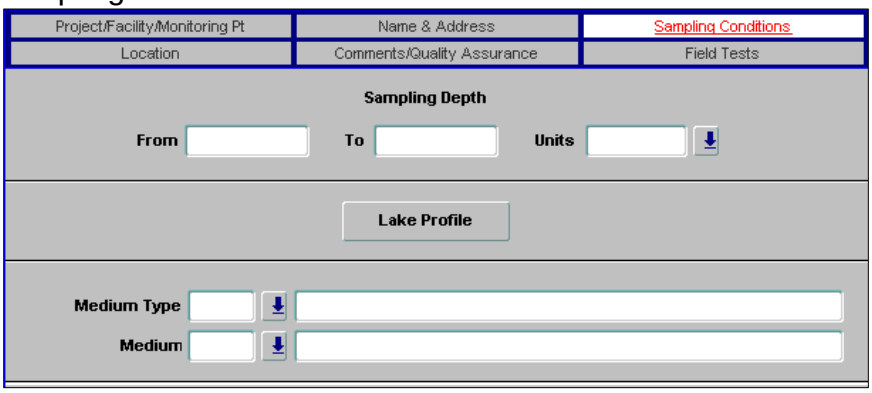


Figure 19: The Sampling Conditions TAB.

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.

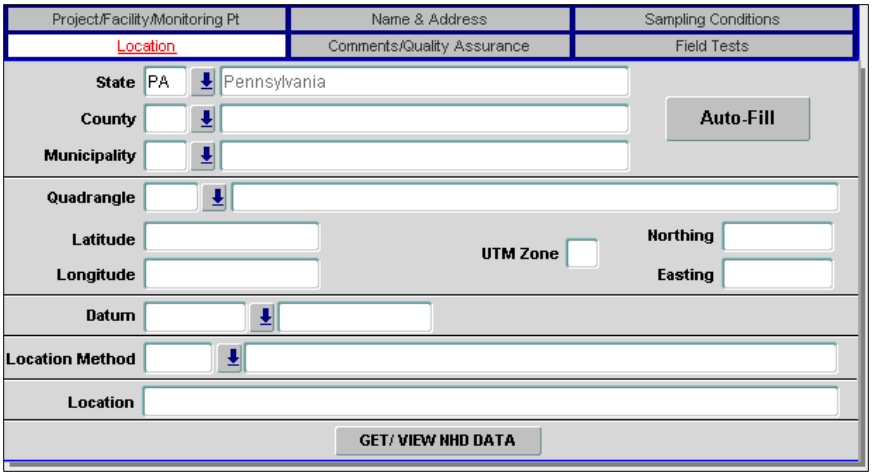


Figure 20: The Location TAB.

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.

<u>Linking the Sample to a NHD Record</u>

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.

1. Click the Location TAB.
2. 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.

3. Click the GET/VIEW NHD DATA button at the bottom of the Location TAB. The NHD pop-up window displays.

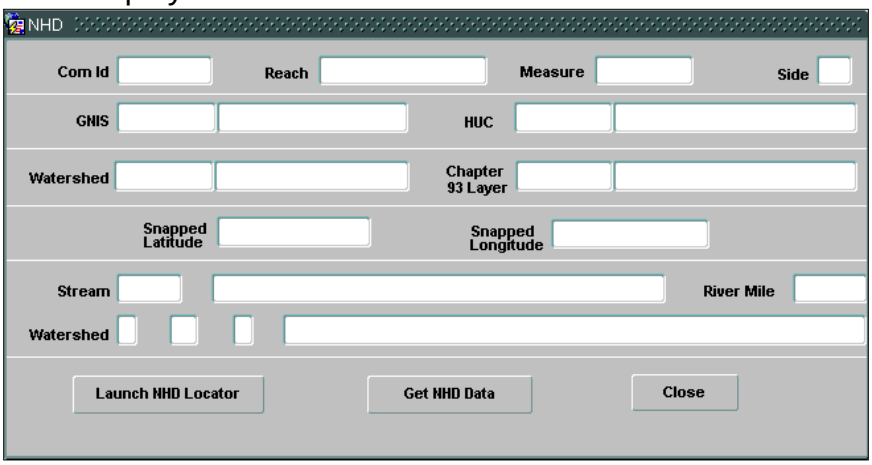


Figure 21: The NHD pop-up window.

