Sampling Protocols for Pennsylvania's Wadeable Streams

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Preface

Through the Divisions of Environmental Services, Fisheries Management and Habitat Management, the Pennsylvania Fish and Boat Commission's Bureau of Fisheries is responsible for a wide range of field and laboratory sampling activities on wadeable stream resources. These activities are shared with biologists working throughout the Commonwealth. While each Division has specific responsibilities and areas of expertise within the agency some overlap in sampling methods occurs. The purpose of this document is to provide the standard PFBC protocols for sampling wadeable streams. Wadeable stream sampling may consist of fish sampling, habitat analysis, water quality analysis, social measures, and benthic macroinvertebrate analysis. Any or all of these components may be sampled as part of a wadeable stream survey. The components sampled depend upon the objective of the stream survey. Please refer to the appropriate module for a description of the approved protocols for each type of sampling to be conducted. Strict adherence to the protocols identified in this document is vital to assure that quality data are collected and available for use in Pennsylvania Fish and Boat Commission decisions regarding the management and protection of our aquatic resources.

MODULE A

Standard Electrofishing Protocols for Sampling Pennsylvania Wadeable Streams

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1. Introduction

Electofishing is one of the most efficient and widely utilized methods that the Pennsylvania Fish and Boat Commission employees use for collecting data on fish species assemblage and biomass. Module A details the field sampling protocols to be used for the majority of data collection needs. Occasionally, specific program evaluations require the development of specialized sampling protocols. Specialized protocols that address ongoing program evaluations are available in Modules B, C and D. Finally, field activities and electrofishing in particular carries inherent risks to both the participants and the environment. Strict adherence to safety protocols and biosecurity protocols are a must when conducting any field activity within the waters of the Commonwealth.

1.1 SAFETY PROGRAM

Pennsylvania Fish and Boat Commission (PFBC) field staff sample throughout Pennsylvania in locations where medical facilities may not be readily available. All employees must follow safety precautions when using sampling equipment. The Bureau of Fisheries has a Safety Committee which is responsible for maintenance and development of current safety procedures. The Committee also maintains the safety standard operating procedures document with which all personnel should be familiar. All personnel involved in electrofishing should be trained in first aid and CPR and should be familiar with standard electrofishing safety procedures and should have completed the USFWS electrofishing training course.

Sampling conditions are the primary safety factor to be considered during field activities. If field conditions, such as high flows or thunderstorms, raise the question of whether a sample can be safely collected, then decisions should always be made with the safety of personnel of primary concern. This same concern for safety of staff must be of primary importance when scheduling the amount of time to be spent in the field. Long days combined with strenuous physical activity increase the probability of accidents occurring. "**Safety first**" must always be the rule.

Employees should promptly report on-the-job accidents to the unit supervisor. If an accident occurs during field operations, the first responsibility of the team leader is to get first aid treatment for the injured employee; their second responsibility is to promptly notify the Division Supervisor.

1.2 BIOSECURITY

PFBC staff must clean all sampling gear following PFBC biosecurity protocols described in Module K.

1.3 PERSONNEL TRAINING

Most training (especially for seasonal employees/interns) is achieved on the job. However, it is recommended that a “dry run” be conducted for new employees at the office to help familiarize them with the equipment and techniques used during wadeable stream surveys. Inexperienced crewmembers should be assigned to less technical tasks until they become familiar with the operation. Dry run training should include the familiarization of equipment use, a review of biosecurity protocols, and a review of electrofishing safety protocols. All employees engaged in

electrofishing operations must be familiar with the Policy for Electrofishing Operations (Module E).

2. Fish Sampling Protocols for Wadeable Streams

Pennsylvania contains approximately 138,400 km (86,000 miles) of flowing water (Pennsylvania Department of Environmental Protection 2009). A great majority of these flowing waters are streams that are typically wadeable throughout the year. For the protocols outlined below, wadeable streams are defined as waters in which the majority of the habitat can be adequately sampled without the need for a boat. In 1976, the Pennsylvania Fish Commission (now Pennsylvania Fish and Boat Commission, PFBC) began to evaluate the fisheries of wadeable streams. Since that time, our wadeable streams' efforts have included estimating population densities of trout as well as conducting stock assessments of warmwater game fish; in particular, smallmouth bass.

Objectives of sampling often include: (1) assessment of the status and trends of sportfish populations important to anglers, (2) characterizing the biological integrity of the stream or river using fish metrics, (3) identifying and coarsely characterizing fish populations of interest to anglers, (4) assessing year class strength and (5) assessing outward physical condition by determining the percentage of fish with disease, fin erosion, lesions and tumors (DELT's) (Appendix A). Each of these approaches employs procedures and strategies that may target all species, a group of species, or a certain lifestage, depending upon the objectives of the study. Characterizing fish populations often involves assessing fish numbers and computing a variety of biological statistics which describe the population in terms of density, growth, mortality, condition, trophic guild, and other measures.

Due to financial and manpower constraints, it is usually extremely difficult to sample an entire length of a wadeable stream. Therefore, it is necessary to sample a representative reach(es) of a wadeable stream in order to depict the conditions of its entire length. It is necessary to devise a sampling design to provide as accurate an assessment of the true conditions as possible, with acceptable cost and effort. The purpose of sampling a stream is often to determine specific population characteristics of a target fish species. Sampling a portion of the population provides the ability to estimate population characteristics including but not limited to abundance, density, and biomass, and the means, variance, standard errors and confidence intervals of these estimates that can be used to define the reliability of the estimates (Platts et al. 1983). It is important to note that any sample is an estimate of the population and is subject to error. However, sampling does not always cause a reduction in reliability just because fewer measurements are taken. Rigorous data collected on a small portion of a population can often provide more reliable estimates than spurious data collected on a large portion of the population (Platts et al. 1983).

Stream sections are the primary unit used to define management objectives within flowing waters in Pennsylvania. Stream sections vary in length, but are usually a minimum of 3 km, except when management objectives dictate otherwise (e.g., special regulation areas). Various parameters are currently used by fisheries management staff to divide streams into homogeneous sections including, but not limited to, biological data from previous surveys (specifically, trout biomass estimates and the associated biomass classification), instream habitat, physical size, gradient, riparian ownership, fish community structure (i.e., cold water vs. warm water), water quality, and established or potential special fishing regulations. Generally, a combination of the

preceding parameters is used to determine the stream section boundaries. In some small streams, the entire stream may be managed as one section. For the purposes of probability sampling, a section, because it is considered relatively uniform biologically and physically (albeit habitats vary) may be considered a statistical stratum.

Some streams in Pennsylvania that support wild trout populations are also stocked with trout of hatchery origin. For most surveys, it is necessary to distinguish between wild trout and hatchery trout. An experienced biologist distinguishes wild trout from hatchery trout based on distinct physical characteristics; such as deformed fins, fin wear, and to some extent lack of coloration especially when present with fin irregularities. It is also advantageous to know if the water being surveyed receives stockings of hatchery trout prior to the beginning of the survey, regardless of origin PFBC, Coop Nursery, or private club, organizations or citizens, so that crew members will be better prepared to focus on these distinguishing characteristics.

2.1 SAMPLE SITE SELECTION

All stream surveys begin in the office. The first step is to establish a clear survey purpose, because the survey purpose dictates the type and intensity of data to collect. In the case of general inventories, investigators should also review the stream's history, its current sectioning strategy, and possibly determine the location of National Pollution Discharge Elimination System (NPDES) outfalls prior to field sampling. NPDES discharges can influence sample site selection and data collection procedures. On-line sources for NPDES information include:

eMapPA: http://www.emappa.dep.state.pa.us/emappa/viewer.htm
eFacts: http://www.ahs2.dep.state.pa.us/eFactsWeb/
ECHO: http://www.epa-echo.gov/echo/compliance_report_water.html

Sample site selection within a stream section is a critical step in collecting a sample that is representative of the fish populations and habitat in the stream section of interest. Important management decisions are based on the results of stream surveys; therefore, it is imperative that sample sites are selected properly. Once it is determined that water quality is similar throughout a stream section, instream habitat is one of the major factors that drive the abundance of fish present. For example, deep pools with good physical structure usually harbor much higher densities of trout than do shallow riffles. Thus, to obtain a representative sample of fish populations inhabiting a stream section, we must obtain a representative sample of the available instream habitat. Sample sites that should be selected reflect conditions that are representative of the stream section as a whole. Unless it is required for a specific survey purpose, the survey leader should avoid intentionally selecting the very best or poorest habitat for sampling within a stream section. When possible, a reconnaissance trip should be conducted by field staff to become familiar with the stream section and the diversity and distribution of instream habitat prior to conducting the fish survey or sufficient time should be allotted to conduct reconnaissance the day of the survey.

In most cases, sample sites that have been previously sampled should receive priority over sites in which no data have been collected to maintain long-term datasets that have been established for specific sample sites. The use of fixed sites allows managers to control for considerable site-level variability in fish populations that can occur due to physical differences between sites including hydrology, local channel characteristics, and woody debris abundance (Wills et al.

2006). The use of fixed sites minimizes such differences and increases our ability to detect and describe temporal trends in fish abundance (Wills et al. 2006).

2.2 SAMPLING CONSIDERATIONS ON PUBLIC AND PRIVATE OWNED WATERS

Stream sections flow through riparian corridors where ownership ranges from public to private to a mix of public and private. In some cases stream sections flow through areas of private ownership that are closed to trespass. Since these waters are closed to the angling public, there would be limited circumstances where sampling would be required by PFBC staff. In these cases, the survey leader should obtain landowner permission prior to conducting any sampling activities on stream segments that are closed to trespassing. To maintain good landowner relations, when possible, it is recommended that the survey leader contact the landowner for permission to conduct stream examinations on private owned stream sections that are open to public angling. If public property is available within the stream section and the habitat therein is similar to the remainder of the section, it is recommended that the sample site(s) be established on the public property to increase the likelihood of long-term access to the site.

2.3 SAMPLE SITE LOCATIONS AND NUMBER OF SAMPLE SITES

The location of sample sites within a stream section will be based on the instream habitat and other physical characteristics to ensure that the minimum number of mesohabitat units are surveyed and a representative sample is collected. Mesohabitat refers to geomorphically defined habitat areas in a stream such as pools, riffles, runs, and glides (Roni 2005). When only one site is being sampled in a stream section, the site should be located approximately in the middle of the section in an area that contains the minimum number of mesohabitat units and the overall characteristics of the site are as representative of the entire section as possible. If two or more sites are being sampled in a section, the sites should be spread throughout the section to provide samples of the fish populations throughout the length of the stream section. If distinct differences are present in the habitat and physical features within the section, then one site should be located within each of the different reaches. For example, if a stream section is channelized for a considerable portion of the section, then one site should be sampled in the channelized portion and one site should be sampled in the free-flowing portion of the section. When possible, sample sites should not be established directly adjacent to the upstream or downstream limits of the section in case the section limits need to be adjusted.

When possible, sites should be located at easily identifiable and readily accessible locations (e.g., small tributaries, trail crossings, power line crossings, etc.) that will aid in future site identification, but these landmarks should not impact instream habitat and fish populations. With the use and widespread availability of GPS units the need to establish sites at readily identifiable locations has somewhat diminished; however, in areas of rugged terrain where satellite acquisition may be difficult, well defined landmarks may be preferred. Typically, sample sites should not include road or bridge crossings. Often, the effect of road crossings creates deep-water areas beneath and adjacent to bridges (Copeland et al. 2001). These "bridge pools" are typically deeper and have habitat that does not represent the rest of the stream section. While selecting sites long distances away from roads may be impractical, the impacts of road crossings may be minimized by sampling at least 100 m upstream of bridge crossings. In addition, areas where small outfalls can have a localized influence on water quality and fish populations should be avoided. There are some instances where sampling at a bridge crossing may be warranted. These include situations where the habitat created at the bridge crossing is representative of the

habitat in the remainder of the stream section, the habitat at a bridge crossing is so unique as to be the only habitat in the stream capable of supporting target species, and in situations where it is impossible to avoid sampling at a bridge crossing (i.e., urban areas).
Transitional and warmwater streams in Pennsylvania are located lower in the watershed, have less variation in instream habitat and are generally considerably longer than coldwater streams. For these reasons the PFBC recommends investigators sample 10% of the section length when surveying coldwater systems and 5% of the section length when surveying transitional or warmwater systems when conducting general status and trend inventories in wadeable streams during an initial inventory of the stream or stream section. Investigators may choose to sample less than the 10% and 5% recommended lengths when conducting a reinventory of the same stream or stream section when it is apparent that conditions have not changed since the initial survey. This should be accomplished by examining one or more sample sites (also referred to as stations) of at least 300 m in length. Thus, a 12-km long coldwater section would have four sites of 300 m each. A 12-km long transitional or warmwater section would involve sampling two sites of 300 m each. Depending on the homogeneity of habitat types the Area Fisheries Managers have the option to sample four sites of 150 m each. Sampling four short stations rather than two long ones in transitional or warmwater systems may increase the chance of sampling all habitat types, obtaining a more complete assessment of species composition, and discerning differences in stream reaches that may lead to changes in management strategies. Additionally, if a substantial change in fish species composition or habitat occurred from one sample site to the next, it is recommended that the researcher conduct an investigation of the stream between the two divergent sample sites to determine where the physical or biological change occurred. The determination of the appropriate sample site length and number of sites sampled is ultimately at the discretion of the survey leader and based upon the local characteristics of the individual stream to be surveyed. Many streams that are transitional or warmwater in nature in their lower reaches support wild trout in their headwaters. When sampling previously unsurveyed waters with the purpose of conducting a wild trout population estimate, investigators may want to consider marking the station at 150 m and at the full 300 m site length. As electrofishing proceeds and it becomes clear that the system is either transitional, warmwater in nature, or that a sufficient number of trout will not be collected to conduct a population estimate, the crew leader can then terminate electrofishing at the 150 m mark. Otherwise, the completion of the full 300 m long site is recommended to gather species occurrence data. Therefore, abundance estimates conducted on sample sites that meet these suggested minimum length limits and adequately sample the available instream habitat types should provide a reliable estimate of the abundance of the species of interest inhabiting the stream section.

Additional approved options, depending upon survey objectives, for determining appropriate station lengths are available through the U.S. Environmental Protection Agency's Environmental Monitoring and Assessment Program (EMAP) and the U.S. Geological Survey's National Water-Quality Assessment Program (NAWQA). These options provide standardized sample site lengths that increase in proportion to stream width and are long enough to incorporate local-scale habitat variability and large-scale assessments. To obtain representative samples of fish assemblages and available habitats, EMAP specifies that stream sample reaches should be 40 times their low-flow wetted width (Lazorchak et al. 1998) while NAWQA suggests that sample reaches should be 20 times their wetted width (Fitzpatrick et al. 1998).

Either the PFBC standard 300 m station length or a site length determined utilizing the EMAP or NAWQA are acceptable for use when conducting routine wadeable stream surveys with the exception that the minimum site length shall not be less than 300 m long on a coldwater stream

or 150 m on a transitional or warmwater stream regardless of stream width. Recent work conducted by Sweka (2010) determined that in order to obtain brook trout biomass estimates within ± 25% of the assumed true population size, a minimum station length of 350 m was required. The mean width of the streams sampled in this study was ≤ 4 m (John Sweka, personal communication). Similarly, Mason (2009) determined that a minimum station length of 250 m was required to obtain a representative sample of the available habitat types based on indices derived from thalweg profiles (e.g., gradient, residual pool length, and residual pool depth) in five central Pennsylvania wild brook trout streams. The recommended standard sample site lengths may be slightly adjusted so that the upstream and downstream limits of the sample sites utilize habitat breaks and natural barriers, such as shallow riffles, waterfalls, bedrock slides, etc., that aid in minimizing fish movement. In some cases, especially in low gradient streams that are commonly characterized by uniform habitat consisting of long runs or pools absent of riffles, it may not be possible to sample the minimum number of mesohabitat units recommended below. In these cases, the site may be considered complete and adequately sampled when a distance of ≥ 20 times mean wetted channel width or the minimum site length recommended below for each stream category is sampled, whichever is greater. Regardless of the method used to determine station length, when possible, a minimum of 10% of the section length should be sampled on coldwater streams and 5% on transitional or warmwater streams. When the survey purpose is to determine population estimates of warmwater gamefish species the minimum station length of 300 m should be examined and a minimum of 10% of the stream or stream section should be surveyed. Variations to the minimum site length may occur during the completion of special projects.

2.3.1 Small Streams: ≤ 5 m in mean width

Sample sites for streams ≤ 5 m in mean width should include at least three mesohabitat units and be a minimum of 300 m in length. Sample sites that are at least 300 m in length for streams ≤ 5 m in mean width will provide a site length that is > 40 times mean wetted channel width and should provide a representative sample of the fish assemblage, habitat types, and abundance estimate of trout or other specie(s) of interest within the sample site that can be extrapolated to a per km basis.

2.3.2 Medium streams: > 5 to 15 m in mean width

Sample sites for streams > 5 – 15 m in mean width should include at least three mesohabitat units and be a minimum of 300 m in length. Sample sites that are at least 300 m in length for streams > 5 – 15 m in mean width will provide a site length that is > 20 times mean wetted channel width and should provide a representative sample of the fish assemblage, habitat types, and abundance estimate of trout or other specie(s) of interest within the sample site that can be extrapolated to a per km basis.

2.3.3 Large streams: > 15 to 20 m in mean width

Sample sites for streams > 15 – 20 m in mean width should include at least two mesohabitat units and be a minimum of 400 m in length. Sample sites that are at least 400 m in length for streams > 15 – 20 m in mean width will provide a site length that is ≥20 times mean wetted channel width and should provide a representative sample of the fish assemblage, habitat types, and abundance estimate of trout or other specie(s) of interest within the sample site that can be extrapolated to a per km basis. Consideration should also be given to conducting multiple 300 m stations depending upon the complexity and length of the management section. A more representative assessment of the stream section would likely be obtained using multiple 300 m stations as opposed to fewer, longer stations.

2.3.4 Very large streams: > 20 m in mean width

Sample sites for streams > 20 m in mean width should include at least two mesohabitat units, when possible, and be a minimum of 500 m in length. The stream sections that fall into this category usually require considerably more manpower to sample and can be very difficult to sample due to their size and increased depth. Sample sites that are at least 500 m in length for streams > 20 – 25 m in mean width will provide a site length that is ≥ 20 times mean wetted channel width and should provide a representative sample of the fish assemblage, habitat types, and abundance estimate of trout or other species of interest within the sample site that can be extrapolated to a per km basis. Sample sites in streams wider than 25 m in mean width should include at least two mesohabitat units, when possible, and be a minimum of 500 m in length as well. Although the sample site length may not be ≥ 20 times mean wetted channel width, the physical demands on the crew and increased time involved, make it impractical to sample sites > 500 m in length. A more representative assessment of the stream section would likely be obtained using multiple 300 m stations as opposed to fewer, longer stations.

2.4 SAMPLING PERIOD

To maintain consistency with PFBC historical sampling and optimize gear efficiency, fish abundance estimates should be conducted during summer low-flow conditions. This is typically from early to mid-June through late September. Late fall surveys, although not typical, may be utilized when deemed necessary by an Area Fisheries Manager. Sampling during the same time period from year-to-year helps to reduce seasonal sampling bias. Additionally, young-of-year (YOY) trout are generally too small to be efficiently captured before late May or early June. Smallmouth bass YOY are typically vulnerable in mid-July to mid-August. Individual streams that are part of a long-term monitoring program should be sampled at approximately the same time each year to reduce as much temporal variability as possible. Procedures exist to characterize DELTs in YOY fish collected in warmwater streams (Appendix A).

2.5 SAMPLING CONDITIONS

Sampling should not be conducted during high flows. If a rain event occurs during the sample that results in increased flow and turbidity, the sample should be abandoned and rescheduled. Avoiding poor water clarity will improve sampling efficiency and minimize sampling bias caused by changing water conditions. The crew leader will be responsible for making the decision as when to abandon a sample based on experience.

2.6 GENERAL ELECTROFISHING PROCEDURES

Sampling should start at the downstream limit of the site and proceed upstream. All personnel actively engaged in electrofishing should wear polarized sunglasses to aid in visibility and capture of fish. During most surveys, a crew uses one backpack electrofisher and consists of three persons - a data recorder/fish processor/bucket carrier and two electrofishers/netters. Members of the team are cross-trained and rotate tasks from site to site. As the team proceeds upstream, all habitats are sampled with the exception of pools that are too deep to work. Sample sites (or portions of a site) in which stream width exceeds the maximum that can be adequately sampled by the crew should be electrofished in a sinuous pattern or by electrofishing along one side of the stream followed by dropping back and electrofishing the opposite side of the stream.

The same general procedures used for backpack electrofishing samples are also used for tow-boat electrofishing samples. One crewmember pulls the tow-boat using a harness and is responsible for operating the remote safety switch in the event that the electrofishing unit must be immediately powered off; while the other two crew members operate the anode probes and electrofish and net the fish. The crew member pulling the tow-boat also carries a net and assists with netting fish. Depending upon distance and stream conditions between the electrofishers and the tow-boat live well the fish collected by the two electrofishers are passed to the crewmember pulling the boat, who in turn places them in the livewell held within the tow-boat. When appropriate the two electrofishers may also place netted fish directly into the livewell. When the livewell becomes full, the crew stops the sample at a habitat break such as a shallow riffle, and places the fish into live cars before proceeding upstream. Alternately, the crew may elect to float the tow-boat downstream from the stopping point and process the fish from the livewell and return them directly back into the water before continuing the electofishing run. In these instances the processed fish should be marked via fin clip to avoid double counting should some fish be captured during the continuation of the survey.

Effort should be directed toward netting the species targeted for the abundance estimate, such as trout. Otherwise efficiency will be reduced. Ideally, other non-target species should be collected during the time elapsed between shocking and netting the targeted species. If the fish species occurrence cannot be adequately determined due to a high abundance of the targeted species, a separate, shorter site should be established to determine species occurrence and the relative abundance of non-target species. Measures of relative abundance are based on the number of fish collected or observed in a 300 m sampling station. When sampling sites are shorter than 300 m the number of non-target species should be normalized to a 300 m site length. The ratings of relative abundance are as follows:

Length of Site	Rare	Present	Common	Abundant
100 meters	1	2 - 8	9 - 34	> 34
200 meters	1	2 - 17	18 - 67	> 67
300 meters	1 - 2	3 - 25	26 – 100	> 100

2.6.1 Gear Selection – Electrofishing

Most PFBC sampling directed at estimating fish abundance in wadeable streams is conducted using electrofishing as the primary tool. In general, alternating current (AC) electrofishing units are more efficient in low-conductivity waters (< 100 *u*s/cm) and direct current (DC) units are more efficient in intermediate to high-conductivity waters (> 100 *u*s/cm). Pulsed-DC units can work effectively in both high and low-conductivity water. Extreme conductivity, whether low or high, usually exceeds the capacity of most power sources and reduces efficiency (Reynolds 1996).

Prior to the start of any electrofishing operation, water conductivity should be measured to provide guidance regarding the necessary power that will be needed to adequately stun the fish. For backpack electrofishers, it is best to set the voltage to a low level, such as 75 volts, and test the effectiveness downstream of the sample site. Starting at an initially lower setting and adjusting the output upward provides a safeguard to overloading the equipment and adversely impacting the resident fishes within the electrical field should water conductivity be higher than anticipated. Electrofishing units should be turned off prior to making voltage adjustments to avoid damage to the electrofishing unit, such as arcing from contact to contact as the dial is

turned. Determining water conductivity in advance helps to provide a general idea of what voltage to use. Generally, the higher the conductivity, the lower the voltage needed to produce the required power (amperage; amps). Voltages should be adjusted to achieve an output of about 0.5 - 1.0 amps (amps = watts/volts) on the older Coffelt® backpack units, which is usually adequate for effectively stunning the fish based on staff observations. For other backpack units, follow the manufacturer's recommendations. It is recommended to use the lowest power that effectively stuns the fish. When using a pulsed-DC backpack electrofisher that allows for frequency control, the unit should be set at < 60 pulses per second (pps) or Hertz (Hz) to sample trout and 120 pps should be used to sample spiny-rayed fishes such as centrarchids.

The "straight" DC T&J manufactured tow-boat units have only two settings, 125 and 250 volts, and the engine throttle on the generator is used to control the output amps. These units are typically used to survey moderate to large streams with moderate to high conductivity and will not effectively stun fish in low (< 100 *u*s/cm) conductivity water. In general, staff's experience suggests that fish are effectively stunned with the T&J units at outputs of 5-8 amps.

There are currently three tow-boat configurations in use; 1) the "straight" semi-variable DC T&J Manufacturing (now BALDOR Generators) units, 2) the Smith-Root 1.5KVA pulsed AC (60 Hz)/DC (120 pps) variable voltage electrofisher, and 3) the Smith-Root 2.5 gpp pulsed AC and DC (7.5-120 Hz) variable voltage electrofisher. The "straight" DC T&J tow-boats have only two settings, 125 and 250 volts whereas the Smith-Root 1.5KVA pulsed AC/ DC unit's variable voltage settings range from 50 to 400 for AC mode and 75 to 566 for DC mode. The Smith-Root 2.5 gpp voltage settings range from 0 to 700 for AC mode and 0 to 1,000 for DC mode.

The following criteria are suggested for determining the number and type of electrofishing units to be used for conducting abundance estimates in wadeable streams. These are provided as guidelines and consideration should be given to crew experience and fitness and to the complexity of the habitat being surveyed.

≤ 5 m mean width: one battery-powered AC/DC backpack electrofisher **OR** one gasoline-powered AC backpack electrofisher

5 – 10 m mean width: one-two battery-powered AC/DC backpack electrofisher **OR** one gasoline-powered AC backpack electrofisher

10 – 20 m mean width: one-two gasoline-powered AC backpack electrofishers **OR** one DC tow-boat electrofisher with 2-3 anodes each (T&J tow-boats are generally inefficient in water conductivities < 100 *u*s/cm)

≥ 20 m mean width: two-three (or more) gasoline-powered AC backpack electrofishers **OR** one-two (or more) DC tow-boat electrofishers with 2-3 anodes each

(T&J tow-boats are generally
inefficient in water conductivities
< 100 *u*s/cm)

Whenever possible, sample gear should remain consistent through time at specific sites to reduce bias between gear types. Efficiency can vary considerably between electrofishers, which can in turn add a great deal of variability to abundance estimates. Therefore, it is important to use the same gear type from year-to-year, which will help to minimize these differences and increase our ability to detect temporal trends in fish abundance. Backpack and towed boat electrofishing field equipment check lists are available in Appendix B.

2.7 POPULATION ESTIMATES

The PFBC uses fish population estimates and the associated biomass estimates as benchmarks for species specific management programs and to alert the Pennsylvania Department of Environmental Protection of sensitive or important fish populations that merit special protection. The PFBC recognizes a variety of scientifically acceptable estimators of fish abundance and the choice of the appropriate estimator is left to the discretion of the biologist. The biologist may take into consideration the distance from the office to the survey station, accessibility to the survey station, instream habitat, water flows and the required precision of the estimate.

2.7.1 Block Nets

Most population estimates, including Petersen mark-recapture and removal/depletion type estimates, assume that the target population is closed to immigration and emigration during the sample period. Thus, when natural barriers such as shallow riffles, waterfalls, bedrock slides, etc., are not present or considered to be inadequate to minimize fish movement, block nets should be set at the top and bottom of the sample site. Block net mesh size should be no larger than 3/8" bar-measure to prevent movement of young-of-the-year (YOY) fish. As a general rule-of-thumb, block nets should be used when the aforementioned natural or man-made barriers are absent or inadequate.

2.7.2 Absolute Abundance Indices – Population Estimates

The two most common methods used to estimate fish abundance in wadeable streams in Pennsylvania are mark-recapture and removal/depletion estimates. Either method is appropriate for small, shallow streams that can be waded and thoroughly sampled with electrofishing gear. Mark-recapture estimates are also used in wider, deeper streams with complex habitat that makes them more difficult to sample. Removal/depletion estimates are generally not recommended for stream sections with these characteristics, as it can be very difficult to adequately deplete the population during each electrofishing pass, which reduces the precision and reliability of the estimate. Mark-recapture estimates for smallmouth bass on mid-size wadeable streams are not recommended as recapture rates have proven consistently low. Regardless of method, population estimates are calculated by the PFBC Agency Resource Database (ARD) for each 25-mm length group.

2.7.3 Petersen Mark-Recapture - Population Estimates

The standard estimator used for mark-recapture population estimates is the Chapman (1951) modification of the Petersen index (omitting –1, which has no practical significance; Ricker 1975)**:**

$$\hat{N} = \frac{(M+1)(C+1)}{(R+1)},$$

where M is the number of fish marked and released during the first electrofishing pass (i.e., marking run), C is the number captured and examined for marks during the second electrofishing pass (i.e., recapture run), and R is the number of recaptures (i.e., previously marked fish) collected in the second pass (Van Den Avyle and Hayward 1999). The Chapman modification provides an unbiased population estimate when $(M + C) \geq N$ and a nearly unbiased estimate if there are at least seven recaptures per 25-mm length group of marked individuals ($R \geq 7$) (Krebs 1989). If the condition $(M + C) > N$ is not met, N has negative bias (Ricker 1975). However, Ricker (1975) reported that the probability of statistical bias can be ignored if recaptures number 3-4 or more. Population estimates are calculated electronically by the ARD for each 25-mm size group when there are at least 3 recaptures (minimum required to ignore statistical bias and to calculate confidence intervals (Ricker (1975)). If there are fewer than 3 recaptures in a 25-mm size-group, the population estimate is calculated by summing the catch of unique individuals captured during the marking and recapture runs and no confidence intervals are computed.

Assumptions of Petersen mark-recapture estimates are (1) marked fish do not lose their marks prior to recapture, (2) marked fish are not overlooked in the recapture sample, (3) marked and unmarked individuals have an equal probability of being recaptured, (4) equal mortality rates of marked and unmarked individuals between the marking and recapture samples, (5) random distribution of marked and unmarked individuals, and (6) closed population (i.e., no immigration or emigration; Van Den Avyle and Hayward 1999).

Preferably, a 24 hr period should pass between the marking and recapture runs to provide for a sufficient recovery period to allow adequate mixing of marked and unmarked individuals (Mesa and Schreck 1989; Petersen et al. 2004). If environmental conditions, logistics, access, or other factors warrant that the recapture run be completed during the same day, a minimum of one hour should be retained between the first and second electrofishing pass to allow the marked fish to recover and become mixed with the unmarked fish. This approach is to be used only when unavoidable and should not be considered standard procedure.

During wild trout specific surveys when fewer than 30 trout are collected per 300 m of stream sampled during the marking run (first pass of a Petersen estimate), the survey is considered complete and catch per unit effort (CPUE) is used to provide an index of relative abundance rather than a population estimate. Capturing fewer than 30 wild trout per 300 m of stream during the marking run indicates the presence of a sparse population and even if a population estimate was conducted, the wild trout biomass classification for the stream section would be moderate at best. Thus, the additional staff time required to complete a recapture event is not warranted. The survey leader may also break the typical 300 m survey site into shorter segments (i.e., two, 150 m sites or three, 100 m sites) and may consider terminating the survey when it is clear that fewer than 30 targeted fish species will be collected over a 300 m site. Site length should not be less than 100 m.

2.7.3.1 Confidence Intervals for Petersen Mark-Recapture Population Estimates

Confidence intervals (95%) for Petersen population estimates are calculated by the PFBC ARD application and are based on the Poisson distribution. Ricker (1975) reported that the probability of a systematic statistical bias could be disregarded if there at least 3 – 4

recaptures. Thus, a confidence interval is calculated for each 25-mm size group with three or more recaptures.

2.7.3.2 Fish Processing Petersen Mark-Recapture Population Estimates

During the marking run (first electrofishing pass of a Petersen mark-recapture estimate) all fish species should be collected and identified, when possible. Targeted fish that are captured are measured to 25-mm size groups, marked with a small caudal fin clip and released in an area of low current near their location of capture. During the recapture run (second electrofishing pass of a Petersen mark-recapture estimate), trout are collected, held in live bags, live cars or the livewell of the tow-boat and processed at the end of the survey or at the discretion of the crew leader. In some cases, enough staff may be available that a separate processing crew can be used to conduct processing at the same time the remainder of the crew is conducting the electrofishing. Target fish are measured to 25-mm size groups and examined for fin clips (i.e., marks). Maximum total lengths (nearest mm) and weights (nearest gram) are taken from a sub-sample of 10 individuals per 25-mm size group for each species of targeted fish collected. The weights are used to calculate a mean weight for each size group to obtain biomass estimates. Field staff should use electronic scales for weight data collection to increase accuracy and efficiency.

Mean weights for each 25-mm size group of trout species can also be obtained from the PFBC ARD. The mean weights generated by the ARD are based on hundreds to thousands of weight measurements, providing precise estimates for each 25-mm size group that can be used to calculate biomass estimates. In these instances, the trout collected during the recapture run are measured to 25-mm size groups, examined for a caudal fin clip, recorded as "marked" or "unmarked", and released back into the stream after the crew has proceeded sampling upstream. However, if the survey purpose dictates or the crew leader feels that collecting weights from fish collected from the survey stream may be important, weights may be collected from a sub-sample of 10 individuals per 25-mm size group for each targeted fish species collected.

2.7.4 Removal/Depletion - Population Estimates

Two or three-pass removal estimates are also used to estimate fish abundance in wadeable streams. Generally, three-pass removal estimates are used, but occasionally two-pass removals are implemented when time limitations, a rain event, or other factor(s) prevent conducting a third electrofishing pass. Removal estimates are best suited for small streams in which an adequate number of fish can be removed on each sampling pass so that measurably fewer fish are available for capture and removal on each subsequent pass. Adequate depletion is generally defined as removing at least 50% of the population during each removal pass (Armour et al. 1983). Population estimates are calculated for two-pass removals using the formulas developed by Armour et al. (1983) and multiple pass removals (i.e., three or more passes) using a slightly modified version of the formulas developed by Zippin (1958) as described by Armour et al. (1983). Population estimates are calculated electronically by the PFBC ARD for each 25-mm size group. The removal method is most commonly used by PFBC staff in small, more remote streams that are logistically difficult for sampling.

Assumptions of the removal/depletion population estimates are (1) all individuals of the target population are equally vulnerable to capture, (2) vulnerability is constant between electrofishing passes, (3) equal sampling effort is exerted during each electrofishing pass, and (4) closed

population (i.e., no immigration or emigration; Van Den Avyle and Hayward 1999). Although it may be difficult and more time consuming to do so, field crews are reminded that it is critical that these assumptions are met to ensure that a reliable population estimate is obtained. Three electrofishing passes should be conducted unless (1) estimated probability of capture is 0.80 or higher on the first and second electrofishing pass (i.e., at least 80% of the fish present were captured during each electrofishing pass) or (2) fewer than 30 targeted fish species or family of fish are captured during the first electrofishing pass.

Capture probability is calculated as:

$$\hat{p} = 1 - \frac{(C_2)}{(C_1)},$$

where C_1 is the total number of fish captured during the first electrofishing pass and C_2 is total number of fish captured during the second electrofishing pass (Armour et al. 1983). If the field crew calculates the capture probability once the second pass is completed and it is 0.8 or higher, the sample can be considered complete and the population estimate can be considered reliable (Armour et al. 1983). When zero trout are captured during the second pass (i.e., estimated capture probability of 100% during first electrofishing pass) the sample is considered complete and a third pass is not conducted. In practice, capture probabilities of 0.80 and higher are uncommon even in small streams; thus, field crews should plan and allow time for three electrofishing passes.

Following the same guidelines as Petersen estimates, when fewer than 30 individuals of targeted fish species or family of fish are collected per 300 m of stream during the first pass, the survey is considered complete and catch per unit effort (CPUE) is used to provide and index of relative abundance rather than a population estimate.

If a crew is sampling a relatively wide, deep site and there is uncertainty as to whether the population can be adequately depleted, the option of computing a mark-recapture estimate, while conducting removal/depletion sampling is available (Lockwood and Schneider 2000). This is accomplished by marking and releasing fish during the first electrofishing pass, noting their recapture during subsequent passes, and ignoring marked fish for depletion estimate analysis or counting them as "recaptures" for mark-recapture analysis (Lockwood and Schneider 2000). This is not the preferred method but is available should circumstances arise where the crew leader feels that this is the only option available to collect a population estimate. If it is felt that the population can't be adequately depleted it is recommended that a crew conduct a standard Petersen mark-recapture population estimate.

If the field crew suspects that their capture efficiency was low during the first electrofishing pass and that they likely did not adequately deplete the population, the crew should conduct a mark-recapture estimate and return to the site the following day to conduct the recapture run. If this is the case, all fish captured during the first electrofishing pass should be marked and redistributed throughout the site in preparation of the recapture run the following day. If environmental conditions, logistics, access, or other factors warrant that the recapture run be completed during the same day, a minimum of one hour should be retained between the first and second electrofishing pass to allow the marked fish to recover and become mixed with the unmarked

fish. In this case, the sample would be completed after the second electrofishing pass and a mark-recapture estimate would be calculated.

2.7.4.1 Confidence Intervals for Removal/Depletion – Population Estimates

Confidence intervals (95%) are also calculated electronically by the PFBC ARD for removal estimates based on the formulas described by Armour et al. (1983). Again, a confidence interval is calculated for each 25-mm size group. If a 25-mm size-group is not depleted (i.e., more individuals collected during one of the subsequent electrofishing passes than in an earlier pass), the lower confidence limit is calculated as the sum of the total number of individuals caught during each electrofishing pass (i.e., $C_1 + C_2 + C_3$ for a three-pass estimate).

2.7.4.2 Fish Processing Removal/Depletion – Population Estimates

Fish processing methods used during removal estimates are similar to those employed during Petersen estimates. All trout collected during each electrofishing pass are placed in live bags/cars and processed downstream of the sample site by a crewmember, preferably below a barrier to prevent their migration back into the sample site or they are processed in total at the end of the survey. When possible, the processed fish should be redistributed back into the sample site when the survey is completed. Total lengths (nearest mm) and total weights (nearest gram) are recorded for a sub-sample of 10 individuals per 25-mm size group for each trout species. Once 10 individual lengths and weights are recorded for a 25-mm size group, the size group is considered to be "filled" and the remaining fish are simply tallied for that size group. Alternately, the crew leader may choose to not collect individual fish length and weight data. In this circumstance, a mean weight will be calculated for each 25-mm size group by the PFBC ARD based on all data within the database for that species and size group.

2.7.5 Relative Abundance Indices – Catch per Unit Effort (CPUE)

Single-pass electrofishing can be used to provide an index of relative abundance. The PFBC ARD calculates CPUE for all electrofishing samples, including population estimates, and is based on the number of fish captured during the first electrofishing pass (i.e., number/electrofishing hr). Single-pass electrofishing allows for reduced sampling time while limiting potential harmful impacts of electrofishing and handling stress on the fish.

Physical characteristics of the sample site play an important role in determining precision of relative abundance indices. Generally, those characteristics that increase habitat complexity, such as deep water, abundant woody debris, and wide streams, decrease capture efficiency which can make it more difficult to directly compare data from one site to another. CPUE is generally not the best choice if rigorous comparisons are needed to detect small changes in fish abundance.

CPUE can be used to assess the relative abundance of all species in the community or a particular species within the community (Filipek et al. 1994). In terms of gamefish, CPUE is most commonly used by PFBC staff to measure smallmouth bass abundance. Smallmouth bass are particularly difficult to recapture; thus, mark-recapture estimates are not recommended (Lyons and Kanehl 1993). In addition, most smallmouth bass populations in Pennsylvania occur in relatively large streams that are usually not suitable for removal/depletion population estimates either; however, satisfactory results have been obtained by incorporating the use of block nets to prohibit movement out of the survey reach.

2.7.5.1 Fish Processing Relative Abundance Indices - CPUE

The same sampling procedures should be followed as when conducting a Petersen mark-recapture population estimate, except that only the first electrofishing pass will be conducted.

2.7.6 Water Quality and Habitat Measurements

In addition to the biological data collected during stream assessments all general stream surveys conducted by the Division of Fisheries Management should also include physical and chemical measurements of the water. These measurements should include pH, total alkalinity, total hardness, specific conductance, and water temperature. When biological oxygen demand is expected to be high measures of dissolved oxygen should also be taken. The protocols for sampling the physical and chemical properties of water are available in Module J.

An assessment of both the instream habitat and riparian cover is also required when conducting general stream surveys. At a minimum, habitat should be assessed at each survey station using the U.S Environmental Protection Agency's Rapid Bioassessment Protocols for use in Wadeable Streams: Periphyton, Benthic Macroinvertebrates and Fish, second edition (Barbour et al. 1999; available in Module F). Furthermore, more general assessments of habitat including substrate type and riparian vegetation cover have proven useful in providing an understanding of the conditions of the stream at the time of the survey. Measures of substrate type, bank vegetation and shading are also available in Module F.

Report writing often occurs months after the actual survey date and recalling specifics about the survey is often challenging. In-depth field notes taken at the time of the survey also prove invaluable when it comes time to write the formal report of the survey.

2.7.7 Custom Surveys

There are circumstances that require specific sampling designs to be developed to meet the needs of the research question being addressed. These projects could be of short-term duration lasting only a single field season or may be long-term and span several field seasons. The Pennsylvania Fish and Boat Commission has conducted custom surveys to determine fish population abundance, CPUE and estimates of YOY abundance for certain species. In these instances the survey leader is required to follow the protocols developed for the individual custom projects. The Division of Fisheries Management currently has two active custom surveys including fourth and fifth order wadeable warm water streams (Module B), and smallmouth bass young-of-year in wadeable lotic habitats (Module C).

2.8 ANESTHETICS

MS-222 is widely used by the PFBC to reduce handling stress while processing fish. Gilderhus and Marking (1987) and Keene et al. (1998) reported that MS-222 concentrations of 60 - 80 mg/l effectively anesthetized rainbow trout in about 3 – 5 minutes, while allowing for fast recoveries (< 6 min) at water temperatures of 9 and 12^{o} C. Thus, a 60 mg/l concentration can be used as a benchmark when anesthetizing salmonids during sampling and the dosage adjusted from there based on the response of the fish. It is important that fish not be over-anesthetized, as the resulting effects may cause more stressful conditions than not using anesthetics and in some cases, mortality can occur. MS-222 may not be used when there is a potential for the fish to be harvested for human consumption within 21 days of the survey. Any other FDA approved methods to anesthetize fish may be used.

3. Field Data Recording and Office Data Entry

3.1 FIELD DATA FORMS

Standard data forms ensure that all necessary data fields are represented and data collected, provide the means of sharing data between areas more easily, and improve the efficiency of QA:QC. The following data forms should be used when conducting field surveys and are included as appendices:

Appendix C: Mark-recapture population estimates
Appendix D: Removal/depletion population estimates

All data should be entered into the PFBC ARD as soon as possible after completing the survey. Due to the requirements of field sampling, adequate time to enter the data may not be available until the field season is completed in early fall. Data entry into the PFBC ARD should receive priority once the field season is complete.

3.2 FISH IDENTIFICATION AND VOUCHERING

Most samples for population estimates require minimal fish sample processing in the office. Occasionally, uncommon species will be collected that need to be identified back at the office or sent to a professional icthyologist for identification. The samples should be preserved in the field by placing them live (after anaesthetization) in a 10% buffered formalin solution. Specimens larger than about 150 mm should be injected with the formalin solution using a syringe to ensure proper preservation. Fish should be kept in the formalin solution for 5-7 days and then thoroughly rinsed with water and allowed to soak in water overnight (Copeland et al. 2001). After one night in water, the specimens should be thoroughly rinsed again with water and placed in a 40% alcohol (ethyl or isopropyl) solution for long-term storage (Copeland et al. 2001).

3.3 REPORTING

Management reports should be formatted following the template provided by the PFBC central office (Appendix E). Reports should contain a brief introduction that provides pertinent background information and objectives of the project; methods including estimator, gear, site description, and statistical analyses used; results; discussion of current results and how they compare to previous surveys or other similar studies and implications for fisheries management decisions, management recommendations, literature cited, and tables and figures as needed to help explain the results.

Minimum reported information should include: sample date, abundance estimator used, sampling gear, site description, estimated number of legal-size fish per site length, estimated number of legal-length fish per km, estimated total biomass of targeted species (kg/ha), percentage of the total biomass comprised of legal-length fish, relative abundance of non-game species, current management program, ownership (percent public vs private) road access, and management recommendations**.** The reports should be prepared and submitted through the Agency Resource Database's electronic report submission system during the fall and winter months before the start of the next field season.

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Appendix A

Methods for Examinations of Fish External Abnormalities – Adopted from the OHIO EPA, *copied with permission verbatim from IDNR 2001.*

External Abnormalities – All fish that are captured are examined for the presence of gross external anomalies and their occurrence is recorded in the fish data sheet and subsequently entered into the FINV database. In order to standardize the procedure for counting and identifying anomalies the following criteria should be followed.

All fish are examined for gross external anomalies. These are anomalies that are visible to the naked eye when the fish are captured, identified, and counted. Table 1 lists the types of anomalies which are recorded on the fish data sheet and subsequently entered into FINV. Exact counts of anomalies present (i.e. the number of tumors, lesions, etc. per fish) are not made; however, light and heavy infestations are noted for certain types of anomalies (Table 1). An external anomaly is defined as the presence of an externally visible skin or subcutaneous disorder. Ultimately, the number and percentage of DELTs and non-DELTs are computed and recorded in the FINV database. Then the total percent anomalies for a specific type of anomaly or group of anomalies can be calculated for 1 or more sites.

