

Exhibit C

Sampling Procedures for Unassessed Streams in Pennsylvania

May 27, 2021

Background

Pennsylvania is fortunate to have a vast flowing water resource comprised of over 83,000 miles of flowing water (Pa. DEP 2006). As of January 2020, the PFBC and its partners have surveyed, 11,562 streams comprising 36,985 miles. Of this total, there have been 5,324 stream sections (16,256 miles) in which wild trout have been documented and designated by the PFBC (PFBC 2020).

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. 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. 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.

Although Pennsylvania contains 62,725 streams totaling 83,286 miles of flowing water in Pennsylvania, the PFBC has been able to conduct surveys and implement management strategies on 9,926 streams totaling 33,836 miles. Of the waters remaining, 74% are less than a mile in length and some have only intermittent flow or will be dry and unable to support a fishery. However, many will 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.

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 strategies. A positive response in wild trout populations resulting from these activities could lead to an elevation in water quality status and increased protection.

Sampling Procedures

A prioritized listing of waters to be worked will be provided by the PFBC. The majority of these waters will be small (< 5 m in width) wadable streams, generally less than 1 m deep. Sample sites should be located within 0.5 miles of the mouth when possible. Sample sites should be located in representative habitat 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 not take place prior to May 20th and must be completed before October 1st.

Sampling will require the collection of physical, chemical, and fisheries data at each sample site. 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. All GPS units should be set to NAD 83. Site lengths 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 a minimum of 100 meters and end at a natural break point where fish movement out of the survey station would be minimal. Wetted stream channel width measurements are taken with a fiberglass tape 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. If only 1 or 2 trout are captured in the initial 100 m site, the site should be extended upstream up to an additional 300 m to attempt to capture additional trout. Total site length in this case could be up to 400 m.

Chemical data collection is normally done in the field. Standard analyses must include time of day, air temperature (°C), water temperature (°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). 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.

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 the 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 is provided in Appendix A. These guidelines should be strictly followed.

Electrofishing setups will consist of battery powered backpacks using either pulsed direct current (DC) or 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 should then be used. Generally, electrofishing using DC current is much safer for the fish compared to AC. Other electrofishing systems may only be used with prior PFBC approval.

Electrofishing proceeds upstream and may be done in a sinuous manner when stream width exceeds the maximum that can be adequately sampled. Alternatively, electrofishing can be conducted while wading upstream on one half of the stream at a time and then returning to the downstream starting locations and proceeding upstream on the unsampled portion of the stream. During sampling, the electrofishing crew should make every attempt to properly identify all fish species present at a site and record the common name on the provided data sheet. 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 (PFBC 2007). Sculpin species should be recorded as Sculpin Spp. The rating criteria are as follows:

- < 2 = RARE
- 2-8 = PRESENT
- 9-33 = COMMON
- > 33 = ABUNDANT

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 sport fish 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. Fish must be immediately released and redistributed throughout the sample site once processing is complete. If high numbers of trout are collected, they must be processed periodically throughout the sample site to reduce handling time and stress. Digital photographs of trout species < 50 mm total length should be provided to the PFBC for verification of species if needed. This is in recognition of the difficulty to identify trout species at lengths < 50 mm. Voucher specimens can and should be collected when species identification is questionable.

If the sampling crew conducts a site within 0.5 miles of the mouth and doesn't find trout, but, based on professional opinion, believes that the watershed characteristics indicate that the stream may support trout further upstream, they are strongly encouraged to establish a second sample site further upstream.

All sampling will utilize single pass electrofishing. There is no need to conduct population estimates for the unassessed waters program.

Data Recording and Data Submission

A field data form has been developed and is provided in Appendix B. All parameters discussed above, except macro invertebrate data, will be recorded on this form. Legible copies of the original data forms must be sent to the PFBC Central Office for data entry and archival. Forms should be sent to: The Pennsylvania Fish and Boat Commission, C/O Robert Weber, 595 East Rolling Ridge Drive, Bellefonte, PA 16823. The original data forms must be kept by the collector. These forms will serve as back-up should the copies be lost in the mail.

Future entry of all data will be the responsibility of the collecting party via the Scientific Collectors database. Field data will be entered into the Scientific Collectors database prior to November 1st. Following data entry, a more formal report will be required from the collector. This report will, at minimum, include a verbal description of every stream surveyed, including primary land uses and any identifiable limiting factors, along with complete data summaries for each stream surveyed for the project. The final report will be sent to the contractor for completion of the contract and a copy sent to the name and address above.

