AOS PROTOCOL AND PROCEDURE: FISH SAMPLING IN WADEABLE STREAMS

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Change Record

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A	02/18/2014	ECO-01394	Initial release
B	01/22/2015	ECO-02632	Protocol migration
C	12/16/2015	ECO-03402	Major updates to include IACUC requirements and input from technicians

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1 OVERVIEW

1.1 Background

Aquatic organisms have long been used to understand natural and anthropogenic changes to environmental conditions. Fish are commonly used as environmental indicators in bio monitoring assessments because the diversity of tolerances and life histories of fish are well known for most species (Simon 1998). Consequently, fish assemblage assessments can quantify assemblage structure and function in aquatic environments and provide a temporally integrated measurement of ecosystem health. Fish are used to assess alterations to ecosystem health because they are a diverse taxonomic group with a broad range of habitat requirements and life history strategies. For example, fish assemblages are commonly composed of species representing a variety of feeding guilds, reproductive strategies, life spans, and tolerances to environmental degradation. Additionally, fish are a highly visible taxonomic group that can be easily sampled by biologists.

However, the same characteristics that make fish excellent indicators (e.g., diversity of species and associated habitat requirements in lotic ecosystems) can be problematic when trying to adequately quantify fish presence and abundance in streams. Specifically, capture efficiencies of standard methods to sample fish in wadeable streams are often less than 100% for fish abundance (i.e., biased) and are influenced by species and body size, stream habitat and hydrologic conditions, and the sampling method used (Bayley and Dowling 1990, Bayley and Peterson 2001, Price and Peterson 2010). Sampling bias for a particular method (e.g., gear) can also be influenced by sample timing. These biases can be particularly problematic for monitoring fish populations, because sampling efficiencies can potentially change temporally due to sample timing (e.g., season, diel period) and fish behavior (e.g., spawning movements). Therefore, it is necessary to consider how sampling methodology can influence the results of fish assessments when developing fish monitoring protocols.

1.2 Scope

This document provides a change-controlled version of Observatory protocols and procedures. Documentation of content changes (i.e. changes in particular tasks or safety practices) will occur via this change-controlled document, not through field manuals or training materials.

1.2.1 NEON Science Requirements and Data Products

This protocol fulfills Observatory science requirements that reside in NEON's Dynamic Object-Oriented Requirements System (DOORS). Copies of approved science requirements have been exported from DOORS and are available in NEON's document repository, or upon request.

Execution of this protocol procures samples and/or generates raw data satisfying NEON Observatory scientific requirements. These data and samples are used to create NEON data products, and are documented in the NEON Scientific Data Products Catalog (RD[03]).

1.3 Acknowledgments

The design and implementation of stream fish sampling methods was based on the guidance from the NEON Fish Sampling Workshop (6-8 March, 2013, Boulder, CO) and attendees D. C. Dauwalter, A. J. Davis, E. A. Frimpong, G. D. Grossman, K. G. Gerow, R. M. Hughes, C. P. Paukert, and D. M. Walters. Abraham Karam (NEON Field Operations) provided advice on minnow trapping methods. The sampling protocols herein follows the guidelines recommended by the American Fisheries Society (AFS; Bonar et al. 2009) and have been chosen to align with those of USGS National Water-Quality Assessment (NAWQA; Meador et al. 1993) and USEPA National Aquatic Resources Survey (NARS; Peck et al. 2006, USEPA 2013).

2 RELATED DOCUMENTS AND ACRONYMS

2.1 Applicable Documents

Applicable documents contain higher-level information that is implemented in the current document. Examples include designs, plans, or standards.

AD[01]	NEON.DOC.004300	EHS Safety Policy and Program Manual
AD[02]	NEON.DOC.004316	Operations Field Safety and Security Plan
AD[03]	NEON.DOC.000724	Domain Chemical Hygiene Plan and Biosafety Manual
AD[04]	NEON.DOC.050005	Field Operations Job Instruction Training Plan
AD[05]	NEON.DOC.014051	Field Audit Plan
AD[06]	NEON.DOC.000824	Data and Data Product Quality Assurance and Control Plan

2.2 Reference Documents

Reference documents contain information that supports or complements the current document. Examples include related protocols, datasheets, or general-information references.