The following is a review of some anomalies commonly encountered in freshwater fishes. These characteristics should be used in determining the types of external anomalies present and in coding the fish data sheets.

1. Deformities – These can affect the head, spinal vertebrae, fins, stomach shape, and have a variety of causes including toxic chemicals, viruses, bacteria, (e.g. *Mycobacterium* spp.), infections, and protozoan parasites (e.g. *Myxosoma carebaiis*, Post 1983). Fish with extruded eyes (see Popeye disease) or obvious injuries should not be included.
2. Eroded fins – These are the result of a chronic disease principally caused by flexibacteria invading the fins and causing a necrosis of the tissue (Post 1983). Necrosis of the fins may also be caused by gryodactylids, a small trematode parasite. When necrosis occurs in the tissue at the base of the caudal fin, it is referred to as peduncle disease. Erosions also occur on the preopercle and operculum and these should be included. In Ohio streams and rivers this anomaly is generally absent in least impacted fish communities, but can have a high incidence in polluted areas. It occurs most frequently in areas with multiple stresses, particularly low or marginal dissolved oxygen

(D.O.) or high temperatures in combination with chronic toxicity (Pippy and Hare 1969, Sniezko 1962).

3. Lesions and ulcers – These appear as open sores or exposed tissue and can be caused by viral (e.g. *Lymphocystis* sp.) and bacterial (e.g. *Flexibacter columnaris*, *Aeromonas* spp., *Vibrio* sp.) infections. Prominent bloody areas on fish should also be included. Small, uncharacteristic sores left by anchor worms and leeches should not be included unless they too, are likewise infected. As with eroded fins, lesions often times appear in areas impacted by multiple stresses, particularly marginal D.O. in combination with sublethal levels of toxics.
4. Tumors – These result from the loss of carefully regulated cellular proliferative growth in tissue and are generally referred to as neoplasia (Post 1983). In wild fish populations, tumors can be the result of exposure to toxic chemicals. Baumann et al. (1987) identified polynuclear aromatic hydrocarbons (PAHs) as the cause of hepatic tumors in brown bullheads in the Black River (Ohio). Viral infections (e.g. *Lymphocystis*) can also cause tumors. Parasites (e.g. *Glugea anomala*, and *Ceratomyxa hasta*; Post 1983) may cause tumor like masses, but these should not be considered as tumors. Parasite masses can be squeezed and broken between thumb and forefinger; whereas true tumors are firm and not easily broken (P. Baumann, personal communication).
5. Anchor worm (*Lernaea cyprinacea*) – This is a common parasitic copepod and can be identified by the presence of an adult female which appears as a slender worm-like body with the head attached (buried) in the flesh of the fish. A small, characteristic sore is left after the anchor worm detaches. Attachment sites are included in the determination of light and heavy infestations. If the formed attachment site becomes infected and enlarged as the result of an infection, it should be recorded as a lesion.
6. Black spot – This disease in common to fish in Ohio and is caused by the larval stage of a trematode parasite (e.g. *Uvulifer ambloplitis* and *Crassiphiala bulboglossa*). They are easily identified as small black cysts (approximately the size of a pin head) on the skin and fins. Black spot has been reported as being most prevalent on fish inhabiting relatively shallow stream and lake habitats which have an abundance of aquatic vegetation with snails and fish eating birds, 2 of

its intermediate animal hosts. It may also increase in frequency in mildly polluted streams or where fish are crowded due to intermittent pooling.

7. Leeches – These parasites belong to the family Piscicolidae and are usually greenish brown in color and 5-25 mm long (Allison et al. 1977). Leeches can be identified by the presence of 2 suckers (one on each end) and the ability to contact or elongate their body. They may occur almost anywhere on the external surface of the fish, but are most frequently seen on the anterioventral surface of bullheads (*Ictaluras* spp.). Field investigators should become familiar with the small sores or scars left by leeches as these are included in the determination of light and heavy infestations. If these sores become enlarged and infected they are also regarded as lesions. Leeches are seldom harmful to fish unless the infestation is very heavy.
8. Fungus – There is a growth that can appear on a fish's body as a white cottony growth and is most frequently caused by *Saprolegnia parasitica*. This fungus usually attacks an injured or open area of the fish and can eventually cause further disease or death.
9. Ich or *Icthyophthirus multifilis* – This is a protozoan that manifests itself on a fish's skin and fins as a white spotting. This disease rarely occurs in wild fish populations.
10. Popeye – This disease is generally identified by bulging eyes and can be caused by gas accumulation in areas where the water is gas supersaturated. It occurs most frequently in Ohio as the result of fluid accumulation from viral infection, nematodes (*Philometra* sp.), or certain trematode larvae (Rogers and Plumb 1977).

Information on external anomalies is recorded because many are either caused or exacerbated by environmental factors and often times indicate the presence of multiple, sublethal stresses. Komanda (1980) found that morphological abnormalities are uncommon in unimpacted, natural fish populations. The effects of temperature, salinity, dissolved oxygen, diet, chemicals, organic wastes, etc, especially during the ontogeny and larval stages of fished can be the cause of many types of anomalies (Berra and Au 1981). The presence of anomalies on fish may act as an index of pollution stress. A high frequency of DELT anomalies (deformities, eroded fins, lesions, and tumors) is a good indication of stress caused by sublethal stresses, intermittent stresses, and chemically contaminated substrates.

The percent DELT anomalies is a metric of the IBI (Ohio EPA 1987). Field investigators are urges to refer to texts on fish health for further information and pictures of specific anomalies. If necessary, affected fish should be preserved for laboratory examination.

Table 1. Anomaly codes utilized to record external anomalies on fish.

Anomaly code	Description of the anomaly
D	Deformities of the head, skeleton, fins, and other body parts.
E	Eroded fins.
L	Lesions, ulcers.
T	Tumors.
M	Multiple DELT anomalies (e.g. lesions, tumors, etc.) on the same individual fish.
AL	Anchor worm – light infestation: fish with 5 or fewer attached worms and/or previous attachment sites.
AH	Anchor worm – heavy infestation: fish with 6 or more attached worms and/or previous attachment sites.
BL	Black spot – light infestation: spots do not cover most of the body with the average distance between spots greater than the diameter of the eye.
BH	Black spot – heavy infestation: Spots cover most of the body and fins with the average distance between spots less than or equal to the eye diameter.
CL	Leeches – light infestation: Fish with 5 or fewer attached leeches and/or previous attachment sites.
CH	Leeches – heavy infestation: Fish with 6 or more attached leeches and/or previous attachment sites.
F	Fungus.
I	Ich (*Icthyophthirus multifilis*).
N	Blind – one or both eyes; includes missing and grown over eyes (does not include eyes missing due to Popeye disease).
S	Emaciated (poor condition, thin, lacking form).
P	External parasites (other than those already specified).
W	Swirled scales.
Y	Popeye disease.
Z	Wound, other, not included above.

River Fish Community Data Sheet: DATE:_____ OBS:_____
LOCATION:_____ Water body name:_____Run:____
Sampling method: ___Shock boat; _____Trawl line; _____Other
Actual shock time:____sec; Volts:____; Amps:____; Waveform: (AC)(DC)(Pulsed DC)
Trawl: distance sampled:_____(m); Mesh size:____(in): # hauls:____
% clouds:_____; Secchi depth:_____; Flow level:_____
Start time:_____Start temp:_____; End time_____ End temp:_____
Comments:_____

Species	Count (tally)								Anomaly code	# affect.
	0-3"	4-6"	7-9"	10-12"	13-15"	16-18"	19-21"	22+"		

Anomaly codes: D=deformities, E=eroded or frayed fins, L=lesions or ulcers, T=tumors, M=multiple DELTS on same fish, AL=anchor worm-light, AH=anchor worm-heavy, BL=black spot-light, BH=black spot-heavy, CL=leeches-light, CH=leeches-heavy, F-fungus, I=Ich, N=blind, S=emaciated, P=external parasites, Y=popeye, W=swirled scales, Z=wound, other (describe)

Macroinvertebrate Community survey data sheet. DATE:_____
OBS:_____ Water Body name:_____
LOCATION:_____ START TEMP:_____END TEMP:_____ Rain:_____
GPS Coordinates of downstream starting point:_____ % CLOUDS:____
Turbidity:_____ Overall sampling effectiveness:_____ Flow level:_____

Semi-Quantitative (Modified-Hess / Surber / Artificial Substrate) Sampling:
Sampling gear used:_____
Preservative used:_____

Replicate sample ID #	#1	#2	#3
Unique sample ID #			
Dominant form of periphyton growth*			
Amount of periphyton growth**			
Amount of sedimentation/embeddedness**			
Amount of macroinvertebrate colonization**			
Other comments			

* FA=Filamentous Algae Growth; NF=Non-filamentous Algae Growth.
** LT (light) < 25% of substrate surface effected; MD (moderate) 25-50% effected; MH (moderately heavy) 51-75% effected; & HV (heavy) > 75% effected.

Qualitative, Multi-Habitat Sampling
Sampling gear used:_____
Begin time:_____ End time:_____ Total sampling minutes:_____

Photo Voucher Cards for Fish Photo IDs

PHOTO FISH VOUCHER

Voucher Number:________________
Date:__________________________
Wetland Name:_________________
Specific location:_______________
Run:________
Common Name:__________________________

PHOTO FISH VOUCHER

Voucher Number:________________
Date:__________________________
Wetland Name:_________________
Specific location:_______________
Run:________
Common Name:__________________________

PHOTO FISH VOUCHER

Voucher Number:________________
Date:__________________________
Wetland Name:_________________
Specific location:_______________
Run:________
Common Name:__________________________

PHOTO FISH VOUCHER

Voucher Number:________________
Date:__________________________
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Specific location:_______________
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Common Name:__________________________

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Voucher Number:________________
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Specific location:_______________
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Common Name:__________________________

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Voucher Number:________________
Date:__________________________
Wetland Name:_________________
Specific location:_______________
Run:________
Common Name:__________________________

PHOTO FISH VOUCHER

Voucher Number:________________
Date:__________________________
Wetland Name:_________________
Specific location:_______________
Run:________
Common Name:__________________________

PHOTO FISH VOUCHER

Voucher Number:________________
Date:__________________________
Wetland Name:_________________
Specific location:_______________
Run:________
Common Name:__________________________

Appendix B

Backpack and Towed Boat Electrofishing Equipment List

Backpack Stream Electrofishing Equipment List

1) Coffelt-type gas backpack shocker with probes (anode and cathode)
2) Gas can with mixed fuel for gas backpack
3) Spare generator and parts for gas backpack
4) Smith Root or Appalachian Aquatics type battery backpack shocker with probes (anode and cathode)
5) Batteries and charger for battery backpack
6) Pack basket(s) or similar container(s) & lid(s) for storage and transport of the following field processing gear:
   live bags, fin clippers/scissors, flagging, electronic scales, small landing net, plastic bottles for water samples, measuring boards, measuring tapes and/or hip chain, scale envelopes, knife, thermometer, pencils, small assortment of tools, spare parts, anesthetic, first aid supplies, etc…
7) 2.5 and/or 5 gallon buckets
8) Hip boots & chest waders for crew
9) Conductivity meter
10) Chemistry kit with supplies for routine water chemistry measurements
11) Fish kit for collection and preservation of voucher/unknown specimens
12) Belly boards for mark & recapture estimates
13) Live cars for holding fish
14) Dip nets for collecting fish
15) Clip board & data sheets
16) GPS unit, range finder, camera, and other electronics with spare batteries for each

Towed Boat Stream Electrofishing Equipment List

1) Towed Boat with chord reels and probes (anode)
2) Generator and electrofisher combination
3) Gas can with fuel for generator
4) Live well (galvanized half tub)
5) Pack basket(s) or similar container(s) & lid(s) for storage and transport of the following field processing gear:
   live bags, fin clippers/scissors, flagging, electronic scales, small landing net, plastic bottles for water samples, measuring boards, measuring tapes and/or hip chain, scale envelopes, knife, thermometer, pencils, small assortment of tools, spare parts, anesthetic, first aid supplies, etc…
6) 2.5 gallon bucket or other container to fill live well
7) Hip boots & chest waders for crew
8) Conductivity meter
9) Chemistry kit with supplies for routine water chemistry measurements
10) Fish kit for collection and preservation of voucher/unknown specimens
11) Live cars for holding fish (optional)
12) Dip nets for collecting fish
13) Clip board & data sheets
14) GPS unit, range finder, camera, and other electronics with spare batteries for each

Appendix C

Mark Recapture Population Estimate Field Data Sheet

Gear:		Volt/Amp:	EF Time: min/sec
Spp:	MARKED	R MARKED	R UNMARKED
25			
50			
75			
100			
125			
150			
175			
200			
225			
250			
275			
300			
325			
350			

Stream:	Sec:	Date:
Trib to:		SSB:
Site Description:		RM:
		Site Length (m):

Lat/Lon:		County:		Widths:
		Topo Map:		1)
Flow: High/Norm/Low				
Water Chems	Time:	Temp:	SC:	2)
	pH: Electic/Color	TA:	TH:	3)

RBP Habitat (High Gradient, 1999) Parameter	Score (0-20; *0-10)	Shade: Dense >75% Partial 25-75% Open <25%	4)
1)SUBSTRATE/COVER			5)
2)EMBEDDEDNESS			
3)VELOCITY/DEPTH			6)
4)SEDIMENT DEPOSITION			
5)CHANNEL FLOW STATUS		Dominant Substrate: Bedrock Boulder Rubble Gravel Sand Silt Clay	7)
6)CHANNEL ALTERATION			
7)FREQ RIFFLES/BENDS			8)
8)LFT BANK STABILITY*			
8)RT BANK STABILITY*			9)
9)LFT BANK VEG PROTECT*			
9)RT BANK VEG PROTECT*			10)
10) LFT BANK VEG WIDTH*			
10)RT BANK VEG WIDTH*			AVG
Time: ***Total Score:***			

Comments:

Crew:

Fish Species Collected:

Appendix D

Removal/Depletion Population Estimates Field Data Sheet

STREAM:		**Sec:**	**Date:**
TRIB TO:			**SSB:**
SITE DESCRIPTION:			**RM:**
			SITE LENGTH(M):
LAT/LON:	**COUNTY (s):**		**WIDTHS(M)**
			1)
			2)
WATER CHEMISTRY	**ELECTROFISHER**	**TOPO MAP (s):**	**3)**
			4)
TIME:	**MODEL:**		**5)**
AIR: °F/°C			**6)**
WATER: °F/°C	**VOLTS: AC/PDC**		**7)**
pH: COLOR/ELEC	**PPS:**	**Flow:**	**8)**
T. ALK: MG/L	**AMPS:**	**HIGH**	**9)**
T. HARD: MG/L	**C1 TIME: SEC/MIN**	**NORMAL**	**10)**
SPEC.COND: µMHOS	**EFFICIENCY:**	**LOW**	**Avg:**

RBP HABITAT [OCT 99] for HIGH GRADIENT STREAMS

PARAMETER	Score 20-0 *10-0	COMMENTS
1)SUBSTRATE/COVER		
2)EMBEDDEDNESS		
3)VELOCITY/DEPTH		
4)SEDIMENT DEPOSITION		
5)CHANNEL FLOW STATUS		
6)CHANNEL ALTERATION		
7)FREQ RIFFLES/BENDS		
8)LFT BANK STABILITY*		
8)RT BANK STABILITY*		
9)LFT BANK VEG PROTECT*		
9)RT BANK VEG PROTECT*		
10) LFT BANK VEG WIDTH*		
10)RT BANK VEG WIDTH*		
TIME: TOTAL SCORE:		

OTHER HABITAT

Shade:
DENSE >75%
PARTIAL 25-75%
OPEN <25%

Dominant Substrate:
BEDROCK
BOULDER
RUBBLE
GRAVEL
SAND
SILT
CLAY

COMMENTS:

CREW:

MM	**CATCH 1** **(mm--grams for first 10 fish / 25 mm Length Group)** **SPP:**	*C 2*	*C 3*
25			
50			
75			
100			
125			
150			
Fish Species Collected:			

Appendix E

Biological Report Template

PA FISH AND BOAT COMMISSION
COMMENTS AND RECOMMENDATIONS
February 18, 2016

WATER: Dubois Creek (404E) Susquehanna County

EXAMINED: June 15, 2007

BY: Wnuk, Frey, Koser, and Bendock

Bureau Director Action:_____
Date:_____

Division Chief Action:_____
Date:_____

WW Unit Leader Action:_____
Date:_____

CW Unit Leader Action:_____
Date:_____

==========

AREA COMMENTS:

The Susquehanna County Conservation District has applied for grants to conduct habitat improvement on Dubois Creek. Habitat improvement is needed because of impacts from the June 2006 flood and subsequent extensive channelization. The Area 4 Fisheries Management Office conducted this survey at the request of the Conservation District to collect baseline data on the fishery prior to restoration. We found a small wild brook trout population that was limited, in part, by poor physical habitat in channelized areas of stream.

AREA RECOMMENDATIONS:

1. Manage Dubois Creek for its natural fish populations under statewide angling regulations.

2. Add Dubois Creek to the list of stream sections that support natural reproduction of trout.

3. The Susquehanna County Conservation District should pursue restoration efforts on Dubois Creek.

4. Re-survey Dubois Creek when restoration efforts are complete.

Warmwater Unit Comments and Recommendations:

Dubois Creek is a small stream of low productivity characterized by cold, cool and warmwater habitats. Area personnel reported impaired habitat near the mouth and indicated that the site, if improved, could provide spawning and nursery habitat for smallmouth bass and white suckers. Smallmouth bass were not recorded at this site during summer sampling. I concur with all Area recommendations and encourage our Habitat Division to lend support to this project as the Habitat Division Chief deems appropriate.

This work made possible by funding from the Sport Fish Restoration Act Project F-57-R Fisheries Management.

Pennsylvania Fish & Boat Commission
Bureau of Fisheries
Division of Fisheries Management

Dubois Creek (4E)
Section 01
Fisheries Management Report

Prepared by:
Robert Wnuk, Aaron Frey, and Scott Koser

Fisheries Management Database Name: Dubois Creek
Lat/Lon: 415812754452

Date Sampled: June 15, 2007 Date Prepared: August 2007

Introduction

Dubois Creek is a 9.6 km long tributary to the North Branch Susquehanna River in Susquehanna County. The stream originates at the confluence of two unnamed tributaries near Franklin Corners and flows generally northeast to its mouth in the borough of Hallstead (Figure 1). Interstate 81 and State Route 11 provide major road access to Dubois Creek, while the United States Geological Survey's Franklin Forks, PA and Great Bend, PA 7.5 minute quadrangles provide topographic coverage.

The Pennsylvania Fish and Boat Commission (PFBC) manages Dubois Creek as a single section extending from the headwaters downstream to the mouth under statewide angling regulations. The PFBC does not stock Dubois Creek and has never previously surveyed the stream. The Pennsylvania Department of Environmental Protection (DEP) classifies Dubois Creek as coldwater fishes in its 25 Code, PA Chapter 93 water quality standards. There are no known permitted discharges in the Dubois Creek watershed.

Dubois Creek drains an area of 33.67 km^2. Land use in the basin is a mixture of agriculture, woodlots, ponds, and single family rural residences. More concentrated residential development exists in the borough of Hallstead. The geology of the watershed consists of Devonian Age sandstones, siltstones, shales, claystones, and conglomerates from the Catskill and Lock Haven Formations. The Catskill Formation underlies the stream valleys while the Lock Haven Formation underlies the hillsides of the drainage. The sandstones of the Lock Haven Formation in Susquehanna County were originally laid down as sediments by shallow, fast moving rivers that fed an ancient sea. When the sea receded and the sediments turned to rock, they formed "Pennsylvania Bluestone", a highly valued building material. Susquehanna County is the center of Pennsylvania Bluestone mining, and numerous bluestone quarries line the hillsides of the Dubois Creek watershed.

The flooding of June 2006 and subsequent cleanup efforts degraded physical habitat in Dubois Creek. Following the cleanup, the Susquehanna County Conservation District applied for funding to begin stream restoration efforts. The conservation district also asked the PFBC to survey fish populations in Dubois Creek as a measure of restoration success. The PFBC initiated the present survey of Dubois Creek to collect baseline information on the fishery prior to restoration.

Methods

We surveyed Dubois Creek on June 15, 2007. All procedures of the survey followed Marcinko et al. (1986). We collected physical and some social data for Section 01 but did not quantify parking.

We assessed three sampling stations. Station 0101 (River Mile 4.65) was located at the SR 1037 bridge. We only collected water chemistry data here because there was little flow. Station 0102 (River Mile 2.83) was located at the upstream end of a breached reservoir and was 150 m long. Station 0103 (River Mile 0.10) was located at a railroad trestle in Hallstead and was 150 m long. We collected physical habitat, water chemistry, and fish population data at Stations 0102 and 0103.

Physical habitat evaluations followed the United States Environmental Protection Agency's Rapid Bioassessment Protocols for high gradient streams (Barbour et al. 1999). All chemical parameters were measured in the field using a colorimetric method for pH, a mixed indicator for total alkalinity, and EDTA titration for total hardness. We used a Smith-Root battery powered backpack electrofishing unit (Model 12-A POW, 300 volts pulsed direct current) with a single anode and a rat-tail cathode to assess fish populations. We identified the fish captured at each site to species with the exception of sculpins *Cottus sp*. Sculpins were only identified to genus because it was difficult to accurately separate mottled sculpins *Cottus bairdii* from slimy sculpins *Cottus cognatus* in the field. Additionally, we assigned abundance ratings to all fish species according to the warmwater stream sampling protocols of Young (2007).

We classified all of the trout we captured as being of wild or hatchery origin based on species, coloration, size, and fin wear. We measured the wild trout to 25 mm length groups and gave them an upper caudal fin clip. We were unable to capture at least 30 wild trout at an individual site, so the number of trout captured was considered the total population present. We obtained wild trout population abundance and biomass estimates for Section 01 using state average weights calculated on August 27, 2007.

Results and Discussion

Section 01 of Dubois Creek possessed a moderate gradient of 18.7 m/km and a rural human population density of 23 persons/km^2 (Table 1). Physical habitat scores ranged from 83 (marginal) at Station 0103 to 139 (suboptimal) at Station 0102 (Table 2). Physical habitat problems at Station 0103 were primarily the result of stream channelization following the June 2006 flood. The channel was uniformly straight and wide. It possessed no water depth and little if any cover for fish. Bank vegetative protection was also poor but the banks had been stabilized with rip-rap. The stream at Station 0102 had not been channelized, but it was silted by the former dam.

Water chemistries in Section 01 reflected the geology and land use of the basin. Total alkalinity ranged from 20 to 26 mg/l (Table 3) and was sufficient to buffer the stream against acid precipitation. The lowest pH value we documented was 6.6 at Station 0102. Organic acid input from a swampy, ponded tributary just upstream from the station caused this minor pH depression.

We documented 10 different fish species at our two sampling stations (Table 4). The only species we rated abundant were blacknose dace *Rhinichthys atratulus* at both stations and central stonerollers *Campostoma anomalum* at Station 0102. White sucker *Catostomus commersonii* young were rated present at both stations but adult white suckers were absent.

Wild brook trout *Salvelinus fontinalis* were rated present at Station 0102 but were absent from Station 0103. We estimated total wild brook trout biomass in Section 01 at 8.76 kg/ha (Class D; Table 5), and we estimated there were 154 legal-length and larger wild brook trout in the stream. Wild brook trout at Station 0102 ranged from 50 to 324 mm total length (Figure 2).

Dubois Creek supported a modest wild brook trout population that was limited, in part, by poor physical habitat in heavily channelized areas. Stream restoration work in these areas could allow this population to expand, and we encourage the Susquehanna County Conservation district to pursue habitat improvement. High summer water temperatures could limit wild brook trout populations near the mouth of Dubois Creek, but habitat improvement in this stretch could allow it to serve as nursery water for juvenile smallmouth bass *Micropterus dolomieu* and white suckers from the North Branch Susquehanna River.

MANAGEMENT RECOMMENDATIONS

1. Manage Dubois Creek for its natural fish populations under statewide angling regulations.

2. Add Dubois Creek to the list of stream sections that support natural reproduction of trout.

3. The Susquehanna County Conservation District should pursue restoration efforts on Dubois Creek.

4. Re-survey Dubois Creek when restoration efforts are complete.

LITERATURE CITED

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

Marcinko, M., R. Lorson, and R. Hoopes. 1986. Procedures for stream and river inventory information input. Pennsylvania Fish and Boat Commission, Bellefonte, PA.

Young, L. 2007. Memo to Area Fisheries Managers re: DFM sampling protocols for wadeable warmwater streams. Pennsylvania Fish and Boat Commission files, Bellefonte, PA.

Table 1. Standard physical and social data for Dubois Creek (4E) Section 01.

Measurement	Value
Upper Limit	Confluence two unnamed tributaries
Lower Limit	Mouth
Length	9.62 kilometers
Mean Width	4.35 meters (based on 2 sites)
Area	4.19 hectares
Gradient	18.7 meters per kilometer
County Location	100% Susquehanna
USGS Quadrangles	Franklin Forks, Great Bend
WCO District	3073
DEP Classification	Coldwater Fishery
Ownership	100% Private
2000 Human Population Density	23 Persons per square kilometer
Road Access:	
% Within 100 meters	69
% Within 300 meters	100
% Within 500 meters	100

Table 2. Physical habitat ratings determined on June 15, 2007 for stations located in Section 01 of Dubois Creek (4E).

	Station	
Parameter	0102	0103
Substrate/Cover	13	3
Embeddedness	12	12
Velocity/Depth	14	8
Sediment Deposition	10	12
Channel Flow Status	11	4
Channel Alteration	11	1
Riffle Frequency	16	16
Bank Stability:		
Left Bank:	9	8
Right Bank:	9	8
Vegetative Protection		
Left Bank:	9	1
Right Bank:	9	1
Riparian Zone Width:		
Left Bank:	8	4
Right Bank:	8	5
Total Score:	139	83

Table 3. Physical and chemical data collected from Dubois Creek (4E) on June 15, 2007.

	Station		
Measurement	0101	0102	0103
Time (24 hour)	1225	1120	1050
Air Temperature (^{o}C)	25.0	20.0	19.0
Water Temperature (^{o}C)	16.1	17.2	20.9
pH	6.8	6.6	7.0
Total Alkalinity (mg/l)	20	23	26
Total Hardness (mg/l)	26	28	36
Specific Conductance (umhos)	103	95	123

Table 4. Abundance ratings for fish species captured from Dubois Creek (4E) on June 15, 2007.

		Station	
Scientific Name	Common Name	0102	0103
Salvelinus fontinalis	Brook trout	P	
Campostoma anomalum	Central stoneroller	A	
Exoglossum maxillingua	Cutlips minnow		R
Rhinichthys atratulus	Blacknose dace	A	A
Rhinichthys cataractae	Longnose dace		C
Semotilus atromaculatus	Creek chub	C	
Nocomis micropogon	River chub		P
Noturus insignis	Margined madtom		P
Catostomus commersonii	White sucker	P	P
Cottus sp.	Sculpins	C	C
	Total Species:	6	7

Abundance ratings (based on number of individuals seen in 300 m of electrofishing):
A = Abundant (> 100); C = Common (26 - 100); P = Present (3 - 25); R = Rare (< 3).

Table 5. Mean biomass estimate for wild brook trout in Section 01 of Dubois Creek (4E) determined on June 15, 2007.

Length Group (mm)	Population Estimate	Estimated Number per Hectare	Estimated Number per Kilometer	Estimated Kilograms per Hectare
50 - 74	1	8	3	0.02
75 - 99	0	0	0	0.00
100 – 124	1	8	3	0.11
125 – 149	1	16	7	0.39
150 – 174	1	16	7	0.65
175 – 199	1	8	3	0.51
200 – 224	0	0	0	0.00
225 – 249	0	0	0	0.00
250 – 274	1	16	7	2.84
275 – 299	1	8	3	1.80
300 – 324	1	8	3	2.44
Totals:	**8**	**88**	**36**	**8.76**
< 150 mm	3	32	13	0.52
> 174 mm	4	40	16	7.59

Figure 1. Dubois Creek (4E) drainage basin.

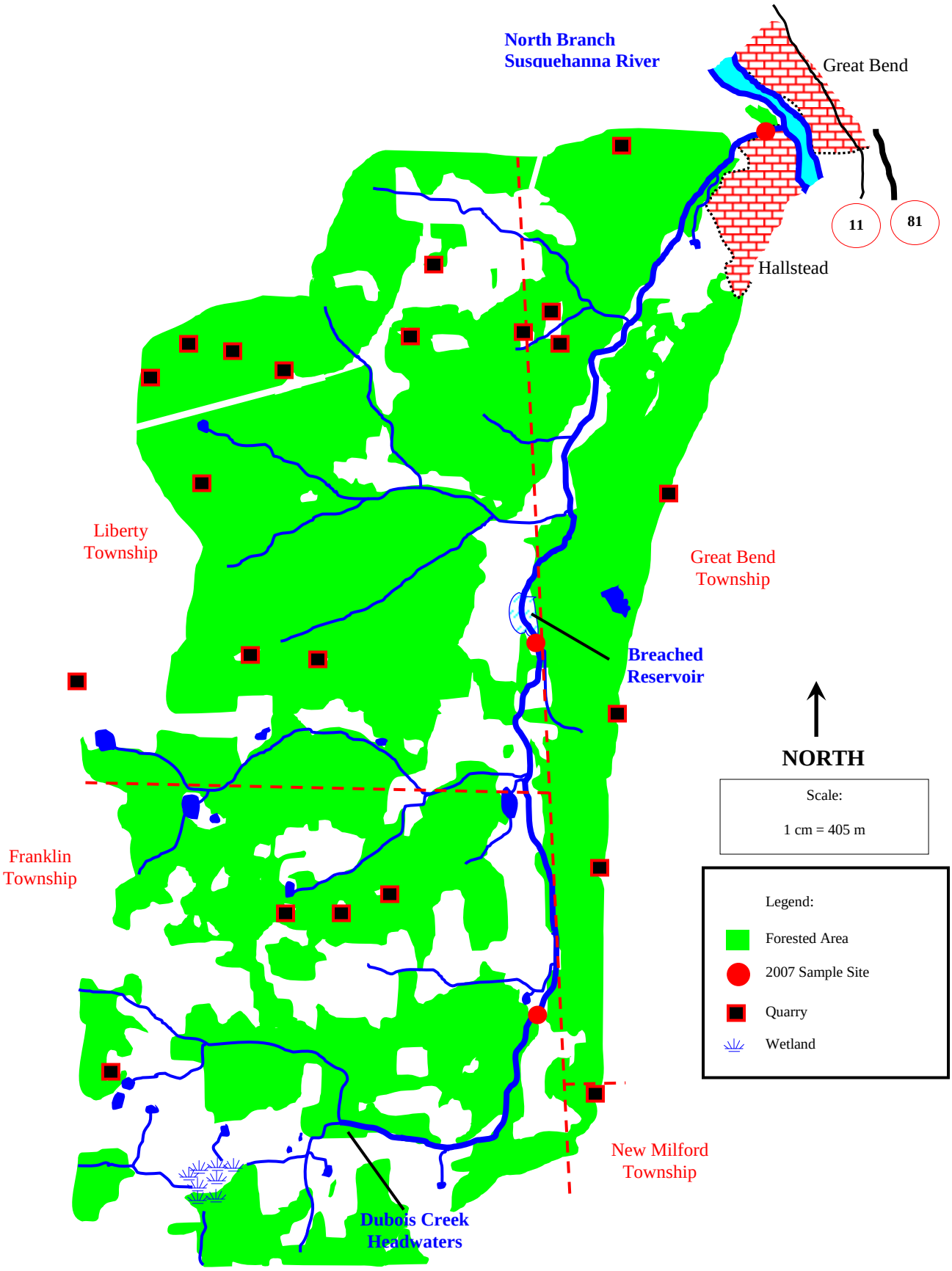
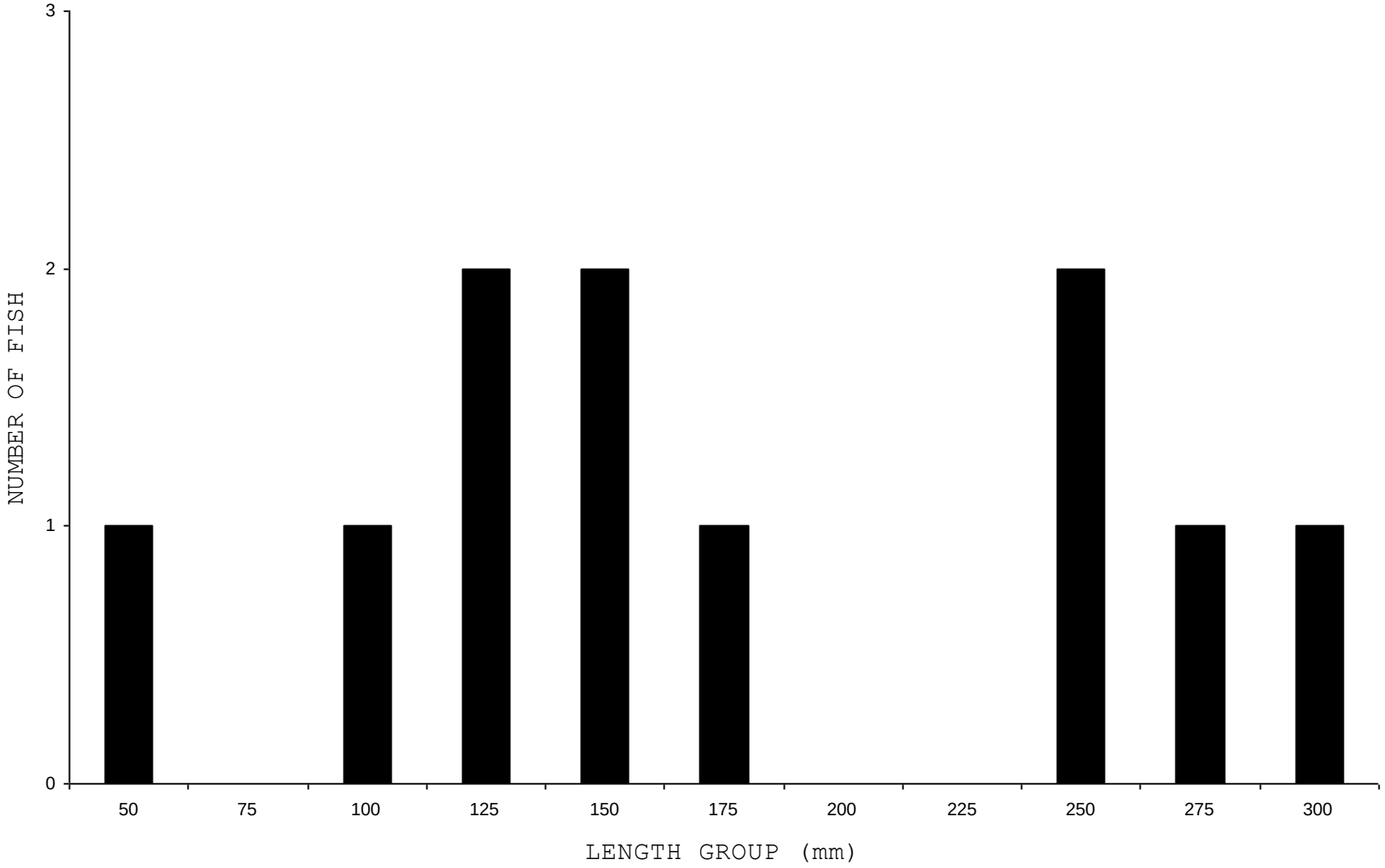


Figure 2. Length-frequency distribution of wild brook trout captured at Station 0102 of Dubois Creek (4E) on June 15, 2007.



DISTRIBUTION

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MODULE B

Sampling Protocols for Fourth and Fifth Order Wadeable Warmwater Streams

Prepared by:
Leroy Young

1. Introduction

This module summarizes the field data collection techniques used by Pennsylvania Fish and Boat Commission (PFBC), Division of Fisheries Management (DFM) staff for determining and documenting the fish species assemblage and the population structure of game fish in fourth and fifth order wadeable warmwater streams. The field data collection techniques (the protocols) described below are designed to be cost-effective, broadly applicable, objective, and repeatable techniques for qualifying and quantifying fish species assemblages and game fish population characteristics. Furthermore, the protocols are designed to be applicable statewide and provide standardized techniques for conducting monitoring activities on wadeable warmwater streams to meet DFM goals and objectives.

The general steps outlined in Module A (Sampling Protocols for Pennsylvania Wadeable Stream Electrofishing) should be followed when conducting surveys as part of Pennsylvania's wadeable warmwater streams initiative. The following specific protocols supersede those in Module A when conducting wadeable warmwater stream surveys.

- Conduct field water chemistry analysis as described in Module J

- Choose sampling gear according to Module A. Due to the larger nature of the fourth and fifth order streams, cooperation with other fisheries management areas may be required to meet the electrofishing gear requirements defined in Module A.

- Sampling stations will occur approximately every 4 miles on a systematic basis.

- Consideration should be given to landowner issues and physical accessibility when identifying sampling stations as described in Module A.

- Sample stations will be between 300 m and 500 m in length with a minimum of 2 stations being sampled.

- Field crews should identify a riffle or other physical barrier to function as a station end point or utilize a block net if a natural barrier is unavailable. The use of block nets is discussed in Module A.

- The first sampling station located upstream of the mouth should be out of the backwater influence of any river but also located within 1 mile of the mouth. An exception can be made when the river elevation influences the stream elevation for more than 1 mile upstream from the mouth. In this instance, move the sampling station upstream to the nearest stream reach not influenced by the river.

- Concentrate sampling on the wadeable portions of the identified stream reach. Avoid reaches that contain a mix of both boatable and wadeable water.

- All game fish species, including sunfish species, catfish, and bullheads, should be measured to the nearest 25 mm length group and recorded on an appropriate field data sheet (Module A; Appendix C, D).

- An index of relative abundance will be established for each non-game species encountered. The index will be based on a count of all non-game species in the first 300 m of each station sampled. The rating criteria is as follows: <3 = RARE; 3-25 = PRESENT; 26-100 = COMMON; >100 = ABUNDANT. For example: In the first 300 m of a station the sampling crew counts 34 white suckers, 100+ tessellated darters, and 2 creek chubs – white suckers would be rated common, tessellated darters would be rated abundant and creek chubs would be rated rare.

- Specific attention to the number of quality length carp and quillback (≥400 mm), suckers and redhorse (≥300 mm), and fallfish (≥ 250 mm) needs to be made so that the survey crew can document on the station data sheet the percentage of the carp, quillback, sucker and redhorse that were greater than or equal to quality length with respect to the relative abundance rating. For example: If the survey crew counts 70 carp in the first 300 m of a station and they estimate that 25 were ≥400 mm in length then a note should be made on the data sheet that carp were rated common and 36% were greater than or equal to quality length.

MODULE C

Sampling Protocols for Smallmouth Bass Young-of-Year (YOY) in Wadeable Lotic Habitats

Prepared by:
Robert Lorantas

1. Introduction

Young-of-year (YOY) smallmouth bass have been annually monitored at set index sites in Pennsylvania rivers and warmwater streams since 1987. Initially collected as part of an effort to determine if YOY smallmouth bass abundance was a reliable predictor of adult smallmouth bass densities in future years, monitoring of these index sites has proven useful in monitoring the presence and expansion of a fish disease that first appeared in the Susquehanna River basin in 2005. The disease, which has been most prevalent during low water years, has been associated with fish kills of primarily YOY smallmouth bass. The YOY monitoring efforts have been expanded in recent years to assist in the understanding of the biotic and abiotic factors that contribute to the occurrence of the disease.

2. Objectives

(1) Estimate density of black bass YOY per **300 meter of shoreline** in selected rivers and streams. Assessments should occur at historic sites or sites identified by USGS partners or River Biologists.
(2) Assess disease incidence (count) among smallmouth bass YOY and collect other diseased fish species encountered and observed regardless of age class.
(3) To the extent possible develop a model that predicts adult black bass density based upon young of year density and other population characteristics (total length) of young of year black bass. Does the YOY index have utility in predicting adult density (2009 DRAFT completed, poster in progress).
(4) Document any changes in density or trends in density through time. Trends are detailed only for those sites where a series of 5 consecutive years of data have been collected (annual).
(5) Assess sample sizes (number of sites) necessary to reliably quantify young of year density in Pennsylvania rivers and stream sections (PSU Coop 2006).
(6) To the extent possible develop a model that predicts young of year density based upon flow data and other environmental characteristics (temperature) rivers and streams. (Completed 2003, update to occur in the future).

2.1 WATER SELECTION & SAMPLING SITES

Historic criteria generally require collecting YOY adjacent to adult black bass sampling sites previously established on selected major rivers and warmwater streams. Based upon Pennsylvania State University sample size evaluations conducted in 2006 a minimum of ten 300 m long sites per major river must be sampled. This represents the total number of 300 m sites for multiple areas where a river spans multiple management areas.

2.2 SAMPLING DATES

Sampling should take place from late June though July. In central Pennsylvania July 10 represents a typical initial sampling date for rivers. A "rule of thumb" sampling target date for YOY smallmouth bass is when bass are in the 40 mm – 70 mm length range. Sampling on or near the same calendar date is important in making annual comparisons since at this early life-

stage daily mortality can influence density measurements. Also, bass greater than 70 mm are more readily capable of eluding the sampling technique and gear defined below thereby influencing density measurements. Waters within the state have shown wide variation as to when smallmouth bass reach this target size and specific sampling dates will have to be determined by Area/Unit personnel.

2.3 SAMPLING EFFORT

The 300 m site distances should be accurately measured with a hip-chain or rangefinder. Smallmouth bass YOY catch should be reported for the entire 300 m site. Reporting catch by 50 m reach is acceptable for comparison to historically collected data.

2.4 SITE MEASUREMENT

Record the latitude, longitude (determined by GPS using NAD83 datum, and decimal degrees format), river mile (determined by Terrain Navigator or ArcGIS at the office), date, and water name of the downstream-most location of each 300 m sampling site. In addition, it is encouraged that proximity to a physical landmark be recorded on a field data form using a rangefinder or other measuring device to identify the downstream-most starting point for the electrofishing site (e.g., start 350 m upstream of SR 22 bridge). Also, it is good practice to list the name of the nearest town in the database (EXCEL sheet) associated with each 300 m site to assist those without knowledge of where the site is located to know the approximate location without having ever been there or without having the burden of plotting the latitude and longitude or river mile to determine location.

2.5 SAMPLE TARGETS

Primary targets are YOY smallmouth bass and other black bass (these included spotted and largemouth bass). Black bass whose age cannot be determined by size should be scale sampled for future age determination. Any diseased or dead black bass, regardless of age, or any diseased or dead fish species (again regardless of age) should be captured and assigned the appropriate condition scale as defined within the **Disease Incidence** section below. The condition should be recorded on the field datasheet.

2.6 DISEASE INCIDENCE

The disease incidence in your catch sample must be quantified by recording the number of black bass YOY and "other fish" with: (1) fungus (*saprolegnia)* or whitish-bleeched appearing skin evident, (2) sore or necrotic tissue (red wound) evident, (3) both a sore and fungus evident, (4) tail or fin eroded, (5) tail eroded and sore, (6) anchor parasite (7) dead, (8) clean fish (no disease or parasite problems). Should dead or dying SMB YOY be observed during sampling only collect and enumerate the ones that are drifting in the current while following your conventional YOY sampling route, do not seek out or move to areas containing dead SMB YOY as this will serve to artificially inflate the number of black bass collected.

2.7 GEAR

Backpack electrofisher–with two 12" diameter electrodes constructed of 3/8"o.d. stainless steel or aluminum. The electrofishing unit should be capable of producing 75-125 watts of output power using Alternating Current (AC). Electrofishing should take place at near shore locales from 0 m depth to approximately 1.25 m depth and at a typical distance of 3-4 m or more from the shoreline. If available the Coffelt back pack units with TAS generators should be used for this work to match the historical sampling gear.

2.8 SAFETY

Personnel should follow all PFBC electrofishing safety guidelines outlined in Module E.

2.9 DATA COLLECTION

Record the total length by species or simply tally by 25 mm length groups all black bass collected. Record the time electrofished for each 300 m site. This can be accomplished either by noting the start and stop time or by incorporating the use of a stop watch. Note any issues that may have affected the electofishing efficiency. These would include situations such as, poor water clarity, higher than average flows, debris accumulation at the historic site, etc.

2.10 DATA TRANSFER AND PROCESSING

Data entry is the responsibility of the Area Managers or their designees. Specific instructions for entering the data into an Excel spreadsheet are provided annually. Upon completion of data entry into the Excel spreadsheet that data is to be returned via email to the Warmwater Unit Leader, the Warmwater Unit Technician, and the River Biologists.

Any questions should be directed to the Warmwater Unit Leader.

MODULE D

Protocols for Conducting Biological Assessments of Unassessed Trout Waters

Prepared by:
Robert Weber, R. T. Greene, Dave Miko

1. Introduction

Pennsylvania is fortunate to have a vast flowing water resource comprised of 86,000 miles of flowing water (PA DEP 2006). To date the Pennsylvania Fish and Boat Commission (PFBC) has surveyed, 3,175 streams comprising 21,654 miles. Of this total there have been 1,709 streams (9,372 miles) in which wild trout have been documented by PFBC staff. There are another 1,702 streams, comprising 3,305 miles, that by PFBC policy are classified as wild trout streams by virtue of the fact they lie upstream of documented wild trout waters (58 Pa. Code §57.11; Figure 1). This results in a total of 3,411 designated wild trout streams comprising 12,677 miles (PFBC 2009).