Qualification of Waters Sampled

Only waters sampled from the prioritized lists provided by the PFBC will qualify for meeting the contractual obligations of the partner.

If a stream is suspected to have water quality conditions that prevent fish from living there, chemical sampling should precede fish sampling. If the pH is found to be less than 4.5 su, subsequent fish sampling does not need to be conducted. All chemical data shall be recorded and submitted as previously outlined to meet the contractual obligation of sampling this assigned water. An adequate field review should be conducted to possibly identify stream reaches on the same water not impacted by poor water quality and the crew is encouraged to conduct sampling at these location(s).

If wild trout are documented within the first 100 meters of an established site but not in great enough abundance to qualify the section for listing the site will be extended past the minimum survey length in order to capture enough wild trout to qualify. The presence of barriers (i.e. perched culverts, waterfalls) or loss of streamflow as the survey progresses upstream are suitable end locations if the site has been extended and qualifying abundances of wild trout were not documented. As long as streamflow remains conducive for electrofishing, the site will be extended until the qualifying minimum criteria are met.

If a stream/site is found to be dry (no flow) near the mouth, further investigation should be conducted at least one additional location up to one mile from the mouth. All sites investigated for flow are to be considered as a sampling location and data sheets completed as previously outlined to adequately document dry streams and to meet the contractual obligation of sampling this assigned water.

Surveys with poor water quality or no surface flow can be considered as a sampled stream to meet the contractual obligations as long as the above protocols are followed.

Travel Allowance

It is anticipated that travel cost will be incurred while completing the Unassessed Waters project. Guidelines for travel cost have been developed and provided in Appendix C. These guidelines can be used to develop your project budget if necessary.

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 nuisance species (ANS) 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 Appendix D 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 must be properly cleaned and disinfected. Particular attention should be given to situations where ANS are known or suspected to occur.

Training

Project leaders and identified key field personnel must attend the PFBC provided classroom and field training sessions each year prior to the beginning of sampling waters for the unassessed waters program. This training is intended to provide survey crews with the classroom theory surrounding the importance of the project and the necessary sampling requirements. The field portion of the training will cover PFBC field sampling protocols and provide hands on training and supervision in PFBC acceptable collection techniques.

Quality Assurance/Quality Control

A trained PFBC employee will accompany each survey crew at times throughout the survey season to assure PFBC protocols are being adhered to and to answer any questions that may have arisen during sampling. The anticipated visitation schedule is as follows: Once

during the first two weeks of sampling and once a month thereafter as long as the PFBC is confident in the sampling crews' ability to follow the Unassessed Water program's protocols.

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

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.
- Armour, C.L., K.P. Burnham, and W.S. Platts. 1983. Field methods and statistical analysis for monitoring small salmonids streams. FWS/OBS-83/33. United States Fish and Wildlife Service, 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 Department of Environmental Protection. 2009. An Index of Biotic Integrity for Wadeable Freestone Riffle-Run Streams in Pennsylvania. PA DEP file report, Harrisburg, PA.
- Pennsylvania Fish and Boat Commission (PFBC). 2020. List of Stream Sections that Support Natural Reproduction of Trout. PFBC Files, 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.
- Pennsylvania Fish and Boat Commission. 2007. DFM sampling protocols for wadeable warmwater streams. PFBC files, Bellefonte, PA.

Appendix A

Policy for Electrofishing Operations

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

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.

GENERAL PREREQUISITE FOR ELECTROFISHING SAFETY

A. 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. All temporary employees or interns who will work on electrofishing crews shall be instructed in the functions of the system they will be using.

B. Gear Inspections

1. All electrofishing units shall be inspected before sampling commences and frequently during the sampling period.
2. All electrofishing systems shall be inspected prior to each operation by the designated Crew Leader. Electrofisher output should also be tested with vohm and megohm meter to determine if voltage leaks are present.

C. Operations Review

1. An annual electrofishing operations review meeting should be conducted involving all permanent staff.
2. All crew members should review operating procedures and systems prior to beginning each electrofishing operation.

BACKPACK ELECTROFISHING GUIDELINES

I. GEAR

Electrical System

Power Source

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

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.

Accessories

1. Electrodes shall be circular stainless steel or aluminum rods.
2. Electrode probe handles will be constructed of non-conductive material, preferably fiberglass, 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.