4. Click the LAUNCH NHD LOCATOR button at the bottom of the screen.

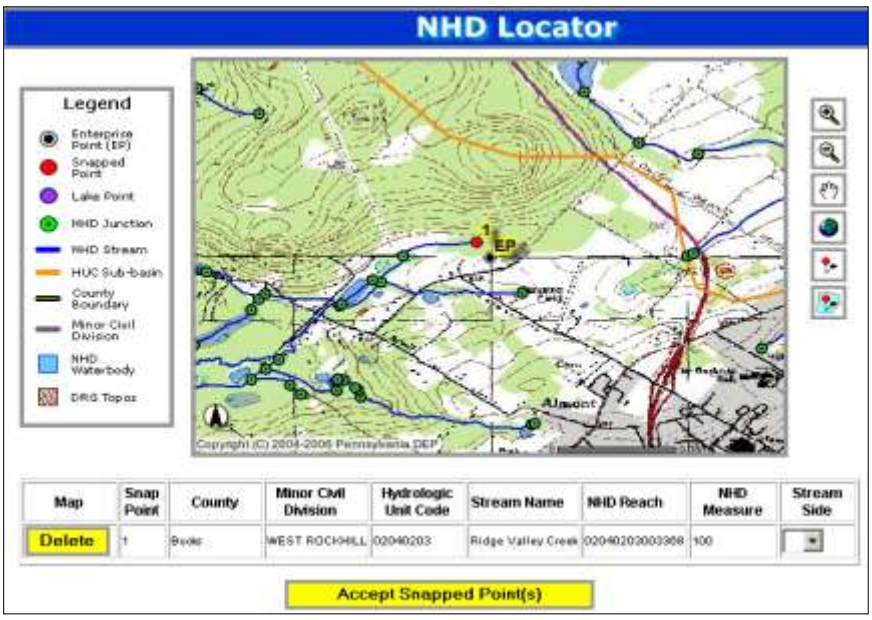


Figure 22: The NHD locator.

5. 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.

6. 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.
7. Click the GET NHD DATA button to add the NHD record created via the NHD Locator Tool to the sample.
8. 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.

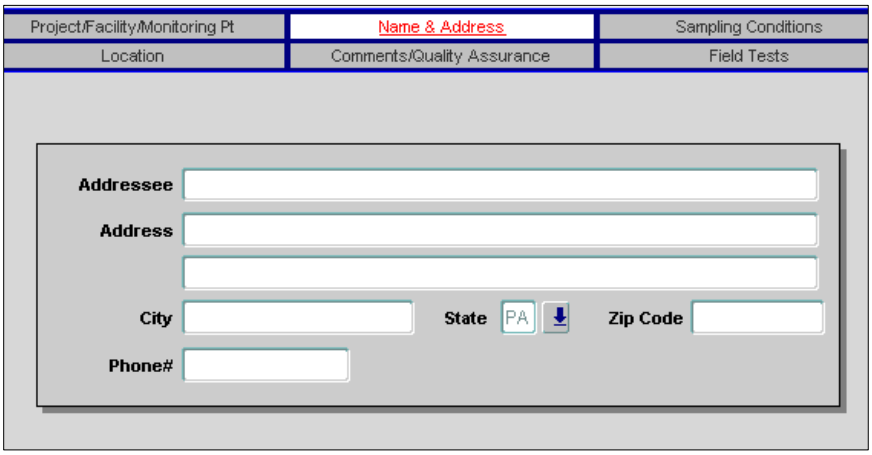


Figure 23: The Name & Address TAB.

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.

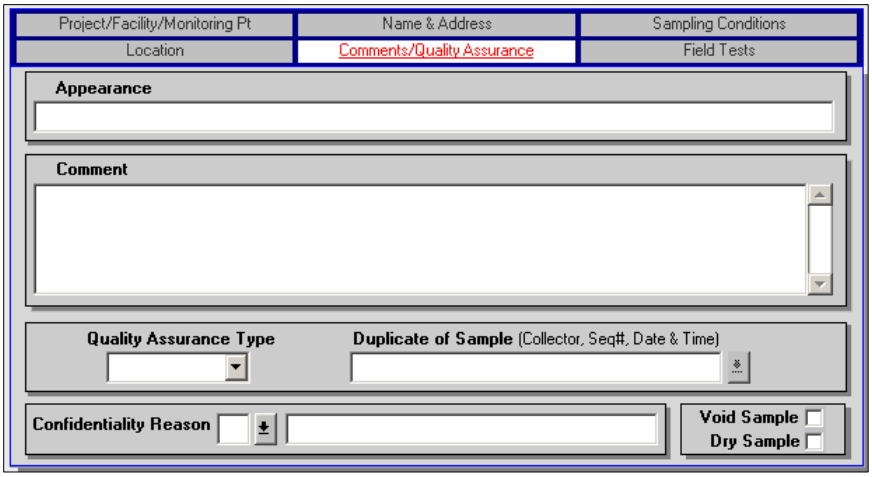


Figure 24: The Comments/Quality Assurance TAB.

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.