All Commonwealth waters have a designated use, which determines the protection standards that the Pennsylvania Department of Environmental Protection (DEP) uses to permit development activities in watersheds. Wild trout streams should be protected at a minimum under the Cold Water Fishes (CWF) designation in 25 Pa. Code Chapter 93 because of their ability to support or maintain a population of wild trout. DEP independently confirms that streams are wild trout waters by reviewing and verifying the PFBC's data. Wetlands located in or along the floodplain of wild trout streams are protected as Exceptional Value Wetlands in 25 Pa. Code Chapter 105. This is the Commonwealth's highest level of wetland protection.

Some wild trout streams receive additional protection under the Commonwealth's special protection waters program (PA DEP 2003) and are designated as either High Quality Cold Water Fishes (HQ-CWF) or Exceptional Value (EV) based upon their biological and social characteristics. Stream and wetland encroachment permits issued for development in watersheds that contain wild trout populations often include a seasonal restriction (no work from October 1 to December 31) to minimize impacts during the time trout are spawning.

Although Pennsylvania contains 64,345 streams totaling approximately 86,000 miles of flowing water in Pennsylvania, the PFBC has only been able to conduct surveys and implement management strategies on 4,877 streams totaling 24,959 miles. As a result, only 8% of the streams and 29% of the total stream miles are being actively managed. While the amount of water surveyed may not seem like much compared to the total amount of available resource, it does amount to sampling and actively managing an average of 163 new waters annually for the past 30 years. Of the waters remaining, many likely support wild trout populations.

The primary threat to unassessed wild trout waters is inadequate water quality protection due to the unknown condition of the trout population and the resulting permitting actions that are not properly conditioned to protect wild trout. The importance of adequately protecting streams has increased dramatically with the recent expansion of Marcellus Shale Gas Extraction throughout much of the state.

Non-point source pollution impacts unassessed waters as well. Proper stream classification is vital as the likelihood that these streams will be impacted by stressors will increase in the future. The PFBC's statement of policy at 58 Pa. Code §57.11 states that "It is the policy of the Commission to accurately identify and classify streams supporting naturally reproducing

populations of trout as wild trout streams." This will continue to be a focus of future wild trout management in the Commonwealth.

Opportunities exist to protect known wild trout populations as well as expand the number and miles of streams officially designated as wild trout. The opportunities to expand the number and quality of wild trout waters include the examination of waters that have not been inventoried to date, promotion of best management practices in watersheds that have been impacted by poor land use, and the application of fisheries management regulations on waters where angler harvest and fishing mortality are significant enough to inhibit trout fisheries from achieving their full potential. A positive response in wild trout populations resulting from these activities could lead to an elevation in water quality status and increased protection.

2. Sampling Procedures

A prioritized listing of potential waters to be worked will be provided by the PFBC. The majority of these waters will be small (< 10 meters in width) wadable streams, generally less than 1 meter deep. Potential sampling sites will be chosen by the survey leader and should contain physical habitat which is representative of the entire stream. For example, if the stream contains a mix of pools and riffles then the site should be chosen to include both of these types of physical habitat. It is routine to review several possible sites before choosing the best sampling site, but unless the characteristics of the stream change drastically along its length, then only one site needs to be surveyed. Sample sites should be located in easily identifiable and readily accessible locations whenever possible to aid in re-sampling. Investigators should avoid sampling at bridge pools unless this habitat type is characteristic of the stream. Sampling should take place during summer low-flow conditions, which usually occur from mid-June to late September. This minimizes sampling bias and allows capture of young-of-the-year trout that are generally not vulnerable to electrofishing at earlier times of the year. Sampling during high flows should be avoided due to reduced sampling efficiency and greater safety concerns.

Sampling will require the collection of physical, chemical, and fisheries data at each sample site. Additionally, aquatic macroinvertebrate data may be collected at the discretion of the survey leader. Aquatic macroinvertebrate data would be useful in describing the effects of pollution sources and in petitioning for an upgrade in a stream's Chapter 93 water quality classification. Aquatic macroinvertebrate data collection will follow standard protocols developed by the Pennsylvania Department of Environmental Protection (Module I).

Physical data collection includes taking extensive field notes and determining site length and width. Field notes should include a written description of the downstream starting point of the sample station detailed enough to allow future investigators to repeat the sample site. Extensive field notes often assist in the explanation of anomalous data discovered after sampling is complete. Latitude and longitude of the beginning of the site should be recorded using a hand-held GPS unit and converted to decimal degrees. Site lengths and site widths are obtained by measuring with a fiberglass tape, hip chain, or range finder. Length measurements are taken through the center of the stream, and are recorded to the nearest whole meter. For this study, site lengths should be approximately 100 meters and end at a natural break point where fish movement out of the survey station would be minimal. Width measurements are taken at a

minimum of five transects perpendicular to the wetted channel and are recorded to the nearest tenth of a meter. Width measurements should be taken at near equal distances throughout the length of the survey station. For example, if a 100 meter station is being surveyed widths should be taken at the downstream starting point and every 20 meters thereafter until 5 widths have been collected.

Chemical data collection is normally done in the field. Standard analyses must include time of day, air temperature (^{o}C), water temperature (^{o}C), pH (standard units), total alkalinity (mg/l), total hardness (mg/l), and specific conductance (umhos). These measurements must follow approved protocols (United States Environmental Protection Agency 1976; American Public Health Association et al. 1980). Approved protocols are provided in Module J. Additionally, the investigator may choose to measure dissolved oxygen (mg/l) if biological oxygen demand is expected to be high. A variety of approved equipment exists to collect chemical data. Users must adhere to proper use and calibration of the equipment based upon the user's manual that accompanied the equipment. Water samples for chemical analyses must be taken from the mid-point of the stream and at mid-depth. Additionally, the sample point should be at a location where the stream is completely mixed rather than at a point that is dominated by flow from a tributary or outfall. If a stream is wider than 20 m, a composite sample obtained at two or three points across the width or through the depth of the stream may be necessary.

Fisheries data will be collected through electrofishing. Persons who participate in electrofishing operations should have completed a certified course provided by the United States Fish and Wildlife Service. At a minimum, one person on the crew, who will act as survey leader, will be required to complete this course prior to sampling for this project. Completion of a First Aid/CPR course is also strongly recommended. A copy of the PFBC Electrofishing Safety Guidelines are provided in Module E.

Electrofishing setups will consist of battery powered backpacks using either pulsed DC (direct current) or unpulsed (straight) DC. Battery backpacks using pulsed DC are the most preferred gear since they tend to be more effective at capturing fish and potentially less harmful to the fish. However, some battery backpack units do not have pulsed DC capability so straight DC would then be used. Overall, electrofishing using DC current is a much safer sampling method for the fish being sampled, however, alternate electrofishing systems may be used with prior PFBC approval.

Electrofishing usually proceeds straight upstream but may be done in a sinuous manner when stream width exceeds the maximum that can be adequately sampled. During sampling, the electrofishing crew will make every attempt to identify all fish species present at a site and record the common name on the provided data sheet. Additionally, the crew should at a minimum assign a subjective abundance rating to each species based on a count of all non-game species found in each station sampled. The rating criteria is as follows: <3 = RARE; 3-25 = PRESENT; 26-100 = COMMON; >100 = ABUNDANT (PFBC 2007) (Module A). Collection of fish species abundance criterion increases in difficulty as fish species richness increases within a specific survey site. The crew should collect and hold all sportfish until sampling is complete and measure them to the nearest 25 mm length group. Fish lengths may also be recorded as total length to the nearest millimeter, but weights do not need to be taken. Digital

photographs of trout species < 50 mm total length must be provided to the PFBC for verification of species. This is in recognition of the difficulty to identify trout species at lengths < 50 mm.

If following normal sampling a significant number of trout are captured, then additional sampling should be completed to determine total abundance of the population. If the number of trout captured in a 100 m site is greater than 35 with at least 5 individuals exceeding 125 mm in length then extending the site up to 300 meters should be completed. At this point a population estimate could be completed using a 3-pass Zippen estimator (Module A).

2.1 DATA RECORDING AND DATA SUBMISSION

A field data form has been developed and is provided in Appendix A. All parameters discussed above, except macroinvertebrate data, will be recorded on this form. Following data entry into the Agency Resource Database, a more formal report will be prepared for all waters where wild trout were documented according to Module A; Appendix E. For waters where no wild trout were collected or too few wild trout were collected to qualify the stream or stream section for consideration as a wild trout water, a memo may be prepared listing all of the waters surveyed. A table listing the water name, county, survey date, and site latitude/longitude along with a map identifying all survey locations should be prepared and placed into individual stream files.

2.2 BIOSECURITY PROTOCOLS

Routine biological sampling requires sampling crews to regularly move sampling equipment between water bodies. As such, it is important for crews to follow proper procedures to minimize the likelihood for transport of non-native plant and animal species from one water body to another or across watersheds. These procedures are in response to ever increasing threats posed by aquatic invasive species (AIS) to the Commonwealth's aquatic resources and recreational users. Recent examples include the introduction of Viral Hemorrhagic Septicemia (VHS) to the Great Lakes, discovery of didymo *Didymosphenia geminata* in the upper portion of the Delaware River, and the spread of Zebra mussel *Dreissena polymorpha* to various waterways in Pennsylvania. The procedures provided in Module K will be followed when fieldwork necessitates the movement of equipment between waterways or across watershed basins. To the extent practical, all susceptible equipment moved between watersheds should be properly cleaned and disinfected. Particular attention should be given to situations where AIS are known or suspected to occur.

2.3 BASIC SAMPLING EQUIPMENT REQUIRED

Backpack electrofisher with nets
Field chemical kit - pH, air & water temp, total alkalinity, hardness and specific conductance
Measuring tape, hip chain, or range finder
Holding buckets and live bags
Fish measuring board
Clipboard with data sheets, blank paper and pencils
Topographic maps
GPS unit

Digital camera
Waders, non-porous gloves, polarized sunglasses
Disinfectant and rinse water

3. Literature Cited

American Public Health Association, American Water Works Association, and Water Pollution Control Federation. 1980. Standard methods for the examination of water and wastewater, 15th edition. Washington, D.C.

Pennsylvania Department of Environmental Protection (PA DEP). 2003. Water Quality Antidegradation Implementation Guidance, Document Number 391-0300-002.

Pennsylvania Department of Environmental Protection. 2006. Pennsylvania Integrated Water Quality Monitoring and Assessment Report. PA DEP file report, Harrisburg, PA.

Pennsylvania Fish and Boat Commission (PFBC). 2009. Strategic Plan for Management of Trout Fisheries in Pennsylvania, 2010-2014. PFBC Files, 450 Robinson Lane, Bellefonte, PA.

United States Environmental Protection Agency. 1976. Methods for chemical analysis of water and wastes. EPA-625/6-74-003a. Environmental Research Center, Cincinnati, Ohio.

Appendix A

Unassessed Water Field Data Sheet

Appendix C
Unassessed Waters Surveys - 2010

Survey Leader: _____ Scl Collector Permit # _____ Sample Date _____

Water Name: _____ Mouth Latitude: _____

Tributary to: _____ Mouth Longitude: _____

Site Latitude: _____ Site Longitude: _____

Site Length: _____ Widths: _____ _____ _____ _____ _____

Site Location: Avg _____

Gear Used: (check all) Backpack AC _____ Backpack DC _____ Other (specify) _____ Sampling Effort (min): _____

Size Group	Species: Number Caught	Species: Number Caught
25 - 49 mm		
50 - 74 mm		
75 - 99 mm		
100 - 124 mm		
125 - 149 mm		
150 - 174 mm		
175 - 199 mm		
200 - 224 mm		
225 - 249 mm		
250 - 274 mm		
275 - 299 mm		
300 - 324 mm		

Species Occurence:

Time of Day: _____ Water Temp: _____ C pH: _____ (Electronic / Colorimetric)

Tot Alk: _____ mg/l (Electronic / Colorimetric) Tot Hard: _____ mg/l (Electronic / Colorimetric) Spec Cond: _____ umhos

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Comments:

MODULE E

Policy for Electrofishing Operations

Prepared by:
Robert Weber and Dave Spotts

Preface

PFBC employees that are actively involved in electrofishing operations will comply with the following safety procedures as they apply to the use of boat, towed boat, and/or backpack electrofishing units.

1. At least one full-time permanent employee on each electrofishing crew will be required to have successfully completed the course "Principles and Techniques of Electrofishing" as offered by the United States Fish and Wildlife Service (Online course offered). Training should only be provided to employees who are routinely involved in electrofishing operations as part of their job responsibilities and duties.

2. All permanent employees who participate in electrofishing are required to have current CPR and First Aid certification. It is strongly recommended that at least two of the electrofishing crewmembers should be CPR and First Aid certified.

3. A trained fulltime permanent employee will conduct a field safety briefing for the entire crew before any electrofishing operations commence.

4. All of the safety procedures contained in the PFBC's Electrofishing Safety Guidelines (see attachment) will be followed. These procedures were historically developed by the Division of Fisheries Management Electrofishing Committee and will be updated as needed.

PENNSYLVANIA FISH AND BOAT COMMISSION
ELECTROFISHING SAFETY GUIDELINES

1. Introduction

Electrofishing has been practiced by personnel of the Pennsylvania Fish and Boat Commission at least since the early 1960's as reported by Miller (1962). Electrofishing can be a hazardous operation. The voltages and currents used are more than sufficient to electrocute a person. The environmental conditions in which these operations are conducted further increase the risks. Fortunately, no disabling electrofishing accidents have occurred in PFBC operations, but there have been accidents of varying degrees of seriousness. As with most states, the evolution of electrofishing as a fish sampling technique in Pennsylvania proceeded largely without the benefit of technical electrical expertise. Safety considerations were not foremost in the minds of those developing the procedures. Perhaps this is understandable, at least in part, due to the lack of operational standards. Pennsylvania's Bureau of Occupational and Industrial Safety has no electrofishing standards. Efforts to address safety in electrofishing have been presented by Coffelt (1978), Lazauski and Malvestuto (1984), Novotny and Priegel (1974), Rawston (1978), Reynolds (1996), and Vincent (1971).

The Division of Fisheries Management's Electrofishing Committee was formed to address concerns about the priority of safety in these operations. The committee agreed upon a goal "to ensure that no person is injured through Pennsylvania Fish and Boat Commission electrofishing operations by providing persons engaged in those operations with safe electrofishing equipment and operational procedures." The following guidelines, adapted from recent published guidelines, are intended to develop a safety first attitude in conducting electrofishing operations.

The PFBC uses three basic approaches to electrofishing—backpack, towed boat and flat- bottom jon boat. Each of the operations uses unique equipment applied to different situations. Separate guidelines were developed for each approach.

2. General Prerequisite for Electrofishing Safety

2.1 WATER SELECTION & SAMPLING SITES

2.1.1 Personnel Training

1. All permanent staff, required to use electrofishing, should complete the course "Principles and Techniques of Electrofishing" as offered by the United States Fish and Wildlife Service (Online Course Available).

2. All permanent staff, required to use electrofishing, should annually receive First Aid and CPR training.

3. In addition to completing the USFWS online electrofishing safety course, all temporary employees or interns who will work on electrofishing crews shall be instructed in the functions of the system they will be using.

2.1.2 Gear Inspections

4. Prior to spring sampling operations, all electrofishing units shall be inspected following the Electrofishing Gear Inspection Checklist (Supplement A). The completed checklist shall be sent to one of the Bureau of Fisheries representatives on the Executive Safety Committee.

5. All electrofishing systems shall be inspected prior to each operation by the designated Crew Leader (Supplement B). Electrofisher output should also be tested with vohm and megohm meter to determine if voltage leaks are present.

6. All outboard motors and boats shall have a thorough annual inspection to determine fitness for operation.

2.1.3 Operations Review

7. An annual electrofishing operations review meeting should be conducted involving all permanent staff.

8. All crew members should review operating procedures and systems prior to beginning each electrofishing operation.

3. Boat Electrofishing Guidelines

3.1 GEAR

3.1.1 Boat

1. Flat bottom, aluminum hull, preferably $\geq$ 18' length and 6' width.

2. Boat load will not exceed maximum load limit specified on the manufacturers capacity plate. Load includes personnel, equipment, outboard motor, water in tubs, and gasoline in cans.

3. Boats will have a bow rail(s), which extends back along the sides at least as far as the forward deck. The bow rail will be waist height, preferably 42", constructed of properly reinforced 1" steel or 1-1/2" aluminum pipe and adequately padded.

4. Forward deck flooring will be coated with non-skid material and repaired as necessary when worn.

5. Boats should be equipped with a steering console and electric outboard engine starter. All electric controls should be on or beside the console to allow the boat operator to face forward at all times.

3.1.2 Boat Accessories

1. All U.S. Coast Guard and Commonwealth required safety equipment for operation of an 18' boat as referenced in Section Y of this AIPP manual. Safety equipment must include the following:

    a. Lights – One all-round white light above gunwales, plus red and green running lights.

    b. At least one B-1 type approved portable marine fire extinguisher. Located away from combustible sources.

    c. Bell, whistle or air horn capable of making an efficient sound signal.

    d. Properly displayed registration.

    e. As per Commission Policy, all persons will wear an approved personal floatation device at all times while on board a Commission boat. Additionally, all boats over 16 feet must also have a type IV throwable device on board.

    f. Visual distress signal (flare) when operating on Lake Erie, including Presque Isle Bay.
2. Outboard motor of sufficient horsepower to maneuver boat and load in heavy wind and waves and to travel distances necessary in reasonable short time. Outboard grounded to boat hull.
3. Paddles, oars, electric motor or second gasoline motor as a back-up in case of failure of primary outboard.

4. Properly vented gasoline tanks with sufficient capacity to complete operations.

5. Twelve volt weatherproof floodlight system to provide visibility for netters.

6. Twelve volt search or spot light for use by boat operator.

7. When operating on a river system, anchor(s) of sufficient size, weight and configuration to hold boat in river currents shall be on board.

8. Bailer or bilge pump shall be used to remove excess water from hull.

3.1.3 Electrical System

3.1.3.1 Generator

1. 3500-5000 watt, 230 volt AC generator with a 110 outlet. Compatiable with electrofisher being used.

2. Generator with neutral or ground wire on the 230 volt outlet attached to the generator frame must be disconnected unless design provisions are made, for example, incorporation of an isolation transformer to prevent lethal shock and equipment damage. Refer to Supplement C on how to check this!

3. Generator with fuel tank large enough to run at least one hour.

4. High quality muffler system.

5. Exhaust should be vented over the side or rear of boat.

6. All hot parts should be screened or insulated.

7. Additional soundproofing desirable by partial covering of generators, but air must not be restricted from engine cooling fan or carburetor intake.

8. Generator shall be fused or have a circuit breaker to prevent an overload.

9. Generator will be grounded to the boat hull.

3.1.3.2 Wiring Connectors, Switches

1. All wire should be multi-strand gasoline and weather resistant neoprene and/or PVC insulated and rated for the maximum current and voltage that can be generated by the system.

2. All wiring shall be encased in metal or plastic conduit and grounded to the boat hull or in plastic watertight conduit. All conductors in given conduits shall be rated at the maximum voltage of any conductor in the conduit.

3. All connections should be made in a plastic watertight junction box.

4. All wire splices should be made with a crimp-on connector, solder and shrink tubing, or screw terminals on barrier strips/terminal board wire connectors and rated for the same current and voltage as the wire.

5. All switches should be weatherproof and operate on low voltage (less than 24 volts) circuits carried in conduits separate from that of high voltage wiring.

6. Pressure positive high voltage circuit activation switches shall be available to a bow netter and the boat operator. All switches will be connected in series with option to use parallel connection in the event of switch failure.

7. All lights, aeration system, and other electrical accessories should be run with a 12 volt DC system.

8. The 12 volt system should be powered by a 12 volt deep cycle marine battery which is protected in a non-metallic, acid resistant case and continuously charged with a battery charger from the 110 VAC outlet on the generator.

9. Each electrical circuit should be fused separately.

3.1.3.3 Transformer-Pulser

1. Should provide the full range of voltage and current desirable for a variety of operations and conductivities.

2. Should have on-off kill switch.

3. Should have meters to show output voltage, amperage and power.

4. Must be grounded to boat hull.

5. Meters and dials should be illuminated for night electrofishing.
6. Meters and dials should be oriented so that the boat operator can see them while steering the boat.

3.1.3.4 Electrode Configurations

1. The best electrode material is stainless steel, followed by flexible conduit, copper tubing and aluminum tubing.

2. All electrodes in AC operations and both anodes in DC operation shall be electrically isolated from metal boat hulls.

3. Fixed electrodes or anodes shall be supported forward of the bow on the end of non-conductive poles or booms, preferably fiberglass.

4. Fixed electrodes or anodes commonly used are approximately one meter in diameter horizontal ring or a cross member configuration from which

up to 12 dropper cylinders are suspended to dangle into the water. Other configurations are suitable and can be constructed using procedures and guidelines developed by the Fisheries Management Division.

5. If using suspended cathodes, they should be constructed of flexible conductive material (conduit) and should be suspended from gunwales as far aft as possible without interfering with outboard motor operations.

3.2 OPERATIONS

3.2.1 General Operations

1. One person will be crew leader for electrofishing operations. The crew leader must have completed the USFWS "Principles and Techniques of Electrofishing" Course.

2. As per Commission Policy, all persons on electrofishing boat shall wear approved personal floatation devices at all times.

3. No alcohol is permitted on electrofishing boats; no intoxicated persons are allowed on electrofishing boats.

4. The boat operator should always be facing forward with netters in full view.

5. Crews should take a 10-15 minute break every hour of electrofishing to prevent fatigue.

6. The person operating the boat is in charge of the controls.

7. All crew members must wear chest or hip waders to insulate them from electrical shock. Suitable waders are generally constructed of neoprene, PVC, silicon, etc. If you use breathable waders, then you must wear long pants under the waders (US Fish and Wildlife Service 2010).

8. Electrofishers and netters should wear rubber gloves.

9. All crew members should consider using ear plugs or mufflers as sound arrestors. Additionally, Full VOX radios for operator and a netter should be used to enhance crew communications and safer operations.

10. Pre-arrange start and stop signal.

11. Avoid operating near people, pets or livestock that are in or near the water.

12. Discontinue operation at the first sign of lightening, heavy rain, rough water, or crew fatigue.

13. Electrofish slowly and deliberately. Aggressively chasing fish should be avoided.

14. Never touch water or electrodes.

15. Shut down electrical field and generator for repairs, crew change, refuels, connections and disconnections.

16. Refuel generator carefully at or on shore with no fuel spillage.

17. No unnecessary passengers shall be on electrofishing boats.

18. Carry all necessary spare equipment.

19. The generator should be located to the rear of the operator and crew, with exhaust vented off the stern.

20. Net handle shall be constructed with non-conductive materials, preferably fiberglass.

21. Set up for night electrofishing operations should be conducted prior to darkness.

22. No smoking in electrofishing boats.

23. Carry the minimum extra gasoline needed to do the job.

24. Netters should wear polarized type sunglasses for daylight electrofishing.

4. Towed Boat Electrofishing Guidelines

4.1 GEAR

4.1.1 Boat

1. Vessels made from suitable material that is capable of floating all electrofishing gear and negotiating rough water without taking on water or otherwise compromising safety. Designs are available from the Fisheries Management Division.

2. Boat bottom covered with form fitting galvanized steel sheet metal or suspended droppers to serve as cathode.

4.1.2 Electrical System

4.1.2.1 Generator and transformer-pulser

1. 3500-5000 watt, 3-phase, 230 volt AC generator.

2. Generator with neutral or ground wire on the 230 volt outlet attached to the generator frame must be disconnected unless design provisions are made, for example, incorporation of an isolation transformer to prevent lethal shock and equipment damage. Refer to Supplement C on how to check this!

3. Generator with attached or remote transformer and pulsator.

4. Transformer must be electrically isolated from generator.

5. High quality muffler system.

6. All hot parts should be screened or insulated.

7. Generator shall be fused or have a circuit breaker to prevent overload.

4.1.2.2 Wiring, Connectors, Switches

1. All wire should be multi-strand gasoline and weather resistant neoprene and/or PVC insulated and rated for the maximum current and voltage that can be generated by the system.

2. All connections should be made in a plastic watertight junction box.

3. All wire splices should be made with a crimp-on connector, solder and shrink tubing, or screw terminals on barrier strips/terminal board wire connectors and rated for the same current and voltage as the wire.

4. All switches should be weatherproof and operate on low voltage (less than 28 volts) circuits of the same rating for current and voltage as the power output circuit.

5. Should have adequate meters to show output voltage, amperage and power

6. Pressure positive high voltage circuit activation switches should be installed in series on all probes and a push-button type switch will be available to the towed boat operator.

7. Anodes should be circular form of stainless steel or aluminum.

8. Boat Bottom covered with form-fitting or stainless steel sheet metal or stainless steel cables to serve as cathode.

9. Retractable electric cord reels should be used to connect probes to junction box.

4.1.2.3 Electrofishing Accessories

1. Anode or electrode probe handles will be constructed of non-conductive material, preferably fiberglass.

2. Net handles shall be constructed of non-conductive material, preferably fiberglass.

3. Buckets carried by netter shall be of non-conductive material.

4.2 OPERATIONS

4.2.1 General Operations

1. One person will be crew leader for electrofishing operations. The crew leader must have completed the USFWS "Principles and Techniques of Electrofishing" Course.

2. All crew members must wear chest or hip waders to insulate them from electrical shock. Suitable waders are generally constructed of neoprene, PVC, silicon, etc. If you use breathable waders, then you must wear long pants under the waders (US Fish and Wildlife Service 2010).

3. Rubber gloves will be made available and should be worn by all crew members.

4. No alcohol is permitted at electrofishing operations; no intoxicated persons are allowed to participate in electrofishing operations.

5. The crew member towing the boat should wear sound arrestors.
6. The crew member towing the boat is in charge of the controls.

7. Crews should take a 10-15 minute break after every hour of electrofishing to prevent fatigue.

8. Avoid operating near people, pets or livestock that are in or near the water.

9. Discontinue operation at the first sign of lightening, heavy rain, or crew fatigue.

10. Electrofish slowly and deliberately; aggressively chasing fish should be avoided.

11. Shut down all electrofishing operations for repairs, crew change, refuels, connections and disconnections.

12. Refuel generator carefully at or on shore with no fuel spillage.

13. All crew members should wear polarized sunglasses during electrofishing operations.

5. Backpack Electrofishing Guidelines

5.1 GEAR

5.1.1 Electrical System

5.1.1.1 Power Source

1. The electrical system for backpack units shall be one manufactured for the purpose of electrofishing by a qualified supplier.

2. The manufactured electrical system shall not be modified.

3. Should have adequate meters to show output voltage, power or amperage.

4. A gasoline powered generator shall have a high quality muffler system and be equipped with kill switch.

5. The transformer shall be electrically isolated from the generator and produce high voltage AC and/or DC current.

6. The electrofishing control units shall have built-in low voltage safety circuits.

5.1.1.2 Wiring, Connectors, Switches

1. All wire should be multi-strand gasoline resistant and rubber insulated and rated for the maximum current and voltage that can be generated in the system.

2. Connectors must be interlocking, weatherproof type.

3. Weatherproof low voltage (less than 28 volts) switches to activate high voltage output should be on each electrode probe.

5.1.1.3 Accessories

1. Electrodes shall be circular stainless steel or aluminum rods.

2. Electrode probe handles will be constructed of non-conductive material, preferably figerglass, and strong enough to support the weight of the person electrofishing.

3. Net handles shall be constructed of non-conductive material and strong enough to support the weight of the person electrofishing, preferably fiberglass.

4. Buckets carried by netter shall be of non-conductive material.

5.2 OPERATIONS

5.2.1 General Operations

1. One person will be crew leader for electrofishing operations. The crew leader must have completed the USFWS "Principles and Techniques of Electrofishing" Course.

2. All crew members must wear chest or hip waders to insulate them from electrical shock. Suitable waders are generally constructed of neoprene, PVC, silicon, etc. If you use breathable waders, then you must wear long pants under the waders (US Fish and Wildlife Service 2010).

3. Any crew member who gets excessive water in the boots shall remove them, dry the boots, and any wet clothing before continuing with electrofishing.

4. No alcohol is permitted at electrofishing operations; no intoxicated persons are allowed to participate in electrofishing operations.

5. The crew members should consider wearing ear plugs.

6. The crew member wearing the backpack electrofishing unit should not operate an electrofishing probe in hazardous situations.

7. A crew member other than the one wearing the backpack electrofishing unit shall be in charge of the controls.

8. Crews should take a 10-15 minute break every hour of electrofishing to prevent fatigue.

9. Avoid operating near people, pets or livestock that are in or near the water.

10. Discontinue operation at the first sign of lightning, heavy rain, or crew fatigue.

11. Electrofish slowly and deliberately; aggressively chasing fish should be avoided.

12. Shut down electrical field and generator for repairs, crew change, refuels, connections and disconnections.

13. Refuel with the backpack shut off, on the ground, and with the engine cooled.

14. All crewmembers should wear polarized sunglasses during electrofishing operations.

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SUPPLEMENT A

ANNUAL BOAT ELECTROFISHING GEAR SAFETY INSPECTION

MANAGEMENT AREA OR UNIT _____ DATE _____

GEAR DESCRIPTION AND PFBC ID# _____

_____1. Boat of adequate size and material.

_____2. Bow rail of sturdy construction.

_____3. Flooring of non-skid material.

_____4. Electrical controls positioned for easy operator viewing.

_____5. Lighting meets minimum U.S. Coast Guard and PFBC requirements for night operation.

_____6. Approved fire extinguishers.

_____7. Bell, whistle or air horn sound signaling device.

_____8. Properly displayed registration.

_____9. Accessory boat power equipment (oars, paddle, motor).

_____10. Outboard motor of sufficient size and operating condition to handle wind, waves and boat weight.

_____11. Properly vented gasoline tanks.

_____12. 12 volt exterior lighting system with search light for operator.

_____13. Anchor.

_____14. Bilge pump.

_____15. High quality muffler on generator properly vented.

_____16. Hot parts of generator properly screened or insulated.

_____17. All metal equipment properly grounded to boat hull.

_____18. Wiring multi-strand high voltage.

SUPPLEMENT A (Cont'd)

ANNUAL BOAT ELECTROFISHING GEAR SAFETY INSPECTION

MANAGEMENT AREA OR UNIT _____ DATE _____

GEAR DESCRIPTION AND PFBC ID# _____

_____19. Wiring and generator with proper fusing or with circuit breakers.

_____20. Wiring, connections and switches are properly grounded.

_____21. All wire splices made with crimp-on connector, solder and shrink tubing, or screw terminals on barrier strips/terminal board.

_____22. Pressure positive high voltage activation switches available for netters and operator.

_____23. Transformer-pulser illuminated for night work.

_____24. Electrodes, anodes and cathodes electrically isolated from boat hull.

_____25. Electrode booms and net handles of nonconductive material.

_____26. Batteries properly enclosed.

_____27. Proper PFD's in boat.

_____28. Sufficient boat cushions for crew.

_____29. Protective rubber gloves for netters.

_____30. Hearing protection for crew.

SUPPLEMENT B

OPERATIONAL BOAT ELECTROFISHING SAFETY INSPECTION

MANAGEMENT AREA OR UNIT _____ DATE _____

GEAR DESCRIPTION AND PFBC ID# _____

_____1. Boat in good repair and not leaking.

_____2. Bow rail of sturdy construction.

_____3. Lighting system functioning.

_____4. Charged fire extinguisher on board, located away from combustible sources.

_____5. First aid kit on board.

_____6. Sound producing device on board.

_____7. Oars, paddle or accessory motor on board.

_____8. Bilge pump functional.

_____9. Muffler in good repair and properly vented.

_____10. Hot parts of generator properly screened or insulated.

_____11. All metal equipment properly grounded to boat hull.

_____12. Circuit fuses in good condition with replacements.

_____13. All wiring, connectors and switches in proper location, interlocking, and in good condition.

_____14. Pressure positive high voltage activation switches functioning.

_____15. Electrodes, anodes and cathodes electrically isolated from boat hull.

_____16. Batteries properly enclosed.

_____17. PFD's worn by all crew members and at least one type IV throwable on board.

_____18. Sufficient boat cushions for crew.

SUPPLEMENT B (Cont'd)

OPERATIONAL BOAT ELECTROFISHING SAFETY INSPECTION

MANAGEMENT AREA OR UNIT _____ DATE _____

GEAR DESCRIPTION AND PFBC ID# _____

_____19. All crew members with rubber boots.

_____20. Protective rubber gloves for netters.

_____21. Hearing protection for crew.

_____22. Crew briefed on electrofishing system and imminent operations.

_____23. Generator fully fueled.

SUPPLEMENT C

Grounding the Generator

1. Check to make sure the ground circuit is isolated from the neutral (internal ground) of the power circuits or if they are bonded as in household applications. The ground circuit should be isolated from all other circuits (see Dia. 1, no. 1). The AC voltage from the generator should be isolated from the ground system (USFWS, March 2004). This means that the neutral (internal ground) of the 230-V outlet be isolated from the ground that is attached to the generator frame.

2. Use 10-gauge wire or larger to ground the generator to the hull of the boat, insuring that the frame of the generator is grounded to the generator itself (see pics. 1-3). Newer generators have rubber mounts separating the two. (Use a bolt and a wire connecter to connect to the boat hull, and a wire connector to a motor mount bolt on the generator).

3. Test using an ohmmeter, you should have continuity between any metal part of the generator and the boat hull. Make sure there is no paint hindering your reading, there should be clean metal where you are testing. Also make sure that the ground is isolated by making sure there is no continuity between any of the ground receptacle contacts and any of the power or neutral receptacle contacts in the power outlets (see Dia. 1, no. 2 and pics. 4-7).

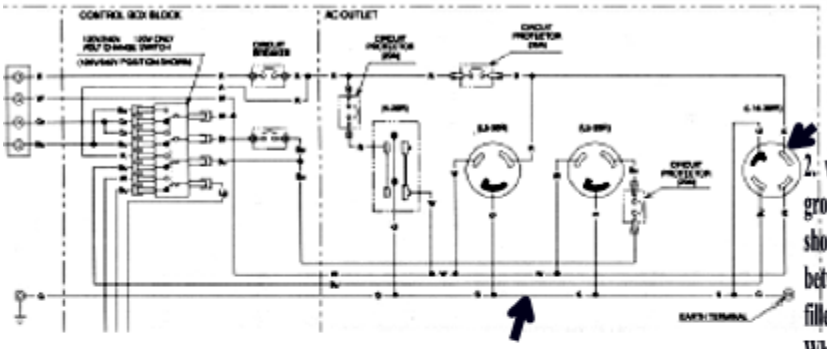
4. If it is not clear whether there is isolation of the ground circuit, contact a certified electrician to check for isolation. On a generator that is not isolated, modifications should be done by an electrician to insure the process is completed properly and to avoid electrical shock hazards.

5. It is also important to note that once a generator is modified or set up for boat electrofishing with the ground circuit isolated from the neutral (internal ground) that it is no longer suitable for normal use on the ground. For normal use such as in household applications the neutral and the ground circuit must be rebounded, which should be done by a certified electrician.

SUPPLEMENT C (cont)

Diagram 1.

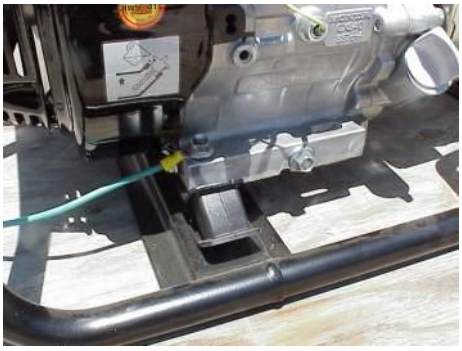
Wiring diagram from Honda EG5000X generator owner's manual



1. Notice that the ground circuit is isolated from all other circuits

2. when testing continuity, the ground receptacle (black fill) should not have any continuity between any of the other white-filled receptacle contacts. Also the White filled contacts should not have continuity between any metal parts of the generator and black filled contacts should have continuity with all metal parts of the generator.

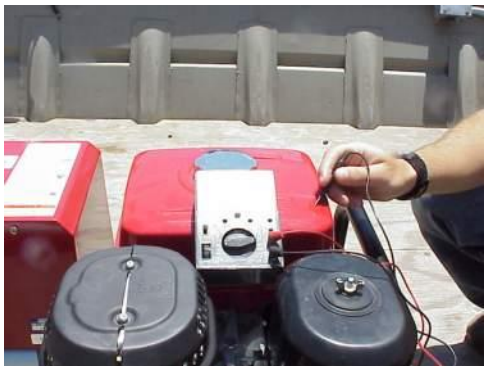
Pic. 1 – grounding to boat hull from generator



Pic. 2 – grounding from to generator from boat hull

Pic. 3 – grounding generator to generator frame

Pic. 4 – checking continuity between generator and Boat hull. (There is continuity)



Pic. 5 – checking continuity between generator And boat hull. (NO continuity due to paint)

Pic. 6 – continuity check between ground receptacle contact and generator. (there is continuity)

Pic. 7 – continuity check between power receptacle contacts And generator. (NO continuity)

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MODULE F

Monitoring Protocols for Habitat Enhancement Projects on Wadeable Streams

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1. Introduction

This document summarizes the field data collection and data analysis techniques, and indices proposed for use by Pennsylvania Fish and Boat Commission (PFBC), Division of Habitat Management (DHM) staff for monitoring the effectiveness of habitat enhancement activities on Pennsylvania wadeable streams. The field data collection and data analysis techniques (the protocols) described below are designed to be cost-effective, regionally appropriate, objective, and repeatable techniques for quantifying physical habitat, fish cover, and fish population and/or community responses to stream habitat enhancement activities. Furthermore, the protocols are designed to be applicable statewide, and provide standardized techniques for conducting monitoring activities appropriate for evaluating the attainment of habitat enhancement goals and objectives. Additional intended uses of the data generated through these monitoring activities will be to obtain information that will effectively show which habitat enhancement activities work, which do not work, where modifications need to be made, and to provide recommendations for future habitat enhancement efforts.

The physical habitat and fish cover monitoring protocols discussed throughout this document were selected after conducting a somewhat extensive review of existing literature. During the literature review phase of this project, three documents containing information relevant to the goals of the project were identified. These documents include the U.S. Department of Agriculture's Guidelines for Evaluating Fish Habitat in Wisconsin Streams (Simonson et al. 1993), the U.S. Environmental Protection Agency's (EPA) Wadeable Streams Assessment Field Operations Manual (USEPA 2004), and the EPA's Rapid Bioassessment Protocols for Use in Wadeable Streams and Rivers (Barbour et al. 1999).

Kaufmann (1993) identified seven general physical habitat attributes important in influencing stream ecology:

1. Stream size – channel dimensions: Kaufmann (1993) recommended that monitoring programs make field measurements of thalweg depth, depth cross-sections, wetted and bankfull width, and discharge as indicators of stream size.
2. Channel gradient: Gradient is a very important determinant of the potential energy in a stream that can be converted into water velocity.
3. Channel substrate size and type: Bottom characteristics are major controls on the species composition of macroinvertebrates, periphyton, and fish assemblages in streams and are often sensitive indicators of potential stressors.
4. Habitat complexity and cover: Complexity and cover determine niche diversity and cover from predation. When other needs are met, complex habitat with abundant cover should generally support greater biodiversity than simple habitats that lack cover. Kaufmann (1993) recommended the following components should be assessed: habitat type and distribution, large woody debris count and size, in-channel cover, residual pools, channel complexity, hydraulic roughness, width variance, and bank sinuosity.
5. Riparian vegetation cover and structure: Riparian vegetation is important to channel structure, cover, shading, nutrient inputs, large woody debris, wildlife corridors, and as a buffer against anthropogenic perturbations. Kaufmann (1993) recommended evaluating channel shading and riparian vegetation structure.

6. Anthropogenic alterations: Land use, buildings, and other human activities in the stream channel and riparian zone may serve as habitat quality indicators and diagnostic indicators of anthropogenic stress.
7. Channel-riparian interaction: Anthropogenic activities can result in the separation of streams from their floodplains and riparian zones. Thus, channel characteristics can be altered, which can affect biotic integrity of stream ecosystems.

Simonson et al. (1993) reported that given adequate chemical conditions, certain physical habitat features are particularly important in determining the occurrence and abundance of fish species at a given location within a stream. With regard to evaluating fish habitat in Wisconsin, Simonson et al. (1993) identify a set of 10 key instream and channel variables for summarizing general "macrohabitat" conditions for fish:

1. Stream size (e.g., width, drainage area, stream order, etc.)
2. Stream gradient
3. Stream temperature
4. Depth
5. Discharge
6. Substrate
7. Cover or shelter
8. Channel habitat units (e.g., pools, riffles, runs, etc.)
9. Bank erosion
10. Riparian conditions (e.g., adjacent land use, buffer width)

Simonson et al. (1993) defined "macrohabitat" assessments of physical habitat as using several values from a site to arrive at an overall picture of habitat conditions or availability for a given segment of stream. PFBC's DHM monitoring protocols are designed for use at the macrohabitat scale, as defined by Simonson et al. (1993). The protocols include the use of 10 key instream and channel variables for summarizing general "macrohabitat" conditions for fish reported by Simonson et al. (1993) in conjunction with the general physical habitat attributes important in influencing stream ecology as identified by Kaufmann (1993), to generate information that can be used to make general conclusions about a given stream reach's ability to support fish, and in turn, evaluate the effectiveness of habitat enhancement projects.

Roni et al. (2005) provided the basic steps for designing a monitoring and evaluation program for aquatic restoration. These steps include: 1) defining project goals and monitoring objectives; 2) defining key questions and monitoring scale; 3) selecting an appropriate monitoring design; 4) determining parameters to monitor; 5) determining the number of sites and years to monitor; 6) determining sampling scheme; 7) implementing the monitoring program; and 8) analyzing and reporting results. PFBC staff use these basic steps as general guidelines when developing reach-scale, site-specific monitoring programs, as well as, watershed-scale monitoring programs for evaluating the effects of habitat enhancement projects on in-stream physical habitat, fish cover, and fish populations and/or communities.

1.1 PROCESS USED FOR DEVELOPING A REACH-SCALE MONITORING PROGRAM

The following hypothetical example illustrates how PFBC staff use the basic steps outlined in Roni et al. (2005) when developing a reach-scale monitoring program for evaluating the effectiveness of a stream habitat enhancement project to enhance local trout productivity and physical habitat conditions in a shallow, over-widened stream channel with heavy deposits of silt and sand, actively eroding streambanks, and very limited cover for adult trout.

Step 1. Define Project Goals and Monitoring Objectives

Project Goal: To enhance fish habitat conditions and increase trout productivity within the project area.

Monitoring Objectives:

1. To determine if the project increased wetted channel depth and reduced channel width;
2. To determine if the project increased thalweg depth and variability;
3. To determine if the project increased residual pool depth and longitudinal pool area;
4. To determine if the project resulted in a change in the dominant substrate particle size and the amount of sand or finer substrate material deposited on the streambed;
5. To determine if the project had a positive effect on the amount, type, and spatial distribution of fish cover;
6. To determine if the project reduced the amount of actively eroding streambanks; and
7. To determine if the project resulted in an increase in trout productivity.

Step 2. Define Key Questions and Monitoring Scale

Key Question: What is the effect of the project on local physical habitat and fish cover conditions and trout productivity in the project area?

Step 3. Select an Appropriate Monitoring Design

Monitoring Design: A Before-After Control-Impact (BACI) design will be used to evaluate the treatment (impact) site and a control site both before and after treatment. A control site will be used to account for environmental variability and temporal trends found in both the control and treatment sites to increase the ability to differentiate treatment effects from other variability.

Step 4. Determine Appropriate Monitoring Parameters

Monitoring Parameters:

1. Wetted Channel Width
2. Water Depth
3. Thalweg Depth
4. Water Surface Slope
5. Substrate Particle Size Class Distribution
6. Amount, Type, and Spatial Distribution of Fish Cover
7. Linear Distance of Actively Eroding Streambank
8. Trout Species Abundance, Size Class Distribution, and Biomass
9. Ancillary Parameters (water temperature, alkalinity, stream discharge, EPA RBP visual-based habitat assessment, watershed land use information, etc.)

Step 5. Determine the Number of Monitoring Sites and Monitoring Frequency, and Duration

Number of Monitoring Sites: One treatment site and one appropriate control site.

Monitoring Frequency: A minimum of one pre-treatment monitoring survey will be conducted at both the treatment and control site. All monitoring data will be collected as close together in time as possible. If time allows, more than one pre-treatment survey will be conducted at both sites prior to implementing the habitat enhancement activities.
Monitoring Duration: Post-treatment surveys at the treatment and control sites will be conducted 1, 3, 5, and 10 years after implementation of the project.

Step 6. Determine Sampling Scheme
Sampling Scheme: Most physical habitat parameters including fish cover will be measured along evenly spaced transects. Bank stability, surface water slope, and fish surveys will be conducted along the entire reach at the treatment and control sites. All data will be collected in accordance with PFBC's Monitoring Protocols for Habitat Enhancement Projects on Wadeable Streams.

Step 7. Implement Monitoring Program
Program Implementation: The monitoring program will be implemented by PFBC DHM Regional Habitat Biologist Section staff with occasional assistance from other PFBC staff and/or project partners. Monitoring activities will be conducted under "summer/fall" (June – November) base-flow conditions, in accordance with PFBC's Monitoring Protocols for Habitat Enhancement Projects on Wadeable Streams. Ultimately, monitoring data will be entered and stored in an agency database that is consistent with the field forms.