Operations

1. One person will be crew leader for electrofishing operations. The crew leader should have completed the USFWS “Principles and Techniques of Electrofishing” Course.
2. All crew members shall wear watertight hip boots or chest waders with non-skid soles.
4. Any crew member who gets excessive water in the boots shall remove them, dry the boots and any wet clothing before continuing with electrofishing.
5. No alcohol is permitted at electrofishing operations; no intoxicated persons are allowed to participate in electrofishing operations.
6. The crew members should consider wearing ear plugs.
7. The crew member wearing the backpack electrofishing unit should not operate an electrofishing probe in hazardous situations.
8. A crew member other than the one wearing the backpack electrofishing unit shall be in charge of the controls.
9. Crews should take a 10-15 minute break every hour of electrofishing to prevent fatigue.
10. Avoid operating near people, pets or livestock that are in or near the water.
11. Discontinue operation at the first sign of lightning, heavy rain, or crew fatigue.
12. Electrofish slowly and deliberately; aggressively chasing fish should be avoided.

13. Shut down electrical field and generator for repairs, crew change, refuels, connections and disconnections.
14. Refuel with the backpack shut off, on the ground, and with the engine cooled.
15. All crewmembers should wear polarized sunglasses during electrofishing operations.

TOWED BOAT ELECTROFISHING GUIDELINES

I. GEAR

A. Boat

- Flat bottom vessel 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.
- Boat bottom covered with form fitting galvanized steel sheet metal to serve as cathode.

B. Electrical System

Generator

- 3500-5000 watt, 3-phase, 230 volt AC generator. (Need Updated)
- Generator with neutral ground wire of 230 outlet must not be attached to frame. See Appendix C
- Generator with attached or remote transformer and pulsator.
- Transformer must be electrically isolated from generator.
- High quality muffler system.
- All hot parts should be screened or insulated.

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

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 in the boat should be with heavy duty PVC insulation.
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 28 volts) circuits of the same rating for current and voltage as the power output circuit.
6. Should have adequate meters to show output voltage, amperage and power. Pressure positive high voltage circuit activation switches shall 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. Boat Bottom covered with form-fitting or stainless steel sheet metal or stainless steel cables to serve as cathode.
8. Retractable electric cord reels should be used to connect probes to junction box.

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.

II. OPERATIONS

A. 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 shall wear chest waders with felt soles or cleats in good condition.
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.

References

- Coffelt, J. 1978. Basic electrofishing safety considerations. Electrofishing Workshop. St. Paul, MN.
- Lazauski, H.G. and S.P. Malvestuto. 1984. Electrofishing: A national survey with recommendations for configuration, construction and safety. Auburn University, Auburn, Ala.
- Miller, J.G. 1962. The use of electricity in fish management in Pennsylvania. The Pennsylvania Angler – October.
- Novotny, P.W. and G.R. Priegel. 1974. Electrofishing boats: Improved designs and operational guidelines to increase the effectiveness of boom shockers. Wis. Dept. Nat. Res. Tech. Bull. No. 73.
- Rawston, R.R. 1978. Boat Electrofishing and ?. OSHA. Electrofishing Workshops. St. Paul, Minnesota. March 9-10, 1978.
- Reynolds, J.B. a1983. Electrofishing. In: Nielsen, L.J. and D.L. Johnson, eds. Fisheries Techniques. Southern Printing Co., Inc. Blacksburg, Va.
- Vincent, R. 1971. River electrofishing and fish population estimates. Prog. Fish Cult. 33(3):163-169.

Appendix B (Double click on image to open pdf document)

Unassessed Waters Surveys - 2018

Survey Leader: _____ Sol Collector Permit # _____ Sample Date _____
 Water Name: _____ Mouth Latitude: _____
 Tributary to: _____ Mouth Longitude: _____
 Crew: _____

Site Latitude: _____ Site Longitude: _____

Site Length: _____ Width: _____
(meters) (meters)
 Site Location: _____ Avg _____

Flow: (Circle One) Dry Low Normal High

Gear Used: Pulsed DC: _____ volts
 Straight DC: _____ volts Sampling Effort (min): _____
 Other (specify): _____

Size Group	Species: Number Caught:	Species: Number Caught:	Species Occurrence:
25 - 49 mm	<input type="text"/>	<input type="text"/>	
50 - 74 mm	<input type="text"/>	<input type="text"/>	
75 - 99 mm	<input type="text"/>	<input type="text"/>	
100 - 124 mm	<input type="text"/>	<input type="text"/>	
125 - 149 mm	<input type="text"/>	<input type="text"/>	
150 - 174 mm	<input type="text"/>	<input type="text"/>	
175 - 199 mm	<input type="text"/>	<input type="text"/>	
200 - 224 mm	<input type="text"/>	<input type="text"/>	
225 - 249 mm	<input type="text"/>	<input type="text"/>	
250 - 274 mm	<input type="text"/>	<input type="text"/>	
275 - 299 mm	<input type="text"/>	<input type="text"/>	
300 - 324 mm	<input type="text"/>	<input type="text"/>	