1. Click the Field Tests TAB.

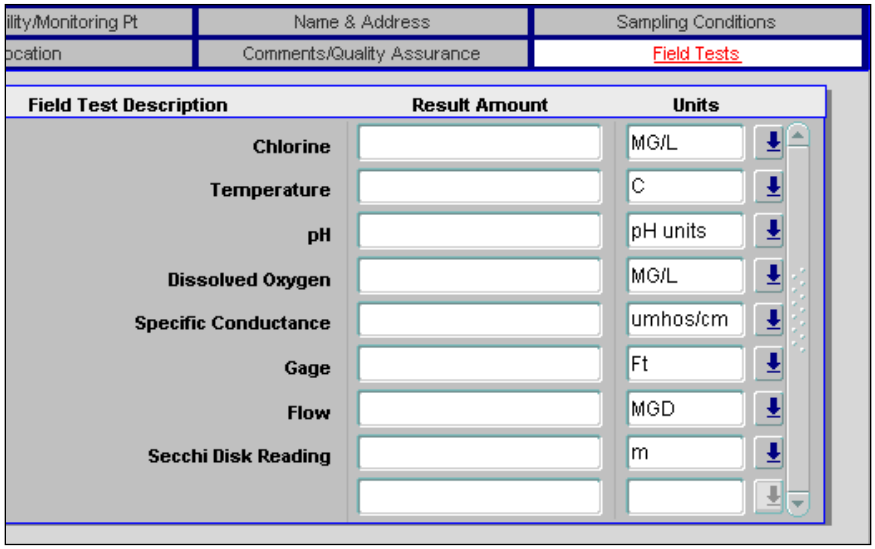


Figure 25: The Field Tests TAB.

2. 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.

3. Enter the amount for the test. Press the [TAB] key.
4. Update the unit of measurement if necessary.
5. Repeat the previous steps all applicable field tests are entered.
6. 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.

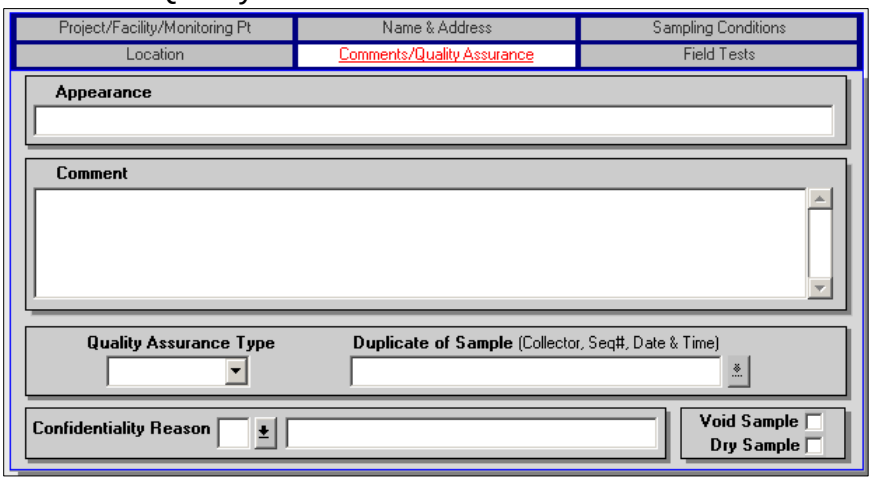


Figure 26: The Comments/Quality Assurance TAB.

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.

1. Access the SAMPLE ENTRY Screen by clicking the Samples Menu and then clicking the Sample Entry command.
2. Click the ENTER QUERY button on the toolbar and query for a sample as described in the
3. *QUERYING FOR SAMPLES* section of the user guide.
4. Update the fields described in the *CREATING SAMPLE RECORDS* section of the user guide.
5. 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.

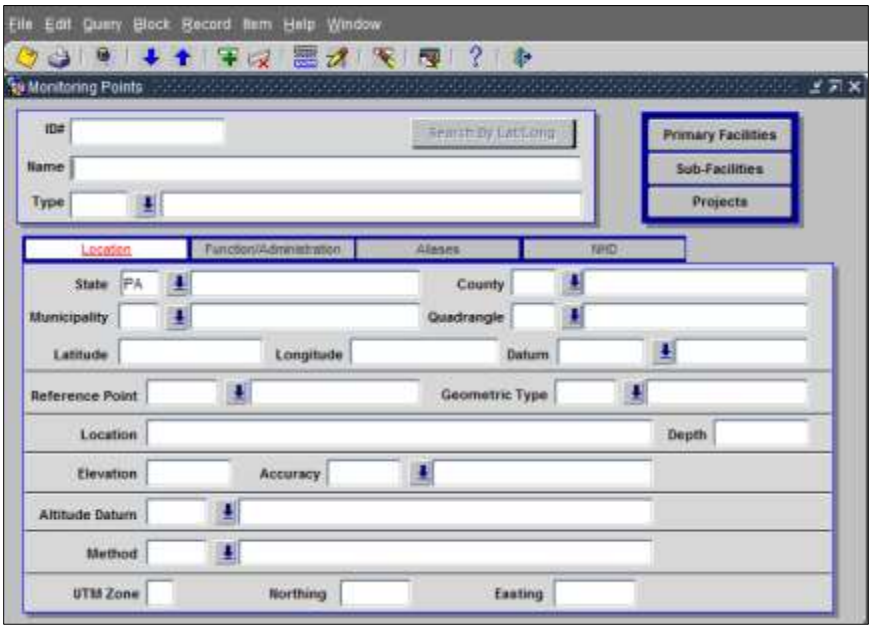


Figure 27: The MONITORING POINTS Screen.