Step 8. Analyzing and Reporting Results.
Data Analysis and Reporting – Descriptive statistics will be generated from the physical habitat and fish monitoring data and used to evaluate the effectiveness of the project with respect to the Monitoring Objectives and Key Question outlined in Steps 1 and 2 above, respectively. Ultimately, a standard reporting format will be developed for reporting the results of PFBC habitat enhancement project monitoring activities.

1.2 PROCESS USED FOR DEVELOPING A WATERSHED-SCALE MONITORING PROGRAM

Concurrent evaluations of multiple stream habitat restoration activities to assess watershed-scale habitat efforts on biota, especially fish populations, are extremely rare, and can be expensive (Roni et al., 2005). Nevertheless, these authors also reported that monitoring programs that assess the cumulative effect of restoration activities on a watershed-scale recovery of both habitat conditions and fish populations are essential for restoration planning, evaluating, and developing a predictive understanding of restoration effectiveness.

The following hypothetical example illustrates how PFBC staff propose to use the basic steps outlined in Roni et al. (2005) when developing a monitoring program to evaluate the effects of multiple habitat enhancement projects on watershed-scale physical habitat and fish cover conditions and trout productivity.

Step 1. Define Project Goals and Monitoring Objectives
Project Goal: To enhance fish habitat conditions and increase trout productivity within the project watershed.

Monitoring Objectives:

1. To determine if the project increased wetted channel depth and reduced channel width;
2. To determine if the project increased thalweg depth and variability;
3. To determine if the project increased residual pool depth and longitudinal pool area;
4. To determine if the project resulted in a change in the dominant substrate particle size and the amount of sand or finer substrate material deposited on the streambed;
5. To determine if the project had a positive effect on the amount, type, and spatial distribution of fish cover;
6. To determine if the project reduced the amount of actively eroding streambanks; and
7. To determine if the project resulted in an increase in trout productivity.

Step 2. Define Key Questions and Monitoring Scale

Key Question: What is the cumulative effect of all habitat enhancement projects within the watershed on physical habitat and fish cover conditions and trout productivity in the project watershed?

Step 3. Select an Appropriate Monitoring Design

Monitoring Design: A Before-After Control-Impact (BACI) design will be used to evaluate the treatment (impact) watershed and a control watershed both before and after treatment. A control watershed will be used to account for environmental variability and temporal trends found in both the control and treatment sites to increase the ability to differentiate treatment effects from other variability.

Step 4. Determine Appropriate Monitoring Parameters

Monitoring Parameters:

1. Wetted Channel Width
2. Water Depth
3. Thalweg Depth
4. Water Surface Slope
5. Substrate Particle Size Class Distribution
6. Amount, Type, and Spatial Distribution of Fish Cover
7. Linear Distance of Actively Eroding Streambank
8. Trout Species Abundance, Size Class Distribution, and Biomass
9. Ancillary Parameters (water temperature, alkalinity, stream discharge, EPA RBP visual-based habitat assessment, watershed land use information, etc.)

Step 5. Determine the Number of Monitoring Sites and Monitoirng Frequency, and Duration

Number of Monitoring Sites: Roni et al. (2005) reported that even where multiple reach-scale restoration projects exist within a watershed, results from a particular study site should not be extrapolated to the other sites, and that generalizations to effects at other sites are not statistically supported. Thus, in order to accurately address the Key Question identified in Step 2 above, all habitat enhancement sites in the treatment watershed should be monitored; however, staff and time limitations will most likely require subsampling of the treatment sites.

In addition to monitoring all or some habitat enhancement sites in the treatment watershed, several control sites are to be established within the treatment reach and several in an appropriate control watershed. Control sites should be as similar as possible to treatment sites with respect to land use, geology, hydrology, biology and other physical features.

Monitoring Frequency: A minimum of one pre-treatment monitoring survey will be conducted at each treatment and control site. All monitoring data will be collected as close together in time as possible. If time allows, more than one pre-treatment survey will be conducted at both sites prior to implementing the habitat enhancement activities.

Monitoring Duration: Post-treatment surveys at the treatment and control sites will be conducted 1, 3, 5, and 10 years after implementation of the project.

Step 6. Determine Sampling Scheme

Sampling Scheme: Most physical habitat parameters including fish cover will be measured along evenly spaced transects. Bank stability, surface water slope, and fish surveys will be conducted along the entire reach at the treatment and control sites. All data will be collected in accordance with PFBC's Monitoring Protocols for Habitat Enhancement Projects on Wadeable Streams.

Step 7. Implement Monitoring Program

Program Implementation: The monitoring program will be implemented by PFBC DHM Regional Habitat Biologist Section staff with occasional assistance from other PFBC staff and/or project partners. Monitoring activities will be conducted under "summer/fall" (June – November) base-flow conditions, in accordance with PFBC's Monitoring Protocols for Habitat Enhancement Projects on Wadeable Streams. Ultimately, monitoring data will be entered and stored in an agency database that is consistent with the field forms.

Step 8. Analyzing and Reporting Results.

Data Analysis and Reporting – Descriptive statistics will be generated from the physical habitat and fish monitoring data and used to evaluate the effectiveness of the project with respect to the Monitoring Objectives and Key Question outlined in Steps 1 and 2 above, respectively. Monitoring results from individual treatment sites will be summarized, and accumulated where appropriate, and reported in the form of a watershed-scale summary. Ultimately, a standard reporting format will be developed for reporting the results of PFBC watershed-scale habitat enhancement project monitoring activities.

2. Physical Habitat and Fish Cover Monitoring Protocols for Habitat Enhancement Projects on Wadeable Streams

2.1 GENERAL SAMPLING PROCEDURES

The DHM's physical habitat and fish cover monitoring protocols include a combination of systematic measurements and less-intensive, visual-based field assessment procedures. The protocols are designed for use on wadeable streams (most areas < 1.2 m deep), and field data is collected under "summer/fall" (June – November) base-flow conditions.

When using PFBC's protocols to monitor the effectiveness of habitat enhancement activities, similar data should be collected in a control reach. Control reaches should have chemical, physical, and biological characteristics similar to those of the treatment reach, and should effectively function as independent replicates of treatment reaches (Rona et al. 2005). Control reaches should be located upstream of treatment reaches, if possible, to avoid being affected by changes made to the treatment reaches.

Monitoring reach length is defined by the mean wetted width of the channel and the boundaries of the habitat enhancement project being evaluated. Mean wetted width is determined based on at least 10 regularly spaced measurements of the wetted width of the channel, measured perpendicular to the direction of stream flow, excluding islands, exposed bars, backwaters, and adjacent wetlands. The length of monitoring reaches are to be between 20 and 40 times the mean wetted channel width, and for streams with a mean wetted width of < 4.6 m (15 ft), a monitoring reach length of at least 91.5 m (300 ft) is used. For example, the length of a monitoring reach with a mean wetted width of 10 m, can range from no less than 200 m, to no more than 400 m, with the actual length being determined by the longitudinal extent of the habitat enhancement project being evaluated.

Once the upstream and downstream boundaries of a given monitoring reach have been determined, these same boundaries will be used in subsequent surveys, regardless of changes in mean wetted width values over time, to ensure the length of stream evaluated remains constant between all surveys conducted at the project site. Thus, it is important to accurately document, or mark, the boundaries of each monitoring reach. The upstream and downstream boundaries should be marked with an orange-capped, 2-foot long, ½" diameter rebar on each side of the stream.

Within the monitoring reach, systematic measurements are taken along 21 evenly spaced transects across the wetted channel. Simonson et al. (1994) recommended measuring habitat variables along approximately 20 transects, spaced 2 mean stream widths apart based upon a study of Wisconsin streams. The upstream-most transect is located at the head of a riffle and the remaining 20 transects are evenly spaced throughout the remainder of the reach. The evenly spaced transects will be between 1 and 2 mean stream widths apart. For example, a monitoring reach with a mean wetted width of 10 m, and a length of 300 m, will have a transect established at the head of a riffle at the upstream end of the reach, and a transect established every 15 m downstream, with the downstream-most transect being located 300 m downstream of the transect at the upstream end of the reach. Additional measures, such as streambank erosion/stability and surface water slope measurements, are made over the entire length of the monitoring reach, and visual-based assessments are made based on features observed throughout the entire monitoring reach.

The PFBC DHM's length of monitoring reaches between 20 and 40 times the mean wetted width and the number of transects used are similar to other protocols. U.S. EPA's Wadeable Streams Assessment (WSA) (USEPA 2004) and Environmental Monitoring and Assessment Program (EMAP) (Kaufmann et al. 1999) lengths are 40 times their low flow wetted width. USGS's National Water Quality Assessment (NAWQA) program lengths are 20 times wetted width (Fitzpatrick et al. 1998). Simonson et al. (1993) specifies 35 times wetted width for Upper Midwest streams. These programs define a reach length proportional to stream width and employ transect measurements that are systematically spaced.

Habitat Assessment Program	Reach length	Number of Transects	Distance between transects	Minimum reach length
PFBC DHM	20 – 40 times mean wetted width	21	1 to 2 channel widths	92 meters

EPA EMAP/WSA	40 times mean wetted width	11	4 channel widths	150 meters
USGS NAWQA	20 times mean wetted width	11	2 channel widths	150 meters
Wisconsin	35 times mean wetted width	≥ 18	2 channel widths	100 meters

2.2 HABITAT SAMPLING PROCEDURES AND DATA GENERATED

2.2.1 Thalweg Profile and Residual Pool Characteristics

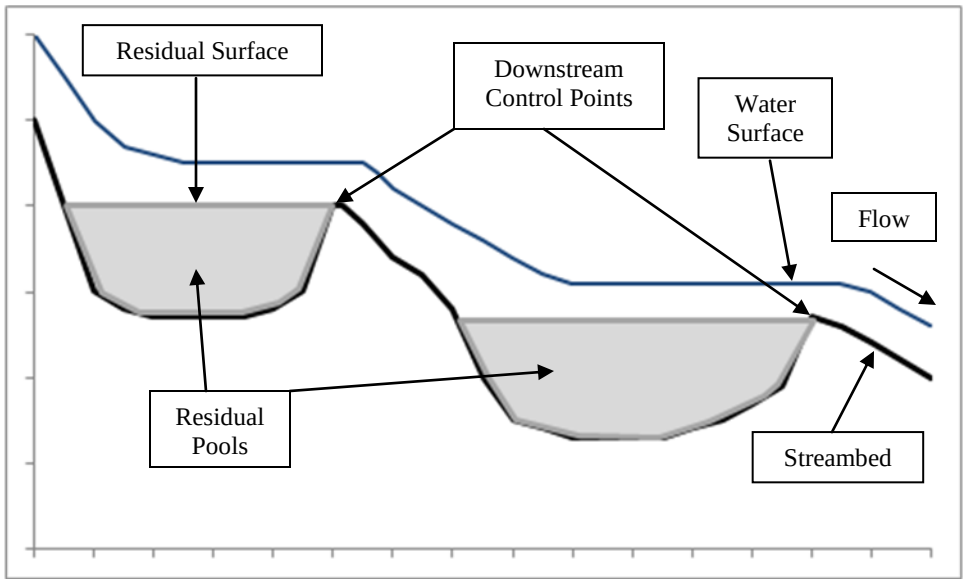
Once the monitoring reach length and transect spacing (monitoring reach length / 20) has been determined, a thalweg profile is conducted. The thalweg profile is a longitudinal survey of depth in the flow path of the deepest water in the stream channel. A minimum of 101 equally spaced thalweg depth measurements are recorded between the head of the riffle located at the upstream boundary of the monitoring reach and the head of a riffle located near the downstream boundary of the monitoring reach. Thalweg profile measurement spacing is the monitoring reach length / 100.

A 300 ft fiberglass measuring tape or surveyor's rope is stretched downstream along the middle of the wetted channel starting at the head of the riffle at the upstream boundary of the monitoring reach. Starting at the upstream boundary of the monitoring reach and proceeding downstream, thalweg depths are taken to the nearest 0.01 foot, and along with the distance on the tape, are recorded on the Thalweg Profile Field Data Sheet (Appendix A). The locations of the 21 transects are marked with a flag on each stream bank while conducting the thalweg profile. Since the thalweg profile must end at the head of a riffle, in most cases the downstream limit of the thalweg profile will extend beyond the location of the last of the 21 transects (Transect #1). However, if the location of the first riffle downstream of Transect #1 is located excessively far downstream, the thalweg profile can be terminated at Transect #1.

After completing the thalweg profile, water surface (WS) slope measured from the head of the riffle located at the upstream limit of the thalweg profile to the head of the riffle located at the downstream end of the profile. Surface water slope is measured using a laser level and calculated as follows:

$$\text{Water Surface Slope} = \frac{\text{WS Elevation Upstream (ft)} - \text{WS Elevation Downstream (ft)}}{\text{Length of Thalweg Profile (ft)}}$$

In the office, thalweg profile and surface water slope data are used to calculate the mean and standard deviation of thalweg depth measurements and to summarize the residual pool characteristics of the monitoring reach. Robison (1997) describes residual pools as depressions along the streambed that contain water even if there is no actively flowing water, and that residual pools are formed by downstream controls that act as dams causing back-watering.

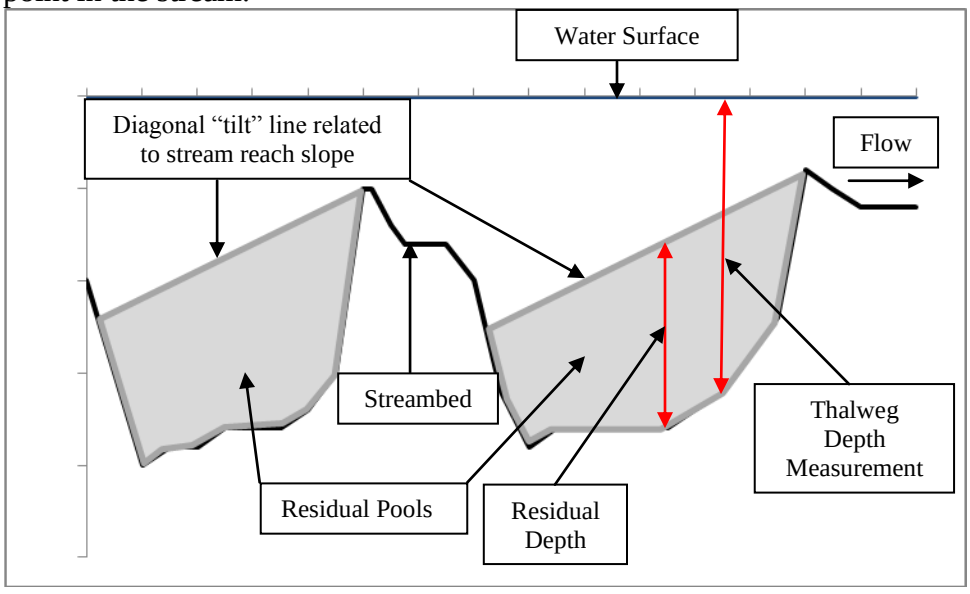


The residual pool characteristics of each monitoring reach are summarized using the Rapid Streambed Profile (RSP) method developed by Stack (1989) and modified by Robison (1997). Residual pool depths are calculated using the RSP method by first plotting the longitudinal distances recorded from the measuring tape stretched along the middle of the wetted channel on the horizontal axis of the plot vs. the corresponding thalweg depth measurement on the vertical axis of the plot. This plot represents the streambed.

Next, the residual surface of each pool is defined by projecting a downward diagonal line upstream from the downstream control point of each pool. This line passes through the deeper areas upstream of the control point until the line intersects a shallow depth measure upstream. The downward slope of the diagonal line (DLS) is related to the actual slope of the stream as follows:

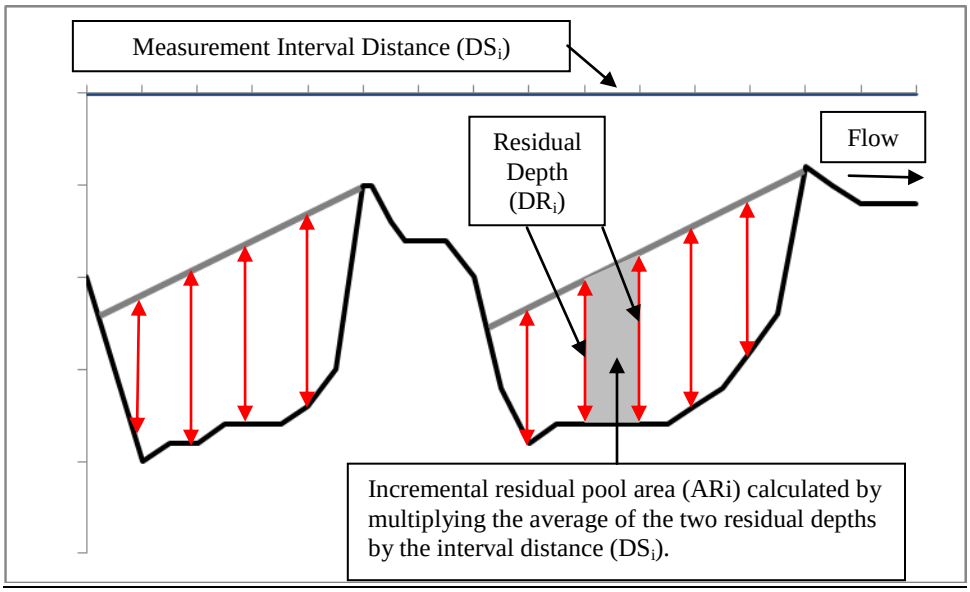
$$DLS = 0.4454 * Slope^{0.942}$$

The distance from the diagonal line to the streambed represents the residual depth (DR_i) at that point in the stream.



Once residual depths are determined, incremental longitudinal areas (AR_i) are calculated by multiplying the mean of the residual depths bordering the increment by the incremental distance (DS_i):

$$(AR_i) = (\text{mean } DR_i) * (DS_i)$$



Once residual depth and incremental longitudinal areas are determined, the residual pool characteristics of the monitoring reach are summarized as follows:

- Mean Incremental Residual Pool Depth
- Total Incremental Longitudinal Pool Area (ft^2) per 100 Feet of Stream Channel
- Total Incremental Longitudinal Pool Area (ft^2) ≥ 1 Foot Deep per 100 Feet of Stream Channel

2.2.2 Transects – Width, Depth, Substrate, PA/MD IFIM

After the thalweg profile is completed and transect locations are marked, transect and fish cover data are collected along each transect starting at the downstream boundary of the reach. The downstream boundary transect is recorded as Transect 1. A transect is set up by driving a 4-foot ½" diameter rebar in each stream bank with a 100 foot fiberglass tape stretched and attached by spring clamps to the rebar perpendicular to flow. The end of the tape should be placed on the left descending side of the channel. At each of the 21 transects, the following information is collected and recorded on the Transect Field Data Sheet (Appendix A): (1) wetted width, (2) depth, substrate, and Pennsylvania/Maryland Instream Flow Incremental Methodology (IFIM) cover codes at seven points, and (3) habitat unit type. Fish cover is also measured along each transect and is discussed under Section 2.3.3.

The distance on the 100 foot fiberglass tape of the left and right edge of water is recorded to the nearest 0.1 ft. The wetted width of the channel is calculated by subtracting the left edge of water from the right edge of water to the nearest 0.1 ft. If a mid-channel bar or island is present, the edges of the bar or island should be recorded and the width of the bar or island should be subtracted from the wetted width of the channel.

Water depth, substrate, and PA/MD IFIM cover codes are measured/noted at evenly spaced intervals across the transect. Water depth is measured to the nearest 0.01 ft at distances corresponding to 0%, 20%, 40%, 60%, 80%, and 100% of the measured wetted width, and at the deepest point (thalweg) along the transect, generating seven depth measurements per transect, and a total of 147 depth measurements within the monitoring reach. Wetted width, water depth, substrate composition, IFIM cover code data, habitat unit type from each transect is recorded on a field data sheet, a portion of which is included below.

Transect: 15	**Wetted Width (REW-LEW):**	43.7	**20% of Wet Width:**	8.7	**Wet Width Mid-Point (LEW + 50%):**		27.9
	LEW	**Left (LEW+20%)**	**L Ctr (LEW+40%)**	**R Ctr (LEW+60%)**	**Right (LEW+80%)**	**REW**	**Thalweg (Max Depth)**
Distance (XX.X ft)	6.0	14.7	23.4	32.1	40.8	49.7	39.9
Depth (XX.XX ft)	0.00	1.08	1.00	2.06	2.19	0.20	2.45
Substrate Size Class	CG	CG	CB	FG	SB	FN	SB
IFIM Cover Code	1	1	1	2	1	2	1
Hab Unit Type: POOL		**Comments:**					

In the field, the predominant substrate particle size class is determined in an ~10 cm diameter plot at distances corresponding to 0%, 20%, 40%, 60%, 80%, and 100% of the measured wetted width, and at the deepest point (thalweg) along each transect, generating seven substrate composition evaluations per transect, and a total of 147 substrate composition evaluations within the monitoring reach. Substrate particle size classes are as follows (USEPA 2004):

Substrate Size Classes and Codes		
Substrate Size Class	**Intermediate Axis Size**	**Code**
Non-Woody Organic	Moss, grass, etc. on sand or smaller substrate particles	ORG
Silt / Clay / Muck	<0.06 mm - Not gritty	FN
Sand	>0.06 to 2 mm – gritty	SA
Gravel (Fine)	>2 to 16 mm – up to marble-size	FG
Gravel (Coarse)	>16 to 64 mm – marble to tennis ball	CG
Cobble	>64 to 250 mm - tennis ball to basketball	CB
Boulder (Small)	>250 to 1000 mm - basketball to meter stick	SB
Boulder (Large)	>1000 to 4000 mm – meter stick to car	XB
Concrete / Asphalt	Any Size	RC
Bedrock (Rough)	>4000 mm – Rough surface rock larger than a car	RR
Bedrock (Smooth)	>4000 mm – Smooth surface rock larger than a car	RS
Wood	Any Size	WD
Other	Write Comment	OT

Transect substrate data are used to determine the dominant substrate particle size class and to quantify the extent of deposition of sand and finer materials (silt, clay, & muck) in each assessment reach. The dominant substrate particle size class is defined as the particle size class which has provided the largest number of observations.

Substrate Size Class	Conococheague Creek		Conoy Creek	
	%	Cumulative %	%	Cumulative %
% Organic	0.0	0.0	0.0	0.0
% Fines (Silt, Clay, Muck)	0.0	0.0	14.3	14.3
% Sand	10.5	**10.5**	**41.9**	**56.2**
% Fine Gravel	10.5	21.0	21.0	77.2
% Coarse Gravel	35.2	56.2	11.4	88.6
% Cobble	**39.0**	95.2	10.5	99.1
% Small Boulder	3.8	99.0	0.0	99.1
% Large Boulder	0.0	99.0	0.0	99.1
% Concrete/Asphalt	0.0	99.0	0.0	99.1
% Bedrock (Rough)	0.0	99.0	0.0	99.1
% Bedrock (Smooth)	0.0	99.0	0.0	99.1
% Large Woody Debris	1.0	100.0	1.0	100.1
Dominant Particle Size Class	**Cobble**		**Sand**	
% Sand or Finer Particles	**10.5**		**56.2**	

The PA/MD IFIM cover code is determined in an ~30 cm diameter plot at distances corresponding to 0%, 20%, 40%, 60%, 80%, and 100% of the measured wetted width, and at the deepest point (thalweg) along the transect, generating seven PA/MD IFIM cover codes per transect, and a total of 147 PA/MD IFIM cover codes within the monitoring reach. PA/MD IFIM cover codes are defined as follows (Susquehanna River Basin Commission 1998):

PA/MD IFIM Cover Code	Cover Description
1	No cover
2	Object at least 6” high and with cross-section horizontal measurement of at least 1 foot
3	Undercut object along bank
4	Aquatic vegetation
5	Terrestrial vegetation < 1 foot above water surface

In the office, substrate, cover codes, and depth field measurements are used to calculate PA/MD IFIM substrate/cover and depth Habitat Suitability Criteria (HSC). The 147 substrate field measurements are placed into three PA/MD IFIM substrate types as defined as follows (Susquehanna River Basin Commission 1998):

PA/MD IFIM Substrate Code	Substrate Description	Substrate Size Class
1	Diameter of < 3 mm	FN, SA
2	Diameter of 3 mm – 64 mm	FG, CG
3	Diameter of > 64 mm	CB, SB, XB

The substrate and cover codes are combined and used to obtain PA/MD IFIM substrate/cover HSC values for adult, juvenile, spawning, and fry brook trout and brown trout at 147 sampling points along the 21 transects. Mean substrate/cover HSC values for each life stage of brook trout and brown trout are computed for the monitoring reach and range from 0 to 1. The PA/MD IFIM substrate/cover HSC values are (Susquehanna River Basin Commission 1998):

	Adult		Juveniles		Spawning		Fry	
PA/MD IFIM Substrate/Cover Codes	Brook Trout HSC	Brown Trout HSC	Brook Trout HSC	Brown Trout HSC	Brook Trout HSC	Brown Trout HSC	Brook Trout HSC	Brown Trout HSC
1.1	0	0.1	0.3	0.3	0.2	0	1	1
1.2	0.6	0.4	1	0.8	0.2	0	1	1
1.3	1	1	1	1	0.2	0	1	1
1.4	0.6	0.4	1	0.8	0.2	0	1	1
1.5	0.6	0.4	1	0.8	0.2	0	1	1
2.1	0	0.1	0.3	0.3	1	1	0.6	0.6
2.2	0.6	0.4	1	0.8	1	1	0.6	0.6
2.3	1	1	1	1	1	1	0.6	0.6
2.4	0.6	0.4	1	0.8	1	1	0.6	0.6
2.5	0.6	0.4	1	0.8	1	1	1	1
3.1	0	0.1	0.3	0.3	0	0.1	0.1	0.1
3.2	0.6	0.4	1	0.8	0	0.1	0.1	0.1
3.3	1	1	1	1	0	0.1	0.1	0.1
3.4	0.6	0.4	1	0.8	0	0.1	0.1	0.1
3.5	0.6	0.4	1	0.8	0	0.1	1	1

Depth measurements are used to obtain PA/MD IFIM depth HSC values for adult, juvenile, spawning, and fry brook trout and brown trout at 147 sampling points along the 21 transects. Depth HSC values for depths not listed in the table below are extrapolated. Mean depth HSC values for each life stage of brook trout and brown trout are computed for the monitoring reach and range from 0 to 1. The PA/MD IFIM depth HSC values are (Susquehanna River Basin Commission 1998):

	Adult		Juveniles		Spawning		Fry		
PA/MD IFIM Depth (ft)	Brook Trout HSC	Brown Trout HSC	Brook Trout HSC	Brown Trout HSC	Brook Trout HSC	Brown Trout HSC	PA/MD IFIM Depth (ft)	Brook Trout HSC	Brown Trout HSC
0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.13	0.04	0.00	0.11	0.15	0.40	0.49	0.06	1.00	1.00
0.38	0.08	0.09	0.21	0.15	1.00	1.00	0.19	1.00	1.00
0.63	0.26	0.17	0.64	0.53	1.00	1.00	0.31	1.00	1.00
0.88	0.50	0.32	0.68	0.67	1.00	0.58	0.44	1.00	1.00
1.13	1.00	0.62	1.00	1.00	1.00	0.46	0.56	1.00	1.00
1.38	1.00	0.83	1.00	1.00	1.00	0.33	0.81	0.50	0.50
1.63	1.00	1.00	1.00	0.82	0.00	0.26	0.94	0.20	0.20
1.88	1.00	1.00	1.00	0.64	0.00	0.18	1.06	0.10	0.10
2.13	1.00	1.00	0.80	0.27	0.00	0.00	1.19	0.10	0.10

2.38	1.00	1.00	0.75	0.27	0.00	0.00	1.31	0.10	0.10
2.63	1.00	1.00	0.70	0.27	0.00	0.00	1.44	0.10	0.10
2.88	0.45	0.56	0.50	0.27	0.00	0.00	1.56	0.10	0.10
3.13	0.45	0.56	0.00	0.27	0.00	0.00	1.69	0.10	0.10
3.38	0.45	0.56	0.00	0.00	0.00	0.00	1.81	0.10	0.10
3.63	0.45	0.56	0.00	0.00	0.00	0.00	1.94	0.10	0.10
3.88	0.45	0.56	0.00	0.00	0.00	0.00	2.06	0.00	0.00
4.13	0.45	0.56	0.00	0.00	0.00	0.00	2.19	0.00	0.00
4.38	0.45	0.56	0.00	0.00	0.00	0.00	2.31	0.00	0.00
4.63	0.45	0.56	0.00	0.00	0.00	0.00	5.94	0.00	0.00
4.88	0.45	0.56	0.00	0.00	0.00	0.00			
5.13	0.45	0.56	0.00	0.00	0.00	0.00			

The habitat unit type (riffle, run, glide, or pool) along the transect is recorded. A habitat unit type is a discrete channel unit based on fluvial geomorphic descriptors, including flow patterns and channel bed shape. Visual determination of habitat units can be subjective with poor precision because they are rarely separated by clearly defined boundaries (Platts 1982). Habitat units are defined as follows (Overton et al. 1997):

Habitat Unit Type	Definition
Riffle	Habitat unit in which water flows swiftly over completely or partially submerged obstructions to produce surface agitation. No defined thalweg.
Run	Habitat unit that is deep and fast (greater than 1 ft/sec) with a defined thalweg and limited surface agitation.
Glide	Habitat unit that has low to moderate velocities, no surface agitation, and no defined thalweg. The channel is a uniform U-shape with a smooth, wide bottom. Glides can appear to be pool-like, but are distinguished by having no significant scour depressions.
Pool	Habitat unit in which scouring water has carved out a non-uniform depression in the channel bed. Water velocity is usually slow. Pools are usually deeper than riffles and runs. Streambed gradient is often near zero and streambeds are often concave in shape. Water surface gradient at low flow is close to zero. Pools often contain large eddies with widely varying directions of flow, compared to riffles and runs, where flow is nearly exclusively downstream. Pools usually are formed around bends or around large-scale obstructions that laterally constrict the channel or cause a sharp drop in the water surface profile.

Transect habitat unit data are used to characterize the diversity of habitat conditions present in the assessment reach. The percent composition of each habitat unit type in the assessment reach provides a summary of the degree of riffle/pool sequence development as a general indicator of habitat diversity. Percent composition of each habitat unit type is calculated as:

% Habitat Unit Type A = Total # of transects located in Habitat Unit Type A / Total # of Transects X 100

2.2.3 Fish Cover

Fish cover is instream objects, channel features, or riparian/bank features that provide complete shelter from the current or visual isolation from predators. Determining fish cover can be

subjective and ambiguous, because cover requirements vary by species, life stage, and season (McMahon et al. 1996). Therefore, we clearly defined what constitutes cover to promote consistency. Cover is divided into two categories: Large Fish cover for fish 200 m or greater (~ ≥ 8 inches) in total length and Small Fish cover for fish less than 200 m. This fish cover criteria is geared toward trout species but may be applicable to other lotic fish species.
Large Fish (L) cover features must be at least 0.3 m (1 ft) long, 0.3 m (1 ft) wide, and 0.3 m (1 ft) high (equals about 1 ft^3) and in or just above water at least 0.3 m (1 ft) deep. **Small Fish (S)** cover features must be at least 0.15 m (0.5 ft) long and 0.15 m (0.5 ft) wide with no minimum depth.

Fish cover includes riparian and bank features, instream structures, and channel features. Fish cover features or types are undercut banks, overhanging vegetation, root wad, woody debris, boulder, submerged & emergent macrophytes, pool, and other debris.

Undercut Banks - Stable banks that overhang the water by at least 1 foot (Large Fish) or 6 inches (Small Fish), at a point where the water is at least 1 foot deep (Large Fish) or no minimum depth (Small Fish). The bottom of the undercut bank must be no more than 12 inches above the water surface. However, if undercut extends 24 inches or more under the bank, the distance from the bottom of the undercut bank to the water surface, may be up to 18 inches. Undercut banks should be recorded on the data sheet as **UB**.

Overhanging vegetation - Thick vegetation overhanging the water that meets the above criteria for cover. This cover type is divided into two categories: OV1 and OV3. **OV 1** is overhanging vegetation that is ≤ 0.3 m (1 ft) above the water surface. **OV 3** is overhanging vegetation > 0.3 m (1 ft) and ≤ 0.91 m (3 ft) above the water surface. Overhanging vegetation should be recorded on the data sheet as either **OV1** or **OV3**.

Root wad - Large root wad or aggregations of smaller root wads located in or in contact with water at least 1 foot deep for Large Fish or no minimum depth for Small Fish. Root wads should be recorded on the data sheet as **RW**.

Woody debris - Large pieces (minimum of 0.1 m (4 inches) in diameter) or aggregations of smaller pieces of wood (e.g., logs, large tree branches) located in or in contact with water at least 0.3 m (1 ft) deep for Large Fish or no minimum depth for Small Fish. Woody debris should be recorded on the data sheet as **WD**.

Boulder - Rocks with an intermediate axis ≥ 0.51 m (20 inches) for Large Fish or ≥ 0.3 m (1 ft) for Small Fish that are located in or in contact with water, and provide cover as defined above. Large pieces of concrete and other artificial rocky aggregates also belong in this category. Boulders should be recorded on the data sheet as **B**.

Submerged & emergent macrophytes - Vascular plants that normally have all or nearly all their biomass below the surface of the water **(SM)** or a significant portion of their biomass above the surface of the water **(EM)**. To count as cover, macrophytes must be rooted in water and be thick or dense enough to provide shelter or visual isolation for fish and provide cover as defined above. Aquatic macrophytes should be recorded on the data set as either **SM** or **EM**.

Pool - Pool size and depth are sufficient to provide a low velocity resting area for large fish, with an adequate portion of the pool bottom being obscured (visually isolated) due to water depth

and/or surface turbulence. Pools with a depth of 3 feet or more are considered cover for large fish, regardless of surface turbulence and visual isolation conditions. Pools as cover should be recorded on the data sheet as **P**. The minimum pool depth without surface turbulence that provides visual isolation should also be recorded on the data sheet.
Other debris - Pieces of human-made debris found in or in contact with water at least 0.3 m (1 ft) deep for Large Fish or no minimum depth for Small Fish that provide shelter or visual isolation for fish. Examples include old tires, abandoned farm implements, and discarded home appliances. Other debris should be recorded on the data sheet as **OD**.

2.2.3.1 Fish Cover Methods

The most commonly used method to assess cover is to measure the cover types and express the total amount of cover as a percentage of the study area (McMahon et al. 1996). All the cover in the stream reach can be measured or cover can be assessed in a portion of the stream reach such as along transects. We elected to use the transect method because this method can be used efficiently even in streams with abundant cover. Fish cover based upon the criteria above is conducted with two methods.

1) Fish Cover Measured Along Transects - The first method is the transect method similar to Simonson et al. (1993). At each of the 21 transects, the length of Large Fish and Small Fish
cover that intercepts the transect line or within 0.3 m (1 ft) upstream and downstream of the transect line is measured. The exception to examining cover within 1 ft on each side of the transect line is the first (downstream boundary) and last (upstream boundary) transects of the site reach. This is done to ensure that fish cover is only measured within the site reach. At the first (downstream boundary) transect, cover is only measured along the transect line and 1 ft upstream of the line. At the last (upstream boundary) transect, cover is only measured along the transect line and 1 ft downstream of the line. Each cover feature is measured with a calibrated rod or pole parallel to the transect line to the nearest tenth of a foot. The following data is recorded on the Fish Cover Field Data Sheet for each fish cover feature encountered along the transects: transect number, length of transect, length of cover, size class (Large Fish or Small Fish), and cover type. Output generated from the data will provide the relative quantity of cover available to fish in the stream reach.

Total fish cover (Large Fish and Small Fish combined) is determined by the sum of the lengths of cover from each transect divided by the sum of the lengths of transects (wetted widths) and is expressed as a percentage. Total Large Fish cover and total Small Fish cover are determined the same way but only using the lengths of cover for the appropriate size class. The percentage of fish cover by cover type (e.g., % boulder) can be calculated the same way by only using the appropriate cover type.

The example in the table below is an abbreviated example of fish cover collected from six transects (typically 21 transects are done). Total fish cover was 21.8% with 17% as Large Fish cover and 4.8% as Small Fish cover. Boulders provided the most Large Fish cover (5.5%) and Small Fish cover (3.8%).

Data Collected in the Field				
Transect	**Length of Transect (Wetted Width) in ft**	**Length of Cover (ft)**	**Size Class (L or S)**	**Cover Type**
1	43.6	0.0	-	-
2	43.8	2.5	S	B
2		1.0	S	B
3	111.8	3.5	S	B
3		0.5	S	WD
3		6.0	S	B
3		1.3	L	B
3		1.0	L	B
3		2.2	L	B
3		5.7	L	P
4	46.0	1.0	S	WD
4		10.0	L	SM
4		12.0	L	P
5	57.2	0.5	S	WD
5		0.5	S	WD
5		3.0	L	B
5		5.0	L	SM
5		1.0	L	B
5		2.4	L	B
5		4.0	L	OV1
6	39.3	1.0	S	UB
6		2.5	L	OV3
6		8.0	L	B
Total	341.7	74.6		

Output Generated From Data			
	Cover Length (ft)	**% Cover**	**Cover Type**
Total Fish Cover	74.6	**21.8**	ALL Cover
Total Large Fish Cover	58.1	**17.0**	ALL Cover
	18.9	5.5	B
	0.0	0.0	EM
	0.0	0.0	OD
	4.0	1.2	OV1
	2.5	0.7	OV3
	17.7	5.2	P
	0.0	0.0	RW
	15.0	4.4	SM
	0.0	0.0	UB
	0.0	0.0	WD
Total Small Fish Cover	16.5	**4.8**	ALL Cover
	13.0	3.8	B
	0.0	0.0	EM
	0.0	0.0	OD
	0.0	0.0	OV1
	0.0	0.0	OV3
	0.0	0.0	RW
	0.0	0.0	SM
	1.0	0.3	UB
	2.5	0.7	WD

2) Distance to Closest Large Fish Cover from Transects - The second fish cover method is distance to closest Large Fish cover similar to a method used by Dr. Todd Petty, West Virginia University. At each of the 21 transects, the distance to the nearest Large Fish cover is measured from the wetted width mid-point of the transect line. Once the wetted width mid-point is determined, find the closest Large Fish cover whether it is along the transect line, upstream, or downstream of the transect line as long as you stay within the site reach. Measure the distance from the mid-point to the closest Large Fish cover with a calibrated rod/pole or fiberglass tape to the nearest tenth of a foot. Record the distance and Large Fish cover type for each of the 21 transects on the Fish Cover Field Data Sheet.

Output generated from the data will provide mean distance to closest Large Fish cover and mean relative distance (adjusted to mean wetted width) to closest Large Fish cover. Mean distance to closest Large Fish cover is determined by averaging the 21 distances obtained from the 21 transects. In the example given below the mean distance to closest Large Fish cover was 15.6
feet. Therefore, a Large Fish in the middle of the channel would need to move 15.6 feet, on average, to get to cover. Mean relative distance to closest Large Fish cover is determined by taking the distance divided by the wetted width for each transect and then obtaining the average. In the example given below the relative distance was 1.0, which means that a Large Fish in the middle of the channel would need to travel, on average,

one channel width to get to cover. The percentage of closest Large Fish cover by cover type (e.g., % boulder) can be calculated by the frequency of the cover type divided by 21.

Data Collected in the Field				
Transect	**Wetted Width (ft)**	**Distance to Closest Large Fish Cover (XX.X ft)**	**Rel. Dist. to Large Fish Cover (Dist/Wet Width)**	**Cover Type**
1	15.9	13.6	0.9	B
2	10.6	6.8	0.6	UB
3	12.2	0.9	0.1	B
4	22.9	19.5	0.9	WD
5	14.0	15.3	1.1	B
6	9.9	16.2	1.6	B
7	13.3	41.6	3.1	B
8	19.0	50.8	2.7	WD
9	25.6	20.4	0.8	WD
10	16.3	5.3	0.3	P
11	17.8	10.4	0.6	WD
12	16.3	19.8	1.2	WD
13	22.0	7.2	0.3	WD
14	26.8	9.3	0.3	OV1
15	10.9	4.7	0.4	WD
16	9.4	3.5	0.4	WD
17	8.6	10.8	1.3	UB
18	20.7	17.7	0.9	UB
19	16.2	20.3	1.3	WD
20	10.7	9.2	0.9	WD
21	13.8	24.6	1.8	UB

Output Generated From Data			
Mean (ft)	15.9	**15.6**	**1.0**
Std Dev (ft)	5.4	12.1	0.8
		%B	23.8
		%EM	0.0
		%OD	0.0
		%OV1	4.8
		%OV3	0.0
		%P	4.8
		%RW	0.0
		%SM	0.0
		%UB	19.0
		%WD	47.6

2.2.4 Streambank Stability

The entire length of both streambanks within the assessment reach are assessed and the total length of stable or unstable bank (whichever appears to be less common) is measured along each streambank and recorded on the Bank Stability / RBP Data Sheet. We use the bank stability guidelines provided in Barbour et al. (1999) and in Overton et al. (1997). In general, relatively steep banks that are not covered by vegetation in vigorous condition or by cobble or larger material, are likely to collapse and suffer from erosion, and therefore are considered unstable. Stable streambanks show **no evidence of breakdown** (clumps of bank broken away and banks are exposed), **slumping** (banks have slipped down), **tension cracking or fracture** (a crack is visible on the bank), or **vertical and eroding** (bank angle is steeper than 80 degrees from the horizontal, and less than 50 percent covered by perennial vegetation, roots, rocks of cobble size or larger, or logs of 0.1 m in diameter or larger).

The streambank stability condition of each bank is expressed as the percentage of the streambank that is stable:

% Stable Streambank = Total length of stable streambank / Total length of streambank X 100

2.2.5 Stream Discharge

During each monitoring event, stream discharge is measured using the midsection, current-meter method, commonly used by the United States Geological Survey at gaging stations, described in detail in Buchanan and Somers (1969) and Rantz et al. (1982). In general, this measurement is the summation of the products of the partial areas of the stream cross-section and their respective average velocities. Velocity and depth is measured at a minimum of 20 verticals across the cross-section. At each vertical, velocity is measured at 0.6 of the depth below the surface. At verticals with depths greater than 2.5 feet, velocity is measured at 0.2 and 0.8 of the depth below the surface, and the average of these measurements is taken as the mean velocity in the vertical.

If a current meter is unavailable, a discharge measurement using Global Water flow meter, model # FP101 (range 0.3 – 15 fps) can be used. Flow measurements should be taken in one or two foot intervals (depending on average wetted width of the stream in question, this may have to be taken to 3 feet in some instances). Water depth should be taken at every interval and the edges of water. With the meter recording average velocity, move the probe side to side and up and down within the specific one or two foot interval you are measuring for roughly 45-60 seconds or until the reading becomes steady. The probe does not measure velocities below 0.1 fps accurately, in circumstances where the user may not get a velocity when some flow is obvious, use .05 ft/sec to compensate. To determine total discharge multiply the area of the interval (Area = depth × length of interval) by the average velocity. This is the discharge for that specific interval, compute all other intervals measured in the same manner and add all the values up to obtain total stream flow.

2.3 RBP HABITAT ASSESSMENT (Visual-Based Assessment)

Stream habitat should also be assessed at each site using the U.S. Environmental Protection Agency's Rapid Bioassessment Protocols (RBP; Barbour et al. 1999). The high-gradient habitat assessment data sheets should be used for riffle/run prevalent stream sections in moderate to high

gradient landscapes. High gradient streams generally have substrates dominated by coarse sediment particles (i.e., gravel or larger). The low-gradient habitat assessment data sheets should be used for glide/pool prevalent stream sections in low to moderate gradient landscapes. Low gradient streams usually have substrates comprised of fine sediment or infrequent aggregations of more coarse (gravel or larger) sediment particles. Habitat assessments are first made on instream habitat, followed by channel morphology, bank structural features, and riparian vegetation. The habitat evaluation process involves rating the 10 parameters as optimal, suboptimal, marginal, or poor based on the criteria included on the habitat assessment field data sheets. All parameters are scored on a numerical scale of 0 (lowest) to 20 (highest). The ratings are then totaled and compared to a reference condition to provide a final habitat rating. Scores increase as habitat quality improves (Barbour et al. 1999).

The habitat assessment should be performed after the detailed habitat and biological sampling is completed. The habitat assessment data sheet should be completed, by a team of 2 or more biologists, if possible, to come to a consensus on the rating.