Time of Day: _____ Water Temp: _____ ° C pH: _____
Electrometric
Colorimetric
 Tot Alk: _____ mg/l Tot Hard: _____ mg/l Spec Cond: _____ umhos
Electrometric
Colorimetric Electrometric
Colorimetric

Comments: _____

Appendix C

Unassessed Waters RFP – Travel Allowance

Overnight Travel Allowance

- One way travel exceeding 75 miles from home location to approximate mid-point of HUC 12 watershed to be sampled to qualify for overnight travel allowance.
- At least 4 sites sampled daily while on overnight status
- Travel allowance request must be provided on RFP
- Receipts should be retained.
- Overnight travel allowance of \$400 per day to accommodate a 3-4 person crew for overnight travel.
- Example - 20 stream contract and all 20 streams exceed 75 miles from home location. If you planned to work all of these streams using overnight travel, you could request \$400/day for up to a total of 5 days. Total travel request would be \$2,000.
- Example: 20 stream contract and 8 streams exceed 75 miles from home location. If you planned to work the 8 streams using overnight travel, you could request \$400/day for up to a total of 2 days. Total travel request would be \$800.

Non-Overnight Travel Allowance

- One way travel exceeding 75 miles from home location to approximate mid-point of HUC 12 watershed to be sampled
 - o At least 4 sites sampled daily to qualify for non-overnight travel allowance
 - o Travel allowance request must be provided on RFP
 - o Receipts should be retained.
 - o Non-overnight travel allowance of \$150 per day if returning to home location without overnight stay to cover travel costs

- One way travel less than 75 miles from home location to approximate mid-point of HUC 12 watershed to be sampled
 - o At least 4 sites sampled daily to qualify for non-overnight travel allowance
 - o Travel allowance request must be provided on RFP
 - o Receipts should be retained.
 - o Non-overnight travel allowance of \$100 per day if returning to home location without overnight stay to cover travel costs

Appendix D

Pennsylvania Fish and Boat Commission Division of Fisheries Management Survey, Boat and Equipment Bio-Security Protocol

Purpose

This document establishes procedures to be implemented by the Pennsylvania Fish and Boat Commission's field biologists to help prevent the spread of known aquatic nuisance species (ANS) and/or other potentially harmful aquatic organisms into waterways in which they do not occur naturally. Routine biological sampling requires staff to regularly move boats and sampling equipment between water bodies. As such, it is important for personnel 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 ANS 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 following procedures should be followed when fieldwork necessitates the movement of boats and equipment between waterways, across watershed basins or in/out of hatcheries. To the extent practical, all susceptible equipment moved between watersheds should be properly cleaned and disinfected. Particular attention should be given to situations where ANS are known or suspected to occur. These guidelines were developed following biosecurity protocols currently being used in Wisconsin, New York, and other states.

I. General Surveys and Sampling Guidance

1. All stream, river, and lake surveys should be conducted with thoroughly dry or disinfected equipment. All protocols shall be followed when working in a hatchery environment. Speak with the Hatchery Manager to determine additional site specific biosecurity measures.
2. Staff should not assume that ANS will eventually affect all waterways or all locations within a given watershed; therefore, only properly treated gear should be used during each survey when a risk of transporting ANS to a new location is present.
3. Staff should identify waters in their respective regions or areas of responsibility where ANS or other potentially harmful organisms occur so that precautionary measures can be taken to prevent translocations of ANS into non-infected waters. Depending on the type of work being done, it may be possible and desirable to work with other agencies or lake volunteers to use

equipment located on-site to collect samples. This would potentially limit the amount of gear required for disinfection.

4. Staff should purchase extra boots, nets and other gear to ensure that disinfected or dry equipment is available during the height of the field season. If having duplicate gear items is not practical, then all susceptible survey gears should be properly treated prior to use, especially when both infected and non-infected waterways are scheduled for surveys. In situations when both types of waters are to be sampled, then non-infected waters should be worked prior to working infected waters. **Do not work infected water first!**
5. If a high percentage of work activities are done in waters with ANS or other potentially harmful organisms, staff should consider dedicating certain gear for use only in those waters.
6. For survey work in waters where the status of potentially harmful organisms is unknown sampling should start at the upper most reach and then proceed in a downstream or down lake direction. This will ensure that non motile organisms are not transported on boots or other gears to uninfected up-stream or up-lake locations.
7. If a water body is known to contain ANS but the extent of infestation is not clear, then efforts to replace or disinfect boots and gears before subsequent surveys shall be made.
8. In waterways where occurrences of ANS are system-wide, survey order and preventative measures are less important.