RD[01]	NEON.DOC.000008	NEON Acronym List
RD[02]	NEON.DOC.000243	NEON Glossary of Terms
RD[03]	NEON.DOC.005003	NEON Scientific Data Products Catalog
RD[04]	NEON.DOC.001271	NEON Protocol and Procedure: Manual Data Transcription
RD[05]	NEON.DOC.001646	General AQU Field Metadata Sheet
RD[06]	NEON.DOC.001152	NEON Aquatic Sample Strategy Document
RD[07]	NEON.DOC.001154	AOS Protocol and Procedure: Aquatic Decontamination
RD[08]	NEON.DOC.00690	AOS Protocol and Procedure: Macroinvertebrate Sampling in Wadeable Streams
RD[09]	NEON.DOC.001153	AOS Protocol and Procedure: Wadeable Stream Morphology Mapping
RD[10]	NEON.DOC.002494	Datasheets for AOS Sample Shipping Inventory
RD[11]	NEON.DOC.003107	Datasheets for Fish Sampling in Wadeable Streams
RD[12]	NEON.DOC.001151	AOS Protocol and Procedure: Aquatic DNA Barcode

2.3 External References

ER[01]	Smith Root LR-20 Series Backpack Electrofisher Operation and Maintenance User's Manual

2.4 Acronyms

Acronym	Definition
A	Ampere or Amp
AFS	American Fisheries Society
AQUI-S20E	10% eugenol; fish anesthetic
cm	Centimeter
DC	Direct Current

EMAP	Environmental Monitoring and Assessment Program (USEPA)
Hz	Hertz
km	Kilometer
m	Meter
mL	Milliliter
mm	Millimeter
MS-222	Tricaine methanesulfonate
NARS	National Aquatic Resources Survey (USEPA)
NAWQA	National Water-Quality Assessment (USGS)
NRSA	National River and Streams Assessment (USEPA)
SL	Standard Length
TL	Total length
USEPA	US Environmental Protection Agency
USGS	US Geological Survey
V	Volt
W	Watt

2.5 Definitions

Amperage: A measure of electrical current strength expressed as amperes.

Ampere (Amp or A): A standard unit of electrical current used to measure strength. Current (A) = Power (W) / Voltage (V).

Anode: A positive electrode that is commonly a ring on a fiberglass pole for backpack electrofishing (Figure 1).

Figure 1. Electrode pole (anode) for backpack electrofishing unit (photo: store.smith-root.com)

Baseflow: Sustained stream flow that consists primarily of groundwater flow, rather than surface water runoff.

Bout: Refers to a series of days when similar sampling will occur at a site (i.e., a five-day fish sampling period = 1 bout).

Capture Efficiency: The proportion of the estimated number of individuals present at a defined site (e.g., water body, reach, macrohabitat) that is sampled with a single gear and specified amount of effort.

Cathode: A negative electrode that is commonly a stainless steel cable that is dragged behind the operator for backpack electrofishing (Figure 2).

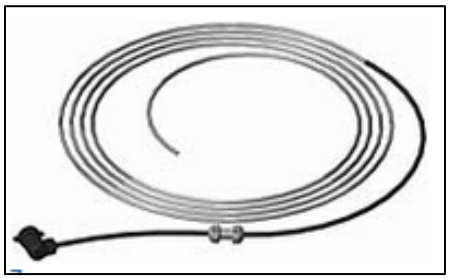

Figure 2. Cathode for backpack electrofishing unit (photo: store.smith-root.com)

Direct Current (DC): The unidirectional flow of electricity.

Duty Cycle: The fraction of time an entity is considered active. In relation to pulsed-DC electrofishing, the duty cycle refers to the proportion of electrical waveforms the current is on and is expressed as a percentage.

Electrode: A metallic conductor through which electrical current leaves (i.e., anode) or enters (i.e., cathode).

Electrofishing: The use of electricity to temporarily immobilize fish for collection of biological data (e.g., taxonomic identification, length weight).

Frequency: The number of times an occurrence repeats. In relation to pulsed-DC electrofishing, the frequency is measured in pulses per second (i.e., Hz) and can be adjusted. High frequency pulses are associated with increased injuries to, or mortality of, fish.

Gear: Type of equipment or method used (e.g., electrofishing).

Hertz (Hz): Frequency of electrical wave cycles per second.

Power: The product of amperage (i.e., current) and voltage and measured in watts.

Pulsed DC: Direct electrical current that is interrupted rapidly.

River Left: The left bank of the stream as viewed while looking downstream.

River Right: The right bank of the stream as viewed while looking downstream.

Sampling Efficiency: A measure of the ability of an individual sampling method to capture fish in a waterbody with a specified amount of effort. Commonly expressed as capture efficiency for individual species at particular sites (e.g., reach).

Thalweg: The portion of the stream where the majority of water flows, the deepest part of the stream.

Volt (V): A standard unit used to measure the difference in potential electrical energy between two points. Voltage (V) = Power (W) / Current (A).

Voltage: The potential electrical difference between two points in a circuit expressed as volts.

Watt (W): A measure of electrical power. Power (W) = Current (A) * Voltage (V).