2.4 SUMMARY OF PHYSICAL HABITAT AND FISH COVER FIELD MEASUREMENTS AND OFFICE CALCULATIONS

Channel Morphology and Channel Features

Field Measures

- Discharge
- Wetted width (n = 21)
- Depth (n = 147)
- Maximum depth (thalweg profile) (n = 101)
- Dominant substrate particle size class (n = 147)
- Transect habitat unit class (riffle, pool, etc.) (n = 21)
- Surface water elevation at the upstream end of two riffles, one riffle located at the upstream end and one near the downstream end of the assessment reach
- Embeddedness (EPA RBP Visual Assessment)
- Sediment Deposition (EPA RBP Visual Assessment)
- Velocity/Depth Regime (EPA RBP Visual Assessment)
- Channel Flow Status (EPA RBP Visual Assessment)
- Channel Alteration (EPA RBP Visual Assessment)
- Frequency of Riffles or Bends (EPA RBP Visual Assessment)

Office Calculations

- Drainage Area
- Stream Order
- Discharge (including water yield (cfs/mi^2 drainage area))
- Mean depth (n = 147)
- Mean width / depth ratio (n = 21)
- Mean thalweg depth (n = 101)
- Standard deviation of thalweg depth (n = 101)
- Mean Incremental Residual Pool Depth

- Total Incremental Longitudinal Pool Area (ft^2) per 100 Feet of Stream Channel
- Total Incremental Longitudinal Pool Area (ft^2) ≥ 1 Foot Deep per 100 Feet of Stream Channel
- Percent sand or finer substrate
- Dominant substrate particle size class (sand, fine gravel, coarse gravel, cobble, etc.)
- Percent habitat unit composition (% of transects in riffle, pool, etc.)
- Surface water slope
- Pennsylvania/Maryland IFIM mean depth habitat suitability criteria value for adult, juvenile, spawning, and fry brook trout and brown trout
- Pennsylvania/Maryland IFIM mean substrate/cover habitat suitability criteria value for adult, juvenile, spawning, and fry brook trout and brown trout

Fish Cover

Field Measures

- Pennsylvania/Maryland IFIM Cover Code (n = 147)
- Length and type of cover for large (fish ≥ 8" in length) along each of the 21 transects
- Length and type of cover for small (fish <8" in length) along each of the 21 transects
- Distance from the mid-point of each transect to the closest large fish cover, and the type of large fish cover (n = 21)
- Epifaunal Substrate/Available Cover (EPA RBP Visual Assessment)

Office Calculations

- Percent large fish cover (broken down by cover type)
- Percent small fish cover (broken down by cover type)
- Percent total fish cover (percent large and small fish cover combined, broken down by cover type)
- Mean distance from the mid-point of each transect to the closest large fish cover
- Pennsylvania/Maryland IFIM mean substrate/cover habitat suitability criteria value for adult, juvenile, spawning, and fry brook trout and brown trout

Streambank, Riparian, and Watershed Land Use Conditions

Field Measures

- Length of unstable (actively eroding) streambank (left bank, right bank)
- Bank Stability Score (EPA RBP Visual Assessment)
- Bank Vegetative Protection Score (EPA RBP Visual Assessment)
- Riparian Vegetative Zone Width (EPA RBP Visual Assessment)

Office Calculations

- Percent Stable Streambanks (left bank, right bank, both banks combined)
- Watershed Land Use (% composition)

3. Biological and Chemical Monitoring Protocols for Habitat Enhancement Projects on Wadeable Streams

3.1 INTRODUCTION

The biological component of the monitoring program is extremely important. Habitat projects are conducted to improve conditions for aquatic life. Thus, physical habitat may have improved, but did it result in better aquatic life. The biological monitoring protocols will include only fish sampling due to staff and time constraints. If aquatic macroinvertebrate monitoring is desired, outside assistance from PA DEP or other project partners would be required.

In most cases, the goal of monitoring fish populations as part of habitat enhancement and restoration projects is to determine if a positive response occurs in the fish population(s) as the condition of the habitat improves. Thus, fish can be used as an indicator of improved conditions and can provide for measures of project success.

A shortcoming of most early evaluations of stream enhancement projects is that they sampled and focused on one or two fish species (usually salmonids; Reeves et al. 1991; Roni et al. 2002). This can be problematic because non-salmonid species may be impacted by enhancement and restoration activities (Roni and Quinn 2001) and in some cases may be more sensitive to habitat alteration (Roni et al. 2005). Thus, monitoring the response of various species and the structure and diversity of the fish community can provide important information about the success of the project. Depending on the scope and specific objectives of a project, monitoring the response of trout or other gamefish may be adequate to measure project success or it may be necessary to collect all fish during the first electrofishing pass to obtain CPUE data for all species (Index of Biotic Integrity (IBI) type survey).

The type of fish sampling required for monitoring will depend on the type of habitat project. For most habitat projects, an IBI survey should be conducted if enough staff and time are available. If wild trout or other gamefish are present in significant numbers, then a population estimate (absolute abundance indices) should be conducted in conjunction with an IBI survey if possible. If a population estimate is conducted without an IBI survey, then noting the presence and categorizing relative abundance of nongame fish should be conducted during the first pass of the population estimate. The least desirable fish survey for monitoring is a single pass catch per unit effort (CPUE) (relative abundance index) of gamefish and noting the presence and categorizing relative abundance of nongame fish.

3.2 GENERAL SAMPLING PROCEDURES

The general fish sampling procedures for monitoring habitat projects are similar to those found in Module A. Please refer to Module A for fish sampling protocols in wadeable streams.

Monitoring reach length is defined by the mean wetted width of the channel and the boundaries of the habitat enhancement project being evaluated. Further details for site length are provided in the General Sampling Procedures section of the Physical Habitat and Fish Cover Monitoring Protocols chapter. Beginning and ending points of the fish sampling reach should be located at

natural barriers (e.g., riffles); if natural barriers are not available block nets should be used to minimize fish movement out of the sampling reach.

3.3 FISH SAMPLING PROCEDURES AND DATA GENERATED

Index of Biotic Integrity (IBI)

The objective is to acquire a representative sample of the fish population in a wadeable stream by sampling all physical stream habitats in relative proportion to their availability. The collected sample will contain most of the species in the stream at the time of sampling in numbers proportional to their actual abundance. The sampling crew will attempt to collect as many fish as possible. All fish will be held in buckets or live cars, identified to species, and enumerated. Approved fish IBI protocols are available in Module H.

Absolute Abundance Indices – Population Estimates

The two most common methods used to estimate fish abundance in wadeable streams in Pennsylvania are mark-recapture and removal/depletion estimates. Please refer to Module A for detailed population estimate protocols.

When it is clear that fewer than 30 targeted fish species will be collected over a 300 m site during the marking run of a Petersen estimate or the first pass of a removal/depletion estimate, a population estimate should not be conducted at the site. A CPUE survey is used to provide an index of relative abundance rather than a population estimate.

Population estimates from Petersen mark-recapture and removal/depletion estimates are calculated electronically by the PFBC Agency Resource Database (ARD) for each 25-mm size group. Wild trout total abundance (#/km) and biomass (kg/ha) estimates can be used as measures of project change.

If the Petersen estimate is conducted in conjunction with an IBI, all fish are captured during the marking run and then only targeted gamefish are collected during the recapture run (second electrofishing pass of a Petersen mark-recapture estimate). If the removal estimate is conducted in conjunction with an IBI, all fish are captured during the first pass and then only trout are collected during subsequent passes.

Relative Abundance Indices - CPUE

For single-pass CPUE surveys, gamefish of interest are netted, measured, and recorded in 25-mm length groups. During the survey, the presence of each nongame fish species is recorded. After the survey has been completed, each nongame fish species should be categorized into one of four groups: Rare, Present, Common, and Abundant based upon the number observed per length of stream sampled. Module A provides detailed protocols for relative abundance indicies.

Length of Site	Rare	Present	Common	Abundant
100 meters	1	2 - 8	9 - 34	> 34
200 meters	1	2 - 17	18 - 67	> 67
300 meters	1 - 2	3 - 25	26 – 100	> 100

3.4 WATER QUALITY SAMPLING PROCEDURES

During each monitoring event, a core set of water quality parameters (temperature, pH, total alkalinity, and specific conductivity) is assessed at or near the downstream end of the monitoring reach. Temperature can be measured by either a field meter or by field thermometer. Field meters should be used for pH and specific conductivity measurements and be calibrated on a regular basis. Alkalinity measurements should be done with a field titration kit. Reagents and pH buffer solutions should not be used after their expiration date. Optional water quality parameters such as nitrate, phosphate, or dissolved oxygen can be assessed at each site. Module J provides the approved protocols for collecting water quality parameters.

3.5 SUMMARY OF BIOLOGICAL AND CHEMICAL FIELD MEASUREMENTS AND OFFICE CALCULATIONS

Biological Conditions

Field Measures – IBI (single pass, collecting all fish)

- Fish species occurrence
- Catch for each individual fish species

Field Measures – Absolute Abundance Indices (multiple passes: Petersen mark-recapture or Removal/depletion of targeted species)

- Fish species occurrence
- Multiple catch of targeted fish species
- Relative abundance (Rare, Present, Common, or Abundant) of nontargeted fish species

Field Measures – Relative Abundance Indices - CPUE (single pass, collecting targeted species)

- Fish species occurrence
- Catch of targeted fish species
- Relative abundance (Rare, Present, Common, or Abundant) of nontargeted fish species

Office Calculations – IBI (single pass, collecting all fish)

- Fish species occurrence
- CPUE for each individual fish species
- IBI metrics regarding: fish species richness and composition, number and abundance of indicator species, trophic organization and function, and reproductive behavior

Office Calculations – Absolute Abundance Indices (multiple passes: Petersen mark-recapture or Removal/depletion of targeted species)

- Fish species occurrence
- Population estimates: abundance (#/km) and biomass (kg/ha) of targeted fish species

Office Calculations – Relative Abundance Indices - CPUE (single pass, collecting targeted species)

- Fish species occurrence
- CPUE of targeted fish species including CPUE abundance and biomass estimates

Chemical Water Quality Conditions

Field Measures

- Temperature
- pH
- Total Alkalinity
- Specific Conductivity

4. Staff Resource Requirements

On small streams (mean wetted channel width <30 ft), chemical water quality, stream discharge, physical habitat, fish cover, and visual-based habitat assessment data can usually be completed in one day (excluding travel time) per site with a staff of three adequately-trained personnel. On larger streams, this data can be collected in one day with a five-person crew.

Fish data collection staff resource requirements vary based on the size of the stream and the type of survey being conducted. If fish survey work is limited to a CPUE survey on a small stream (mean wetted channel width <30 ft), this type of survey can be conducted by a three-person crew in a ½ day per site. On small streams that warrant more-intensive mark-recapture or depletion surveys, fish data collection will typically require one full day with an adequately-trained three-person crew. Mark-recapture or depletion surveys conducted on large streams can require up to a full day with a five-person crew.

In the office, data entry of all field data (including fishery data) typically requires 1.5 person-days per site. Thus, total staff resource requirements for data collection and entry range from 6.0 to 11.5 person-days per site. Since each treatment site monitoring event typically requires monitoring of a control site, these staff resources will by multiplied by a factor of 2, and the total staff requirements associated with monitoring one treatment-control pair is approximately 12 to 23 person-days.

5. Habitat Assessment During Biological Assessments of Wadeable Streams

5.1 INTRODUCTION

Biological assessments of wadeable streams designed to characterize a component of the fish community must include a measure of basic physical and habitat parameters. Some physical habitat measurements are mandatory as they have a direct impact on management decisions or are a required parameter in determining fish species biomass. Other physical habitat parameters, while not required to be collected, have proven useful in explaining fish population trends, especially when multiple surveys completed over multiple years have been conducted.

5.2 MANDATORY HABITAT PARAMETERS

Sampling Field Procedures: Pre-Electrofishing Preparation and Site Assessment

Assuming that a sampling site has been selected either previously or on the day of the stream survey, upon arrival at the site the sampling crew members, usually two, should receive various individual assignments from the crew leader. Tasks to be accomplished and an example of crew member assignments prior to electrofishing include the following:

- site length measurement (crew member #1)
- site width measurements (crew member #1)
- stream and riparian physical attribute ratings (crew member #1 with possible contributions from crew members #2 and #3; completed after reviewing the site)
- fish habitat and stream channel characteristics notations (crew member #1 with possible contributions from crew members #2 and #3; completed after reviewing the site)
- physical and chemical characteristics measurement and notations (crew members #2 and #3)
- optional aquatic macroinvertebrate collection and identification (crew members #2 and #3)
- electrofishing equipment preparation and assembly (crew member #2)

When sampling with a typical three-person crew, one crew member, immediately upon arriving at the site, begins to establish the downstream and upstream site limits and measure the assigned minimum sampling site length while other crew members begin the assigned tasks noted above. As the site is being measured it is acceptable to extend or reduce the site length to find a suitable blockage to fish movement or a suitable location for a blocking net. Sites are generally 300 m long or longer and should be measured down the center of the stream following the current and not the bank. Measurements should be conducted using a fiberglass measuring tape, hip-chain, or digital range finder and be recorded to the nearest meter. The end points are flagged with surveyor's tape or some other temporary marker, if necessary. Intermediate points may also be flagged, such as at the 150 m point. This is done so that electrofishing may be terminated early in cases where a population estimate is desirable for a particular species in a 300 meter long or longer site, but it is clear by the time that the electrofishing crew reaches the 150 m flag that the successful collection of at least 30 individual fish of that species or species group (trout) is unlikely in the full 300 m site.

The same crew member records the mean wetted channel width, which is necessary for calculating the surface area of the sample site. This usually occurs at the same time as the site length is being measured. The mean wetted channel width should be measured at a minimum of 10 evenly spaced transects per site using a fiberglass measuring tape, hip-chain or range finder, and recorded to the nearest 0.1 meter. For example: if a sample site is 300 m long, start at one end of the site, measure the width to the nearest 0.1 m, pace off approximately one tenth of the site length (30 m) and take another measurement (Marcinko et al. 1986). Continue in this manner until 10 measurements have been recorded. Be sure that the distance between measurements does not correspond to a natural pattern within the stream, such as a riffle at every measuring point with pools in between. If there is a high amount of variability among the widths, measurements should be taken at 15 transects per site.

While measuring the sampling site, the crew member makes mental notes or records information that will later be used in describing in-stream and riparian habitats on data sheets. The goal is for physical habitat descriptions at the site (or sites) to be adequate to represent the section in which the sampling site is located. Information may also be recorded on stream flow, bank erosion, shade, bank vegetation, and substrate composition. It is suggested that stream channel features (pools, riffles, runs, glides) and their relative lengths also be characterized. If the sample site is measured in conjunction with electrofishing, such as using a hip-chain, the physical characteristics should be recorded after the electrofishing survey along with the RBP Habitat form (Appendix C).

Flow will be designated as high, normal, or low, which will be defined as follows:

High flow:	The stream is bank full or approaching bank full. Water is above the normal water line with grasses and other vegetation possibly submerged.
Normal flow:	The stream is within the limits of the normal water line. The flow may be further described as being on the high or low side of normal (i.e., high-normal, low-normal) if the general description of "normal" is inadequate for a given situation.
Low flow:	The stream is below the normal water line and portions of the stream channel are exposed, such as sand bars and rubble. It should be noted if the stream is nearly dry.

Bank erosion will be described by one of four terms, heavy, moderate, light, and none, which will be defined as follows:

Heavy:	Frequent raw banks occur on the outsides of bends and often between pools. Over 50% of the banks are raw.
Moderate:	Raw banks occur along 25% to 50% of the stream.
Light:	5% to 25% erosion.
None:	Less than 5% erosion.

Shade will be described by one of three terms, dense, partial, and open, which will be defined as follows:

Dense:	More than 75% of the stream is covered by a canopy.
Partial:	Between 25% and 75% of the stream is covered by a canopy.
Open:	Less than 25% covered.

Bank vegetation refers to the predominant vegetation on the banks and within 100 m of the stream. More than one of the following four terms may be used to describe bank vegetation in some limited cases, especially when a narrow line of trees provides shade to the stream along otherwise shade-free riparian land:

Agricultural:	This refers to cultivated land, row crops, nurseries, orchards, and pastures.
Grasses and sedges:	This refers to grasses, tufted marsh plants, and plants having solid, non-woody stems.
Shrubs:	This refers to woody vegetation less than 4.5 meters in height.
Trees:	This refers to hardwoods and conifers over 4.5 meters in height.

Substrate composition refers to the predominant substrate(s) in the stream channel. Two to three substrate types from the following list may be selected to describe the predominant substrates.

Bedrock	
Boulders:	256 mm (10 in.) in diameter or larger.
Rubble:	64 to 255 mm (2.5-10.0 in.) in diameter.
Gravel:	2 to 64 mm (0.08 – 2.5 in.) in diameter.
Sand:	0.06 to 2.0 mm in diameter; gritty texture between fingers.
Silt:	0.004 to 0.06 mm in diameter.
Clay:	< 0.004 mm in diameter; smooth, slick texture between fingers.

Stream channel features, specifically pools, riffles, runs, and glides, along with a description of their depths and their relative lengths may also be recorded by the sampling crew. For example, a site may be described as having long, 0.5 to 1.0 meter deep pools and short, very shallow riffles, with an occasional run and very deep pool.

Along with stream channel features, it is appropriate to make notations about fish habitat and habitat impacts. While the aforementioned features, such as pools, may provide good fish habitat, other forms of fish habitat that the investigators feel are noteworthy should be listed or described as the site is being measured or after it is electrofished. These habitat types and descriptions may include, but are not limited to, large boulders, deep pools, pocket pools, cascading or tumbling water, undercut banks, tree roots, woody debris (snags), fallen trees, submerged logs, overhanging shrubs, bedrock crevasses, aquatic vegetation, rip-rap, mud-sills, deflectors, and bridge holes. Additionally, it is appropriate to note when stream bank fencing projects are in place or other forms of habitat improvement have been implemented.

In addition to positive habitat features, particularly poor habitat should be noted as well. It is appropriate to indicate when the stream channel and its habitat have been degraded by stormwater runoff, channelization, agriculture, mining, road maintenance, and logging activities, as well as impacts from sewage or other discharges, such as accumulations of solids, algae, or iron precipitate, to list a few. When specific and substantial sources of habitat degradation are observed, including dams, they should be recorded. This information can then be provided to the Division of Habitat Management for consideration for habitat enhancement.

While previously described notations are about the sampling site and upstream impacts on the sampling site, it is suggested that the survey forms also include some general notations about the drainage basin. These will primarily focus on land use as well as sources of pollution and habitat degradation if they have not already been covered in notes that pertain to the sampling site. The notes may include recommendations that will later appear or be further developed in the stream or stream section report. For some assessments such as, warmwater streams and life stage (young of year) assessments, habitat measures need not be a survey component.

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Appendix A

Field Data Sheets Utilized During Habitat Assessments

Thalweg Profile Field Data Sheet

Site:							
Date:		**Crew:**					
Site Length (ft):				**Thalweg Spacing (ft):**			
Thalweg Profile Start Location at Upstream End of Site							
Comments:							
Station	**Distance (ft)**	**Depth (ft)**	**Comment**	**Station**	**Distance (ft)**	**Depth (ft)**	**Comment**
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**			

Thalweg Profile Field Data Sheet

Station	Distance (ft)	Depth (ft)	Comment	Station	Distance (ft)	Depth (ft)	Comment
51				81			
52				82			
53				83			
54				84			
55				85			
56				86			
57				87			
58				88			
59				89			
60				90			
61				91			
62				92			
63				93			
64				94			
65				95			
66				96			
67				97			
68				98			
69				99			
70				100			
71				101			
72				102			
73				103			
74				104			
75				105			
76				106			
77				107			
78				108			
79				109			
80				110			

Transect Field Data Form

Site:		Page ______ of ______
Date:	Time:	Crew:
Site Length (ft):		Transect Spacing (ft):
Upstream Boundary:		
Downstream Boundary:		
Notes:		

Transect:	Wetted Width (REW-LEW):		20% of Wet Width:		Wet Width Mid-Point (LEW + 50%):		
	LEW	Left (LEW +20%)	L Ctr (LEW +40%)	R Ctr (LEW +60%)	Right (LEW+ 80%)	REW	Thalweg (Max Depth)
Distance (XX.X ft)							
Depth (XX.XX ft)							
Substrate Size Class							
IFIM Cover Code							
Hab Unit Type:		Comments:					

Transect:	Wetted Width (REW-LEW):		20% of Wet Width:		Wet Width Mid-Point (LEW + 50%):		
	LEW	Left (LEW +20%)	L Ctr (LEW +40%)	R Ctr (LEW +60%)	Right (LEW+ 80%)	REW	Thalweg (Max Depth)
Distance (XX.X ft)							
Depth (XX.XX ft)							
Substrate Size Class							
IFIM Cover Code							
Hab Unit Type:		Comments:					

Transect:	Wetted Width (REW-LEW):		20% of Wet Width:		Wet Width Mid-Point (LEW + 50%):		
	LEW	Left (LEW +20%)	L Ctr (LEW +40%)	R Ctr (LEW +60%)	Right (LEW+ 80%)	REW	Thalweg (Max Depth)
Distance (XX.X ft)							
Depth (XX.XX ft)							
Substrate Size Class							
IFIM Cover Code							
Hab Unit Type:		Comments:					

Transect:	Wetted Width (REW-LEW):		20% of Wet Width:		Wet Width Mid-Point (LEW + 50%):		
	LEW	Left (LEW +20%)	L Ctr (LEW +40%)	R Ctr (LEW +60%)	Right (LEW+ 80%)	REW	Thalweg (Max Depth)
Distance (XX.X ft)							
Depth (XX.XX ft)							
Substrate Size Class							
IFIM Cover Code							
Hab Unit Type:		Comments:					

Transect:	Wetted Width (REW-LEW):		20% of Wet Width:		Wet Width Mid-Point (LEW + 50%):		
	LEW	Left (LEW +20%)	L Ctr (LEW +40%)	R Ctr (LEW +60%)	Right (LEW+ 80%)	REW	Thalweg (Max Depth)
Distance (XX.X ft)							
Depth (XX.XX ft)							
Substrate Size Class							
IFIM Cover Code							
Hab Unit Type:		Comments:					

Transect:	Wetted Width (REW-LEW):		20% of Wet Width:		Wet Width Mid-Point (LEW + 50%):		
	LEW	Left (LEW +20%)	L Ctr (LEW +40%)	R Ctr (LEW +60%)	Right (LEW+ 80%)	REW	Thalweg (Max Depth)
Distance (XX.X ft)							
Depth (XX.XX ft)							
Substrate Size Class							
IFIM Cover Code							
Hab Unit Type:		Comments:					

Transect:	Wetted Width (REW-LEW):		20% of Wet Width:		Wet Width Mid-Point (LEW + 50%):		
	LEW	Left (LEW +20%)	L Ctr (LEW +40%)	R Ctr (LEW +60%)	Right (LEW+ 80%)	REW	Thalweg (Max Depth)
Distance (XX.X ft)							
Depth (XX.XX ft)							
Substrate Size Class							
IFIM Cover Code							
Hab Unit Type:		Comments:					

Fish Cover Field Data Sheet

Stream Name:		Site ID:
Date:	Min depth for Pool as cover (ft):	Crew:

Cover and Size Classes	**L or S**	**Large Fish (L) Cover** is defined as features that provide shelter from predators and/or strong currents or visual isolation for a fish that is a least 8 inches in total length. Large fish cover features must be at least 1 foot long, 1 foot wide, and 1 foot high (1 cubic foot) and in or just above (< or = 3 ft) water at least 1 foot deep. **Small Fish (S) Cover** features must be at least 6 inches long and 6 inches wide, with no minimum depth.
Undercut Banks	**UB**	Stable banks that overhang the water by at least **1 foot (Large Fish) or 6 inches (Small Fish)**, at a point where the water is at least **1 foot deep (Large Fish) or no minimum depth (Small Fish)**. The bottom of the undercut bank must be no more than 12 inches above the water surface. However, if undercut extends 24 inches or more under the bank, the distance from the bottom of the undercut bank to the water surface, may be up to 18 inches.
Overhanging Vegetation	**OV1 or OV3**	Thick vegetation overhanging the water that meets the above criteria for cover. **OV 1** is overhanging veg that is < or = 1 foot above the water surface, **OV 3** is overhanging veg > 1 foot and < or = 3 feet above the water surface.
Root Wad	**RW**	Large root wad or aggregations of smaller root wads located in or in contact with water at least 1 foot deep for large fish or no minimum depth for small fish.
Woody Debris	**WD**	Large pieces **(minimum of 4 inches (10 cm) in diameter)** or aggregations of smaller pieces of wood (e.g., logs, large tree branches) located in or in contact with water at least 1 foot deep for large fish or no minimum depth for small fish.
Other Debris	**OD**	Pieces of human-made debris found in or in contact with water at least 1 foot deep for large fish, or no minimum depth for small fish, that provide shelter or visual isolation for fish. Examples include **old tires, abandoned farm implements, and discarded home appliances**.
Boulder	**B**	Rocks with an **intermediate axis > or = 20 inches (Large Fish) or 12 inches (Small Fish)** that are located in or in contact with water, and provide cover as defined above. Large pieces of **concrete and other artificial rocky aggregates** also belong in this category.
Submerged & Emergent Macrophytes	**SM or EM**	Vascular plants that normally have all or nearly all their biomass below the surface of the water **(SM)** or a significant portion of their biomass above the surface of the water **(EM)**. To count as cover, macrophytes must be rooted in water and be thick or dense enough to provide shelter or visual isolation for fish.
Pool	**P**	Pool size and depth are sufficient to provide a low velocity resting area for large fish, with an adequate portion of the pool bottom being obscured (visually isolated) due to water depth and/or surface turbulence. Pools with a depth of **3 feet or more are considered cover for large fish,** regardless of surface turbulence and visual isolation conditions.

Transect	Length of Transect (ft) (XX.X)	Length (XX.X ft) of Cover Intersecting Transect Line (within 1 foot of transect line)		Cover Type	Comment		Distance to Closest Large Fish Cover (XX.X ft)	Cover Type	Comment
		Length of Cover	Size Class (L or S)						

Cover and Size Classes	**L or S**	**Large Fish (L) Cover** is defined as features that provide shelter from predators and/or strong currents or visual isolation for a fish that is a least 8 inches in total length. Large fish cover features must be at least 1 foot long, 1 foot wide, and 1 foot high (1 cubic foot) and in or just above (< or = 3 ft) water at least 1 foot deep. **Small Fish (S) Cover** features must be at least 6 inches long and 6 inches wide, with no minimum depth.
Undercut Banks	**UB**	Stable banks that overhang the water by at least **1 foot (Large Fish) or 6 inches (Small Fish)**, at a point where the water is at least **1 foot deep (Large Fish) or no minimum depth (Small Fish)**. The bottom of the undercut bank must be no more than 12 inches above the water surface. However, if undercut extends 24 inches or more under the bank, the distance from the bottom of the undercut bank to the water surface, may be up to 18 inches.
Overhanging Vegetation	**OV1 or OV3**	Thick vegetation overhanging the water that meets the above criteria for cover. **OV 1** is overhanging veg that is < or = 1 foot above the water surface, **OV 3** is overhanging veg > 1 foot and < or = 3 feet above the water surface.
Root Wad	**RW**	Large root wad or aggregations of smaller root wads located in or in contact with water at least 1 foot deep for large fish or no minimum depth for small fish.
Woody Debris	**WD**	Large pieces **(minimum of 4 inches (10 cm) in diameter)** or aggregations of smaller pieces of wood (e.g., logs, large tree branches) located in or in contact with water at least 1 foot deep for large fish or no minimum depth for small fish.
Other Debris	**OD**	Pieces of human-made debris found in or in contact with water at least 1 foot deep for large fish, or no minimum depth for small fish, that provide shelter or visual isolation for fish. Examples include **old tires, abandoned farm implements, and discarded home appliances**.
Boulder	**B**	Rocks with an **intermediate axis > or = 20 inches (Large Fish) or 12 inches (Small Fish)** that are located in or in contact with water, and provide cover as defined above. Large pieces of **concrete and other artificial rocky aggregates** also belong in this category.
Submerged & Emergent Macrophytes	**SM or EM**	Vascular plants that normally have all or nearly all their biomass below the surface of the water **(SM)** or a significant portion of their biomass above the surface of the water **(EM)**. To count as cover, macrophytes must be rooted in water and be thick or dense enough to provide shelter or visual isolation for fish.
Pool	**P**	Pool size and depth are sufficient to provide a low velocity resting area for large fish, with an adequate portion of the pool bottom being obscured (visually isolated) due to water depth and/or surface turbulence. Pools with a depth of **3 feet or more are considered cover for large fish,** regardless of surface turbulence and visual isolation conditions.

Transect	**Length of Transect (ft) (XX.X)**	**Length (XX.X ft) of Cover Intersecting Transect Line (within 1 foot of transect line)**		**Cover Type**	**Comment**		**Distance to Closest Large Fish Cover (XX.X ft)**	**Cover Type**	**Comment**
		Length of Cover	**Size Class (L or S)**						

Notes:

Site:			Date:	
Personnel:			Time:	
Comments:				
Assessment Reach Length (ft):			Transect Interval Length (ft):	

Transects	Left Bank Unstable (ft)	Right Bank Unstable (ft)	EPA High Gradient RBP		
			Parameter	Score (Left)	Score (Right)
1 - 2			1. Epifaunal Sub/ Available Cover		
2 - 3			2. Embeddedness		
3 - 4			3. Velocity / Depth Regime		
4 - 5			4. Sediment Deposition		
5 - 6			5. Channel Flow Status		
6 - 7			6. Channel Alteration		
7 - 8			7. Frequency of Riffles (or bends)		
8 - 9			8. Bank Stability		
9 - 10			9. Vegetative Protection		
10 - 11			10. Riparian Veg Zone Width		
11 - 12			EPA Low Gradient RBP		
12 - 13			1. Epifaunal Sub/ Available Cover		
13 - 14			2. Pool Substrate Characterization		
14 - 15			3. Pool Variability		
15 - 16			4. Sediment Deposition		
16 - 17			5. Channel Flow Status		
17 - 18			6. Channel Alteration		
18 - 19			7. Channel Sinuosity		
19 - 20			8. Bank Stability		
20 - 21			9. Vegetative Protection		
			10. Riparian Veg Zone Width		

Sample Site:					Sample Site:				
Date:			Time:		Date:			Time:	
Personnel:			Meter:		Personnel:			Meter:	
Comments:					Comments:				
Comment	Distance ft	Depth ft	Rev	Seconds	Comment	Distance ft	Depth ft	Rev	Seconds

FISH SURVEY DATA **PFBC HABITAT MANAGEMENT**

Water:_____ **Section:** _____ **Date:**_____

County:_____ **WCO District:**_____ **SubSubBasin:**_____

UPS Latitude:_____ **UPS Longitude:**_____ **River Mile:**_____

UPS Site Descriptor:_____

DNS Latitude:_____ **DNS Longitude:**_____ **River Mile:**_____

DNS Site Descriptor:_____

Site Length:_____ **Mean Width:**_____ **Topo:** _____

Collectors: _____

GEAR

Gear:_____

Volts:_____ **V**

Amps:_____ **A**

Watts:_____ **W**

AC:___ **DC:**___ **Pulsed DC:**___

Electrofishing Time:

_____

Individual Width Measurements

RBP HABITAT

_____ **Gradient**

1

2

3

4

5

6

7

8L

8R

9L

9R

10L

10R

Total Score:_____

WATER QUALITY DATA

Time (24 hr):_____

Air Temperature:_____ **°C**

Water Temperature:_____ **°C**

pH:_____ **SU**

Alkalinity:_____ **mg/L**

Hardness:_____ **mg/L**

Specific Conductance:

_____ **µS/cm**

TDS:_____ **mg/L**

Dissolved Oxygen:_____ **mg/L**

Nitrate (NO_3-N):_____ **mg/L**

Orthophosphate:_____ **mg/L**

Notes:

SPECIES:												
Not Measured												
0 mm												
25 mm												
50 mm												
75 mm												
100 mm												
125 mm												
150 mm												
175 mm												
200 mm												
225 mm												
250 mm												
275 mm												
300 mm												
325 mm												
350 mm												
375 mm												
400 mm												
425 mm												
450 mm												
475 mm												
500 mm												
525 mm												
550 mm												
575 mm												
600 mm												
625 mm												
650 mm												
Measured (mm)												

Appendix B

Habitat Monitoring Equipment List

Transect & Fish Cover:
4 – 4 foot rebar
Sledgehammer (3 – 4 lbs, 18" – 24" handle)
2 – 100 foot fiberglass tape
1 – 300 foot fiberglass tape
1 – 300 foot surveyor's rope
4 spring clamps
46 Wire Flags
Flagging
2 calibrated 5 foot PVC pipes (0.1 ft marks)
Clipboard with calculator
Pencils
Data sheets – Transect
Data sheets – Fish Cover
Data sheets – Thalweg Profile
Data sheets – Bank Stability / RBP
GPS unit
Camera

Surveying:
Laser level
Tripod
Telescoping leveling rod
Rod level
Benchtie
Aluminum dome bench marker
16 – 2 foot rebar
Surveyors markers (orange caps)
Orange spray paint
Rebar driving head
Magnetic locator
Heavy duty lath carrier bag
Machete
Two-way radios
Range finder

Appendix B (continued)

Habitat Monitoring Equipment List

Discharge:
Current meter
4 foot wading rod
Headphones
Data sheets – Stream Discharge

Personal Gear:
Chest waders and wading boots
Polarized sunglasses
Raincoat
Neoprene gloves for cold weather

Appendix C

RBP Habitat Assessment – High and Low Gradient Streams Field Data Sheets

Stream habitat should be assessed at each sampling site using the U.S. Environmental Protection Agency's Rapid Bioassessment Protocols (RBP; Barbour et al. 1999). The high-gradient habitat assessment data sheets should be used for riffle/run prevalent stream sections in moderate to high gradient landscapes. High gradient streams generally have substrates dominated by coarse sediment particles (i.e., gravel or larger). The low-gradient habitat assessment data sheets should be used for glide/pool prevalent stream sections in low to moderate gradient landscapes. Low gradient streams usually have substrates comprised of fine sediment or infrequent aggregations of more coarse (gravel or larger) sediment particles. Habitat assessments are first made on instream habitat, followed by channel morphology, bank structural features, and riparian vegetation. The habitat evaluation process involves rating the 10 parameters as optimal, suboptimal, marginal, or poor based on the criteria included on the habitat assessment field data sheets. All parameters are scored on a numerical scale of 0 (lowest) to 20 (highest). The ratings are then totaled and compared to a reference condition to provide a final habitat rating. Scores increase as habitat quality improves (Barbour et al. 1999).

The following procedures for performing habitat assessments are adapted from those included in the U.S. EPA's RBP technical document: EPA 841-B-99-002 (Barbour et al. 1999) and should be followed by PFBC staff when assessing habitat during a general stream survey.

1. The habitat assessment should be performed after the biological sample is completed on the same reach from which the biological sampling was conducted.
2. The investigators should obtain a close look at the habitat features while measuring out the site and conducting the biological sampling to familiarize themselves with the sample reach.
3. The habitat assessment data sheet should be completed, by a team of 2 or more biologists, if possible, to come to a consensus on the rating.

Habitat Assessment Field Data Sheet – <u>High Gradient Streams (side 1)</u>

Stream Name:	Location:	
Station #: Rivermile:	Basin/Sub-basin:	Agency:
Lat: Long:	Date: Time: am pm	Reason for Survey:
Investigators:	**TOTAL SCORE:**	

Habitat Parameter	*Condition Category*			
	Optimal	**Suboptimal**	**Marginal**	**Poor**
1. Epifaunal Substrate/ Available Cover	Greater than 70% of substrate favorable for epifaunal colonization & fish cover; mix of snags submerged logs, undercut banks, cobble or other stable habitat and at stage to allow full colonization potential (logs/snags that are not new fall and not transient	40-70% mix of stable habitat; well suited for full colonization potential; adequate habitat for maintenance of populations; presence of additional substrate in the form of newfall, but not yet prepared for colonization (may rate at high end of scale.	20-40% mix of stable habitat; habitat availability less than desirable; substrate frequently disturbed or removed.	Less than 20% stable habitat; lack of habitat is obvious; substrate unstable or lacking
SCORE:	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1 0
2. Embeddedness	Gravel, cobble and boulder particles are 0-25% surrounded by fine sediment. Layering of cobble provides diversity of niche space.	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 0
3. Velocity/Depth Regime Note: Deep = > 18"	All four velocity/depth regimes present (slow-deep, slow-shallow, fast-deep, fast-shallow) (Slow is <0.3 m/s, deep is > 0.5 m).	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 low).	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 0
4. Sediment Deposition	Little or no enlargement of islands or point bars and < 5% of the bottom affected by sediment deposition.	Some new increase in bar formation, mostly from gravel, sand or fine sediment; 5-30% of the bottom affected; slight deposition in pools.	Moderate deposition of new gravel, sand or fine sediment on old and new bars; 30-50% 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 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 0
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.
SCORE:	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1 0

Habitat Assessment Field Data Sheet – High Gradient Streams (side 2)

Habitat Parameter	*Condition Category*			
	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 years) may be present, but recent channelization is not present.	Channelization may be extensive; embankments or shoring structures present on both banks; and 40-80% 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.
SCORE:	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1 0
7. Frequency of Riffles (or bends)	Occurrence of riffles relatively frequent; ratio of distance between riffles divided by width of the stream < 7:1 (generally 5 to 7); variety of habitat is key in streams where riffles are continuous, placement of boulders or other large natural obstruction is important.	Occurrence of riffles infrequent; distance between riffles divided by the width of the stream is between 7 to 15.	Occasional riffle or bend; bottom contours provide 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 a ratio of > 25.
SCORE:	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1 0
8. Bank Stability Note: Determine left & right banks by facing downstream**.**	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 healed 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.
Score (LB):	10 9	8 7 6	5 4 3	2 1 0
Score (RB):	10 9	8 7 6	5 4 3	2 1 0
9. Vegetative Protection	More than 90% of the stream bank surfaces and immediate riparian zone covered by native vegetation, including trees, understory shrubs, or non-woody macrophytes; vegetative disruption through grazing or mowing minimal or not evident; almost all plants allowed to grow naturally.	70 – 90% of the stream bank 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 stream bank 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 stream bank surfaces covered by vegetation; disruption of stream bank vegetation is very high; vegetation has been removed to 5 centimeters or less in average stubble height.
Score (LB):	10 9	8 7 6	5 4 3	2 1 0
Score (RB):	10 9	8 7 6	5 4 3	2 1 0
10. Riparian Vegetative Zone Width	Width of riparian zone > 18 meters (58'); human activities (parking lots, roadbeds, clearcuts, lawns or crops) have not impacted zone.	Width of riparian zone 12 – 18 meters (39'-58'); human activities have impacted zone only minimally.	Width of riparian zone 6 – 12 meters (20'-39'); human activities have impacted zone a great deal.	Width of riparian zone < 6 meters (20'); little or no riparian vegetation due to human activities.
Score (LB):	10 9	8 7 6	5 4 3	2 1 0
Score (RB):	10 9	8 7 6	5 4 3	2 1 0

Habitat Assessment Field Data Sheet – Low Gradient Streams (side 1)

Stream Name:		Location:	
Station #: Rivermile:		Basin/Sub-basin:	Agency:
Lat: Long:		Date: Time: am pm	Reason for Survey:
Investigators:		**TOTAL SCORE:**	

Habitat Parameter	*Condition Category*			
	Optimal	**Suboptimal**	**Marginal**	**Poor**
1. Epifaunal Substrate/ Available Cover	Greater than 50% of substrate favorable for epifaunal colonization & fish cover; mix of snags submerged logs, undercut banks, cobble or other stable habitat and at stage to allow full colonization potential (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 newfall, 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
SCORE:	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1 0
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.
SCORE:	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1 0
3. Pool Variability Note: Deep = > 18"	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.
SCORE:	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1 0
4. Sediment Deposition	Little or no enlargement of islands or point bars and < 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.
SCORE:	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1 0
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.
SCORE:	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1 0

Habitat Assessment Field Data Sheet – Low Gradient Streams (side 2)

Habitat Parameter	*Condition Category*			
	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 years) may be present, but recent channelization is not present.	Channelization may be extensive; embankments or shoring structures present on both banks; and 40-80% 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.
SCORE:	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1 0
7. Channel Sinuosity	The bends in the stream increase the stream length 3 to 4 times longer than if it was in a straight line. (Note: channel braiding is considered normal in coastal plains and other low-lying areas. This is not easily rated in these areas).	The bends in the stream increase the stream length 2 to 3 times longer than if it was in a straight line.	The bends in the stream increase the stream length 1 to 2 times longer than if it was in a straight line.	Channel straight; waterway has been channelized for a long distance.
SCORE:	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1 0
8. Bank Stability Note: Determine left & Right by facing downstream**.**	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 healed 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.
Score (LB):	10 9	8 7 6	5 4 3	2 1 0
Score (RB):	10 9	8 7 6	5 4 3	2 1 0
9. Vegetative Protection	More than 90% of the stream bank surfaces and immediate riparian zone covered by native vegetation, including trees, understory shrubs, or non-woody macrophytes; vegetative disruption through grazing or mowing minimal or not evident; almost all plants allowed to grow naturally.	70 – 90% of the stream bank 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 stream bank 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 stream bank surfaces covered by vegetation; disruption of stream bank vegetation is very high; vegetation has been removed to 5 centimeters or less in average stubble height.
Score (LB):	10 9	8 7 6	5 4 3	2 1 0
Score (RB):	10 9	8 7 6	5 4 3	2 1 0
10. Riparian Vegetative Zone Width	Width of riparian zone > 18 meters (58'); human activities (parking lots, roadbeds, clearcuts, lawns or crops) have not impacted zone.	Width of riparian zone 12 – 18 meters (39'-58'); human activities have impacted zone only minimally.	Width of riparian zone 6 – 12 meters (20'-39'); human activities have impacted zone a great deal.	Width of riparian zone < 6 meters (20'); little or no riparian vegetation due to human activities.
Score (LB):	10 9	8 7 6	5 4 3	2 1 0
Score (RB):	10 9	8 7 6	5 4 3	2 1 0

MODULE G

Protocols for Conducting Angler Use and Harvest Surveys on Wadeable Streams

Prepared by:
Tom Greene

1. Introduction

Angler use and harvest surveys are conducted periodically on a variety of wadeable Pennsylvania streams. Information collected from these surveys is used to estimate angler effort, catch, harvest, and the associated catch and harvest rates by species. Estimated angler effort is expressed in terms of angler hours, angler trips or angler days, and estimated angler catch is expressed in terms of the number of fish harvested or the number of fish caught and released by species. As part of these surveys, information is also colleted on angler demographics, angler opinions, and more recently, to assess the economic benefits that fishing has on the Commonwealth's economy.

This module will describe procedures that have been used in the past to conduct angler surveys on wadeable Pennsylvania streams. It will also outline methods that can be used to conduct angler surveys on these waters in the future.

2. Current Methods

To date, angler surveys have been used to estimate angler use, catch, and harvest on individual waters as part of a specific program option, such as; stocked trout streams managed under statewide regulations, stocked trout streams managed under Delayed Harvest regulations, streams managed for wild trout under statewide regulations, streams managed for wild trout under special regulations, and stocked trout streams that support warm/coolwater fish populations. In addition, angler surveys have been conducted to estimate statewide use, catch, and harvest on randomly selected waters from a statewide list of waters such as, wild trout streams and stocked trout streams. Angler surveys could also be used on wadeable streams that are managed for warm/coolwater fish populations in Pennsylvania.

For these surveys, two standard sampling components were used to estimate fishing activity and success over a given time period these included, counts of angler activity (or angler use counts) and angler interview information. Angler use counts were completed to estimate angler effort and angler interview information was used to provide estimates of catch rates by species. Subsequently, the product of estimated angler effort and estimated catch rate was used to equate to the estimated catch (Lockwood 2000).

Angler surveys on wadeable Pennsylvania streams have been completed using different methods. For example, angler use and harvest surveys on trout stocked waters from 1988-1992 were conducted using a method adapted from Fisk (1966) and Butler and Borgeson (1965). This method required collecting angler use count information at two-hour intervals (seven counts per day) over a 12-hour sample day. Aside from the last count of the day, angler interview information was collected at the completion of each use count in the time period remaining before the next use count was scheduled to begin. These surveys were conducted over relatively short eight-nine day time periods that coincided with the opening of regular trout season and periods directly following an inseason stocking. Information collected from these surveys provided an estimate of angler effort and angler harvest on wadeable streams stocked with adult trout.

Since 1992 most angler survey work on wadeable Pennsylvania streams has been conducted using the roving creel survey method (Malvestuto 1983). Sampling units (weekdays or weekend days)

for these angler surveys have been determined using a stratified random sampling design (Malvestuto 1983). Considering the fact that the daylight period (sunrise to sunset) could not be sampled during one survey shift, a randomly selected sub-sampled portion of the day was sampled. The sub-sampled portion of the day has typically corresponded to a 6.5-hour per day sample shift covering a morning/afternoon or afternoon/evening time period. This method required collecting angler use count information at either specified times or 1.5 hour intervals (two - five counts per day). Aside from the last count of the day, angler interview information was collected at the completion of each use count in the time period remaining before the next use count was scheduled to begin. The sample period for these surveys generally extended over a time frame of two months or more. Information collected from these surveys provided an estimate of angler effort, catch, harvest, and release, as well as, information on angler demographics, angler opinions, and in more recent surveys, an estimate of the economic benefits that a specific component of fishing has contributed to Pennsylvania's economy.

3. Final Methods

3.1 PLANNING

Given the variability in access that exists between waters, some planning should be done before an angler survey begins. Items to consider should include, survey mode such as; travel by foot, auto or a combination of both, the number of vantage points available along the stream for making use counts, and the amount of time that will be required to complete one angler count circuit. The time required to complete one count will have a bearing on determining the number of creel clerks that are needed for the survey and the amount of time that is necessary between count intervals that also leaves enough time to interview an adequate number of anglers. In regards to clerk safety, another point to consider would be the time that sampling activity during the afternoon/evening shift should end for the day. These factors have been outlined in a form (Table 1) that has been used as part of the preparation for conducting previous Pennsylvania Fish & Boat Commission angler surveys (Lorantas 2000).

3.2 GENERAL PROCEDURES

Depending upon information needs the selection of waters for angler surveys could be on an individual water basis or a number of waters could be surveyed to collect information on a specific management program. In cases where a number of waters will be surveyed to represent a program, it is advisable to have the specific study waters determined by random selection.