Creating a New Monitoring Point

Step 1: Header Block (required)

Use the header block to identify the monitoring point name and type.

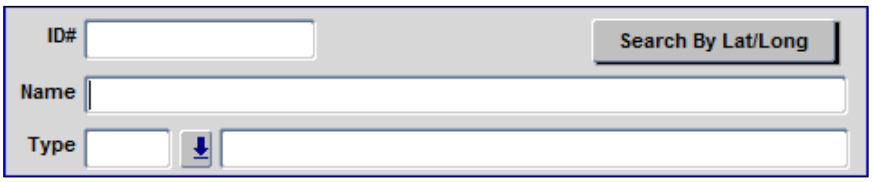


Figure 28: The monitoring point header block.

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.

<u>Step 2: Location TAB (required)</u>

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.

1. Click the Location TAB.

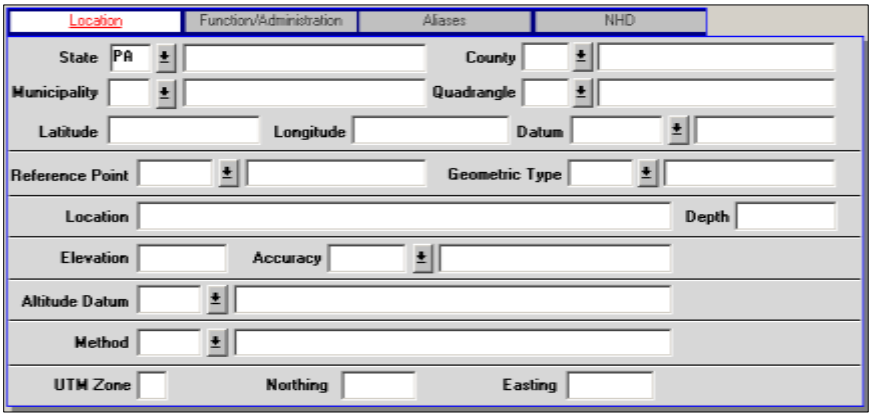


Figure 29: The Location TAB.

2. The state defaults to “PA”. Update if necessary.
3. Select a code identifying the county in which the sample was collected using the list of values button.
4. Optionally, select the code identifying the municipality in which the sample was collected by using the list of values button.
5. Enter the latitude where the sample was taken in Degrees-Minutes-Seconds (e.g. 40-15-42.8394).
6. Enter the longitude where the sample was taken in Degrees-Minutes-Seconds (e.g. -76-52-46.9194).
7. 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.
8. 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.

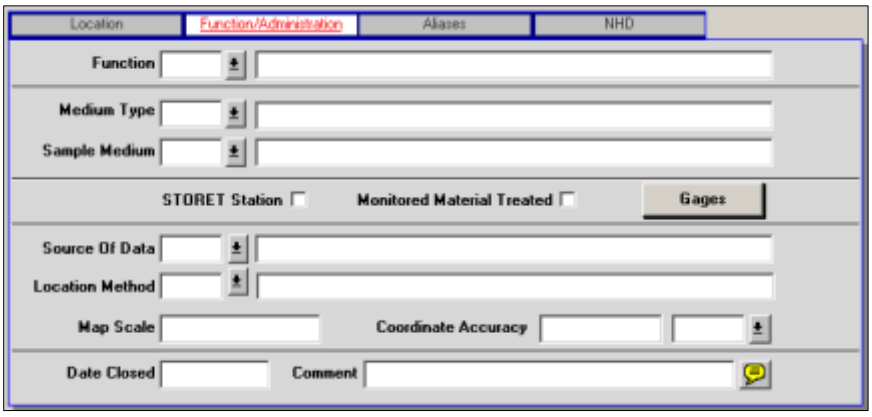


Figure 30: The Function/Administration TAB.

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.