3 METHOD

The objective for this document is to outline the sampling protocol and procedures for sampling fish and other top predators at NEON wadeable stream sites. Other top predators (e.g., salamanders, crayfish), although not targeted in this sampling protocol, will be identified when collected as bi-catch. Sampling protocols designed to understand stream fish assemblage structure are often a compromise between multiple approaches that either qualitatively characterize species richness, or quantify abundances of species that are most susceptible to the particular method. The two most commonly used methods (gears) to sample fish in wadeable streams are electrofishing and seining, both of which are used by the USGS NAWQA (Moulton et al. 2002) and USEPA EMAP or NRSA (Peck et al. 2006) programs. The American Fisheries Society (AFS) has also developed standard freshwater sampling method recommendations for fish in warm water (electrofishing and seining; Rabeni et al. 2009) and cold water (electrofishing and snorkeling; Dunham et al. 2009) wadeable streams.

Although seining has been found to sample certain sizes and species of fish more effectively than backpack electrofishing (Bayley and Dowling 1990, Onorato et al. 1998, Bayley and Peterson 2001), seining is often considered a supplementary sampling method to electrofishing in standard protocols (Moulton et al. 2002, Peck et al. 2006). Seining has not been found to significantly increase estimates of diversity in several studies (Poos 2007, Mercado-Silva and Escandon-Sandoval 2008, Meador 2012). It is also difficult to employ if the site contains many obstructions (e.g., aquatic plants, woody snags, or boulders). Electrofishing is problematic at extremely high and low conductivities and where turbidity is high. Electrofishing employing alternating current (AC) can be more effective, but it increases mortality.

The use of multiple gears to sample fish in wadeable streams would 1) require the estimation of sampling efficiency (i.e., bias) for all gears at each sampling site and 2) likely limit the spatial extent or the number of subsamples that could be effectively conducted in a single visit (e.g., 5 days). Limited sample sizes are particularly problematic when attempting to detect small changes (i.e., 10-25%) in

abundances and often unfeasibly large (e.g., >1000 samples) for wadeable stream fish (Quist et al. 2006, Fischer and Paukert 2009). Therefore, the protocol outlined here describes the use of a single method (i.e., DC or pulsed DC backpack electrofishing) to sample fish in wadeable streams at designated NEON sites (1 km stream) with the use of replicate ~100 m reaches to estimate absolute abundance, and adjust one-pass relative abundance estimates from reaches where multi-pass depletion estimates are not used. If electrofishing is not effective at a site (e.g., shallow intermittent streams), minnow traps may be used.

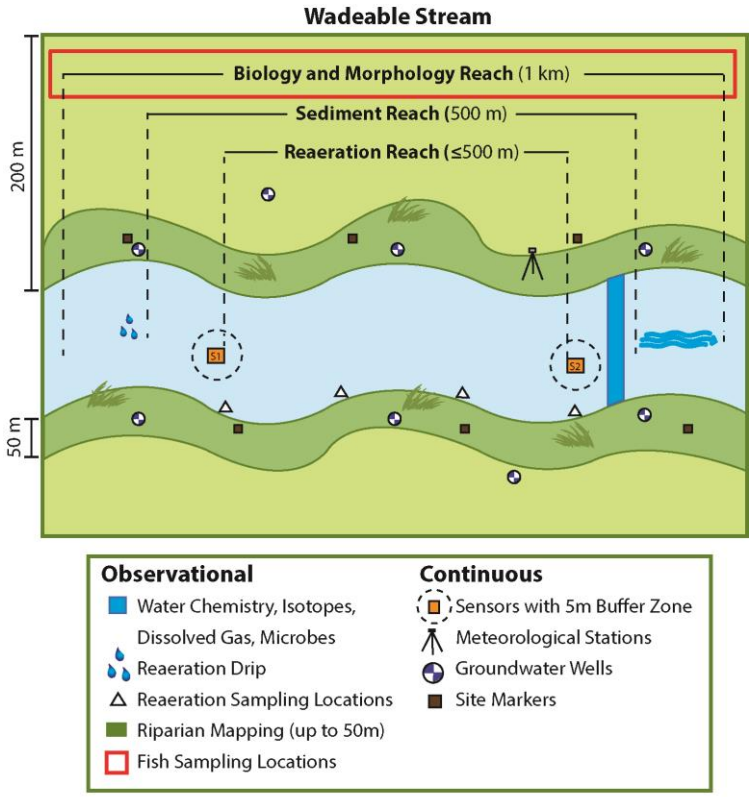

Figure 3. A generic wadeable stream site layout. Fish sampling will occur throughout entire biology and morphology reach.

Standard Operating Procedures (SOPs), in Section 7 of this document, provide detailed step-by-step directions, contingency plans, sampling tips, and best practices for implementing this sampling procedure. To properly collect and process samples, field technicians **must** follow the protocol and associated SOPs. Use NEON's problem reporting system to resolve any field issues associated with implementing this protocol.