Considering the variability in access and physical characteristics between waters in Pennsylvania, a number of survey methods could be used. These include the roving creel survey, the access point survey, or a complemented survey that uses more than one survey method (Malvestuto 1983). Based on staff experience from previous surveys, the roving creel survey method seems to provide a reasonable method to use on many wadeable streams. Sampling units (weekdays or weekend days) for angler surveys should be selected based on a stratified random sampling design. Since the daylight period (sunrise to sunset) of a day cannot be sampled during one survey shift, a randomly selected sub-sampled portion of the day should be sampled. The sub-sampled portion of the day should correspond to a morning/afternoon or afternoon/evening work shift. The

afternoon/evening shift should be designed to end at a time near the average time of sunset for each month. Morning/afternoon shifts should cover the portion of the day in advance of but contiguous with the afternoon/evening shift. Depending upon logistics and travel considerations, survey shifts generally range between 6.0 – 7.0 hours in duration. Survey shift starting and ending times should be defined such that the sampling period encompasses as much of the fishing day (sunrise to sunset) as possible. An example of a monthly calendar based on sampling four survey shifts per week (both weekend days and two randomly selected weekdays) is provided in Table 2.

Daily work shifts generally encompass a 7.5-hour workday. Based on a 6.5-hour survey shift, this allows clerks a time period of 0.5 hours before each survey shift to travel and prepare for survey activities and 0.5 hours after each survey shift to travel then carefully store collected data and survey gear. It is important to point out that the monthly calendars and angler count forms will describe survey shifts and not work shifts. As some time is allotted for travel between the clerks work office (or residence) and the survey location.

3.3 ANGLER USE COUNT PROCEDURES

As pointed out in the planning section, the number of counts per survey shift and count intervals often depend on the amount of time required to complete one count circuit. Typically, four angler counts are conducted within a shift and angler count intervals can be set at fixed intervals such as, 1.5 hours or a random time may be selected for counts within the survey shift. Daily angler count forms will identify the times at which angler counts should be made within a shift. Clerks should adhere to these schedules as close as possible. An example of an angler count form is provided in Table 3.

Accurate angler counts should be made from locations that provide a complete view of the area being counted. The amount of stream that is viewable from a vantage point, such as a road, will vary from stream to stream. In some cases angler counts will be able to be made by viewing the stream from vantage points located along a road. Other cases will require clerks to walk along the stream to accurately collect angler count information. Most cases will require a combination of walking a portion of the stream to count anglers and counting anglers from a vantage point along a road. Access points along the stream may be used to define points for beginning each angler count. Clerks should conduct angler counts in the specified direction of travel until the count is completed for the designated count time. For example, in the simplest cases points for angler counts would be defined as the upstream limit of a stream section (represented by the number 1) and the downstream limit of the section (represented by the number 2). If the beginning point for an angler count is a number 1, the clerk should begin the count at the upstream section limit and count in a downstream direction to the downstream section limit. Conversely, if the beginning point for the angler count is a number 2, the clerk should begin the angler count at the downstream section limit and count in an upstream direction to the upstream section limit. These are noted on the survey schedule calendar as RT = 1 or RT = 2 and on the Daily Schedule/Count Tally form as Begin at Site 1 or Begin at Site 2 (Tables 2 & 3).

3.4 RECORDING ANGLER USE COUNT DATA

Upon the completion of each use count the clerk should record the number of anglers counted. In this case simply enter the total angler count for the designated count time in the space provided on the angler count form. It is very important to remember that accurate angler use count information is essential to the success of the survey, as angler effort cannot be estimated without angler use count information. Angler interview data without the corresponding angler effort information renders the interview information virtually useless. Therefore, clerks should be diligent in conducting and recording accurate angler use count information and be sure to keep the data in a safe location.

When it is necessary to conduct angler use counts by walking portions of the stream, clerks should take care as to how they approach anglers along the stream. Therefore, aside from the necessity to cross the stream from time to time, clerks should avoid wading through the stream for any extended distances. Where possible, clerks should try to travel on paths along the stream that would be conducive for conducting use counts without disturbing anglers in the act of fishing.

3.5 WEATHER CONDITIONS

After the last angler count has been completed for the day, record the weather codes that best describe weather conditions during the survey shift. The codes used to document weather conditions are outlined in Table 4.

Column 1 – record cloud cover, if any.

Column 2 – record precipitation level, if any.

Column 3 – record wind and air temperature levels.

Additional information can be added to the sheet such as, columns recording water temperature, stream flow conditions or turbid water conditions.

3.6 ANGLER INTERVIEW PROCEDURES

Unless instructed otherwise, angler interviews should be conducted continuously between angler use counts within the designated stream section during the survey shift. Again, survey schedule calendars will identify workdays and shifts throughout the duration of the study period. Creel clerks should interview as many anglers possible between angler use count intervals. Both actively fishing anglers and anglers who have concluded their fishing trip for the day should be interviewed. However, priority should be given to collecting completed trip interviews from anglers who have concluded their fishing trip for the day. Unless instructed otherwise, each individual angler within an angling party should be interviewed separately and information pertaining to the number of fish caught, fish harvested, and angler opinions should be recorded separately for each angler.

Actively fishing anglers should be approached cautiously as to not disturb their fishing activity. Prior to initiating an interview, clerks should identify themselves as employees of the Pennsylvania

Fish & Boat Commission and inform anglers that they are collecting survey data using guidelines established by the PFBC. Clerks should politely encourage anglers to participate. If necessary, Clerks should indicate that the information collected will ultimately be used to enhance the fishery resource on this stream and other similar streams statewide. Clerks may also indicate that angler responses will remain confidential and that the name or address will not be asked of the angler. Politely thank anglers who refuse to participate and move on. Bear in mind that the angler has the option of not participating in the interview.

Questions raised by anglers that the creel clerk cannot answer should be recorded on the interview form along with the name, address and or telephone number of the angler so that the question can be referred to appropriate PFBC personnel. Appropriate staff should be alerted to requests for information as soon as possible. Creel clerks should not attempt to answer questions related to Pennsylvania Fish & Boat Commission laws, regulations, policies and procedures if they are unsure. As there could be serious consequences if anglers were misinformed.

3.7 RECORDING ANGLER INTERVIEW INFORMATION

Along with stream identification information, angler interview information will be recorded on angler age group, angler gender, length of time fished, angler zip code, county or state of angler residence, complete or incomplete trip, type of tackle used, species caught, total number caught by species, total number harvested by species, and a series of questions for the anglers.

The following section provides an example of instructions for the type of information that would typically be recorded on an angler interview form (Table 5).

Creel Clerk – The name of the creel clerk conducting the angler interview should be recorded on each interview form. This will be of use if there is any question pertaining to the data entered on the form.

Water Name: - The name of the survey stream.

Sub-subbasin – The number and letter of the sub-subbasin where the survey water is located should be recorded.

Date – The date of the angler interview should be recorded.

Age Group - The age group of the angler should be recorded. Age group is defined as adult, or those 16 years of age or older and required to possess a fishing licensed, or youth for those less than 16 years of age who are not required to possess a fishing license.

Angler Gender – Record the gender of the angler that is being interviewed.

Start Fishing Time – The time that the angler began fishing on the survey stream for the day (defined as when the first lure or bait entered the water) should be recorded. This should reflect the time that anglers were actually in the act of fishing and not their arrival time along the stream

before fishing activity began. All time entries should be recorded in military time (hours and minutes).

Time of Interview or Stop fishing Time – The time the interview was initiated or in the case of a completed trip, the time that the angler reported they stopped fishing (last lure or bait reeled in) should be recorded. Again, all time entries should be recorded in military time (hours and minutes).

Zip Code – Record the zip code of the angler's permanent home residence. This information will be useful in tracking where the anglers lived in relation to their fishing destination and the distance they traveled to the stream.

County or State of Permanent Residence - The county of permanent residence should be recorded for Pennsylvania residents and state of permanent residence should be recorded for out of state anglers. Table 6 provides codes for Pennsylvania counties and other states.

Trip complete – Record the type of trip recorded on the interview based on completed trip (where fishing activity has ended for the day) or incomplete trip (where fishing activity will continue after the interview). These can simply be coded with a number 1 to represent a completed trip interview and a number 2 to represent an incomplete trip interview.

Terminal Tackle – Record the type of tackle used by the angler such as, flies, artificial lures or bait. This can be done by entering a check mark for the type of tackle used by the interviewed angler. If more specific information is required, it can be written in the space provided. For example, worms or minnows could be entered for more specific information on the type of bait used by the angler. If more than one tackle type is being used, place a check mark in each tackle type that is being used by the interviewed angler.

Species Caught – Record all species of fish caught by the angler, including those fish that the angler reports as returned to the water. A list of abbreviations for fish species common to Pennsylvania waters is provided in Table 7. For any species of fish caught that does not appear on the list simply write in the species name in the space provided for species caught on the interview form.

Total Number Harvested – Record the number of each fish species in the angler's possession. Record only the fish that are harvested by the angler being interviewed. All anglers should be interviewed separately. Therefore, do not record harvest information that pertains to an angling party.

Total Number Released – Record the number of each fish species the angler returned to the water, (caught and released). Fish retained on a stringer should be recorded as a harvested fish. If the angler is unsure as to the disposition of the fish (kept or released) record the fish as harvested and circle the number to indicate there is uncertainty.

3.8 RECORDING RESPONSES TO ANGLER OPINION QUESTIONS

Some questions relating to angler opinions and or economic impacts are typically asked as part of the angler interview process. The creel clerk should read all question and answer choices to the angler verbatim, slowly and calmly, and record the response. The interviewer should not attempt to influence the angler's response in any way and should never indicate how others have responded to a question. The creel clerk may repeat the question as many times as necessary or reasonable. Again, all anglers should be interviewed separately. It is acceptable for the creel clerk to ask opinion related questions to young anglers. More time and patience may be required in soliciting responses from youngsters. Responses from parents should not be recorded in lieu of the younger angler's response. If the youngster appears to be unwilling or unable to respond, then the young angler should be thanked and the interview should be concluded. The interviewer should not in any way suggest that responses were immature or that the youngster was not able to provide acceptable responses. The survey should be concluded in a manner that suggests the interview naturally concluded.

3.9 SURVEY MODE

Survey procedures often require that creel clerks make use of a combination of auto and foot travel for angler count and interview purposes. Interviewing departing anglers in an auto-based survey can be accomplished by, parking in a safe location (legal parking along a roadway), carefully exiting the vehicle and then carefully approaching actively fishing or departing anglers. Seat belts must always be worn while traveling in vehicles regardless of the distance traveled. This practice is required of all drivers in the state of Pennsylvania. Patient controlled operation of autos is required during the survey. All state laws and regulations must strictly be adhered to. Under no circumstances should angler counts be made from moving vehicles. Autos must be stopped completely and legally parked or located out of traffic when angler counts are made.

3.10 WEATHER CONDITIONS

Pennsylvania Fish and Boat Commission safety procedures and policies must be adhered to at all times. Schedule deviations due to bad weather must be recorded on angler count sheets and reported to PFBC supervisory personnel as soon as possible. It is the responsibility of the creel clerk to keep abreast of weather conditions while engaged in survey activities and to determine when weather conditions warrant abandoning survey operations. Creel clerks should be expected to work through light to moderate rain events. Creel clerks should plan where to go to seek refuge in instances of severe weather. The clerk should be observant of prevailing local weather conditions and use good judgment in deciding when to return to their auto or places of safety and abandon survey operations.

4. Points to Remember

Providing information to anglers in regards to sampling dates on survey waters could bias the survey. Therefore, survey schedules and dates for sampling specific waters should remain confidential between the clerk and supervisory personnel. Supervisory personnel should provide the information when other PFBC staff members need to be notified of survey schedules.

Angler interviews should be updated periodically for anglers actively in the process of fishing. Precautions should be observed as to the frequency of these updates as to not disturb the angler's trip. Priority should always be given to interviewing anglers who have completed their fishing trip for the day.

In the busiest of situations, a creel clerk may elect to interview every other or every third angler. For example, suppose the shift end time was approaching, yet many uninterviewed anglers were fishing. It would be appropriate to interview every other angler fishing or concluding their fishing trip through the end of the shift to insure that anglers were randomly sampled.

Complete angler use counts should be made and recorded at the designated time. Be aware that interview information without corresponding angler effort information renders the interview information virtually useless. Therefore, accurate angler count information is critically important to the success of the survey.

The catch and harvest portion of the interview form should always be completed. Individual angler catches and responses to questions must remain confidential and not discussed with anyone, especially other anglers, and most certainly those not directly connected with the survey. Persons interested in survey results should be directed to supervisory personnel.

Keep in mind that the ultimate goals of the creel clerk should be to complete the use counts and interview as many anglers who have completed their fishing trips as possible.

The creel clerk is responsible for all aspects of the operation including security. At the end of the survey day, the clerk should carefully stow all gear, and check all data sheets for completeness and legibility. The clerk should provide completed data forms to Supervisory personnel on a weekly basis or as arranged by his or her supervisor. In any case where data is going to be mailed, all such data should be backed up with a duplicate copy maintained by the clerk.

Creel clerks should always drive carefully and conduct themselves in a courteous manner, as they may represent the only contact some anglers may have with the Pennsylvania Fish and Boat Commission. Remember to thank each angler who participated in the interview process.

5. Equipment

Standard equipment needs for creel clerks include, a copy of Creel Survey Procedures that serve as a reference guide for the study, a copy of the Creel Survey schedule(s) for the water or waters where the clerk will be collecting data, and an adequate supply of Angler Count and Angler Interview forms that apply to the survey. Clerks should be provided with a clipboard for the maintenance of data sheets and an adequate supply of pencils. The clerks should be issued a pair of properly fitted hip boots or chest waders along with rain gear for use during the performance of their survey duties. An identification tag should also be issued to the clerk that can be worn on a hat or outer garment to identify them as a creel clerk working for the PFBC. To aid in the angler count process, clerks should be provided with a counter to keep track of the number of anglers counted during a count interval and a set of binoculars to assist in viewing anglers for count purposes. In cases where the length of harvested fish needs to be recorded, clerks should be issued

a ruler. To aid in the location of study waters clerks should also be issued a map with directions to the study area. In some cases a GPS Unit may be required to ascertain the location of study sections based on latitude – longitude coordinates. Creel clerks may be assigned the use of a Commission vehicle for travel or in some cases may use their personal vehicle with reimbursement provided for travel costs in accordance with the current state allowance per mile of travel.

6. Data Entry, Output and Reporting

Data entry and error checking of data should be done at a specified office location under the supervision of permanent PFBC personnel. Data entry programs for PFBC use and harvest surveys have typically been developed in cooperation with PFBC Information Systems staff at the Pleasant Gap office.

Data output has been generated through the use of some in-house programming and through the Pennsylvania Cooperative Fish & Wildlife Unit at the Pennsylvania State University. For future PFBC use and harvest surveys there is a need to have computerized programming developed that would provide standard outputs for use and harvest surveys conducted by staff. Programs should be designed that would generate estimates of angler effort, catch, harvest, and release rates by fish species, as well as, numbers caught, harvested and released by species. Programming should be developed for surveys intended to estimate use and harvest on a group of waters for statewide estimates and to provide estimates on an individual water basis, as well.

At the conclusion of the survey, narrative reports should be prepared based on the results from use and harvest surveys. Use and harvest survey reports can be prepared by Area Office or Central Office staff based on the direction of their supervisor.

The information collected from angler surveys should be used to update water specific management plans and or management programs at the statewide level. Ultimately, these data will be used to form the basis for more informed management decisions on the fisheries resource.

7. Literature Cited

Butler, R. L. and D. P. Borgeson 1965. California "catchable" trout fisheries. California Department of Fish and Game Bulletin, 127. 47 pp.

Fisk, L. 1966. Creel census method for "catchable" trout fisheries. In Calhoun, A. (ed.), Inland Fisheries Management, pp. 187-192.

Lockwood, R. N. 2000. Conducting roving and access site angler surveys. Chapter 14 in Schneider, J. C. (ed.) 2000. Manual of fisheries survey methods II: with periodic updates. Michigan Department of Natural resources, Fisheries Special Report 25, Ann Arbor, MI.

Lorantas, R. 2000. Creel survey schedule/route information needs for PFBC use and harvest surveys. PFBC Files, 450 Robinson Lane, Bellefonte, PA.

Malvestuto, S. P. 1983. Sampling the recreational fishery. Pages 397-419 in L. A. Nielsen and D. L. Johnson, editors. Fisheries Techniques. American Fisheries Society, Bethesda, MD.

Table 1. Creel Survey Schedule/Route Information Needs.

To produce a count and interview schedule for roving creel surveys you will need to provide the information requested below (4 items). Please carefully consider work the clerk will need to complete each day to accomplish his/her tasks. The work site for all clerks is located at the water scheduled to be surveyed. The work day length is 7.5 hours and the survey day length is 6.5 hours, with a total of one hour to be used before and/or after each shift for: travel, fueling, and maintaining equipment, error checking field forms, and in some cases, key entering data. A 6.5 hour survey day, 5 day per week survey schedule will be developed for each water scheduled for sampling.

(1) Water:_____ County:_____

(2) Survey mode: Foot:_____ Auto:_____ Both:_____

Considering clerk safety and fishing activity the late shift should end: _____ hours (before/after) sunset. Most surveys are concluded 0.5 hours after sunset.

(3) Time and location information required for data:

	Number of sites	Time to visit all sites and return to starting location (make complete circuit)
Vantage point sites (for making counts)		
Access/angler use sites (developed and undeveloped locations anglers frequent)		

(4) Equipment needs:

Item	Quantity

Table 2. Angler Survey Calendar.

MAY 2005

SUNDAY	MONDAY	TUESDAY	WEDNESDAY	THURSDAY	FRIDAY	SATURDAY
1 RT= 1-1 ST= 1345 EN= 2015	2	3 RT= 1-1 ST= 1345 EN= 2015	4 RT= 2-2 ST= 1345 EN= 2015	5	6	7 RT= 2-1 ST= 1345 EN= 2015
8 RT= 1-2 ST= 715 EN= 1345	9	10	11 RT= 2-1 ST= 715 EN= 1345	12 RT= 1-1 ST= 715 EN= 1345	13	14 RT= 2-1 ST= 715 EN= 1345
15 RT= 1-2 ST= 1345 EN= 2015	16 RT= 2-1 ST= 1345 EN= 2015	17	18 RT= 1-2 ST= 715 EN= 1345	19	20	21 RT= 2-1 ST= 715 EN= 1345
22 RT= 1-1 ST= 1345 EN= 2015	23	24	25	26 RT= 1-2 ST= 1345 EN= 2015	27 RT= 2-1 ST= 715 EN= 1345	28 RT= 1-2 ST= 1345 EN= 2015
29 RT= 2-1 ST= 715 EN= 1345	30	31				

Table 3. Angler Count Form.

CREEL SURVEY - DAILY SCHEDULE/COUNT TALLYS

Sunday MAY 29, 2005
SURVEY PERIOD : 715 - 1345
BEGIN AT SITE 2-1

		COUNTS		
		SHORE	BOAT	
TIME	DIR	ANGLERS	ANGLERS	BOATS
715	<	[___]	[___]	[___]
845	>	[___]	[___]	[___]
1015	<	[___]	[___]	[___]
1145	>	[___]	[___]	[___]

WEATHER CONDITION CODE(S) [ ][ ][ ]

Table 4. Weather conditions and their corresponding codes.

Code	Cloud Cover	Code	Precipitation	Code	Wind and Temperature
0	sunny (no cloud cover)	0	no precipitation	0	wind calm (<10mph) warm (>65˚F)
1	partly sunny (occasional cloud cover)	1	intermittent rain	1	moderate wind (>10mph) warm (>65˚F)
2	partly cloudy (partial continuous cloud cover)	2	continuous rain	2	wind calm (<10mph) cool/cold (<65˚F)
3	cloudy (total cloud cover – gray)	3	intermittent rain with thunder or lightening	3	moderate wind (>10mph) cool/cold (<65˚F)
		4	continuous rain with thunder or lightening		
		5	intermittent snow		
		6	continuous snow		

On survey dates where turbid (muddy) water conditions are encountered write in turbid water under the weather condition codes.

Table 5. Angler Interview Form.

Clerk: _____

Water Name: _____ SSB _____ Subsection: ____

DATE: _____ (mo, day, yr) Age Group _____ 1 = Adult 2 = Youth Angler Gender _____ 1 = Male 2 = Female

START FISHING TIME: (2400 TIME) _____

TIME OF INTERVIEW: (2400 TIME) _____ ZIP CODE:_____ COUNTY OR STATE (If not in PA)_____

TRIP COMPLETE _____ 1 = Yes; 2 = No

Terminal tackle used: FLIES_____LURES_____ BAIT TYPE_____

SPECIES CAUGHT	*TOTAL # HARVESTED*	*TOTAL # RELEASED*
	/ /	/ /
	/ /	/ /
	/ /	/ /
	/ /	/ /

Size of Fish Harvested (species/inches):

_____ _____ _____ _____ _____

Questions:

1. How often do you harvest (keep) fish when fishing this water?

   _____Always _____Almost Always _____Half _____Rarely _____Never

2. How many days will you be fishing during this trip? _____

3. How many times a year do you go fishing in Pennsylvania? _____

4. Do you have any additional thoughts you would like to share with the PA Fish and Boat Commission?

   _____

   _____

   _____

   _____

Table 6. Pennsylvania Counties with their corresponding numeric codes and United States: State, District, and Territory Codes.

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Pennsylvania County Codes

Code	County	Code	County	Code	County
1	Adams	24	Elk	47	Montour
2	Allegheny	25	Erie	48	Northampton
3	Armstrong	26	Fayette	49	Northumberland
4	Beaver	27	Forest	50	Perry
5	Bedford	28	Franklin	51	Philadelphia
6	Berks	29	Fulton	52	Pike
7	Blair	30	Greene	53	Potter
8	Bradford	31	Huntingdon	54	Schuylkill
9	Bucks	32	Indiana	55	Snyder
10	Butler	33	Jefferson	56	Somerset
11	Cambria	34	Juniata	57	Sullivan
12	Cameron	35	Lackawanna	58	Susquehanna
13	Carbon	36	Lancaster	59	Tioga
14	Centre	37	Lawrence	60	Union
15	Chester	38	Lebanon	61	Venango
16	Clarion	39	Lehigh	62	Warren
17	Clearfield	40	Luzerne	63	Washington
18	Clinton	41	Lycoming	64	Wayne
19	Columbia	42	McKean	65	Westmoreland
20	Crawford	43	Mercer	66	Wyoming
21	Cumberland	44	Mifflin	67	York
22	Dauphin	45	Monroe		
23	Delaware	46	Montgomery		

Table 6 cont.

United States: State, District, and Territory Codes					
Code	**State**	**Code**	**State**	**Code**	**State**
AL	Alabama	KY	Kentucky	OH	Ohio
AK	Alaska	LA	Louisiana	OK	Oklahoma
AZ	Arizona	ME	Maine	OR	Oregon
AR	Arkansas	MD	Maryland	PA	Pennsylvania
CA	California	MA	Massachusetts	PR	Puerto Rico
CO	Colorado	MI	Michigan	RI	Rhode Island
CT	Connecticut	MN	Minnesota	SC	South Carolina
DE	Delaware	MS	Mississippi	SD	South Dakota
DC	District of Columbia	MO	Missouri	TN	Tennessee
FL	Florida	MT	Montana	TX	Texas
GA	Georgia	NE	Nebraska	UT	Utah
GU	Guam	NV	Nevada	VT	Vermont
HI	Hawaii	NH	New Hampshire	VA	Virginia
ID	Idaho	NJ	New Jersey	VI	Virgin Islands
IL	Illinois	NM	New Mexico	WA	Washington
IN	Indiana	NY	New York	WV	West Virginia
IA	Iowa	NC	North Carolina	WI	Wisconsin
KS	Kansas	ND	North Dakota	WY	Wyoming

Table 7. List of species and codes used for angler use and harvest surveys.

Code	Species	Code	Species
ST	Brook Trout	RB	Rock Bass
BT	Brown Trout	RES	Redear Sunfish
RT	Rainbow Trout	SAUG	Sauger
GRT	Golden Rainbow Trout	SMB	Smallmouth Bass
BASS	Bass spp.	SPB	Spotted Bass
BBH	Brown Bullhead	SNF	Sunfish spp.
YBH	Yellow Bullhead	TMKY	Tiger Muskellunge
BC	Black Crappie	WB	White Bass
BG	Bluegill	WC	White Crappie
CARP	Common Carp	WCF	White Catfish
CATS	Catfish spp.	WBC	Crappie spp.
CC	Channel Catfish	WE	Walleye
LMB	Largemouth Bass	WSKR	White Sucker
MKY	Muskellunge	YP	Yellow Perch
NP	Northern Pike		
PAN	Panfish spp.		
PSS	Pumpkinseed		

For any species caught that is not listed write in the name of the species in the space provided for Species Caught on the interview form.

Appendix A

Creel Clerk Guide to Making Angler Contacts During an Interview

Creel Clerks should use the following greeting, transition, and conclusion for contacting anglers during the interview portion of the survey. Slight adjustments of this script will be necessary to accommodate each individual interview situation.

GREETING

Good Morning/Afternoon! My name is John and I represent the Pennsylvania Fish and Boat Commission, we are conducting a fishing survey today and I would like to ask you some questions about your fishing trip today.

1. When did you start fishing or get your line in the water today?

From this response the clerk can quickly determine how long the angler was fishing and whether to continue with the catch and harvest part of the interview.

TRANSITION

Thanks for sharing your catch information today, now I would like to ask you some additional questions concerning your fishing trip. From here the clerk would proceed to ask the series of questions designed for the survey.

CONCLUSION

Thank you for your participation in our survey today. I wish you good luck for the remainder of the season!

MODULE H

Pennsylvania Warm Water Wadeable Index of Biotic Integrity (IBI) Fish Sampling Protocol for Streams

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Preface

This protocol is the result of multiple fish IBI projects conducted or influenced by biologists from many agencies and institutions including the United States Environmental Protection Agency, the Pennsylvania Department of Environmental Protection, the Pennsylvania Fish and Boat Commission, and the Pennsylvania State University. The majority of the text provided herein was adapted from the "Quality Assurance Project Plan" written for the most recent project, "Pennsylvania Fish Index of Biotic Integrity Development and Metrics Verification Project (REMAP)," conducted from 2008-2011.

1. Sampling Procedures and Requirements

The objective of the fish IBI sampling protocol is to acquire a representative sample of the fish population in a warm water wadeable stream or river by sampling all physical stream habitats in relative proportion to their availability. A representative sample is adequate when using the multimetric fish IBI. A sample will contain most of the species in the stream at the time of sampling in numbers proportional to their actual abundance. The sampling method, species identification, and species enumeration must be consistence in application. The accurate identification of each fish collected is essential and species level identification is required. At least one crew member must be capable of accurately identifying fish species of Pennsylvania. Electrofishing crews should have a crew leader experienced with operation of electrofishing gear and appropriate electrofishing tactics for warm water wadeable streams. The sampling crew must extend an adequate effort as measured by distance sampled, time fished and thoroughly covering the habitat types. Time fished can legitimately vary over the same distance as dictated by cover, stream conditions and the number of fish encountered. While it may be impossible, a concerted effort by the various members of the crew should be made to capture every fish sighted. The effort and intensity of the field crew during the collection phase is an important component of the IBI protocol. Since the ability of the netters to see stunned and immobilized fish is partly dependent on water clarity, sampling should only be conducted during periods of "normal" water clarity and flows. The sampling period is from May through October, so a wide variety of stream flow conditions may be encountered. 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, low flow norms. An experienced crew leader should decide if conditions are adequate and direct their crews to ensure an adequate effort is extended and a representative sample is collected.

The reach length in the sampling protocol was well tested during the IBI development. It demonstrated the ability to collect representative samples with very good precision and generated an accurate multimetric IBI. A 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). Warm water streams require additional effort due primarily to the higher number of species and individuals present. Minimum site lengths for wadeable streams are based on average stream width as summarized below. The starting point is first determined and marked on a USGS 7.5' topographical quadrangle map and/or finding the latitude and longitude. In the first 100 meters, 5 wetted channel widths are measured using a graduated measuring tape or a range finder (every 20 meters from the starting point) and averaged. If the calculated reach

length cutoff falls in the middle of a habitat sequence, or excludes a habitat type, then the upstream cutoff must be extended to include the sequence or missing habitat type.

Table 3. Reach lengths.

Average Stream Width (m)	Minimum Site Length
<10m	100m
10 to 40m	10 times the average stream width
>40m	Maximum of 400m

For example: The average stream width is 14 meters. Minimum sampled length would be 140 meters. However, the 140 meters mark falls about 5 meters short of the head of the pool. The upstream cutoff would need to be extended to the head of the pool for a total sampled length of 145 meters. The reach length decision should be based on distance necessary to cover all habitat types. If a field reconnaissance is done close to the time of sampling, the appropriate reach length can be determined and marked off using a highly visible marker such as blaze-orange ribbon or surveyor's flags. For larger streams it may be necessary to use a combination of electrofishing gear. With the concept that field sampling should attempt to collect every fish in the reach it is important to match the electrofishing gear to the stream size. A larger stream could be sampled with a backpack and towboat in tandem. A less preferred method would be to sample half the width of the stream for the length of the reach with a towboat and return to the start of the reach and sample the other half of the stream. A crew large enough to sample the full width of the stream in one pass is preferred because it prevents fish from escaping to uncovered sections of the stream, and if the crew is too small it will be difficult to adequately handle and process the fish. Good desk top reconnaissance and field reconnaissance should correctly assess the crew and gear needs, but stream conditions change and the crew leader must make the final decision in the field. Crew leaders should not sample if they feel a representative sample cannot be collected.

1.1 WADEABLE OR NON-WADEABLE

The warm water wadeable IBI stream sites are limited to streams that would normally be wadeable for an extended period during the sampling window of May through October. The sample site or the wadeable reach must be representative of the general condition of the stream in that area. It should not be an isolated wadeable reach within a section of stream that is not wadeable. The selection of wadeable sites will be determined by desk top reconnaissance and field reconnaissance. A wadeable reach is a reach that the field crew can sample with the expectation that they can collect every fish in the reach. There may be small areas of pools or holes along a bank that cannot be waded safely, but that the crew can surround and collect the majority of fish. This should be determined during field reconnaissance, but again the crew leader will not sample a reach if a representative sample cannot be collected.

2. Sampling Procedures

The warmwater wadeable stream size starts at about third order and dictates most of the electrofishing will be done with a towboat. However, in some cases a backpack electrofishing

unit could be used. Backpack crews will have a minimum of three members. Direct current or pulsed direct current backpack units are preferred. Trends have been toward the use of battery operated backpack units, but generator powered units may also be used. Depending upon the size of the water being surveyed, electrode arrays should consist of either two hand-held probes with ring-type electrodes or an anode probe paired with a rat-tail cathode. Each probe pole should have a power cutoff safety switch.

Channel width and depth should be considered before choosing between backpack and towboat electrofishing methods. Towboat electrofishing units will have a portable generator, pulsator or power control box, multiple hand-held probes and a trailing or hull laden cathode. Electrofishing techniques with towboats require an electrofishing crew consisting of four to six individuals. On the largest streams using multiple electrofishing units with eight or possibly more crew members may be needed during the collection phase of the sampling.

Table 5. Possible combinations of gear and crew requirements.

Electrofishing Gear	Number of Crew
Backpack	Minimum 3
Towboat	4 to 6
One Towboat, 2 - Passes	5 or more
Towboat & Backpack	7 or more
Two Towboats	8 or more

Warm water wadeable streams will have considerable variation in stream width and at times this will make the selection of electrofishing gear and crew size complicated. Crew leaders should be familiar with the sites and select the appropriate electrofishing gear and crew size. The reach should be determined and marked. The beginning and ending points of a site should be at a natural barrier (e.g. riffle) and if no natural barriers exist, a block net can be used. The IBI electrofishing effort should be a one-pass electrofishing effort, providing that the crew can cover both banks. If the crew cannot cover both banks in one pass then another pass on the other bank will be necessary. The exact latitude and longitude of the downstream limit should be recorded for each site. The electrofishing effort time is recorded on the survey sheet. Begin electrofishing in an upstream direction using a side to side sweeping motion to cover all habitats. Electrofishing tactics should be directed by the crew leader. Fish are held in buckets or a large tub for identification and enumeration. The crew will attempt to collect as many fish as possible of all species. Because collection methods are not consistently effective for young-of-the-year fish and because their inclusion may seasonally bias the results, fish less than 25mm in length will not be included in the samples.

Safety

Fish are collected using portable electrofishing units and safety procedures must be followed at all times. The crew leader has primary responsibility for safety while electrofishing. Electrofishing units have a high voltage output and may deliver a dangerous electrical shock. The crew should avoid contact with the water, probes and the towboat unless sufficiently insulated against electrical shock. All crew members should wear waders with non-slip soles

and watertight rubber (or electrician's) gloves that cover to the elbows. All crew members should wear polarized glasses to enhance their ability to see fish and enhance their ability to see bottom structure to avoid tripping and falling. As with any fish sampling method, the proper scientific collector permits are required and must be obtained before commencement of any electrofishing activity.

2.1 FIELD SAMPLE PROCESSING PROCEDURES

Captured fish are immediately placed in a bucket or with a towboat in large tub. Fish may need to be transported to another large tub at the fish identification station. Water is replaced regularly in warm weather to maintain adequate dissolved oxygen levels in the water, reduce waste by-products, and minimize mortality. Aeration can be provided to further minimize stress and mortality. Special handling procedures may be necessary for species of special concern. Fish that are not retained for vouchers or other purposes are released back into the water after they are identified to species and enumerated. Every effort will be is made to minimize holding and handling times. Invasive alien species will be kept and appropriately disposed of out of the water if requested by state collecting permits. Each sample crew must have at least one person that is a taxonomic specialist in fish identification. The majority of captured fish are identified to species in the field; however, any uncertainty about the field identification of individual fish may require preservation or photographing for laboratory identification. Table 6 provides guidance for selecting fish to be returned to the laboratory. Fish are preserved for future identification in buffered 10% formalin and labeled by date, collector's initials, water body, text description of locality, latitude/longitude and geographic identifier (e.g., pool or river mile). Identification is required to the species level at a minimum and may be necessary to the sub-specific level in certain instances. Fish will be transferred from 10% formalin to wash water and then to a series of ethyl alcohol washes from 35% to 50% to 70%. A number of ichthyology keys can be used including Becker (1983), Boschung, et al (2004), Cooper (1983), Etnier, et al (1993), Jenkins, et al (1994), Lee, et al (1980), Page et al (1991), Smith (1985), Stauffer, et al (1995), and Trautman (1981).

Table 6. The following guidelines are recommendations to consider when selecting sub-samples to fix, preserve, and subsequently identify in the laboratory (Walsh and Meador 1998).

1. Species that cannot be positively or reliably identified in the field by the fish taxonomic specialist. Difficulties in making identifications in the field may result from a number of factors, including fish size and age; smaller fish may be more difficult to positively identify in the field than larger fish. Examples of small sized fish that may require close examination in the laboratory to identify include many of the clupeids (herrings and shads), cyprinodontids (topminnows), poeciliids (livebearers), cyprinids (minnows), catostomids (suckers), percids (darters), and cottids (sculpins). A complete size range of specimens should be preserved unless there is suspicion that the species may be protected or rare, in which case photographic documentation should be considered.
2. Specimens that are to be archived in voucher or reference collections, or intentionally sought for independent taxonomic verification. A small sample should be taken upon

consultation with a fish taxonomic specialist, based on suggested need for archiving or when an independent opinion is required. (If the Collection Permit allows or suggests)

3. Suspected or known un-described species of which there is a known paucity of museum material, or of which specimens are otherwise taxonomically valuable (for example, for the purpose of comparing morphological variation), and that are available in reasonable numbers and are not known to be imperiled. (If the Collection Permit allows or suggests)
4. Cryptic taxa or two or more species that co-occur in the same drainage and that cannot be easily separated without closer examination of critical characters, especially those requiring use of a microscope. Unless a procedure is adopted and the time is taken to confidently separate such taxa in the field, it will be necessary to preserve all samples for subsequent identification.
5. New drainage records. Any specimen, or a subset of an entire sample, that is recognized as representing new drainage records or significant range extensions within a drainage should be preserved and identified in the laboratory. It is especially important to save samples of preserved specimens of any fishes that are suspected as being introduced, in order to confirm taxonomic identifications and to document new distributional records. (If the Collection Permit allows or suggests)
6. Samples of common species (for example, mosquitofish, Gambusia affinis or G. holbrooki) that are collected in large numbers and that cannot be processed fully in the field may have to be preserved in their entirety or as a subset. (If the Collection Permit allows or suggests)
7. Samples that provide important life history specimens. The taxonomic specialist can provide advice if a sample yields valuable specimens that are of interest to ecologists or may otherwise be worth preserving and archiving for future research purposes.

2.2 SAMPLE HANDLING AND CUSTODY

The field crew leader will review and initial all completed field forms to prevent loss and assure that all sites are sampled according to the detailed plan of study. An important quality control aspect of this process involves ensuring neat handwriting on the datasheets. Any sheets that show bad handwriting or smudges should be neatly re-written in the office. Any subsequent changes that are made to the fish data sheets are initialed and dated. Specimens retained for laboratory identification will need to be processed to complete each site's species and enumeration sheet. If possible some laboratory identification of samples should be completed during the collection season to prevent the accumulation of a large number samples.

2.3 FIELD CHEMISTRIES

Field chemical data including water temperature, pH, conductivity, dissolved oxygen, and alkalinity should be taken in conjunction with the IBI survey. Temperature can be measured by either a field meter or by field thermometer. Field meters should be used for pH, conductivity, and dissolved oxygen measurements. Alkalinity measurements should be done with field titration kit. The results of the field chemical test should be recorded on the field data sheet.

Table 7. Field instrument calibration specifications.

Instrument	Calibration Activity	Frequency of Calibration	Acceptance Criteria	Corrective Action
Temperature	Check against NIST certified Thermometer	Check prior to beginning of survey	± 1 EC of NIST thermo-meter	Adjust or replace probe/meter
Dissolved oxygen	Calibrate with saturated moist air; check with 0.0 DO std.	Daily prior to use; check at end of day	±0.5 mg/l from 0.0 std.	If DO exceeds criteria prepare fresh 0.0 std., clean probe, change mem-brane; recali-brate; qualify data.
Conductivity	Calibrate with single point standard; check with standard in range of samples.	Daily prior to use; check cali-bration at end of day.	10% of true value of check standard	If conductivity exceeds criteria prepare fresh Standard and re-Calibrate; qualify data accordingly.
Alkalinity	Field titration kit LaMotte DR-A #3467 Hawk Run #901400		 ±0.4 mg/l ±0.2 mg/l	Check titration chemicals
pH	Two buffer calibration of expected range pH 4 -7 or 7-10	Check prior to beginning survey	+/- 0.1 S.U. recalibrate	Check probe for bubbles, etc. New buffers. Replace probe.

2.4 HABITAT FIELD FORMS

A habitat survey must be done in conjunction with all IBI surveys. The PA Modified RBP Habitat Assessment Protocol for wadeable streams and rivers was based on the habitat assessment method found in Rapid Bioassessment Protocols for Use in Wadeable Streams and Rivers Second Edition (Barbour et al. 1999). The PA Modified RBP Habitat Assessment form is located in Module F; Appendix C. The crew leader or a member of the crew should be trained in the visual-based habitat assessment technique needed to accurately complete the habitat assessment. If the crew member completing the habitat assessment form needs guidance they should review the RBP 1999 Manual Chapter 5, "Parameters to be evaluated in sampling reach."

The reach length must be determined before the habitat assessment form is completed. The habitat assessment form maybe completed before the fish sample is collected or after the fish sample is collected. The crew will probably have a better understanding of the habitat characteristics after the fish collection is completed. Most of the habitat parameters are evaluated within the limits of the sample reach, but frequency of riffles, grazing or disruptive pressures and riparian widths may be evaluated based on the area that can be observed from the reach, not just within the reach.

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MODULE I

Monitoring Protocols for Benthic Macroinvertebrates in Wadeable Streams

Reviewed by:
Tom Shervinskie

1. Introduction

The PFBC has cooperated with the Department of Environmental Protection in the development and/or review of sampling protocols for wadeable riffle/run freestone streams (Chapter 2), multi-habitat pool/glide streams (Chapter 3), and limestone streams (Chapter 4). The protocols outlined in the following chapters cover collection methods, preservation techniques and laboratory analysis. The complete documents, which are located on the World Wide Web at: http://www.portal.state.pa.us/portal/server.pt/community/water_quality_standards/10556/technical_documentation_macroinvertebrate_stream_protocols/554005 also include metric analysis, index development and a master taxa list.

Benthic macroinvertebrates are collected and identified as a measure of stream health. While water samples taken at a point in time may be used to describe water quality at any given moment, benthic macroinvertebrates can exhibit multi-voltine to merovoltine reproductive life cycles. Additionally, their varied sensitivities to pollutants, allow biologists to reach conclusions about long-term water quality at a given stream sampling site by examining representative samples of benthic macroinvertebrates. When sampling sites are representative of stream segments or stream sections, samples of benthic macroinvertebrates may be used to describe long-term water quality in those sections as well as locate portions of sections where water quality changes.

Benthic macroinvertebrate assessments include qualitative, semi-quantitative and quantitative sampling protocols. Sampling can occur in a single habitat or multiple habitats depending on the stream. Identification and quantification of benthic macroinvertebrates to the taxonomic levels of genus or species are preferable when conducting ecological studies, cause and effect surveys, or pollution investigations. However, quantitative samples are labor intensive in the field and in the lab. A qualitative sample identified to the family taxonomic level, or to order for some limited taxonomic groups, provides enough insight into stream health to document degraded, moderate, or good to excellent water quality conditions.

When sampling benthic macroinvertebrates, fisheries managers are trying to develop a sense of long-term water quality, which may help explain fish species composition or abundance at a given sampling site and will help support recommendations regarding corrective measures in cases where water quality is degraded. Such recommendations, when implemented, may improve water quality and, therefore, benthic macroinvertebrate and fish populations. They may also allow more intensive fisheries management, such as stocking. Additionally, the identification of stream segments with very good to excellent water quality allows fisheries managers to recommend that the DEP examine streams for special protection classification, regardless of trout biomass. Qualitative samples may also be used to measure the impact of point source discharges in some cases and to focus the attention of the DEP on these discharges if degradation is evident.

A qualitative approach is appropriate when the survey purpose is to assess stream health or for inventory purposes. When collecting qualitative benthic macroinvertebrate samples, the samples should be taken from all habitat types at each sampling site when possible., A sampling crew member or a combination of crew members will, where stream width allows, take two or three

samples across a riffle transect, a pool transect, and along the bank (root wads, twig deposits, leaf deposits, aquatic plant beds, etc) using a "kick screen" or a rectangular macroinvertebrate net. The kick screen should be 3' X 3' with a net mesh size of 800–900µ. Nitex bolt cloth is preferred for net material. Additionally, rocks, organic detritus, and aquatic vegetation should be hand-picked with forceps for attached macroinvertebrates. Samples can be hand-sorted on the screen or net, placed in a lab pan, and where possible, identified streamside. Abundant and/or dominant taxa will receive an additional notation that they are abundant. In cases where field identification is not possible, the unidentified portion of each individual sampling site's specimens will be preserved in a site-specific vial of 70% ethyl alcohol and returned to the office lab for later identification. Into each vial will be placed a waterproof label on which the stream name, site number, and date are written in pencil.

Semi-quantitative and quantitative sampling protocols should be used for cause and effect surveys or pollution investigations. Semi-quantitative sampling requires, but is not limited to a D-frame kick net with a net mesh size of 500µ. Quantitative sampling requires a Surber sampler with a net mesh size of 500µ. To perform population statistics, the investigator should collect a minimum of six individual Surbers across a riffle and analyze them individually. The collection sites should be located upstream (or in a reference stream), in the mixing zone and at a downstream location below the mixing zone.

2. A Benthic Index of Biotic Integrity for Wadeable Freestone Riffle-Run Streams in Pennsylvania

2.1 NET MESH CONSIDERATIONS

In recent years, many state water quality programs, federal agencies (e.g., USEPA, USGS), and other water quality monitoring organizations began using net sampling devices with 500µ □mesh nets. In order to conform to this trend, the 500µ □net mesh size has been adopted for the Department's D-frame sampler used in the DEP-RBP sampling method (described below). Future references to the D-frame sampler in the document assume 500-µ mesh netting. The net mesh size of other screen samplers has not changed and still is to be 800-900 µ. Because of this net mesh size change, the mesh size of the sampler used must be noted on field and bench identification sheets for the collected benthic sample.

2.2 QUALITATIVE METHODS

The type of sampling gear used is dependent on survey type and site-specific conditions. The recommended gear in wadeable streams is 3' x 3' flexible kick-screens and 12-inch diameter round D-frame nets. In larger streams or rivers, grab-type samplers may be used to obtain qualitative samples. While generally thought of as quantitative devices, Eckman, Peterson, or Petite Ponar grab samplers can also be used to obtain qualitative data. The type of gear, dimensions, and mesh size must be reported for all collections. When more than one gear type is used, the results must be recorded separately.

Physical variables should be matched as closely as possible between background and impact stations when selecting locations for placement of the sampling gear within each station. Matching these variables helps minimize or eliminate the effects of compounding variables. Macrobenthos often exhibit clustered distributions, and if the sampling points are selected in close proximity to each other, a single clustered population may be obtained rather than a generalized measure of the overall population within the selected subhabitat. Spacing the sampling points as far apart as possible within the sub-habitat can minimize the problem of clustered distributions.