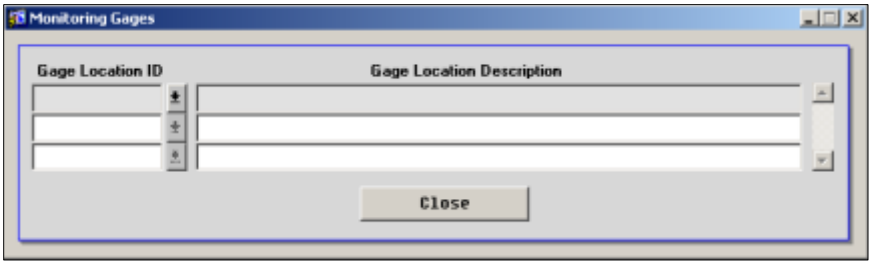


Figure 31: The “Monitoring Gages” pop-up window.

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.

<u>Step 4: Aliases TAB (optional)</u>

Use the Aliases TAB to create identifiers for the monitoring point in addition to the ID generated by SIS.

1. Click the Aliases TAB.

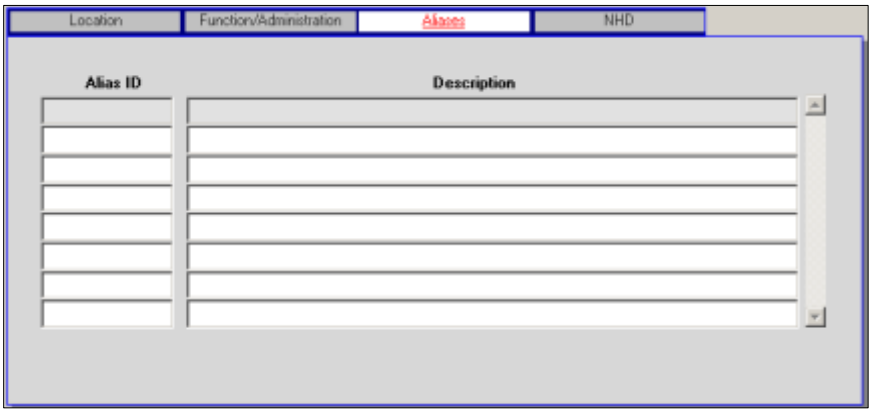


Figure 32: The Aliases TAB.

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.

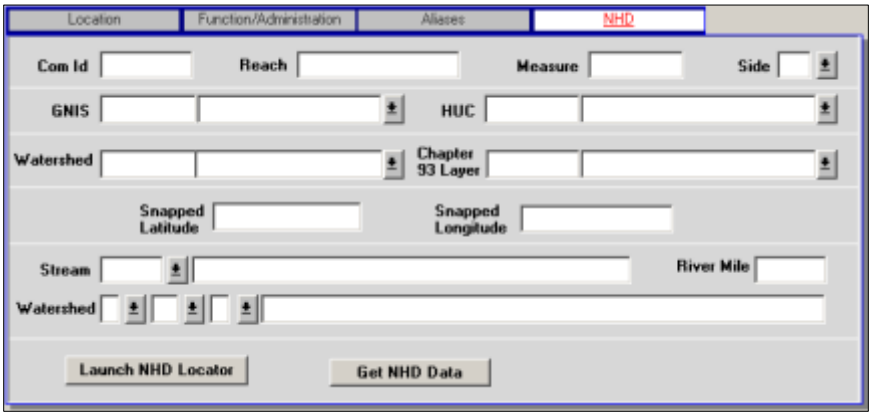


Figure 33: The NHD TAB.