The value of NEON data hinges on consistent implementation of this protocol across all NEON domains, for the life of the project. It is therefore essential that field personnel carry out this protocol as outlined in this document. In the event that local conditions create uncertainty about carrying out these steps, it is critical that technicians document the problem and enter it in NEON's problem tracking system.

The procedures described in this protocol will be audited according to the Field Audit Plan (AD[05]). Additional quality assurance will be performed on data collected via these procedures according to the NEON Data and Data Product Quality Assurance and Control Plan (AD[06]).

4 SAMPLING SCHEDULE

4.1 Sampling Frequency and Timing

Wadeable stream fish sampling will occur two times per year during the growing season at each site, roughly spring and autumn. Ranges of sample timing are provided on a site-by-site basis by Science Operations based on data collected by the aquatic sensors and Field Operations. Sample timing shall be outlined in the NEON Aquatic Sampling Strategy Document (RD[06]).

Sampling corresponds with the first and third sampling windows for Macroinvertebrate Sampling in Wadeable Streams (RD[08]). Fish sampling must occur within a 1 month window of the specified sampling date (2 weeks before – 2 weeks after) depending on weather conditions at the site and should occur after macroinvertebrate sampling (RD[06]).

A minimum of 2 weeks between sampling bouts shall be observed. Sampling bouts should not be longer than 5 days long. All three passes in a fixed sampling reach must be sampled within the same day, with at least 30 minutes between passes to allow fish to resettle in the reach.

4.2 Criteria for Determining Onset and Cessation of Sampling

A range of dates for each site will be determined *a priori*, based on historical data including stream discharge, amount of time since last flood, water temperature (or accumulated degree days), and riparian greenness.

4.3 Timing for Laboratory Processing and Analysis

Samples may be stored for up to 1 month following the preservation guidelines in SOP D.6. For storage and shipping timelines see SOP F. Adipose fin clips may be taken from a maximum of 10 individuals per species per sampling bout for isotope and/or DNA analysis. Fin clips will be collected using scissors that are large enough to clip the fin in one quick motion. The cut should be made perpendicularly to the fin rays and remove half of the fin or less. If the fish does not have an adipose fin, a clip of the left pelvic fin may be collected. Fin clips will be placed in labeled collection vials and returned to the laboratory for storage.

4.4 Sampling Timing Contingencies

Fish sampling shall occur only during daylight hours for safety and consistency of capture efficiency. All three-passes in a fixed reach must occur within the same day, with at least 30 minutes and no more than 2 hours between passes. A minimum of 2 weeks between sample periods shall be observed.

Delay/ Situation	Action	Outcome for Data Products
Hours	If flooding occurs during electrofishing activities, captured fish should be released and sampling discontinued. If an entire pass cannot be completed, abandon data collection and start over on the next appropriate day.	None as long as samples are collected within the pre-determined sampling window. If waiting for flooding to diminish causes sampling to occur outside of the sampling window, data must be flagged.
3-7 Days	If heavy rainfall that affects visibility or flooding occur on or prior to the targeted sampling date (>1.5x above baseflow), or unsafe wading conditions occur (Lane and Fay 1997), wait a minimum of 3-7 days after water level drops to near-baseflow conditions (within 25% of baseflow as determined by sensor data) to allow the fish assemblage to redistribute	None as long as samples are collected within the pre-determined sampling window. If waiting for flooding to diminish causes sampling to occur outside of the sampling window, data must be flagged.

4.5 Sampling Specific Concerns

1. Fish sampling should not occur while other sampling activities are occurring upstream in the NEON reach that may disturb sediments or otherwise affect hydrology of the system.
2. Fish sampling must be completed within a 5-day period per site. If field conditions appear unfavorable (e.g., prolonged thunderstorms, tropical storms, expected flooding) during the proposed sampling bout, postpone sampling until the next appropriate time.
3. Reasonable efforts should be made to minimize mortality to fish during sampling. This includes the use of best fish handling practices (e.g., frequent changes of stream water in buckets, aerators) and limited use of collected specimens.
4. Electrofishing-related injuries should affect < 1% of fish captured. If this number is exceeded at the site, stop sampling and contact the NEON Aquatic Ecologist.

5 SAFETY

This document identifies procedure-specific safety hazards and associated safety requirements. It does not describe general safety practices or site-specific safety practices.

Personnel working at a NEON site must be compliant with safe field work practices as outlined in the Operations Field Safety and Security Plan (AD[02]) and EHS Safety Policy and Program Manual (AD[01]). Additional safety issues associated with this field procedure are outlined below. The Field Operations Manager and the Lead Field Technician have primary authority to stop work activities based on unsafe field conditions; however, all employees have the responsibility and right to stop their work in unsafe conditions. In addition the following general safety guidelines are provided:

1. Technicians are required not to put hands and feet in waters where alligators are present and to make sure a safe distance from hazards is maintained.
2. All employees shall have access to a form of communication with other team members such as a two-way radio.
3. Technicians should be aware of any site-specific hazards and to the waters of that particular location (i.e. current status, tidal charts, etc.).
4. Activities in streams should only be performed when flow conditions are safe. Do not attempt to wade a stream where velocity x depth is ≥ 0.93 m^2/s (10 ft/s; Lane and Fay 1997).
5. Safety Data Sheet information shall be readily available to technicians working with chemicals included in this protocol.