II. Gear Specific Disinfection Protocols

1. Arrival and departure from boat launch sites

Upon arrival and departure from a water body the following procedures shall be followed for boats, trailers, and all other gear that comes in contact with the water.

- Inspect and remove all visible aquatic plants, animals, mud, and other organic material from your boat, trailer, equipment, and gear at the sampling location. If you suspect a new occurrence of an invasive plant or animal, save a specimen for identification and report the sighting (Specimen collection kits should be available on all surveys).
- When departing from a boat launch, drain all water from your boat, motor, live well, bilge, transom well, and all equipment and gear that came in contact or held water. This should include trailers, tubs, buckets, electrofishing droppers and cross supports, boots, hoses, pumps, and all other items that could serve as potential water holdings for aquatic species.

- Do not knowingly transfer any aquatic animals, plants or water from one water body to another.
- Dispose of unwanted aquatic plants and animals in an appropriate way.
- Do not store dissolved oxygen probes or other water chemistry gear in lake water, use distilled or tap water for probes and empty all lake containers and samplers used during chemical or vertical profile assessments.
- Trailers with carpeted bunks should be disinfected after the boat is launched in waters where ANS are known to exist. A spray bottle with a disinfectant solution should be kept in the vehicle so the driver can treat the trailer bunks after the boat is deployed.

2. Disinfecting boats, trailers, and live wells

- Recheck and remove any remaining organic material from boats, trailers, and live wells. Drain water from live well, bilges, and pumps.
- The outside and inside of the boat trailer, live wells, bilges, and pumps should be sprayed with the disinfectant and left wet for the appropriate contact time.
- The inside of the live wells, bilges, and pumps, should be made to contact the solution for the appropriate contact time as well.
- Run pumps so they take in the disinfectant and make sure that the solution comes in contact with all parts of the pump and hose.
- The boat, trailer, bilges, live well and pumps should be rinsed with clean water or water from the next water body after the appropriate contact time. ***Every effort should be made to keep the disinfectant and rinse water out of surface waters and to dispose of used solutions appropriately***

3. Disinfecting motors

- For outboard motor, immerse the lower unit in a bucket of disinfectant and run the motor to ensure contact with all internal parts and allow for appropriate contact time.
- Alternatively, rig a short (6-foot) piece of garden hose to lower unit mufflers. 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.
- Allow the disinfectant to remain in the motor for the appropriate contact time. 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.

- A non-corrosive (Virkon Aquatic) is recommended for use to protect the impeller. Rinse with clean water or water from the next water body.

4. Disinfecting commonly used equipment and gear by staff

- **Large Sampling Nets** – Organic debris should be removed prior to disinfection. Power washing is not required, but nets could be sprayed with a garden hose to remove debris. Nets may be steam cleaned, washed, and dried thoroughly for five days or treated with a disinfectant. To disinfect nets, immerse in disinfectant for the required contact period, rinse, hang to dry, or use immediately. Drying trap nets or seines could be done by setting them up on shore to thoroughly air dry.
- **Dip nets, measuring boards, and other sampling gear:** Remove all organic material from gear. There are several options for disinfecting smaller gear items. Note that dissolved oxygen probes and other sensitive electronic sampling gears can be damaged by disinfectants and should only be rinsed with clean water. For other gear used in water, choose one of the following options:
 - **Option One:** The gear can be sprayed with the disinfectant and a wet surface maintained for the appropriate contact time. The gear should be rinsed with clean water or water from the next water body before it is used again.
 - **Option Two:** Fill a tub with disinfectant and place all equipment in the tub for the appropriate contact time. The gear should be rinsed with clean water or water from the next water body before it is used again.
 - **Option Three:** Use a completely new set of gear for each water body sampled throughout the work day or work week. Disinfect all gear at the end of the survey day or week using option one or two.

5. Disinfecting personal protective gear, including rain gear, gloves, boots and waders

- Scrub personal protective gear with the disinfectant. After scrubbing, the gear should be kept wet with the disinfectant for the appropriate contact time. Rinse with clean water or water from the next water body.
- Alternatively, personal gear may be steam cleaned or dried thoroughly for five days after cleaning with soap and water.