5. Click the LAUNCH NHD LOCATOR button at the bottom of the screen and the NHD Locator Screen displays.

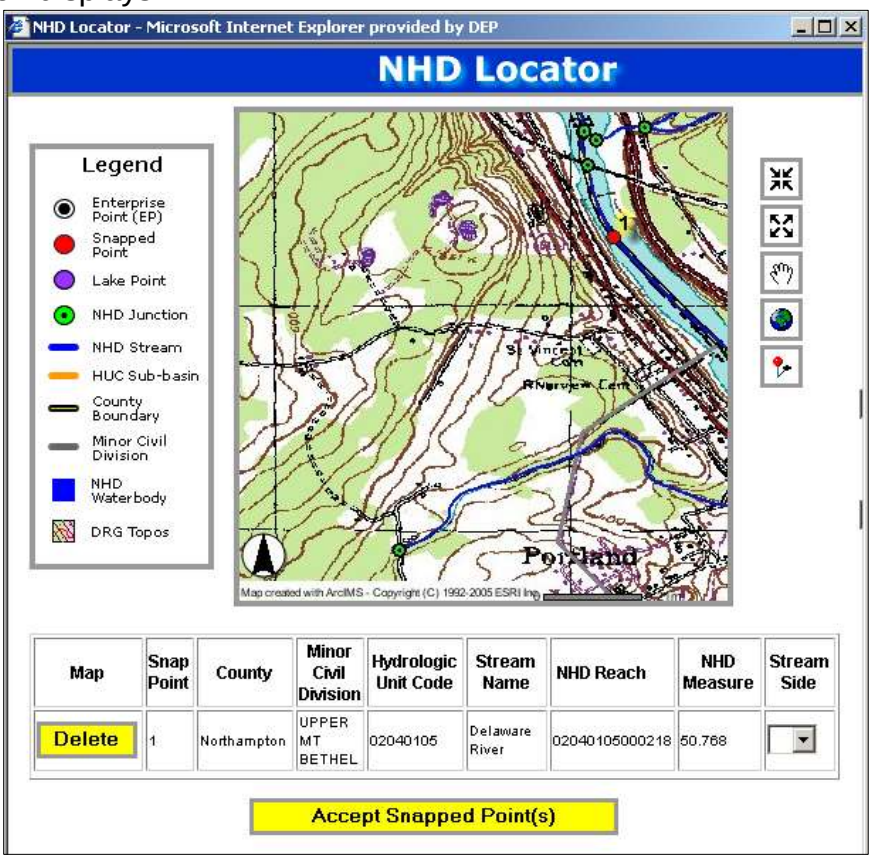


Figure 34: The NHD locator.

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 *e*FACTS 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.*

1. Query an existing monitoring point or create a new one.
2. Click the PRIMARY FACILITIES button and the “Monitoring Point/Primary Facilities” pop-up window displays.
3. Click the CREATE RECORD button on the toolbar or press the [F6] key.

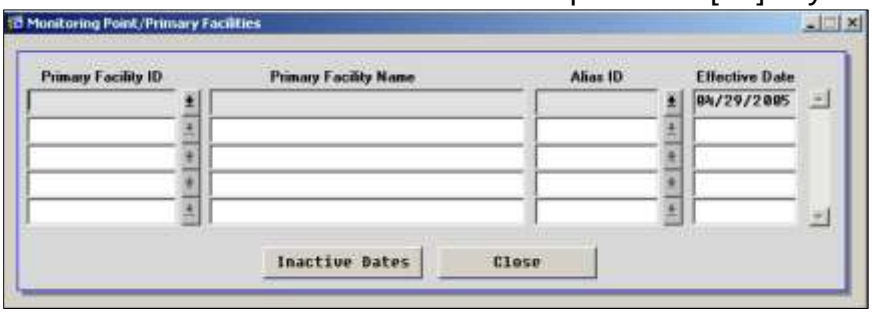


Figure 35: The “Monitoring Point/Primary Facilities” pop-up window.

4. 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:
   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. Update the effective date if necessary.
7. Click the SAVE button on the toolbar or press the [F10] key.
8. 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.

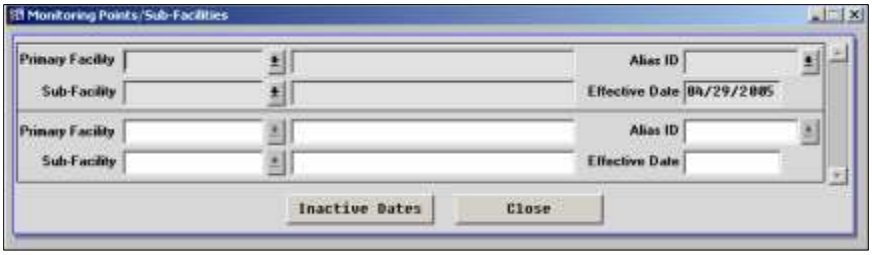


Figure 36: The “Monitoring Point/Sub-Facilities” pop-up window.

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.

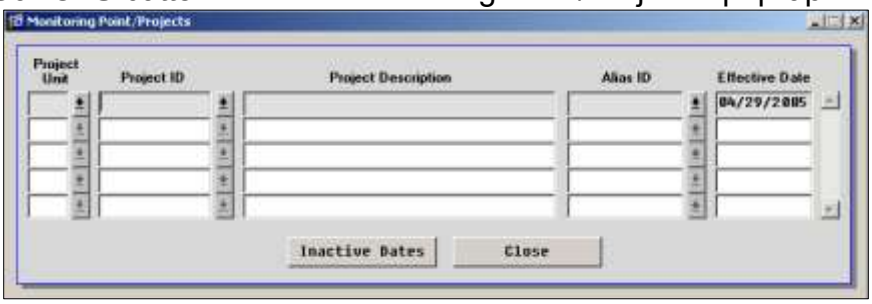


Figure 37: The “Monitoring Point/Projects” pop-up window.

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.