When electrofishing additional safety precautions are required (Reynolds and Kolz 2013):

1. Operator must be fully trained by manufacturer of equipment and all other associated personnel shall be trained on safe operations through established and approved training program (see Section 6.2 Training Requirements).
2. Audible signals must be used to alert technicians when electrofishing equipment is in operation.
3. Chest waders and heavy-duty rubber gloves must be worn while working near an electrofishing unit.
4. Before sampling, inspect waders and gloves for leaks. Leave the water immediately if waders or gloves develop leaks.
5. Avoid operating near bystanders, pets, or livestock that are in or near the water.
6. Electrofishing must be suspended if anyone feels a shock, however minor, for investigation and repair of equipment.
7. Avoid operating an electrofishing unit in heavy rain (light rain is acceptable) as this can increase the probability of electrical shock.

6 PERSONNEL AND EQUIPMENT

6.1 Equipment

The following equipment is needed to implement the procedures in this document. Equipment lists are organized by task. They do not include standard field and laboratory supplies such as charging stations, first aid kits, drying ovens, ultra-low refrigerators, etc.

Table 1. Equipment list – Field preparation

Item No.	R/S	Description	Purpose	Quantity	Special Handling
Durable items					
	R	HDPE bottle, amber, 1 L	Stock solution ofMS-222 container	2	N
	R	Lab safety glasses	Safe handling of chemicals	1 pair	N
	R	Battery charger (electrofishing batteries)	Charging the electrofisher	1	N
Consumable items					
	R	Tricaine methanesulfonate (MS-222)	Euthanizing specimens	20 g	Y
	R	$NaHCO_3$	Buffering agent for MS-222	50 g	N
	R	AQUI-S20E	Anesthetizing specimens	50 mL	N
	R	Nitrile gloves (pair)	Safe handling of chemicals	1	N

Item No.	R/S	Description	Purpose	Quantity	Special Handling
	R	Field data sheets (print on waterproof paper, write in pencil)	Recording data	10	N
	R	Specimen labels (waterproof paper)	Labeling specimens	2 sheets	N

R/S=Required/Suggested

Table 2. Equipment list – Reach establishment

Item No.	R/S	Description	Purpose	Quantity	Special Handling
Durable items					
	R	Site-specific morphology map	Navigating to sampling reaches	1	N
	R	Plot survey markers (aluminum, site-specific)	Establishing sampling reaches	12	N
	R	Meter tape (50 or 100 m)	Establishing sampling reaches	1	N
	R	Handheld GPS (with batteries, ± 1 m accuracy)	Navigating to sampling reaches	1	N
Consumable items					

Item No.	R/S	Description	Purpose	Quantity	Special Handling
	R	Flagging tape	Establishing sampling reaches	1 roll	N

R/S=Required/Suggested

Table 3. Equipment list – Electrofishing

Item No.	R/S	Description	Purpose	Quantity	Special Handling
Durable items					
	R	Steel studded fence posts (i.e., T-post)	Securing block net at reach boundary	8	N
	R	Fence post driver or small sledge	Securing block net at reach boundary	1	N
	R	Fence post puller	Removing block net fence posts at reach boundary	1	N
	R	3 mm mesh block nets with lead lines and top lines with floats	Catching drifting specimens	4	N
	R	L-type block net stakes (e.g., ~45 cm long, 10 cm handle, 1 cm diameter stainless rod)	Securing block net at reach boundary	15	N

Item No.	R/S	Description	Purpose	Quantity	Special Handling
	R	Net repair kit: • needle • string • butane lighter • zip ties	Repairing nets	1	N
	R	Battery-powered backpack electrofishing unit	Electrofishing	1	N
	R	Anode pole (1.5-2.0 m) with attached anode ring	Electrofishing	1	N
	R	Cathode (rattail type; 1-2 m and 10-15 mm in diameter)	Electrofishing	1	N
	R	Electrofisher batteries (rechargeable)	Electrofishing	3	N
	R	Abrasive pad to clean anode rings	Electrofishing	1	N
	R	4.7 mm mesh dip nets with fiberglass handles	Catching immobilized specimens	4	N
	R	Rubber lineman gloves (Class 0, rated for max use voltage 1,000V AC/ 1,500V DC)	Safe handling of electrofishing equipment	1 pair per person	N
	R	5 gallon buckets	Storing specimens	10	N
	R	Hand held conductivity/temperature meter	Measuring conductivity and temperature	1	N