III. Disinfectants and Procedures for Their Use

1. Boat, trailer, equipment, and gear

- Washing with ~ 212°F water using a high temperature pressure washer (steam power washer). This technique is approved for zebra mussel and whirling disease waters. (See Appendix 1 for boat disinfection guidelines).
- Dry equipment thoroughly for 5 days after cleaning with soap and water and/or high pressure water. Nets, anchors, lines and boots can be dried for 5 days. When felt soled waders are used special care needs to be taken to ensure that felts are totally dry or disinfected by soaking in a prescribed chlorine or Virkon Aquatic solution (see next bulleted item for solution recommendations and Appendix 2 for *Didymosphenia geminata* treatment solution).
- Disinfecting with either 200 ppm (10%) Chlorine (0.5oz/gallon of clean water) for 10-minute contact time or 1:100 dilution of Virkon Aquatic (38 grams per gallon of clean water) for 20 to 30 minute contact time. Note: Virkon is not registered to kill zebra mussel veligers nor invertebrates like spiny water flea. Therefore this disinfectant should be used in conjunction with hot water (>104° F or 40°C) application.

2. Safety Precautions for Disinfectant Use

- When handling or spraying 10% 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. It 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 lake 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.

3. Disinfecting Solutions

- **Chlorine** – Household bleach (5.25% Chlorine) can be purchased with VISA. HTH is granular chlorine (70% calcium hypochlorite) and can be purchased from a pool supply company.
- **Sodium Thiosulfate** – Commonly used to neutralize chlorine and iodine. It should be available at a pool supply company or from a chemical supply company.

- **Virkon Aquatic** - Available from Western Chemical. Their number is 1-800-283-5292.

Boat Disinfection Guidelines: (Modified from OWRB 2005)

Boat hulls, anchors, trailers:

- Always 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 from an infested field collection site should be drained of water at the field site, and if possible, flushed with hot water, steam or disinfectant solution and allowed to dry before the next use. If appropriate, the field site water may be drained back into the original body of water, as long as conditions and the decontaminant used are such that this would not cause chemical or biological contamination. Otherwise, such water containing disinfectant solutions must be drained into a suitable container for treatment prior to final disposal. Field crews may elect not to drain the bilge area until they return to the storage lot if they are not going to any other bodies of water until decontamination is completed.
- If the bilge water is drained and collected, it must be disinfected and then disposed of, by suitable means, to avoid causing environmental damage or contamination.
- After draining all contained water from boat compartments, all compartments that held water should be washed with a high temperature pressure washer or disinfectant solution and left open to completely dry prior to use in the next site.
- All boats, anchors, trailers used in field sampling will be cleaned using a high temperature pressure washer working from fore to aft and gunnels to keel in a thorough manner.
- While using the high temperature pressure washer, particular attention should be paid to the cooling water intakes on the lower unit of the motor.
- Particular attention should be paid to the carpeted trailer bunks since they can hold water for extended periods of time. These areas should have already been treated with a disinfectant solution when the boat was unloaded into the lake but should be washed with a high temperature pressure washer anyway.
- Lower the motor to drain all water from the lower unit. Replace the motor into the “transom saver”, if available, when this is accomplished.

Data source: Modified from: OWRB, Oklahoma Water Resources Board. 2005. Decontamination Protocol for Aquatic Nuisance Species. Technical Report 05-157.

Best Recommendations for Decontamination of Outdoor Equipment for Protection of spread of *Didymosphenia geminata*

Cleaning Solutions (** minimum of 1 minute exposure on 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 (~1 cup salt to 1 gallon of water)

Boats and other gears

- “Check, Clean, Dry” all gear
- **Check:** Look for, and remove visible algae and plant material from boot, gear, or anything that has made contact with the water or sediments.
- **Clean:** Soak, scrub, and/or expose all equipment one of the solutions described above for a minimum of 1 minute. Absorbent items like felt-soled waders require 30-40 minutes of soaking.
- **Dry:** Items should be dried completely, and then allowed to dry for an additional 48 hours when possible. *Didymosphenia geminata* can survive for months in moist conditions. If complete drying isn’t possible, restrict use of gear to a single waterway.
- Remember to check trailers, trailer “bunks” with absorbent carpet, engines, paddles/oars, bilge areas, ropes, anchors, etc.

Data Source: Northeast Aquatic Nuisance Species Panel; <http://www.biosecurity.govt.nz/>