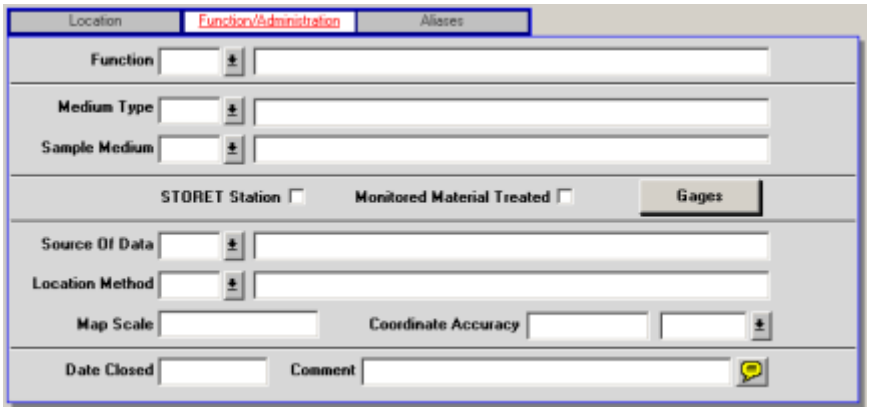


Figure 38: The Function/Administration TAB.

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.

1. Click the Reports Menu, select Sample reports, and then click the Long/Short Sample report command. The LONG/SHORT REPORTS Screen displays.
2. 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.

3. Enter a collector ID.
4. Enter a beginning sample collection date in the *SAMPLE BEGIN DATE* field.
5. If necessary, change the numbers in the *BEGIN* and *END SEQUENCE NO.* fields
6. 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.

1. Click the Reports Menu and then click the Missing sample header/results command. The MISSING HEADER/RESULTS REPORTS Screen displays.
2. 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.
3. Select a Collector Group, Business Unit, or Collector to run the report for from the lists of values.
4. Enter a collection date range for either the Missing Header or Missing Results report.
5. 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:
   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. 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

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:					Bottle Notes: (N-normal; MNF-metals non-filtered; MF-metals filtered; B-bac't; others: indicate)
		Temp (ºC)	DO (mg/L)	pH	Cond. (umhos)	Alkalinity (mg/L)	
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

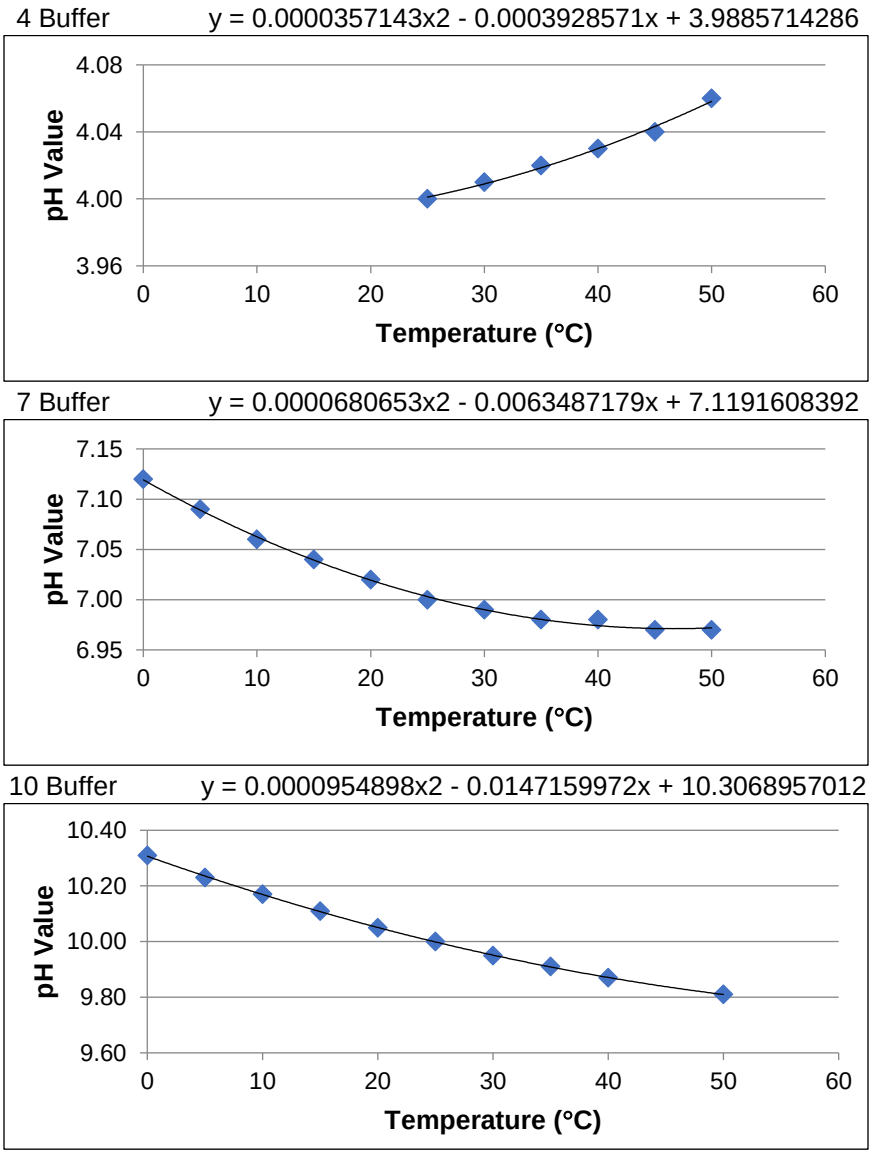


Figure 1. Second-order polynomial regression lines fitted to temperature-corrected pH values for BDH buffers.