2.2.1 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 and still provide adequate results. The kickscreen is constructed with a 3' x 3' piece of net material (800-900 μ mesh size) fastened to two dowel handles (approximately 1"d. X 4' long).

2.2.1.1 Traditional Method

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 3' x 3' area immediately upstream of the net to a depth of 3" - 4" (approximately 10 cm). 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 in order to make valid comparisons. The effort, expressed as area, must be reported for all collections. Collect a minimum of four screens at each site. Initial sampling should be conducted in riffle areas. Collection in additional habitats to generate a more complete taxa list can be conducted at the discretion of the investigator. Initial analysis of the data must be limited to the riffle data for standardization. A second analysis including other habitats may be conducted as needed.

Data observations shall be recorded on a standard field sheet created for each station sampled. Record the relative abundance of each recognizable family in each individual collection in the field. Relative abundance categories, with the observed "total" ranges indicated in parenthesis include: rare (0-3), present (3-10), common (11-24), abundant (25-99), and (occasionally) very abundant (100+). The investigator, at his/her discretion, may elect to enumerate certain target taxa.

Recording the results of each collection has several advantages that are lost if the data are composited for each station:

a. A stressed or enriched community often exhibits little variability in community structure over an area while a healthy community should have a more complex structure. If varied taxa are found on each screen, the community is probably complex, while the presence of only a few dominant taxa on every screen indicates the community is a simple one.

b. Collecting intolerant taxa in a majority of screens is a good indication of an unstressed community. However, collecting intolerant taxa in only one out of four screens may be an indication that the intolerant taxa have only a marginal existence at that location. A comparison of the composited taxa lists for each location may not indicate the rarity of the intolerant taxa, but this rarity would be readily apparent if the taxa lists for individual screens were compared.

c. Separate screen taxa lists provide information concerning the distribution of taxa. For example, mayflies are taken in one of four screens at the background station and in none of the four screens at the impact station. All the other taxa collected at both the stations are tolerant forms. Based on a composited taxa list for each station, one might conclude that the impact station is depressed due to the absence of mayflies. However, the individual screen taxa lists would indicate that the mayflies may have a clumped distribution and there is a possibility that the collector simply missed the clumps at the impact station. This will be apparent to the biologist while in the field and he/she can continue collecting until comfortable that mayflies are indeed absent or less abundant at the impact station. Later, it can be reported, for example, that 4 of 10 screens contained mayflies at the background station while only 1 of 10 screens contained mayflies at the impact station. This is an instance when the collector, while still in the field, may choose to count the mayflies in each screen (especially if the background screens had many mayflies while the impact screens only had one or two).

d. Separate screen data can lend weight to an analysis when classification techniques (ordination or clustering) are used. Results that cluster or score the individual background screens differently than the individual impact screens indicates a difference between the locations. When the classification technique scores background and impact screens in an apparent random manner, then it is likely that there is no impact or that the natural variability is large and masks any impacts. Individuals of representative taxa for a station may be composited in a single vial and preserved for later laboratory verification or identification. Generally, the level of taxonomic identification would follow that as listed in section 2.E.1.

Answers to several questions can be useful in subsequent analysis and can be stored with the taxa lists as remark fields. The answers to the following questions, which require collector judgment, can be recorded in the field on a coded form. What are the dominant and rare taxa? Are there any taxa that are found to be unusually abundant?

2.2.1.2 Assessment Method

This method is used for assessments conducted as part of the Statewide Surface Waters Assessment Program and employs the same kick screen gear, physical disturbance techniques, and relative abundance determinations as the traditional method (2.B.1.a). The main difference is that only two kicks are usually required and macroinvertebrate identifications are done streamside to family level taxonomy with hand-held lens (10X) if necessary. Data are recorded on standard field forms. Refer to the Statewide Surface Waters Assessment Protocol for further details.

2.2.2 D-Frame

The handheld D-frame sampler consists of a bag net attached to a half-circle ("D" shaped) frame that is 1' wide. The net's design is that of an extended, round bottomed bag (500µ mesh size). The methodology is basically the same as with the kick-screen - except for the following points: one person, facing downstream and holding the net firmly on the stream bottom, employs the net. One "**D-frame effort**" is defined as such: the investigator vigorously kicks an approximate area of 1 m^2 immediately upstream of the net to a depth of 10 cm (or approximately 4", as the embeddedness of the substrate will allow) for approximately one minute. All benthic dislodgement and substrate scrubbing should be done by kicks only. Substrate handling should be limited to only moving 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 in order to assure all kicked material flows toward the net. Avoiding areas with crosscurrents, the substrate material from within the square meter area should be kicked toward the center of the area – above the net opening. The concepts and field forms concerning field recording of invertebrate data discussed in the kick-screen method section (2.B.1a) also apply to the D-frame method.

2.3 SEMI-QUANTITATIVE METHOD (DEP-RBP)

In Plafkin (1989), USEPA presented field-sampling methods designed to assess impacts normally associated with pollution impacts, cause/effect issues, and other water quality degradation problems in a relatively rapid manner. These are referred to as Rapid Bioassessment Protocols (RBPs). The DEP-RBP method is a bioassessment technique involving systematic field collection and subsequent lab analysis to allow detection of benthic community differences between reference (or control) waters and waters under evaluation. The DEP-RBP is a modification of the USEPA RBP III (Plafkin, et al; 1989); designed to be compatible with Pennsylvania's historical database. 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/effect approach), the DEP-RBP modifications were designed for unimpacted waters, as well as impacted waters.

2.3.1 Sample Collection

The purpose of the standardized DEP-RBP collection procedure is to obtain representative macroinvertebrate fauna samples from comparable stations. The DEP-RBP assumes the riffle/run habitat to be the most productive habitat. Riffle/run habitats are sampled using the D-frame net method described above. The number of D-frame efforts is dependent on the type of survey conducted as described below:

2.3.1.1 Limestone Streams

For limestone stream surveys, two paired D-frame efforts are collected from each station - one from an area of fast current velocity and one from an area of slower current velocity within the same riffle.

2.3.1.2 Antidegradation Surveys

For Antidegradation surveys, it is necessary to characterize macroinvertebrate fauna communities from an area larger than a single riffle. Therefore, an antidegradation survey station is defined as a stream reach of approximately 100 meters in length. At each station, six "D-frame efforts" are collected. Make an effort to spread the samples out over the entire reach. Choose the best riffle habitat areas and be certain to include areas of different depths (fast and slow) and substrate types that are typical of the riffle. The resulting "D-frame efforts" (six for anti-degradation, two for other survey types) are composited into one sample jar (or more as necessary). Care must be taken to minimize "wear and tear" on the collected organisms when compositing the materials. It is recommended that the benthic material be placed in a bucket and filled with water to facilitate gentle stirring and mixing. The sample is preserved in ethanol and returned to the lab for processing.

2.3.2 Sample Processing

Samples collected with a D-frame net are generally considered to be qualitative. However, the preserved samples can be processed in a manner which yields data that are "semi-quantitative" - data that were collected by qualitative methods but gives information that is almost statistically as strong as that collected by quantitative methods. The following procedure is adapted from USEPA 1999 RBP methodology and used to process qualitative D-frame samples so that the resulting data can be analyzed using benthic macroinvertebrate biometric indices (or "metrics"). Equipment needed for the benthic sample processing are:

- 2 large laboratory pans gridded into 28 squares* (more gridded pans may be necessary depending on the size of the sample);
- an illuminated magnifying viewer;
- slips of paper (numbered from 1 to 28) for drawing random numbers;
- forceps (or any tools that can be used to pick floating benthic organisms); and
- grid cutters made from tubular material that approximates an inside area of 4 in^2 *.

* USEPA's (1989) gridding techniques suggested using "5 cm x 5 cm" (2" x 2") grids. Existing equipment consisted of 14" x 8" x 2" pans which were conducive to dividing into 2" x 2" grids and thus, contained 28 squares. The 4-in2 grid cutters conform to these pan dimensions. While pan size is not critical, the number of grids (28) must be maintained if any basic density comparisons wish to be made between samples. Grid cutters (or similar sub-sampling devices) used with different sized pans should conform to the pans' grid dimensions.

The procedure described below begins with the premise that the collected samples have been properly composited according to the type of survey. For antidegradation surveys, a station sample represents a composition of six D-frame efforts (collected from fast and slow riffle areas in a 100 meter reach). For limestone surveys, a station sample is a composition of two D-frame efforts.

Following the steps listed below; process each composited D-frame sample to render a sub-sample size targeted for the specific survey type. The targeted sub-sample size for antidegradation surveys is 200 benthic organisms and 300 for limestone surveys (±20% for each).

a. The composited sample is placed in a 28-square gridded pan (Pan1). It is recommended that the sample be rinsed in a standard USGS No. 35 sieve (or sieve bucket) to remove fine materials and residual preservative prior to subsampling.

b. The sample is gently stirred to disperse the contents evenly throughout Pan1 as thoroughly as possible. (In order to ease mixing and to minimize "wear-and-tear" on the more delicate organisms, water may be added to the pan to the depth of the sample material before stirring.)

c. Randomly select a grid using the 28 random number set and, using the grid cutters, remove the debris and organisms entirely from within the grid cutter (centered over the selected grid and "cut" into the debris) and place removed materials in a second gridded pan (Pan2).

> i. Float and pick, count, and sub-total all identifiable organisms (excluding pupae, larval bodies missing too many critical structures to render confident IDs, extremely small instar larvae, empty shells or cases, and non-benthic taxa) from each cut grid placed in Pan2. Repeat until at least 4 grids have been sub-sampled from Pan1. If, after 4 Pan1 grids have been sorted, the sub-total is less than the targeted sub-sample (20 ± 20%), then continue to remove and sort grids one at a time until 200 organisms (± 20%) are obtained from Pan2. If the benthic organism yield from the 4 Pan1 grids exceeds the 200 ± 20% target (240+), then proceed to Step ii.
>
> ii. With all of the 240+ identifiable organisms remaining in Pan2, randomly select one grid and "back count" (removing) all the organisms from that grid. Repeat one grid at a time until the bug count remaining in Pan2 satisfies the "200 ± 20%" rule.

d. If not identified immediately, the sub-sample should be preserved and properly labeled for future identification.

e. The benthic material remaining (Pan1) after the target sub-sample has been picked can be returned to its original sample jar and preserved. They shall be retained in accordance with QA retention times as specified for the respective survey type.

f. Any grid chosen must be picked in its entirety.

g. Record the final grid counts selected for each gridding phase (Pan1, Pan2, and Pan2 "back counting" as necessary) on the lab bench ID sheet for the sample.

Processing larger, excessive amounts of D-frame sample debris

Hopefully, the collector will rarely have very large amounts of D-frame materials to process. The reduction of large materials by careful removal, inspection, and rinsing in a bucket or

using a sieve prior to field preservation or at the lab is encouraged. However, if the amount of material composited in the field jars exceeds the functional sorting capacity of Pan1, then follow this guidance:

o Evenly distribute the material between as many pans as necessary.
o From each pan (Pan1a, Pan1b, etc.), remove debris and organisms from 4 random grids and place in Pan2 as described in Step 2.3.2.c above.
o Once the required 4 grids from each Pan1 have been placed in Pan2, evenly and gently redistribute the materials as in Step 2.3.2.b.
o Then, resume processing, again as described in Step 2.3.2.c, selecting a grid from Pan2 and placing the materials into a gridded Pan3.
o Process this material and repeat as described in Step 2.3.2.c.i until the targeted 200 ± 20% sub-sample is obtained from Pan3.
o If, after processing 4 grids, the +20% upper limit (240+) is obtained, follow "back counting" method in Step 2.3.2.c.ii.
o Once the targeted sub-sample is reached, continue with Step 2.3.2.d.

2.4 IDENTIFICATION

2.4.1. Taxonomic Level

The level of identification for most aquatic macroinvertebrates will be to genus. Presently, the identification of Chironomidae, or midges, is to the family level. Some individuals collected will be immature and not exhibit the characteristics necessary for confident identification. Therefore, the lowest level of taxonomy attainable will be sufficient. Certain groups, however, may be identified to a higher taxonomic level as follows:

Snails (Gastropoda) - Family
Clams, mussels (Bivalvia) - Family
Flatworms (Turbellaria)
    identifiable planariids - genus
    or Family Planariidae
    others – Class Turbellaria
Segmented worms (Annelida)
    aquatic earthworms & tubificids - Class Oligochaeta
    leeches - Class Hirudinea
    Moss animacules - Phylum Bryozoa
Proboscis worms – Phylum Nemertea
Roundworms - Phylum Nematoda
Water mites- "Hydracarina" (an artificial taxonomic grouping of several mite superfamilies)

2.4.2 Verifications

For quality assurance purposes, certain laboratory invertebrate processing procedures should be checked routinely. Normally, a colleague may perform these spot checks. These include the floating/picking steps, taxonomic identifications, and total taxa list scans:

a. Sorting. After the floating and picking has been completed for samples that require this treatment (Pa-RBP, Surber-type, multi-plate, and grab samples), the residue should be briefly scanned before discarding to assure that the sample has been sufficiently "picked". This should be done for 10% of the samples (or at least one sample) per survey.

b. Identification. For samples not involving litigation or enforcement issues, laboratory bench ID sheets for all samples should be reviewed. Any unusual taxa or taxa that are not typical to the type of stream or water quality condition that was surveyed, should be checked. For samples involving legal issues, representative specimens of each taxon may need to be verified by independent expert taxonomists.

3. Pennsylvania DEP Multi-habitat Stream Assessment Protocol

TheUnited States Environmental Protection Agency's Rapid Bioassessment Protocols 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 and the "multi-habitat" approach. The single, most productive habitat approach is typically used to assess streams where cobble substrate (riffle/run) is the predominant habitat. The multi-habitat approach involves sampling a variety of habitat types instead of sampling a single habitat, such as cobble substrate in riffles and/or runs.

In April of 2002, the Pennsylvania DEP began developing a macroinvertebrate bioassessment protocol for assessing the Commonwealth's low-gradient streams. Low-gradient waterways consist of pool/glide channel morphology and naturally lack riffles. The multi-habitat field and laboratory methods described in Barbour et al (1999) were used as a starting point for the project. Water chemistry, physical habitat, and aquatic macroinvertebrates were collected at 77 sampling sites in this study. The project goal was to identify practical and regionally appropriate field, laboratory, and data analysis procedures and to develop an index of biological integrity that accurately reflects the ecological conditions of Pennsylvania's low-gradient streams.

3.1 FIELD METHODS

All chemical water quality, physical habitat, and aquatic macroinvertebrate data is collected from a sample reach approximately 100 meters in length. During development of the protocol, water temperature, pH, dissolved oxygen, and conductivity were measured in the field and a chemical sample was collected from each reach for laboratory analysis. This sample was collected under base flow (non-stormwater runoff) conditions.

Field	Lab	
Temperature	pH Total	Organic Carbon
Dissolved Oxygen	Alkalinity	Chloride
pH	Nitrate-N	Sulfate
Conductivity	Total Phosphorus	Iron

Total phosphorus and total organic carbon samples are preserved with 10% sulfuric acid and samples analyzed for metals are preserved with concentrated nitric acid to a pH <2. All samples

are kept on ice and delivered to the DEP laboratory in Harrisburg, PA within 48 hours of collection.

Physical habitat is documented using the EPA Glide/Pool Prevalence Habitat Assessment Field Data Sheet (Barbour et al. 1999). This evaluation divides the habitat of the stream and its adjacent land use into ten parameters. Each parameter is scored on a scale of 0 to 20, with a higher score indicating better conditions. Depending on the score, a parameter can fall into one of four categories: Poor, Marginal, Suboptimal, and Optimal.

For the purpose of this protocol, only nine of the ten parameters are used. Channel Sinuosity is not used because the range of sinuosity as defined in the data sheet is not applicable to Pennsylvania streams. Even the State's most sinuous streams will have low values using this definition. Thus, total habitat site scores can range from 0-180, with 180 being a perfect score.

The majority of macroinvertebrate samples were collected from October to May. A small number of samples were collected outside of this period to test the seasonal variability of the protocol. Seasonal variability analysis results are discussed on pages 6 and 7.

Aquatic macroinvertebrate samples are collected using a multi-habitat sample collection method modified from that described in Barbour et al (1999). Organisms are collected from five different habitat types within the sample reach. A total of 10 "jabs" are collected within each sample reach. Each jab consists of a 30-inch-long sweep of a 0.3-meter wide area, using a D-frame dip net (500 micron mesh). At least two jabs are made in each of the habitat types present within the sample reach.

The biologist first identifies which habitat types are present within the sample reach. 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 extensive habitat type(s) in the reach. All jabs are combined into several 2-liter largemouth jars and preserved in ethyl alcohol. Typically, the combined 10 jabs will fill three to four 2-liter sample jars about 2/3 full with organic and inorganic material. Sample jars are topped-off with 95% ethanol to ensure adequate sample preservation.

3.2 LAB METHODS

In the laboratory, each composited sample is placed into a 3.5" deep rectangular pan (measuring 14" long x 8" wide on the bottom of the pan) marked off into 28 four-square inch (2" x 2") grids. Using an illuminated magnifying lens, macroinvertebrates are picked from a minimum of four grids, selected at random, to generate a 200-organism (+/- 20%) sub-sample. Additional grids may be selected at random until the sub-sample is obtained. The organisms contained in the 200-organism sub-sample are identified to the lowest practical taxonomic level (usually genus). Some individuals collected will be immature and not exhibit the characteristics necessary for confident identification. If the individual cannot be confidently identified to the proper level, it should be discarded. All pupae are discarded. Certain groups are identified to a higher taxonomic level as follows:

Flatworms (Turbellaria) – Phylum Turbellaria
Segmented worms (Annelida), aquatic earthworms, & tubificids – Class Oligochaeta
Proboscis worms – Phylum Nemertea
Roundworms – Phylum Nematoda
Water mites – "Hydracarina" (an artificial taxonomic grouping of several mite superfamilies)
Midges – Family Chironimadae
Weevils – Family Curculionidae
Sand flies\no-see-ums – Ceratopogonidae
Decapoda, Gastropoda, and Pelecypoda are identified to family

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 can not 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 so as 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

3.3 LABORATORY PROCESSING PROCEDURE

3.3.1 Initial Processing of Raw Macroinvertebrate Sample

1. Fill a five-gallon bucket about 2/3 full with cold water.
2. Decant ethanol from samples by gently dumping the contents of sample bottles into a 500-micron sieve.
3. Gently rinse most of the silt and/or very-fine sand from the sample material in the sieve using an abundance of clean, cold water.

4. Gently transfer the rinsed sample material from the sieve into the five-gallon bucket.
5. Repeat step 2 until approximately ½ of the material contained in a given sample is transferred into the five-gallon bucket.
6. Gently agitate the contents of the bucket and decant the water and a portion of the bucket's contents into a 500-micron sieve.
7. Transfer the contents of the sieve into a clean, white, 3.5" deep rectangular pan (measuring 14" long x 8" wide on the bottom of the pan) marked off into 28 four-square inch (2" x 2") grids.
8. Gently fill the five-gallon bucket about 2/3 full with clean cold water and repeat steps 6 & 7 until all organisms are transferred from the bucket into the pan.
9. Repeat steps 1 through 8 until all of the organisms contained in the sample are transferred to the pan.

3.3.2 Picking the 200-Organism Sub-sample

1. Remove a reasonable amount of organic material from a randomly selected grid in the 3.5" deep rectangular pan and place it in a large clear glass or plastic dish (sample-picking dish) containing clean water. The sample-picking dish should be placed on top of a white paper towel or piece of paper.
2. Using an illuminated magnifying lens and forceps, grasp individual large pieces of debris from the sample-picking dish, dip them in a deep dish or bowl of cold water (rinse dish), and discard them. Usually after numerous large pieces of debris are discarded, more material from the selected grid can be placed in the sample-picking dish.
3. After the large pieces of debris are removed from the sample-picking dish, move the organic matter away from the front edge of the dish so that there is an area of the dish that is relatively free of debris.
4. Starting with the debris closest to the debris-free area of the sample-picking dish, start moving small allotments of debris into the previously debris-free area so that individual organisms can be clearly detected and transferred from the sample-picking dish to a 3"-diameter petrie dish or similar dish containing clean cold water or ethanol (sub-sample organism dish). Use a hand held counter and keep track of the number of "identifiable" organisms (i.e., organisms in good enough condition to be identified to genus for most taxa) transferred to the sub-sample organism dish.
5. Continue working from the front edge of the sample-picking dish toward the back edge of the dish until all organisms have been transferred from the sample-picking dish to the sub-sample organism dish. Sometimes the water in the sample-picking dish will become cloudy making it hard to see the organisms in the dish. If this happens, carefully pour off the water in the sample-picking dish, being careful not to pour off organisms and debris during the process, and replace it with clean, cold water. It is best to pour off water between steps 2 and 3 above.
6. Use forceps and netting attached to a pipette, pencil, or similar object, to transfer all of the contents of the randomly selected grid to the sample-picking dish and repeat steps 1- 4 above until all organisms have been placed in the sub-sample organism dish.
7. Repeat steps 1-5 above until a minimum of 4 randomly selected grids are processed. All organisms in the 4^{th} grid are to be transferred to the sub-sample organism dish, even if the 200 +/- 20% criterion is already met. If the estimated number of "identifiable" organisms

in the sub-sample are less than 160, process additional grids until a minimum of 160 organisms are contained in the sub-sample.

8. If the sub-sample contains more than 240 organisms after picking the fourth grid, place the sub-sample in a clean gridded pan containing a small amount of cold water. Using an illuminated magnifying lens, randomly select grids and transfer all organisms from these grids to a separate container, using a hand-held counter to keep track of the number of "identifiable" organisms transferred. Continue selecting grids and transferring organisms until a sub-sample of 200 +/- 20% is produced.

4. An Index of Biological Integrity (IBI) for "True" Limestone Streams

"True" limestone streams, limestone spring streams, or simply limestone streams are very unique. These streams are formed by large alkaline springs or they are streams maintained by many large alkaline springs. Pennsylvania has approximately 83,000 miles of streams and there are probably less than 800 miles of limestone streams. However, this small subset of streams is of great ecological and economical importance. Limestone streams like the Letort Spring Run and Spring Creek are world famous trout fishing streams attracting anglers from around the country and from many nations. The ecological integrity of limestone streams must be assessed correctly if they are going to be properly protected. These streams have fairly low gradient, constant temperatures, high alkalinity and are highly productive. Their unique physical and chemical characteristics produce a unique macroinvertebrate community. The lack of diversity in habitat, temperature and water chemistry produces a macroinvertebrate community with low diversity. The highly productive water chemistry produces a high density of macroinvertebrates. The end result is a community with a low number of taxa that is generally dominated by a few taxa. In fact five taxa, *Lirceus*, *Gammarus*, *Ephemerella*, *Optioservus* and Chironomidae, accounted for about 79.2 % of the total organisms collected in the 188 sample data set. The unique macroinvertebrate communities created by these unique aquatic environments make it essential that a separate Index for Biological Integrity (IBI) be developed for limestone streams. If limestone streams are assessed with an IBI for freestone streams even the very best sites would look impaired. On the other hand, if a freestone stream is assessed using the limestone IBI an impaired stream could easily pass as unimpaired. This makes it very important for streams to be correctly classified as limestone streams. A mistake in stream classification will make it impossible to properly assess the stream's ecological condition. The EPA publications detailing the development of Rapid Bioassessment Protocols (Plafkin et al. 1989; Barbour et al. 1999) were a major source for the development of the limestone stream IBI.

4.1 STREAM CLASSIFICATION AND REFERENCE CRITERIA

Limestone streams are streams formed by large limestone springs or are very strongly influenced by limestone springs. However, a stream located in limestone geology that appears to originate from spring sources does not guarantee it should be classified as a limestone stream. Limestone streams are always in limestone geology, but all streams in limestone geology are not limestone streams. The two most important characteristics in the classification of a limestone stream are temperature and alkalinity. The sampling of Pennsylvania limestone streams indicates the alkalinity should be maintained above 140 mg/l throughout the year. Many streams may yield

high alkalinity results for much of the year, but if there are any periods where the alkalinity fluctuates below 140 mg/l the stream should be examined very carefully. Groundwater temperatures are approximately 50 to 55 degrees Fahrenheit (F). Streams strongly influenced by groundwater will maintain temperatures near 50 degrees F. Many macroinvertebrates need fluctuating temperatures to complete their life cycles so if temperatures fluctuate too much the diversity of the macroinvertebrate community increases and it no longer is a distinct limestone community. These two criteria may require the investigator to have year round data on the stream to correctly classify it as limestone. Table 1 lists the criteria for limestone streams and reference limestone streams. Note for a stream to qualify as reference it must qualify for at least High Quality under the Chapter 93.4b antidegradation requirements.

Table 1.

Limestone Streams Criteria Parameter	**Criterion**	**Explanation**
Alkalinity	Minimum 140 mg/l	Stream must maintain high alkalinity throughout the year
Temperature	40 to 65 deg. F 4 to 18 deg. C	Constant temperatures are very important, check to see if the stream is ice free in the winter
Stream originates from limestone springs or very strongly influenced by limestone springs		
Drainage Area	Maximum 20 sq. miles Surface drainage area	There maybe exception to this parameter as long as all other criteria are met
Designated Water Use	Cold Water Fishes (CWF)	Must be designated a CWF in Chapter 93

4.2 FIELD SAMPLING AND LABORATORY SAMPLER PROCESSING

4.2.1 Net Mesh Considerations

All limestone stream samples collected for the development of this document used net mesh in the 800-900µ range. In recent years, many state water quality programs, federal agencies (e.g., EPA, USGS), and other water quality monitoring organizations began using net sampling devices with 500µ mesh nets. Field sampling comparisons have shown that the 500µ mesh size blocked quickly preventing macroinvertebrates and vegetation from entering the net resulting in a poor sample. In order to insure an accurate assessment 800-900µ net mesh must be used to collect samples.

4.2.2 D-Frame Net

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 such: the investigator vigorously kicks an approximate area of $1m^2$ (1 x 1 m) immediately upstream of the net to a depth of 10cm (or approximately 4", as the embeddedness of the substrate will allow) for

approximately one minute. All benthic dislodgement and substrate scrubbing should be done by kicks only. Substrate handling should be limited to only moving 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 in order 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.

4.2.3 Semi-Quantitative Method (PaDEP-RBP)

In Plafkin (1989), EPA presented field-sampling methods designed to assess impacts normally associated with pollution impacts, cause/effect issues, and other water quality degradation problems in a relatively rapid manner. These are referred to as Rapid Bioassessment Protocols (RBPs). The PADEP-RBP method is a bioassessment technique involving systematic field collection and subsequent lab analysis to allow detection of benthic community differences between reference (or control) waters and waters under evaluation. The PADEP-RBP is a modification of the EPA RBP III (Plafkin, et al; 1989); designed to be compatible with Pennsylvania's historical database. 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 EPA'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 EPA's RBP III method was designed to compare impacted waters to reference conditions (cause/effect approach), the PADEP-RBP modifications were designed for un-impacted waters, as well as impacted waters.

4.2.4 Sample Collection

The purpose of the standardized PADEP-RBP collection procedure is to obtain representative macroinvertebrate fauna samples from comparable stations. The PADEP-RBP assumes the riffle/run habitat to be the most productive habitat. Riffle/run habitats are sampled using the D-frame net method described above. For limestone stream surveys, two paired D-frame efforts are collected from each station - one from an area of fast current velocity and one from an area of slower current velocity within the same riffle. Limestone streams have low gradient often making it difficult to locate well developed riffles. If there is no riffle in the sample area use a run or the best rock substrate available. The resulting "D-frame efforts" (two) are composited into one sample jar (or more as necessary). Care must be taken to minimize "wear and tear" on the collected organisms when compositing the materials. It is recommended that the benthic material be placed in a bucket and filled with water to facilitate gentle stirring and mixing. The sample is preserved in ethanol (95%) and returned to the lab for processing.

4.2.5 Sample Collection Period

Samples must be collected from January through May. All samples used to develop this IBI were collected in this time period. Limestone streams have a low number of sensitive taxa and only a few of these taxa are generally found larger 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 limestone streams emerge in May and June and are normal difficult or impossible to collect from June through December.

Collecting samples from January through May ensures this very important ecological indicator taxa will not be missed.

4.2.6 Sample Processing

Samples collected with a D-frame net are generally considered to be qualitative. However, the preserved samples can be processed in a manner which yields data that is "semi-quantitative" - data that was collected by qualitative methods but gives information that is almost statistically as strong as that collected by quantitative methods.

The following procedure is adapted from EPA 1999 RBP methodology and used to process qualitative D-frame samples so that the resulting data can be analyzed using benthic macroinvertebrate biometric indices (or "metrics"). Equipment needed for the benthic sample processing are:

- 2 large laboratory pans gridded into 28 squares (more gridded pans may be necessary depending on the size of the sample). White polyethylene pans 18"L x 12"W x 3.5"D were used, but any similarly sized pan with 28 equal grids may be used.
- Illuminated magnifying viewer. (optional)
- Slips of paper (numbered from 1 to 28) for drawing random numbers, and
- Forceps (or any tools that can be used to pick floating benthic organisms),
- Grid cutters made from tubular material that approximates an inside area of 4 in^2.

The targeted sub-sample size is 300 for limestone surveys (±□ 20%), (240 to 360 organisms). Samples must be properly prepared for sub-sampling. Macroinvertebrates tend to clump so the sample should be mixed in the sample container or the sub-sample pan to make it as homogenous as possible. If necessary the sample maybe mixed in a bucket prior to being placed in the pan. In order to further reduce the effect of clumping a two-tiered sub-sampling technique is employed. A minimum of 4 grids must be selected from the first pan.

Tier 1 – Rinse the sample in a standard USGS No. 35 sieve to remove fine materials and residual preservative. During the rinse larger rocks, sticks, and leaves maybe removed making sure to retain all the macroinvertebrates. Place the sample in a 28-square gridded pan (Pan1) and add enough water to distribute the sample evenly. Randomly select 4 grids using the 28 random number set and, using the grid cutters, remove the debris and organisms entirely from within the grid cutter and place in a second gridded pan (Pan2). Selecting a minimum of 4 grids reduces the effect of clumping. Do a visual scan of Pan2 to ensure that there are enough identifiable (this excludes pupae, extremely small instar larvae, and empty shells or cases) organisms to reach the targeted sub-sample size (300 +/- 20%). If there do not appear to be enough organisms randomly select additional grids until there appears there are a minimum of 300 +/- 20% organisms.
Note: In limestone streams we have never needed more than 4 grids.

Tier 2 –Randomly select grids from pan2 removing all the organisms from each grid until there is a sub-sample of 300 +/- 20%. If it appears that the number of benthic organisms from the last grid will cause the sub-sample to exceed its target size by more than 20% (>360 organisms), count them and place in a clean gridded pan (Pan3) with enough water to

facilitate gentle stirring and even distribution. Randomly select grids from Pan3 and remove individuals until the count of organisms remaining in Pan3 falls within the +20% upper limit.

Comments:

1. If the sample is too large to fit in pan 1 evenly divide sample into 2 or more pans. Randomly select a minimum 4 grids from each pan and place them in a pan.
2. The benthic material remaining after the target sub-sample has been picked can be returned to its original sample jar and preserved. They shall be retained in accordance with QA retention times as specified for this respective survey type.
3. Any grid chosen must be picked in its entirety.

4.2.7 Identification, Taxonomic Level

The level of identification for most aquatic macroinvertebrates will be to genus. Some individuals collected will be immature and not exhibit the characteristics necessary for confident identification. If an individual cannot be confidently identified to the proper level it should be discarded. All pupae are discarded. Certain groups are identified to a higher taxonomic level as follows:

Flatworms (Turbellaria) - Phylum Turbellaria
Segmented worms (Annelida) aquatic earthworms & tubificids - Class Oligochaeta
Proboscis worms – Phylum Nemertea
Roundworms - Phylum Nematoda
Water mites - “Hydracarina” (an artificial taxonomic grouping of several mite superfamilies)
Midges – Family Chironimadae

MODULE J

Protocols for Sampling Water Quality in Wadeable Streams

Prepared by:
Jason Detar

1. Introduction

Evaluation of the chemical and physical parameters of water is critical in determining the quality and productivity of a fishery as well as identifying pollution sources and intensities (Marcinko et al. 1986). A record of water quality parameters can allow investigators to track or identify changes in waters quality and provide valuable support to affect changes to protect the resource from continued degradation. Numerous water quality parameters may be measured through either field or laboratory analyses. Appendix A provides a list of water quality parameters, units of measurement, required precision of measurements, and whether measurements should occur in the field or laboratory. For common measurements routinely conducted by PFBC staff in the field, analytical methodology is listed.

1.1 WATER CHEMISTRY IN WADEABLE STREAMS

1.1.1 Field Analyses

The following basic water chemistry parameters should be measured in the field during each stream survey: water temperature, pH, total alkalinity, total hardness, and specific conductivity. The standard PFBC "wet" chemistry kits and/or electronic meters should be used. Reagents should be replaced annually before the start of the field season. When not in use, water chemistry kits should be removed from vehicles to prevent reagents from freezing during spring sampling and/or prolonged exposure to high temperatures during summer. The standard methods used by the PFBC to measure the various chemical parameters are adapted from those suggested in the *Standard Methods for the Examination of Water and Wastewater* (American Public Health Association et al. 1980) and *Methods for Chemical Analysis of Water and Waste* (U.S. EPA 1976).

Sampling for onsite field analysis should be conducted by collecting a grab sample and then pouring (**not dipping**) smaller volumes into the test containers or collecting a water sample directly from the source into the test containers. When obtaining a water sample utilizing a grab sample, the container used for collecting the sample should be rinsed with the water being tested at least three (3) times before obtaining the final volume of water to be analyzed. Since the same container is used repeatedly for collecting the grab samples, storage of any chemicals, fish samples, or other potential contaminants should not be placed in the container. If such contamination does occur, the container should be replaced and no longer used for water samples. Similarly, if the water sample is collected directly from the source into the test containers then the test containers should be rinsed three times with the source water prior to collecting the sample.

The actual point in a flowing stream where a water sample is collected is important. In streams less than 20 m wide, grab samples should be collected in mid-stream and at mid-depth, where physically possible. Larger and deeper streams may require that a composite sample be obtained at multiple (generally 2 or 3) points across the width and/or through the depth of the stream. When sampling points are located downstream from the confluence of a major tributary, a complete mixing of both streams should occur upstream of the sampling point. Areas where small outfalls can have a localized influence on water quality should be avoided.

1.1.2 pH – Field

Generally, pH is measured in the field using a Hellige color comparator. Six color wheels are available to provide the ability to measure pH values from 3.0 to 10.2 *su*. A separate reagent is used for each color wheel and pH range. Each bottle of reagent is equipped with a built-in eyedropper with a white measurement line approximately one inch up from the tip. In most cases, the bromthymol blue-D reagent and color wheel, which contains the range of 6.0 to 7.6, should be used for the first measurement. To begin the analysis, the two square glass vials should be thoroughly rinsed (at least 3 times) and then filled to the 10-ml line with the test water. One vial should then be inserted into the left slot of the Hellige color comparator. Next, the eyedropper should be filled to the white measurement line with the appropriate reagent (in this case, bromthymol blue-D) and then added to the second vial. Care should be taken to **never** touch the eyedropper to the side of the vial. This results in water being transferred into the reagent bottle, which dilutes the reagent. The reagent should be mixed with the water sample by rolling the vial between your hands. Do **not** place your finger over the end of the vial and shake, as this will contaminate the sample. Next, place the vial in the right slot of the Hellige color comparator and hold the comparator at arms-length pointed toward the northern sky. Finally, rotate the color wheel to obtain as close a match of the color on the wheel to the color of the sample as possible to obtain the pH measurement. If the color of the sample does not match any of the colors on the wheel, the samples should be emptied, rinsed, and the next appropriate color wheel and reagent should be used to attempt to obtain a color match and pH measurement. Additionally, if the color of the sample appears to match one of the colors at either end of the range for that wheel, the next appropriate color wheel should be used to confirm the pH measurement, as each wheel overlaps the next successive wheel by four measurement units.

1.1.3 Total Alkalinity – Field Mixed Indicator

In most cases, total alkalinity is measured in the field using the buret titration method. A 125-ml Erlenmeyer flask should be rinsed thoroughly (at least 3 times) with test water and then filled with test water to the 100-ml line. Next, 0.10 ml of bromcresol green-methyl red mixed indicator should be added to the test water and mixed by swirling the solution in the Erlenmeyer flask (American Public Health Association et al. 1980). Next, the 5-cc syringe (marked in 0.2-cc increments) should be filled with the sulphuric acid standard solution (H_2SO_4; 0.020N), and slowly added to the solution of bromcresol green-methyl red mixed indicator and test water, taking care not to touch the end of the syringe to the Erlenmeyer flask to prevent contamination. As the H_2SO_4 is added to the solution, the Erlenmeyer flask should be swirled regularly. When the solution turns from a blue-green color to pink, the test is completed and the alkalinity measurement is recorded as 10X the number of H_2SO_4 cc added. The most accurate measurement is obtained by determining the value when the solution turns the lightest shade of pink detectible without returning to a clear solution. For example, if 0.4 cc of H_2SO_4 is added, the alkalinity would be recorded as 4 mg/l. If the entire 5-cc syringe of H_2SO_4 is added, the alkalinity would be recorded as 50 mg/l. For samples in which the test water is thought to have low alkalinity, the H_2SO_4 should be added 1 drop at a time. Each drop is approximately equal to 0.1 cc or 1 mg/l.

For samples in which the test water is thought to have very high alkalinity, the following procedures can be used to reduce the amount of H_2SO_4 required if it is in short supply. Follow the same procedures as listed above, except that the Erlenmeyer flask should be filled to the 50-

ml line with test water rather than the 100-ml line. When the solution turns from a blue-green color to pink, the test is completed and the alkalinity measurement is recorded as **20X** the number of H_2SO_4 cc added. For example, if 0.4 cc of H_2SO_4 is added, the alkalinity would be recorded as 8 mg/l. If the entire 5-cc syringe of H_2SO_4 is added, the alkalinity would be recorded as 100 mg/l.

1.1.4 Total Hardness – Field

In most cases, total hardness is measured in the field using the buret titration method. A 125-ml Erlenmeyer flask should be rinsed thoroughly (at least 3 times) with test water and then filled with test water to the 100-ml line. Next, 1 gram of the UniVer 3^{tm} powder should be added to the test water and mixed by swirling the solution in the Erlenmeyer flask. Next, the 5-cc syringe (marked in 0.2-cc increments) should be filled with the ethylenediamine tetraacetic acid (EDTA; 0.020N) titrant, and slowly added to the solution of UniVer 3^{tm} and test water, taking care not to touch the end of the syringe to the Erlenmeyer flask to prevent contamination. As the EDTA is added to the solution, the Erlenmeyer flask should be swirled regularly. When the water is very cold, such as in early spring or late winter, EDTA must be added at an even slower pace to avoid over-shooting the point of color change. When the solution turns from a purple-red to blue color, the test is completed and the total hardness measurement is recorded as 10X the number of EDTA cc added. The most accurate measurement is obtained by determining the value when the solution turns the lightest shade of blue (may appear blue-gray) detectible without returning to a clear solution. For example, if 0.4 cc of EDTA is added, the total hardness would be recorded as 4 mg/l. If the entire 5-cc syringe of EDTA is added, the hardness would be recorded as 50 mg/l. For samples in which the test water is thought to have low hardness, the EDTA should be added 1 drop at a time. Each drop is approximately equal to 0.1 cc or 1 mg/l. For samples in which the test water is thought to have very high hardness, the following procedures can be used to reduce the amount of EDTA required if it is in short supply. Follow the same methods as listed above, except that the Erlenmeyer flask should be filled to the 50-ml line with test water rather than the 100-ml line. When the solution turns from a purple-red to blue color, the test is completed and the total hardness measurement is recorded as 20X the number of EDTA cc added. For example, if 0.4 cc of EDTA is added, the hardness would be recorded as 8 mg/l. If the entire 5-cc syringe of EDTA is added, the hardness would be recorded as 100 mg/l.

1.1.5 Specific Conductivity - Field

In most cases, specific conductivity is measured in the field using an electronic meter. As with any electronic meter, the manufacturer's directions should be followed regarding calibration and maintenance to ensure that the meter is functioning properly. At a minimum, specific conductivity meters should be calibrated at the beginning and middle of each field season using a series of conductivity standards recommended by the meter's manufacturer. Conductivity should be recorded to the nearest microsiemen (*u*s) or micromho (mmho).

1.1.6 Water Temperature - Field

Water temperature should be measured in the field anytime that water quality measurements are collected. Water temperature can either be measured with a standard pocket thermometer or with an electronic meter. Pocket thermometers should be calibrated with a lab-quality thermometer annually at the beginning of the field season. As with any electronic meter, the manufacturer's directions should be followed regarding calibration and maintenance to ensure

that the meter is functioning properly. At a minimum, electronic thermometers should be calibrated at the beginning and middle of each field season. Water temperature should be recorded to the nearest one degree centigrade (^{o}C).

1.1.7 Dissolved Oxygen - Field

Dissolved oxygen (D.O.) is collected less frequently than the previous six parameters. When collecting in the field, D.O. can be measured using the Azide-Winkler titration method or with a probe and electronic meter. The Azide-Winkler method provides accurate measurements, but requires the use of strong chemicals and is typically no longer used by PFBC staff in the field. As with any electronic meter, the manufacturer's directions should be followed regarding calibration and maintenance to ensure that the D.O. meter is functioning properly. At a minimum, electronic D.O. meters should be calibrated at the beginning and middle of each field season. D.O. should be recorded to the nearest one tenth milligram per liter (mg/l) or nearest one tenth part per million (ppm).

1.1.8 Laboratory Analyses

Water samples collected for laboratory analyses should be collected in clean, individual containers and labeled with the date, stream name, county, site number, and a brief description of sampling point (Marcinko et al. 1986). The type of container, volume of water required, and preservative added to the sample depend on the chemical analyses that will be performed. Refer to American Public Health Association et al. (1980) for details in all cases where special analyses are required. It is also a good idea to contact the lab that will be conducting the analyses to determine if any other special procedures are necessary.

2. Literature Cited

American Public Health Association, American Water Works Association and Water Pollution Control Federation. 1980. Standard methods for the examination of water and wastewater, 15th edition. Washington, D.C.

Marcinko, M., R. Lorson, and R. Hoopes. 1986. Procedures for stream and river inventory information input. Fisheries Management Section, Pennsylvania Fish Commission, Bellefonte, Pennsylvania.

U.S. Environmental Protection Agency. 1976. Methods for chemical analysis of water and wastes. EPA-625/6-74-003a. Environmental Monitoring and Support Laboratory, Environmental Research Center, Cincinnati, Ohio.