Item No.	R/S	Description	Purpose	Quantity	Special Handling
	R	Chest waders (approved for electrofishing)	Safe wading	1 pair per person	N
	R	Chest wader repair kit (e.g., Aquaseal) or extra waders	Safe wading	1	N
	R	Polarized sunglasses	Increasing efficiency of fish capture	1 pair per person	N
Consumable items					
		(none)			

R/S=Required/Suggested

Table 4. Equipment list – Minnow traps (use only if directed to do so by NEON HQ)

Item No.	R/S	Description	Purpose	Quantity	Special Handling
Durable items					
	R	Gee minnow traps, galvanized steel, 6 cm mesh	Catching specimens	20	N
	R	Nylon rope, 1/2 inch diameter	Securing minnow traps	200 m	N

Item No.	R/S	Description	Purpose	Quantity	Special Handling
Consumable items					
	R	Aquamax fish food (container)	Luring fish	1	N
	R	Whirl-pak® sample bags, 24 oz.	Fish food container	40	N

R/S=Required/Suggested

Table 5. Equipment list – Fish processing

Item No.	R/S	Description	Purpose	Quantity	Special Handling
Durable items					
	R	Fish and top predator taxonomic ID key specific to location or region (denotes endangered species)	Identifying specimens	1	N
	R	Portable aerators (batteries, diffusion stone)	Aerating buckets	15	N
	R	Small dip net (3.2 mm mesh)	Handling specimens	5	N
	R	Fish measuring boards (50 cm)	Measuring specimen length	2	N

Item No.	R/S	Description	Purpose	Quantity	Special Handling
	R	Portable digital scale (batteries, charger)	Weighing specimens	1	N
	R	Plastic tray	Weighing specimens	2	N
	R	Digital camera (batteries, memory card)	Photographing specimens	1	N
	R	25-50 mL graduated cylinder, plastic	Mixing anesthetic and euthanizing solution	1	N
	R	Fin clipping scissors	Cutting fish fins for DNA barcode samples	1	N
	R	Tissue containers	For preserving fin clips for DNA barcoding; maybe provided by an external lab	10	N
Consumable items					
	R	Nitrile gloves (pair)	Safe handling of chemicals	10	N
	R	HDPE wide mouth specimen jars (1 L)	Specimen preservation containers	50	N
	R	MS-222 stock solution	Euthanizing specimens	1 L	Y
	R	AQUI-S20E stock solution	Anesthetizing specimens	1 L	Y

Item No.	R/S	Description	Purpose	Quantity	Special Handling
	R	10% buffered formalin (37-40% formaldehyde)	Preserving specimens	20 L	Y

R/S=Required/Suggested

6.2 Training Requirements

All technicians must complete protocol-specific training for safety and implementation of this protocol as required in Field Operations Job Instruction Training Plan (AD[04]).

Personnel are to be trained in fish sampling in wadeable streams and safe working practices for water-based fieldwork. Specific training for fish sampling must also include electrofishing training for all technicians. All lead aquatic technicians within a domain shall be required to receive an electrofishing safety training prior to operating a backpack electrofisher. This training will be provided by the backpack electrofisher manufacturer (Smith-Root) in Vancouver, WA or other location as specified by the Field Operations manager. Additionally, all technicians shall complete the US Fish and Wildlife Service (USFWS) online Electrofishing Safety training course (National Conservation Training Center [NCTC] CSP 2202-OLT) once per season before the first sampling bout. Technicians must pass the Electrofishing Safety Exam with a score >80%. Additional training resources include Wader Safety training which is available through the NCTC.

The overall goal for participating in and successfully completing these training courses is to enhance the safety of all NEON staff and public bystanders in the fish sampling area. Additionally, it is of great importance to limit the health impacts of target fish species and non-target organisms (e.g. amphibians, reptiles) through the proper use of sampling equipment and technique. All field crews participating in fish sampling shall have one member that has received the manufacturer safety training; all crew members shall have completed the USFWS NCTC electrofishing safety training and NEON CPR/AED/First Aid training.

External Training References:

USFWS NCTC CSP2202-OLT Electrofishing Safety course description: http://training.fws.gov/nctcweb/catalog/CourseDetail.aspx?CourseCodeLong=FWS-CSP2202-OLT

NCTC CSP2202-OLT resources include presentation, electrofishing and wader safety videos, safety policies, and the final exam: http://nctc.fws.gov/courses/csp/csp2202/resources/index.html

American Fisheries Society document which provides biologists with health and safety guidelines for conducting fish sampling activities which includes references to OSHA, Smith-Root, and USFWS safety information: http://fisheries.org/docs/policy_safety.pdf

6.3 Specialized Skills

N/A

6.4 Estimated Time

The time required to implement a protocol will vary depending on a number of factors, such as skill level, system diversity, environmental conditions, and distance between sample plots. The timeframe provided below is an estimate based on completion of a task by a skilled two-person team (i.e., not the time it takes at the beginning of the field season). Use this estimate as framework for assessing progress. If a task is taking significantly longer than the estimated time, a problem ticket should be submitted.