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 (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	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	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	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	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	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**

Parameter	Optimal	Suboptimal	Marginal	Poor
7. Riffle Frequency	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	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	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.
_____	**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 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.
_____	**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 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.
_____	**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 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**

TOTAL _____

C-2 RIFFLE/RUN PREVALENCE HABITAT DATA COLLECTION ABBREVIATED (ONE-PAGE) FORM

Physical Habitat Evaluation Form for Riffle/Run Prevalence	GIS Key (YYYYMMDD-hhmm-User):
Waterbody Name:	Location:

Parameter	Optimal	Suboptimal	Marginal	Poor
1. Instream Cover (Fish)	mix of boulders, cobbles, submerged logs, undercut banks or other stable habitat			
	> 50%	50% to 30%	30% to 10%	< 10%
	20 19 18 17 16	**15 14 13 12 11**	**10 9 8 7 6**	**5 4 3 2 1**
2. Epifaunal Substrate	Riffles as wide as stream; lengths extending twice the widths. Well-developed riffle and run. Abundant cobble.	Riffles as wide as stream; lengths less than twice the widths. Abundant cobble. Boulders and gravels common.	Riffles not as wide as stream; lengths less than twice stream widths. Runs may be lacking. Prevalence of gravels, big boulders or bedrocks. Some cobbles.	Riffles or runs rare or absent. Prevalence of big boulders and/or bedrocks. Cobbles rare or absent.
	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 surrounded by fine sediment			
	< 25%	25% to 50%	50% to 75%	> 75%
	20 19 18 17 16	**15 14 13 12 11**	**10 9 8 7 6**	**5 4 3 2 1**
4. Velocity/Depth Regimes	**shallow-fast**	**shallow-slow**	deep-fast	deep-slow
	Four present	Three present	Two present	One present
	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. No dredging.	Some channelization (bridge abutments). Past dredging or channelization (over 20 years ago), but not recent.	New embankments on both banks. 40% to 80% of reach channelized or disrupted.	Banks gabioned or cemented. > 80% of reach channelized or disrupted.
_____	**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. Less than 5% of bottom affected by sediment deposition.	Some new increase in bar formation, mostly from coarse gravel. 5% to 30% of bottom affected. Slight deposition in pools.	Moderate deposition of new gravel and/or coarse sand on bars. 30% to 50% of bottom affected. Deposits at obstructions, constrictions, and bends. Moderate deposition in pools.	Heavy deposits of fine material. Increased bar development. More than 50% of bottom changing frequently. Pools almost absent due to substantial deposition.
_____	**20 19 18 17 16**	**15 14 13 12 11**	**10 9 8 7 6**	**5 4 3 2 1**
7. Riffle Frequency	Distance between riffles divided by stream width			
	5 to 7	7 to 15	15 to 25	> 25
	Riffles relatively frequent. Variety of habitat.	Riffles infrequent.	Occasional riffle or bend. Bottom contours provide some habitat.	Almost all flat water or shallow riffles. Poor habitat.
	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 banks. Minimal channel substrate exposed.	Water fills > 75% of channel. < 25% of channel substrate exposed.	Water fills 25% to 75% of channel and/or riffle substrates 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	Banks stable. No evidence of bank erosion or 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. "Raw" areas frequent along straight sections and bends. On side slopes, 60% to 100% of banks have erosional scars.
	20 19 18 17 16	**15 14 13 12 11**	**10 9 8 7 6**	**5 4 3 2 1**
10. Bank Vegetative Protection	Streambank surfaces covered by vegetation			
	> 90%	90% to 70%	70% to 50%	< 50%
	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	Grazing, mowing, or other vegetative disruption minimal or absent. Almost all plants growing naturally.	Disruption evident, but not greatly affecting full plant growth. More than half of potential plant stubble height remaining.	Disruption obvious. Areas of bare soil and/or closely cropped vegetation common. Less than half of potential stubble height remaining.	Disruption extensive. Vegetation removed to 2" or less in average stubble height.
_____	**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 > 18 meters. No human activities impacting riparian zone.	Width 12 to 18 meters. Human activities minimally impacting zone.	Width 6 to 12 meters. Human activities impacting zone a great deal.	Width < 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**

TOTAL _____

_____

C-3 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. Epifaunal 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-4 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-5 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:**				