Appendix A

Water Quality Measurement Guidelines from Marcinko et al. (1986)

APPENDIX 4

Codes for Additional Chemistries

Parameter	Units	Precision	Where Measured	Code
Stream Flow	CFS	000.0	Field	01
Pipe Flow	GPM	000.0	Field	02
Temperature	C	000.0	Field	03
pH (range only from 0-14 anyway; max can only be _ _._ _--4 digits)	SU	00.00	Field: Colorimetric Field: Electrometric Lab: Colorimetric Lab: Electrometric	04 05 06 07
Dissolved Oxygen (range only from 0-14 anyway; max can only be _ _._ _ --4 digits)	mg/l	00.00	Field: Electrometric Field: Winkler Lab: Electrometric Lab: Winkler	08 09 10 11
Specific Conductance	umhos/cm@25C	0000. 0.	Electrometric	12
Total Alkalinity (will probably never get +9,999 mg/l)	mg/l	000.0	Field: Mixed Indicator Field: Methyl Orange Lab: Mixed Indicator Lab: Methyl Orange Lab: Endpoint Lab: Low Alkalinity	13 14 15 16 17 18
pH_4 (Acidity to pH_4)	mg/l	000.0	Lab	19
pH_8-Hot (Acidity to pH_8)	mg/l	000.0	Lab	20
pH_8-Cold (Acidity to pH_8)	mg/l	000.0	Lab	21
Total Organic Carbon	mg/l	000.0	Lab	22
Chemical Oxygen Demand	mg/l	000.0	Lab	23
Biological Oxygen Demand (5 day)	mg/l	000.0	Lab	24
Total Phosphorus	mg/l	00.00	Lab	25
Total Dissolved Phosphorus	mg/l	00.00	Lab	26
Aluminum	ug/l	0000.	Lab	2 7

APPENDIX 4 (Cont'd)

Parameter	Units	Precision	Where Measured	Code
Cadmium	ug/l	0000.	Lab	28
Chromium	ug/l	0000.	Lab	29
Copper	ug/l	0000.	Lab	30
Total Iron	ug/l	0000.	Lab	31
Iron/Ferrous	ug/l	0000.	Lab	32
Iron/Ferric	ug/l	0000.	Lab	33
Manganese	ug/l	0000.	Lab	34
Nickel	ug/l	0000.	Lab	35
Lead	ug/l	0000.	Lab	36
Zinc	ug/l	0000.	Lab	37
Selenium	ug/l	0000.	Lab	38
Strontium	ug/l	0000.	Lab	39
Barium	ug/l	0000.	Lab	40
Cobalt	ug/l	0000.	Lab	41
Arsenic	ug/l	0000.	Lab	42
Total Solids	mg/l	0000.	Lab	43
Suspended Solids	mg/l	0000.	Lab	44
Settleable Solids	mg/l	0000.	Lab	45
Total Dissolved Solids	mg/l	0000.	Lab	46
Nitrate - N	mg/l	00.00	Lab	47
Nitrate - N	mg/l	00.00	Lab	48
Ammonia - N	mg/l	00.00	Lab	49
Kjeldahl Nitrogen	mg/l	00.00	Lab	50
Total Hardness	mg/l	0000.	Field: EDTA Lab: EDTA	51 52
Calcium	mg/l	000.0	Lab	53

APPENDIX 4 (Cont'd)

Parameter	Units	Precision	Where Measured	Code
Magnesium	mg/l	000.0	Lab	54
Sulfates	mg/l	0000.	Lab	55
Chlorides	mg/l	0000.	Lab	56
Fluoride	mg/l	000.0	Lab	57
Methylene Blue Active Substance	mg/l	000.0	Lab	58
Phenols	ug/l	0000.	Lab	59
Cyanide (probably never >99 instream but 0.09 could mean something)	mg/l	00.00	Lab	60
Sodium	mg/l	0000.	Lab	61
Oil-Grease Freon	mg/l	000.0	Lab	62
Osmotic Pressure	Milliosmoles	000.0	Lab	63
Benzene	ug/l	0000.	Lab	64
Toluene	ug/l	0000.	Lab	65
Ethyl Benzene	ug/l	0000.	Lab	66
Xylene	ug/l	0000.	Lab	67
Total Residual Chlorine	ug/l	0000.	Lab	68
PCB	ug/l	0000.	Lab	69
Kepone	ug/l	0000.	Lab	70
Mirex	ug/l	0000.	Lab	71
Chlordane	ug/l	0000.	Lab	72
Carbon dioxide	mg/l	000.0	Field	73

MODULE K

Pennsylvania Fish and Boat Commission Biosecurity Protocols

Prepared by:
Brian Wisner, Andrew Shiels, Dave Miko, Ken Stark

COMMONWEALTH OF PENNSYLVANIA
PENNSYLVANIA FISH & BOAT COMMISSION

ADMINISTRATIVE POLICY

SUBJECT:	Biosecurity Measures for Commission Operations, Facilities, and Equipment
NUMBER:	2009-001
AUTHORIZED BY:	John A. Arway Executive Director
EFFECTIVE DATE:	March 16, 2011
REPLACES:	Reissued without change, replacing 2009-001 – Biosecurity Measures for Commission Operations, Facilities, and Equipment, dated June 22, 2009

In recent years, introduction of various aquatic invasive species (AIS) into the waters of the Commonwealth, and areas hydrologically connected to Pennsylvania, have been well-documented. AIS include both microscopic and macroscopic organisms, with highly varied distributions. Some macroscopic AIS, such as zebra mussels (*Dreissena polymorpha),* Didymo (*Didymosphenia geminata),* and northern snakehead (*Channa argus),* are already found in Pennsylvania, whereas other species (e.g., bighead carp, *Hypophthalmichthys nobilis,* and silver carp, *H. molitrix*) are in the Ohio River and are expected to eventually reach waters of the Commonwealth. The microscopic AIS, Viral Hemorrhagic Septicemia IVb (VHS) virus, has been identified in the Great Lakes Basin and its occurrence may have widespread implications for wild and hatchery fishes and the aquaculture industry. These organisms pose potentially significant ecological and economic threats to Pennsylvania. For fish production, AIS can pose a serious health issue for reared fish as well as having substantial economic implications for the Commission. In fragile ecosystems, AIS may compete with, or prey upon, native flora and fauna.

To reduce the threat presented by AIS, the Commission has developed the attached protocols for its field operations, fish production, and disease monitoring procedures. These procedures will be implemented to reduce the inadvertent transmission of AIS, especially as a result of activities that require staff to regularly enter or move equipment and materials between water bodies. Commission staff will, to the extent practical, follow the most current protocols for disinfecting equipment and other items moved between waters of the Commonwealth.

This policy remains in effect until revised or rescinded by the Executive Director.

Drafted by: B. Wisner, A. Shiels, D. Miko, K. Stark - 4/14/08

Reviewed by: L. Young - 8/18/08
B. Barner- 8/24/08
L. Shepler - 9/2/08
L. Anders - 9/18/08
A. Shiels - 1/7/09 & 5/13/09
B. Wisner - 6/1/09
D. Miko - 3/24/09
R. Morgan - 4/23/09
K. Edwards - 5/1/09
B. Matscavage - 5/5/09
T. Cochran - 5/5/09
D. Martin - 5/27/09
T. Schaeffer – 6/16/09

Reviewed: Director of Policy, Planning & Communications - _____

Pennsylvania Fish and Boat Commission Biosecurity Protocols:

Procedures to minimize the transfer of aquatic invasive species into or between waters of the Commonwealth

1. Introduction

In recent years, potential environmental problems associated with the introduction of various aquatic invasive species (AIS) have become well-known. The effects of some of these organisms are well-documented. Zebra mussels (*Dreissena polymorpha)* are already present in waters where Pennsylvania Fish and Boat Commission (PFBC) staff operate boats. Didymo (*Didymosphenia geminata)* was recently discovered in the upper portion of the Delaware River and in the Gunpowder River in northern Maryland. Additionally, Viral Hemorrhagic Septicemia IVb (VHS) virus has been identified in the Great Lakes Basin and the efforts to control its spread have had widespread implications for the aquaculture industry. The effects of other, lesser-known AIS are only beginning to be understood. As an example, chytrid fungus (*Batrachochytrium dendrobatidis*), of African origin, is a globally decimating amphibian species and would be a serious threat to Pennsylvania species if it becomes established. Overall, AIS pose threats to the ecological health and the economic benefits of the waters of the Commonwealth, to the state's natural biodiversity, to the operation of PFBC facilities, and to the agency's ability to fulfill its mission. The PFBC has enhanced its fish production and disease monitoring procedures to address this problem. However, additional procedures are needed to further minimize the possible inadvertent spread of AIS through routine PFBC activities which require staff to regularly move boats, sampling equipment, and other items between water bodies. This document establishes procedures to be implemented by PFBC field staff to help prevent the spread of aquatic invasive species and/or other potentially harmful aquatic organisms. The following procedures must be followed when fieldwork necessitates the movement of boats and equipment between waterways or across watershed basins. To the extent practical, all susceptible equipment moved between watersheds must be properly cleaned and disinfected. Particular attention must be given to situations where AIS are known or suspected to occur. These guidelines were developed, in part, from biosecurity protocols currently being used in Wisconsin and New York. Additional information used in the development of this document was obtained from http://www.biosecurity.govt.nz/ and http://www.nwhc.usgs.gov/publications/amphibian_research_procedures/specimen_collection.jsp.

2. Surveys and Sampling Guidance

A. It is assumed that all waterways and all locations within a given watershed are vulnerable to AIS infestation. Therefore, to minimize and avoid transport of AIS as a result of Commission activities, only properly treated equipment shall be used during activities conducted in waters of the Commonwealth. It will be the responsibility of all PFBC field staff to stay current with any announced changes to this Biosecurity Protocol.

B. For the purposes of this document, hatchery waters are considered waters of the Commonwealth. Hatchery protocols are discussed later in this document. All vehicles and boats entering the hatchery areas must be thoroughly disinfected following the

appropriately prescribed protocols described throughout this document. Protocols for each hatchery may need to be developed to address circumstances unique to each facility. Personnel must follow hatchery-specific biosecurity procedures when conducting sampling or marking hatchery fish.

C. For the purposes of this document, wetlands, vernal pools, and similar amphibian and/or reptile habitats are considered waters of the Commonwealth. It is critical that biosecurity protocols, particularly those pertaining to the spread of disease pathogens, be followed when equipment is exposed to or transported between these waters. The below disinfection procedures, particularly those involving the use of chlorine bleach, are effective against the pathogens of concern.

D. As part of the routine scheduling of any PFBC activity that will occur on waters of the Commonwealth or waters located in neighboring states or countries, every reasonable effort will be made to determine if AIS occur in those waters. This will allow precautionary measures to be taken to prevent translocation of AIS into non-infected waters or transmission from infected waters. Depending on the type of work being done, it may be possible and desirable to work with other agencies or partners to use equipment located on-site to collect samples. This would potentially limit the amount of equipment required for disinfection.

E. The Commission shall provide extra equipment to ensure that disinfected or dry equipment is available. If having duplicate gear items is not practical, then all susceptible equipment must be properly treated prior to use. In situations when activities are scheduled to occur in succession on both infected and non-infected waters, then non-infected waters must be worked prior to working infected waters. **Do not work infected waters first!**

F. If a high percentage of work activities are done in waters with AIS, staff shall dedicate certain equipment for use only in those waters.

G. For activities conducted in waters of the Commonwealth where the status of AIS is unknown, work shall start at the upper-most reach and then proceed in a downstream or down lake direction, if feasible. This will ensure that non-motile organisms are not transported on boots or other equipment to uninfected up-stream or up-lake locations.

H. If a water of the Commonwealth is known to contain AIS, but the extent of infestation is not clear, then efforts shall be made to replace or disinfect equipment before beginning subsequent activities.

I. In waters of the Commonwealth where occurrences of AIS are known to be system-wide, work order and preventative measures are less important. It must not be assumed, however, that all waterways within a watershed are infected. When in doubt, disinfection procedures shall be followed when moving between waterways.

J. If a new occurrence of an AIS is suspected, the following steps shall be taken:
   1. Document the location of the suspected AIS. (Collect GPS coordinates if possible.)

2. If possible, secure a specimen for positive identification by qualified personnel. (Fisheries Management staff shall have specimen collection kits available on all surveys.)
3. If specimen collection is not possible, secure a high-quality digital image or color photograph.
4. Notify appropriate PFBC staff: Communications Chief, Bureau Director-Fisheries, Chief of Fish Production, Chief-Fisheries Management, Bureau Director-Boating and Access. No information should be released to the public until a positive identification of specimens is verified.

3. Exposure and Handling of Diseased Specimens

A. Causal assessment of external abnormalities or death in amphibians, reptiles, or fish

First, note whether there are sick, deformed, or dead animals of more than one vertebrate class and phyla (e.g., dead birds, frogs, fish, snails, insects) present in the immediate area; if so, there is a much greater chance the problem was caused by a toxicant (poison). In this case, field personnel should exercise caution to prevent self-contamination. If, however, only one taxon (type of animal) has been affected, it is more likely that the illness, deformities, or deaths are due to an infectious disease.

B. Disease precautions and procedures

Any amphibians, reptiles, or fish (dead or alive) that appear to be "sick" or deformed should be considered contagious specimens. Only handle suspected animals while wearing "rubber" gloves. Dispose of the gloves after handling the animal and do not use them to handle other reptiles, amphibians, or fish at the site. Retained specimens are to be secured in appropriate containers such as tightly capped bottles or doubled zip bags, immediately labeled (date, place, etc.), and the exterior of the container is to be disinfected. Specimen kits appropriate for collecting potentially contagious specimens will be made available to all field units. Affected living animals and any carcasses should never be released or discarded at other sites and should not be taken into laboratory settings with other amphibians, fish, or reptiles. Follow the disinfection guidelines below for any exposed equipment. Contact the Natural Diversity Section (814-359-5237) for further instructions for disposal or transport (for testing, identification, etc.) of the diseased specimen(s).

4. Equipment Disinfection Protocols

These protocols are to be used to reduce the risk of spreading AIS during all Pennsylvania Fish and Boat Commission activities.

A. Boat and Trailer

1. Upon arrival and prior to launching, and upon removal from the water and prior to departure from a boat launch site, the following procedures will be conducted:

   a) Inspect and remove all visible aquatic plants, animals, mud, and other organic material from the boat, trailer, and equipment at the work location. Aquatic plants, animals, mud, and other organic material found on equipment prior to launching that

remained from a previous location shall be collected and placed into an approved container for transport back to regional offices for proper disposal.

b) Drain the bilges of the boat by removing the drain plug. Bilge pumps are not capable of removing all water from those areas. Wet wells, live wells, and any other compartments that could hold water must be drained of water at the field site, when appropriate. If the boat and trailer will not be in contact with other waters of the Commonwealth, the bilge area may be drained upon return to the boat storage facility provided that facility is sufficiently isolated from local waters and hatchery operations as to prevent their contamination.

c) Disinfect trailers equipped with carpeted bunks after the boat is launched, when the boat is not being returned to the trailer, *and* the trailer is being removed from the launch site. The trailer may be decontaminated at the storage facility if there is no potential for contaminating other waters. Disinfect the trailer according to one of the approved methods described in Appendix 1.

2. Upon return to the storage facility and prior to launching into another water of the Commonwealth:

a) Inspect and remove any remaining aquatic plants, animals, mud, and other organic material from the boat, trailer, and equipment at the work location and dispose of properly.

b) Recheck the bilges, wet wells, live wells, and any other compartments for any remaining water. Spray these areas.
   i. If bilge water is drained at the storage facility, the water shall be collected, disinfected, and disposed of properly to avoid causing environmental damage or contamination.
   ii. Pumps must be operated to take in the disinfectant and make sure that the solution comes in contact with all parts of the pump and hose.

c) After draining all water from boat compartments, all compartments that held water shall be washed with a high temperature (200ºF) pressure washer or with an approved disinfectant and allowed to remain wet for the appropriate contact time, as described in Appendix 1. Compartments shall be left open to completely dry prior to next use.

d) All boats and trailers used in field activities will be cleaned using a high temperature pressure washer or through application of disinfectant solution working from fore to aft and gunnels to keel in a thorough manner.
   i. Particular attention must be paid to the cooling water intakes on the lower unit of the motor.
   ii. Particular attention must be paid to the carpeted trailer bunks since they can hold water for extended periods of time.
   iii. Lower the motor to drain all water from the lower unit and disinfect motor according to the procedures described below.

e) After application of disinfectant solution, the boat, trailer, bilges, live well, and pumps must be rinsed with clean water after the appropriate contact time. ***Every***

effort shall be made to keep the disinfectant and rinse water out of surface waters and to properly dispose of the solutions.

B. Boat Motors

1. Upon return to storage facility, and prior to launching into another water of the Commonwealth, boat motors shall be treated in the following manner:

   a) Outboards
      i. Clean all exterior parts of the motor with one of the approved methods described in Appendix 1.
      ii. Immerse the lower unit in a bucket of disinfectant and run the motor to ensure contact with all internal parts allowing for appropriate contact time as described in Appendix 1.
      iii. Attach a short (6-foot) piece of garden hose to lower unit muffs. A pail of the disinfectant can be set in the back of the boat and gravity fed to the lower unit to run the disinfectant through the motor. The hose will need to be primed to start the gravity flow because the lower unit does not create enough suction to prime the hose.
      iv. Allow the disinfectant to remain in the motor for the appropriate contact time.
      v. A non-corrosive disinfectant such as *Virkon Aquatic* is recommended for use to protect the impeller. PLEASE NOTE: Virkon Aquatic® is labeled for use only as a bactericide and viricide! Do not depend on its use against other AIS such as invertebrates (e.g., zebra mussel), plants, vertebrate species, etc. See Appendix 1 for other disinfection methods!
   b) Jet Drives
      i. Clean all exterior parts of the motor with one of the approved methods described in Appendix 1.
      ii. Spray any open and accessible portions of the water intake and nozzle portions of the motor. A non-corrosive disinfectant such as *Virkon Aquatic* is recommended. PLEASE NOTE: Virkon Aquatic® is labeled for use only as a bactericide and viricide! Do not depend on its use against other AIS such as invertebrates (e.g., zebra mussel), plants, vertebrate species, etc. See Appendix 1 for other disinfection methods!

2. After application of disinfectant solution, the motor must be rinsed with clean water after the appropriate contact time. ***Every effort shall be made to keep the disinfectant and rinse water out of surface waters and to properly dispose of the solution.***

C. Commonly Used Equipment

1. After use, and prior to using equipment in another water of the Commonwealth, the equipment must be treated using the following procedures. Careful record keeping and equipment labeling will be necessary to ensure that equipment has been treated for sufficient time with the proper disinfection procedures and to ensure that dedicated equipment will only be used in its assigned waterways.

   a) Large Equipment (e.g., stocking trucks, dredges) – Organic debris must be removed prior to disinfection. Power washing is not required, but large equipment could be sprayed with a garden hose to remove debris. Equipment may be steam cleaned, washed, and dried thoroughly for five days or treated with a disinfectant. When

appropriate, immerse equipment in disinfectant for the required contact period as described in Appendix 1.

i. After application of disinfectant solution, the equipment must be rinsed with clean water after the appropriate contact time. ***Every effort shall be made to keep the disinfectant and rinse water out of surface waters and to properly dispose of the solution.***

b) Small Equipment (e.g., buckets, water sampling equipment, electrofishing equipment) – Remove all organic material from gear and follow ***one*** of the options described below.

i. Spray with disinfectant and maintain a wet surface for the appropriate contact time described in Appendix 1.
ii. Fill a tub with disinfectant and place all equipment in the tub for the appropriate contact time as described in Appendix 1.
iii. Use a completely new set of equipment for each water body sampled throughout the work day or work week. Disinfect all equipment at the end of the activity using option one or two.
    - Dissolved oxygen probes and other sensitive electronic equipment can be damaged by disinfectants and must only be rinsed with clean water. Do not store dissolved oxygen probes or other water chemistry gear in water from the work site. Use distilled or tap water for probes and empty all lake containers and samplers used during chemical or vertical profile assessments at the survey location.

c) Personal Protective Equipment (e.g., rain gear, gloves, boots, waders, and PFDs) – Remove all organic material from gear and follow ***one*** of the options described below.

i. Scrub personal protective equipment with an approved disinfectant. After scrubbing, the equipment must be kept wet with the disinfectant for the appropriate contact time as described in Appendix 1.
ii. Personal equipment may be steam cleaned or dried thoroughly for five days after cleaning with soap and water.
iii. After application of disinfectant solution, the equipment must be rinsed with clean water after the appropriate contact time. ***Every effort shall be made to keep the disinfectant and rinse water out of surface waters and to properly dispose of the solution.***

5. PFBC State Fish Hatchery (SFH) Protocols

Recent outbreaks of Viral Hemorrhagic Septicemia (VHS) in the Great Lakes drainage have drawn attention to the need for biosecurity improvements to prevent or minimize the possible introduction of VHS and other pathogens and aquatic invasive species into our fish production facilities. The needs and abilities of individual production facilities to adopt biosecurity measures will vary and a "one size fits all" approach is not practical, but both short- and long-term efforts must be directed at improvement. In general, each hatchery must be evaluated and, within each hatchery, zones of high and low disease risk must be identified. Each identified zone should have its own equipment and specific

zone isolation and disinfection procedure. The following are areas for consideration when developing individual hatchery biosecurity plans.

A. Hatchery Water Sources

Optimally, water sources should be PFBC-owned, fenced, and free of fish. Water sources need to be as secure as possible within the parameters of each hatchery. Minimally, springs and wells must be fenced and secured where feasible.

1. Hatch Houses

a) Hatch house influent water – Most facilities have equipment for UV treatment and pre-filtration of hatch house influent water. In some cases, it is limited to egg incubators only. As funding becomes available, these systems should be upgraded to include all hatch house production water. These systems must be properly maintained, including the cleaning of quartz sleeves and the replacement of UV bulbs at manufacturer recommended periods. Where feasible, upgrading to ozone treatment should be considered.

b) Egg disinfection – It is important that all production trout eggs be properly disinfected. The modified California method has been tried at many of our trout stations without any significant additional egg mortality. This procedure triples the standard surface disinfection contact time and, at least theoretically, allows iodophor to enter the egg during the hardening process. This procedure must be made a Standard Operating Procedure (SOP) at all trout hatcheries. All eyed eggs should be processed through a mechanical picker to remove dead eggs and then surface disinfected with iodophor before further incubation. Eggs shipped to other SFHs must be disinfected by the receiving facility before being placed into incubation units. Warm/cool water eggs must also be disinfected per instructions from Production Managers based on the results of ongoing egg disinfection studies.

c) Hatch house equipment – Hatch house equipment (nets, brushes, buckets, basins, etc.) must be dedicated for hatch house use only (color-coded) and this equipment must be stored away from the equipment used in outside rearing units. Equipment disinfection containers that are sufficient in depth to submerse nets, brushes, etc., must be present in all hatch houses. These containers must be properly maintained with disinfectant to ensure complete disinfection. Nets, brushes, and other equipment must be allowed a sufficient contact time for complete disinfection. Rearing units should be surface disinfected between lots of fish. Suitable disinfectants may include *Virkon Aquatic* or *Iodophor* solutions, depending on use.

d) Spawning – During spawning activities, brood fish should not be brought into hatch house areas where eggs or juvenile fish are cultured. Disinfectant footbaths must be used when transporting eggs into hatch house/egg incubation areas. If fish must be spawned in proximity to hatch house eggs/fish due to hatchery limitations, a specific spawning area with proper disinfection and isolation must be set up to minimize contamination. Only disinfected materials (e.g., eggs, equipment) are allowed to leave this area and enter other areas of the hatch house. The spawning area must be thoroughly disinfected at the end of each day.

e) Cleaning activities – All rearing units should be cleaned daily. All nets, brushes, and other equipment, especially mortality collection nets, must be disinfected between each tank or rearing unit. As indicated above, sufficient contact time must be permitted for disinfection of the equipment. All mortalities should be removed from rearing units daily, and they must be disposed of properly. Mortalities must not be disposed of in tank drains or in open drain aqueducts.

f) Access to hatch house buildings – Access to hatch house buildings should be restricted to essential staff only. All equipment brought into hatch house buildings must be surface disinfected. All staff must use disinfectant footbaths and wash their hands with disinfectant soap before entering a hatch house building. Hatch houses with garage doors or multiple-use should have a barrier (e.g., simple hanging chain) around tanks to force foot traffic through foot bath areas. These areas may be off-limits to visitors and tour groups.

2. Outside Rearing Units

a) Influent water disinfection – Systems to disinfect influent water for outside rearing units are currently not available in PFBC hatcheries. As funding becomes available, case-by-case consideration should be given to installing such systems.

b) Cleaning activities – Solids collection sections of rearing units must be cleaned regularly according to best management practices. Mortalities should be removed daily from the entire rearing unit, not just at the effluent rack or screen. Unless untreated, recirculated water is used, rearing units should be cleaned and mortalities should be removed in a downstream progression by row, not across rows. Exceptions may be made for limited water flow conditions that would harm the fish by cleaning as stated above. Dedicated sets of nets, brushes, and other equipment must be provided for the raceway area.

c) Predator control – Where available, bird netting and other predator controls must be maintained and operated properly to prevent the entry of predators into rearing units.

d) Brood fish – Brood fish should be held in rearing units that are isolated from production fish. If this is not possible, brood fish should be held at the heads of raceway rows or in rows dedicated to brood fish only and physically separated from adjacent rearing unit rows. Brood fish should not be held in recirculated water.

e) Aqueducts and piping – At least annually, efforts must be made to eliminate escapee fish from pipes and aqueducts. These fish serve as reservoirs for fish pathogens. Escapee fish in downstream piping and polishing ponds should never be moved into upstream rearing units. Open aqueducts should be cleaned at least annually to remove aquatic vegetation and accumulated debris.

f) Cool/warm water areas – Dedicated, color-coded equipment (nets, brushes, etc.) must be maintained for use in outdoor cool/warm water rearing areas. Combination hatcheries (cold-ww/cw) must have separate equipment for both outdoor rearing areas. All potential means of cross contamination between cool/warm water culture areas and coldwater culture areas must be avoided.

g) Hand feeding – Employees engaged in hand feeding must ensure that scoops or other utensils are used to distribute the feed. In instances where utensils are not used, hands should be covered by gloves that are dedicated for use at a specific feeding site or rearing unit.

B. Stocking Procedures

Necessary precautions need to be taken to minimize contaminating stocking equipment (nets, buckets, hoses, etc.) during stocking operations. Waterways Conservation Officers shall inform volunteers to keep buckets out of the receiving waters and dump buckets of fish rather than dip them into waters. Stocking buckets shall be labeled "Dump It Don't Dip It" using commercially available stencils and permanent ink or paint. If volunteers contaminate a bucket, they should be given another bucket if available. Contaminated buckets and equipment must not come into contact with uncontaminated hatchery water within the transport tanks. Any contaminated equipment shall be disinfected by drivers before going to another water body for stocking. A spray bottle pre-mixed with disinfectant will be acceptable for most applications. Disinfected buckets shall be rinsed with transport tank water prior to adding more fish to the bucket. In situations where the transport tank water is tempered by stream or lake water to reduce fish stress and buffer temperature changes, bucket disinfection should be done and rinse water taken from tank compartments that were not tempered before going to another water body. When tempering is necessary, all tempered tank water will be discharged from the stocking truck before moving on to the next water body or returning to the hatchery. Additional disinfection measures should be taken when returning to the hatchery as outlined elsewhere in these protocols.

C. Trucks and Other Equipment

All vehicles and their equipment, including stocking trucks, boats, boat trailers, sludge trucks, construction and maintenance equipment, and other vehicles that contact water bodies outside of a specific fish production site must be disinfected prior to entering the fish production portion of a hatchery. If stocking trucks and associated equipment (e.g., nets, buckets, hoses) come into contact with receiving waters, equipment must be disinfected before moving to the next water body for stocking purposes. All containers and other equipment used to transport fish, fish gametes, or fertilized eggs to or from other facilities, or used by other facilities must be disinfected and all associated transport water must be disinfected prior to discharge at a production facility. Where feasible, this must include vehicle wheel dips at facility entry points and at locations where vehicles pass between identified disease risk zones within a hatchery. Vehicle and equipment disinfections at the hatcheries must be conducted at designated areas and must include external surfaces, empty internal tank surfaces, and equipment carried on the transportation units, such as nets, buckets, etc.

It is a common practice for employees from one facility to assist at another facility in order to complete certain tasks, such as cleaning a polishing lagoon or fin clipping. In these types of situations, special consideration must be given to disinfecting personal equipment and apparel, such as boots, outer weather gear, gloves, etc., that are exposed to fish or transport/receiving waters. This equipment must be disinfected before entering the water or handling fish. There must be several pairs of spare waders, aprons, and gloves on site for use by visiting workers. Occasionally, construction crews may need to

have contact with water sources or production water in the performance of their duties. Their tools and personal equipment must be disinfected following the same protocols as hatchery staff. Felt-soled waders must not be used in hatchery waters.

Trucks or equipment that have been in contact with waters known to contain certain AIS such as zebra mussels and Didymo will undergo additional disinfection methods known to kill those species.

Care must be taken to avoid the discharge of potentially harmful, un-neutralized disinfectants.

D. Fish, Fish Gametes, and Fertilized Fish Egg Transfers

All transfers of fish gametes, fertilized eggs, and fish from within the PFBC fish production system and from other production sources must be approved in advance by the Fish Health Unit and the Fish Production Division Managers and Director.

6. Awareness

Training, oversight, and signage will be needed to maximize opportunities for success.

7. Summary

A biosecurity program can only be effective if it is a priority for administrators, hatchery managers and their staff, field biologists, regional outreach and education staff, water safety instructors, construction and maintenance operators, etc. Reduced flexibility will occur in moving fish from one facility to another to meet short-term production needs. Increased awareness and vigilance will be needed to ensure that oversights or mishaps do not occur that could quickly undo years of biosecurity precautions. Staff from all PFBC divisions will be required to observe the biosecurity restrictions and measures defined in the individual hatchery management plans.

Appendix 1

A. Species-Specific Disinfectants and Procedures for Their Use

Note that many of these methods will be effective against multiple species – but when in doubt, always research which method is best for the particular species and equipment that is to be disinfected. Disinfection procedures for invertebrates are still being developed and evaluated. Thus, try to ensure successful disinfection – use the highest concentration disinfecting agent for the longest duration that won't adversely affect your gear. Always be aware of disposal procedures for disinfectant solutions in order to avoid accidentally polluting waterways!

Zebra/Quagga Mussel – *Dreissena spp.* (and most other invertebrates)

- Wash using a high temperature steam pressure washer at temperatures >200° F or 100°C for 3 – 10 minutes depending on organism lifestage, density, etc. (e.g., thick clusters of adults will take longer to kill than a few scattered larvae)
- Wash in water at a minimum temperature of 120° F (49° C) (e.g., undiluted hot tap water) for at least 20 minutes (note: water must be maintained at 120° F (49° C) or above throughout process)
- Use of chlorine-based disinfection procedures (see below) (precautions necessary)
- Equipment drying procedures (see below) – Note that it can take up to 21 days to kill adult zebra mussels by drying but most will die within one week (must be tested to confirm death)
- Phenol base cleaners (e.g., Lysol) – immersion in full strength for at least 2 minutes
- Ethanol (50%) – immerse for at least 2 minutes or use repeated flooding rinses of ethanol
- Salt solution (saturated salt solution diluted to 5%; e.g., 50 ml saturated salt solution in 950 ml water) – immersion for at least 30 minutes (exact exposure time depends on mussel life stage, density of mussels, etc.)
- Freezing solid for 1 – 24 hours depending on organism lifestage, density, etc.

Whirling Disease

- Wash using a high temperature steam pressure washer at temperatures >104° F or 40°C.

Didymo – *Didymosphenia geminata*

(** minimum of 1 minute exposure to any one (1) of the following):

- Hot water: 140°F
- Dishwashing detergent: 5% solution (~1 cup detergent to 1 gallon of water) ("environmentally friendly" detergents are not considered effective)
- Salt solution: 5% solution (saturated salt solution diluted to 5%; e.g., 50 ml saturated salt solution in 950 ml water)
- Air: *Didymosphenia geminata* can survive for months in moist conditions. If complete drying isn't possible, restrict use of gear to a single waterway.

Boats and Other Equipment – "Check, Clean, Dry"

- Check: Look for and remove visible algae and plant material from boots, gear, or anything that has made contact with the water or sediments.

- Clean: Soak, scrub, and/or expose all equipment in one of the solutions described above for a minimum of 1 minute. Absorbent items like felt-soled waders require 30-40 minutes of soaking.
- Air Dry: Items must be dried "to touch," and then allowed to dry for an additional 48 hours when possible.
    - *Didymosphenia geminate*. Dry: Items must be dried "to touch," and then allowed to dry for an additional 48 hours when possible. Can survive for months in moist conditions. If complete drying isn't possible, restrict use of gear to a single waterway.
    - Check trailers, trailer "bunks" with absorbent carpet, engines, paddles/oars, bilge areas, ropes, anchors, etc.

B. Disinfecting Solutions and Agents

Virkon: 0.5% (1:200) solution of Virkon Aquatic® sprayed on at an application rate of 300 ml per square meter. Virkon is available from Western Chemical. Contact number is 1-800-283-5292. PLEASE NOTE: Virkon Aquatic® is labeled for use only as a bactericide and viricide! Do not depend on its use against other AIS such as invertebrates (e.g. zebra mussel), plants, vertebrate species, etc. See above in Appendix 1 for other disinfection methods!

Chlorine: (NOTE: Chlorine, especially at high concentrations, is highly corrosive and causes degradation of plastics and rubber. Chlorine solutions must be neutralized with sodium thiosulfate prior to flushing.)

- 50% (1:1) household bleach (5.25% liquid sodium hypochlorite) dip, wipe, or spray; or
- 10% (1:9) household bleach (5.25% liquid sodium hypochlorite) immersion for 10 minutes; or
- 200 ppm [150 ml of household bleach (5.25% liquid sodium hypochlorite)/10 gal water or 35 g of granular 70% HTH® (pool chlorine)/26 gal water dip or spray (not for use on nets); or
- 20 ppm [15 ml of household bleach (5.25% liquid sodium hypochlorite)/10 gal water or 3.5 g of granular 70% HTH®/26 gal water complete immersion for 30 minutes.
- Household bleach (5.25% chlorine) can be purchased with VISA through the PFBC's cleaning supply contract (Grainger).
- HTH is granular chlorine (70% calcium hypochlorite) and can be purchased from a pool supply company.
- Sodium thiosulfate should be available at a pool supply company or from a chemical supply company.

Quaternary Ammonium Compounds (follow manufacturer instructions)

- Roccal-D™; or
- BrightWater™; or
- Parvosol™; or
- Formula 409®, 1:2 dilution for soaking or full strength as a spray for 10 minutes.

Heated Water

- 200°F (93°C) poured on gear
- 140°F (60°C) complete immersion for 15 minutes (requires a consistent heat source)
- 113°F (45°C) complete immersion for 60 minutes (requires a consistent heat source)

Salt Solution

- Always start with a saturated salt solution and dilute with water to the desired concentration (e.g., 5% salt solution; saturated salt solution diluted to 5%; 50 ml saturated salt solution in 950 ml water)

Sunlight

- Complete drying in direct sunlight for a minimum of 4-6 hours. Because of the necessarily limited times involved, this method is only recommended for non-absorbent materials.

Freezing

- Items must be frozen for a sufficient duration to kill all AIS life stages – preferably 24 hours or longer.

Air Drying

- Items must be dried long enough to completely dehydrate the organism of concern (many AIS can survive for months in barely damp conditions!). When in doubt, always dry to touch and then continue drying for at least an additional 48 hours. More absorbent materials will take more time to dry thoroughly.

Rubbing Alcohol (Ethanol)

- For wiping down small equipment.

Appendix 2

General Safety Precautions for Disinfectant Use

- When handling or spraying chlorine bleach solution, wear protective equipment (mask, gloves, goggles, rain gear, etc.) and use in a well-ventilated area (follow precautions on MSDS). Stay upwind when spraying. Chlorine will break down in sunlight and when in contact with organic material.

- Chlorine is corrosive to metal and rubber and is toxic to fish at the recommended concentrations. So, rinse well after disinfection or neutralize with sodium thiosulfate. For neutralizing chlorine, spray sodium thiosulfate at 800 ppm solution (3 grams per gallon of water) on all chlorine treated surfaces after the disinfection period is over. Rinse with water from the next waterbody to remove any remaining sodium spray.

- Virkon Aquatic – This is a disinfectant in the peroxygen (hydrogen peroxide) family. It is a powder. It is 99.9% biodegradable and breaks down to water and oxygen and is not corrosive at the working dilution. Wear a dust mask and eye protection if mixing powder. Wear rain gear and gloves if spraying. Stay upwind from the spray.

AIS Biosecurity Protocols Check List (08/08/08)

Equipment	Activity	Checked
AT WORK SITE		
INSPECT	Inspect and remove all visible aquatic plants, animals, mud, and other organic material from the boat, trailer, and equipment.	
DRAIN	Drain bilges or water holding containers.	
DISINFECT	Disinfect equipment.	
AT STORAGE FACILITY		
INSPECT	Inspect and remove all visible aquatic plants, animals, mud, and other organic material from the boat, trailer, or other equipment.	
DRAIN	Drain the bilges or other water holding equipment.	
DISPOSE	Collect disinfected and disposed of bilge water.	
DISINFECT	Operate pumps to take in the disinfectant and make sure that the solution comes in contact with all parts of the pump and hose.	
	Wash water holding compartments with a high temperature pressure washer or with an approved disinfectant.	
	Clean equipment used in field activities using a high temperature pressure washer or through application of disinfectant solution.	
Boat Motors		
INSPECT	Immerse the lower unit in a bucket of disinfectant and run the motor.	
DISINFECT	Allow the disinfectant to remain in the motor for the appropriate contact time.	
Large Equipment		
INSPECT	Inspect and remove all vegetation and other organic debris prior to disinfection.	
DISINFECT	Power washing is not required, but large equipment could be sprayed with a garden hose to remove debris.	
RINSE	After application of disinfectant solution, the equipment must be rinsed with clean water.	
Small Equipment & Personal Equipment		
INSPECT	Inspect and remove all organic material from gear.	
DISINFECT	Spray with disinfectant; maintain a wet surface for the appropriate contact time described in Appendix 1.	
RINSE	After application of disinfectant solution, the equipment must be rinsed with clean water.	

MODULE L

Protocols for Determining Social Factors on Wadeable Streams

Prepared by:
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1. Introduction

The Pennsylvania Fish and Boat Commission has long recognized the importance and utility of incorporating various social factors when administering fisheries management programs throughout the Commonwealth. The evaluation of the social characteristics of a stream or stream section is necessary in determining the present and potential social limitations of the stream or stream section as they relate to angler utilization (Marcinko et al. 1986). Social characteristics may play an increasingly important roll in guiding resource planners with respect to maintaining or opening up new access area or identifying areas where access for fishing is deficient.

The social data currently collected by Pennsylvania Fish and Boat Commission biologists were largely chosen to address the needs of a specific fisheries management program, namely the Catchable Trout Program. Initiated in 1983 Operation Future revolutionized the way in which the Pennsylvania Fish and Boat Commission allocated stocked trout statewide. A system, which assigned the number of hatchery trout stocked into each county based on county quotas and license sales, was replaced with a system that allocated trout on an individual waters basis based on resource classification. This current resource classification based system incorporates key biological and social factors when assigning waters to the appropriate resource category. The individual social factors include public access, the amount of available parking, stream ownership, and human population density. These parameters are combined to derive a recreational use potential score, which is incorporated in a formula to generate stream specific trout allocation rates as outlined in the *Operational Guidelines for the Management of Trout Fisheries in Pennsylvania Waters* (PFBC 2011).

Depending upon the intended management of a stream or stream section the collection of social data may or may not be required during a general inventory of an un-assessed wadable stream. Therefore it requires the investigator to either have a preconceived management plan for an un-assessed stream to know whether or not to collect social data or it requires the investigator to revisit the stream to collect social data in the event an intensive management plan is going to be proposed.

The current methods used by the Pennsylvania Fish and Boat Commission to collect social data are relatively straight forward and no significant changes have been proposed. Over time, however, as fisheries biologists became more familiar with local resources and gained expertise applying these social factors to management programs some situation specific variations to the current methods were independently developed and applied within some individual management areas. In instances where it was determined that these situation specific variations did not alter the original intent of the data being collected those variations have been offered for use within this document. This should allow fisheries biologists the opportunity to collect data more efficiently or better apply fisheries management programs on a water specific basis when intimate knowledge of the resource is available.

1.1 SOCIAL DATA

Interviews with the fisheries biologists responsible for the collection of social data revealed that in seven out of the eight fisheries management areas social data is primarily collected only if a stream or stream section is being considered for inclusion in an intensively managed fishery

program. Most often this would be the Catchable Trout Program, but the collection of social data may also prove beneficial on wadable streams not being considered for inclusion in the Catchable Trout Program, and in fact, one fisheries biologist indicated that social data was collected during all wadeable stream surveys. Some fisheries biologists stated that social data, typically ownership data, was occasionally recorded on general stream inventories where no intensive fisheries management programs were planned. The collection of stream ownership data most often occurred when the stream or stream section was located almost entirely within the boundaries of one landowner and often the landowner was a federal, state, or other local government agency. Fisheries biologists identified time constraints and the applicability of the data over time as the major limiting factors for not collecting social data during all wadeable stream surveys. This was most appropriate to the collection of ownership information as the rate at which property ownership changes often renders tax maps obsolete thus requiring a door to door approach to assessing stream ownership. Additionally, a quick change in the ownership of lands along streams can render the collected data meaningless in a relatively short period of time and as a result most fisheries biologists opt to collect social data only when it is required to implement a specific fisheries program.

1.1.1 Riparian Ownership

Recorded as a percentage of stream or stream section that is open and closed to fishing by the general public. Ownership is determined from plat maps, tax maps, or field investigations requiring the investigator to travel to the county courthouse, township office, or the stream reach in question and may require door-to-door contacts. Ownership can be categorized as defined below. Additionally, if there is a single major landowner it is encouraged that the landowner be identified.

Note: Tax maps are available online for some counties.

To calculate ownership, total the lengths of the banks within the stream reach being evaluated as they fall into the ownership categories listed below and divide by twice the total length of the stream reach. Express the results as percentage rounded to the nearest whole number so that the sum of the percents equals 100 (See Example 1.1).

Owner Categories:

Army Corp of Engineers
Bureau of Forestry
Bureau of Parks
Municipalities, Townships, Counties etc.
Not determined
Other Federal Agency
Pennsylvania Fish and Boat Commission
Other State Agencies
Pennsylvania Game Commission
Private Closed to Fishing
Private Open to Fishing
Public Closed to Fishing
Soil Conservation Service

Example 1.1: Located in Bald Eagle State Park Section 02 of Pine Run is 2 kilometers long. One side of the stream is owned by the Bureau of Parks while the other side is owned by 4 private landowners 3 of which allow fishing. Each private landowner owns 0.50 km of stream bank.

Ownership would be recorded using the Owner Categories as:

Bureau of Parks = $\frac{2\text{ km}}{4\text{ km}}$ = 0.50 or 50%

Private open to fishing = $\frac{1.5\text{ km}}{4\text{ km}}$ = 0.375 or 38%

Private closed to fishing = $\frac{0.5\text{ km}}{4\text{ km}}$ = 0.125 or 12%

Primary Owner:

If desired, identify the primary owner with more detail than available from the Owner Categories (Example 1.2).

Example 1.2: In example 1.1 the Bureau of Parks is the owner but Bald Eagle State Park could be recorded as the **Primary Owner**. The name of a private individual or private corporation could also be used.

1.1.2 Access

Access for wadable streams is divided into two criteria: Proximity to Road and Parking. Proximity to road is the measure of the percentage of stream or stream section that falls within 100 meters, 300 meters, and 500 meters of a public road. Parking is the measure (#/km) of the available legal and safe parking places along a stream or stream section. When combined, proximity to road and parking provide a measure of recreational use potential. Include only the near shore unless 100 meter accessibility is contiguous to the far shore.

1.1.2.1 Proximity to Road

Using a caliper set at a 100 meter equivalent of the map scale, a piece of transparent plastic marked with a 100 meter map equivalent, or computer assisted mapping software (i.e., GIS) mark off on a map those portions of stream or stream section located within 100 meters of public roads or parking lots.

Using a map measure, which could include a map wheel or computer assisted measuring software determine the length of stream within 100 meters of a public road or parking lot and divide by the total length of the section. Express the result as a percentage rounded to the nearest whole number.

Repeat this procedure using map equivalents of 300 and 500 meters. The 300 and 500 meter percentages are cumulative in that the length of stream within 300 meters of a road

includes that which is within 100 meters and the length of stream within 500 meters of a road includes that which is within 100 and 300 meters.

Public roads having limited entry, such as the turnpike and the interstate roads, should not be included. Additionally, if a public road (primarily Game Commission, Bureau of Forestry, or Bureau of Parks) is gated during the angling season, only the portion of road available for unrestricted use should be used to determine proximity to road.

Variations

Some fisheries biologists do not take into consideration road access within the specified parameters when it is explicitly known that access to the stream from a public road is extremely difficult. These situations typically result from geographic structures, which include shear drop-offs, mountains, or other physical features that prohibit access to the stream or stream section from a public road. These difficult access scenarios are not actively investigated on every stream but are excluded from the access calculation when extensive prior local knowledge of the stream is available to the fisheries biologist.

1.1.2.2 Parking

Using a caliper, a piece of transparent plastic, or computer assisted measuring software (i.e., GIS), on the most accurate map available, determine all roads and parking lots located within 500 meters of any portion of the stream or stream section. No distinction should be made between public and private parking. However, for private access points, the investigator should judge the number of parking spaces being used for stream access-generally one for private residences. Through on-site inspection, count the number of spaces where vehicles could safely and legally park. In the case of publicly owned lands it may be necessary to determine where parking is authorized. In the case of developed parking areas, it is not necessary to count parking spaces if this information is available. This information should be recorded as number of parking places/kilometer or total number of spaces per stream or stream section.

Parking capacity that includes counts made on public roads having restricted areas (gated) should not be reported.

Variations

Some fisheries biologists do not count every available legal and safe parking spot once ample parking has been determined. This typically occurs in urban or suburban settings where a stream or stream segment is adjacent to large developments or city blocks. Once ample parking is determined the total number of parking spaces should be derived by extrapolating the parking count over the remaining uncounted portions of road within the 500 m criteria. The fisheries biologist must reach the minimum number of parking spots per kilometer required to achieve the maximum access rating before extrapolating the remainder of the count.

1.1.2.3 Human Population Density

Determine from USGS maps all township(s) and/or other municipalities in which the stream section is located. If the township or municipality includes any part of the section or if the stream section creates a portion of the township or municipality border, it should

be included. Using the most recent Pennsylvania Industrial Census Series for the appropriate county or counties, total the area (converted to square kilometers) of the townships or municipalities and divide the total population by the total area. Express the results as persons per square kilometer rounded to the nearest whole number. Do not derive the human population density by generating an average of the persons per square kilometer within each township or municipality that the stream section comes in contact with (Example 1.3).

Example 1.3: Section 02 of Middle Spring Creek flows through Hopewell, Southampton, and Shippensburg townships in Cumberland County and Southampton Township in Franklin County. Human population density values from the 2000 Census were as follows:

Township	**All Persons**	**Land Area (km^2)**	**Persons per km^2**
Hopewell	2,096	72.6	28.9
Shippensburg	4,504	6.5	692.9
Southampton (Cumb. Co)	4,787	135.9	35.2
Southampton (Frank. Co)	6,138	98.4	62.4

Correct: **(2,096 + 4,504 + 4,787 + 6,138)/(72.6 + 6.5 + 135.9 + 98.4) = 55.9 persons/km^2**

Incorrect: (28.9 + 692.9 + 35.2 + 62.4)/4 = 204.8 persons/km^2

2. Literature Cited

Marcinko, M., R. Lorson, and R. Hoopes. 1986. Procedures for stream and river inventory information input. Fisheries Management Section, Pennsylvania Fish Commission, Bellefonte, Pennsylvania.

Pennsylvania Fish and Boat Commission. 2011. Operational guidelines for the management of trout fisheries in Pennsylvania waters. Pennsylvania Fish and Boat Commission, 450 Robinson Lane, Bellefonte, PA.