This protocol requires three qualified technicians for 5 consecutive field days. There is no lab processing at the Domain Support Facility associated with this protocol. Fish tissue samples may require shipping to a NEON-approved laboratory for DNA barcoding. Additionally, tissue samples and/or voucher specimens (target and non-target species) may be preserved, archived, and maintained by domain staff. Sample preservation and archival may require up to 10 minutes of processing per specimen for domain staff.

7 STANDARD OPERATING PROCEDURES

Preparing for Sampling

Begin preparations at least two days before going to the field to allow batteries to fully charge.

1. **VERY IMPORTANT:** Charge or replace batteries for backpack electrofishing unit, GPS unit, camera, portable scale, temperature/conductivity meter, and portable aerators overnight or longer.
2. Inspect electrofishing unit for normal operation (e.g., no frayed cathode or broken anode, no error message when turned ON, functioning activation switch).
3. Inspect lineman gloves and waders for holes and tears, repair if necessary.
4. Inspect dip nets and block nets for rips, tears, and holes. Repair, if necessary.
5. Inspect portable aquarium pumps, diffusion stones, and batteries.
6. Inspect buckets to ensure handles are present and functioning.
7. Ensure that all equipment has been decontaminated since last use (see RD[07]).
8. Print data sheets (RD[11]) and specimen labels (**Error! Reference source not found.**) on waterproof paper.
9. Select random sampling reaches if this is the first sampling date for the year (SOP B).
10. Preparing fish anesthetic (AQUI-S20E; 10% eugenol) and euthanizing agent (MS-222) in the Domain Support Facility.
    a. **Anesthetic** (AQUI-S20E): This drug is currently available under the USFWS Investigational New Animal Drug (INAD) program; Study Protocol 11-741. As such, NEON must comply with data reporting requirements including drug acquisition and handling as well as fish treatment and disposition. Be sure to bring along the INAD reporting datasheets in the field when conducting fish sampling activities.
        1) AQUI-S20E should be added directly to the treatment water. Do not make stock solutions or other dilute solutions of AQUI-S20E.
    b. **Euthanizing Agent** (MS-222)

        1) Mix stock solution of MS-222 (site-specific, depends on EHS permits) in the Domain Support Facility.
        2) Wear nitrile gloves and eye protection, as MS-222 is a skin and eye irritant.
        3) Weigh 20 g of MS-222 powder and 50 g $NaHCO_3$.
        4) Mix 20 g MS-222 + 50 g $NaHCO_3$ in a bucket with 1 liter tap water.
        5) Pour the stock solution into two 1 L amber HDPE bottles.
        6) Label bottles “MS-222 stock solution”.
        7) MS-222 stock solution must be stored in dark bottles in a room-temperature (~70 ^{o}F) environment. Stock solution may be reused over sampling bouts.

SOP A Establishing Sampling Reaches

Establish sampling reaches during the first year of sampling. Reaches may need to be re-established if significant morphological changes have occurred since the last sample bout (e.g., riffles are now pools).

1. Measure out ten, non-overlapping, 100 m (± 20 m) reaches, starting ~20 m above the downstream permit boundary (Figure 4).
   a. Leave 20 m at the downstream boundary allows space for electrofisher testing and releasing processed fish.
   b. Due to heterogeneous stream morphology and habitat characteristics, sampling reaches may deviate from 100 m in stream length to allow reaches to end at the natural boundaries of channel units (e.g., riffle, run, pool). A minimum length of 80 m and maximum of 120 m sampling reach lengths are acceptable. If natural channel units are longer than 120 m, then end the reach at 100 m.
   c. If the permitted reach is < 1 km, a minimum of eight reaches must be present at the site.
2. Install a permanent aluminum plot survey marker on the bank at river right at each reach boundary (including the permit boundaries; Figure 4). Record the location of each marker on the handheld GPS unit and on the Reach Delineation Data Sheet (**Error! Reference source not found.**).
   a. GPS points should be added to the site-specific stream morphology map (RD[09]) at the Domain Support Facility.
   b. If you are unable to install plot survey markers on the right bank (river right), use the left bank and note in the Reach Delineation Data Sheet (**Error! Reference source not found.**). The right bank is preferred for consistency across sites.

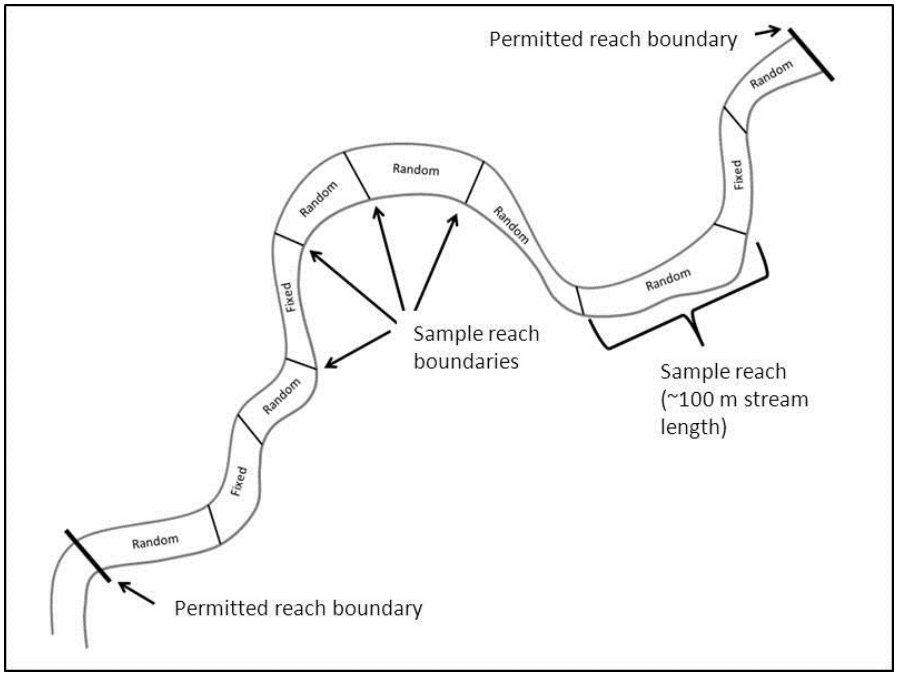

Figure 4. Schematic of 1 km permitted NEON stream site delineated into ten 100 m reaches: 3 fixed and 7 random sampling reaches. Three random reaches will be chosen each year for sampling

SOP B Fixed and Random Sampling Reach Selection

Reach selection occurs during the first year of sampling. Reaches will be revisited over the following years, unless the morphology of the stream significantly changes (e.g., riffles become pools). If stream morphology changes, new reach establishment occurs.

1. Up to six 100 m (± 20 m) reaches (three fixed and three random) will be sampled during each sampling bout (Table 6).
2. Select three of the 10 reaches to be the "fixed" reaches. Fixed reaches will be sampled two times per year throughout the duration of NEON measurements.
   a. The three fixed 100 m (+/- 20 m) reaches should be chosen to best represent the habitat variability throughout the 1 km permitted reach. For example, the inclusion of primary habitat (e.g., pools) and distinctive (e.g., waterfall and plunge pool) habitat features should be a priority when selecting permanent reaches. Fixed reaches will be selected by the NEON Aquatic Ecologist or Domain Aquatic Technician.
   b. Avoid having sensor sets within reaches. Electrofishing must occur ≥5 m away from all in-stream electronics.
3. Select three of the remaining seven random reaches to be sampled annually. Refer to Appendix E for a randomized order of reaches for each wadeable stream site.
4. Use the same three random reaches for the two sampling dates (bouts) within one year (Table 6).
5. For each year of sampling, continue down the list of randomized reaches not sampled previously. In year three (if the stream contains 10 reaches), there should only be one reach that has not yet been sampled. Return to the first random reach on the list when all reaches have been sampled.
6. Follow this pattern for the remainder of the study.

Table 6. Example of fixed and rotating reach design for one site over 10 years. Gray boxes denote when a reach is sampled. Randomized reach order for each site is presented in Appendix E.

	Reach 1	Reach 2	Reach 3	Reach 4	Reach 5	Reach 6	Reach 7	Reach 8	Reach 9	Reach 10
Year	Random	Fixed	Random	Fixed	Random	Random	Random	Random	Fixed	Random
1	■	■		■		■	■		■	
2		■	■	■	■			■	■	
3	■	■		■		■			■	■
4		■		■	■		■	■	■	
5		■	■	■		■			■	■
6	■	■		■			■	■	■	
7		■	■	■	■				■	■
8	■	■		■		■	■		■	
9		■	■	■	■			■	■	
10	■	■		■		■			■	■

SOP C

SOP D Field Sampling

D.1 Site Set-up

1. Navigate to the most downstream sampling reach selected for this sampling bout using GPS points, the morphology map, or the plot survey markers.
   a. Sampling must begin at the downstream sampling reach and proceed upstream to minimize disturbance.
2. Set up fence posts and block nets at downstream and upstream boundaries of the sampling reach.
   a. Always secure the downstream block net first, followed by the upstream net.
   b. Secure a 3 mm mesh block net at the reach boundary using steel fence posts or existing structure (e.g., tree).
   c. Relocate any debris (i.e tumble weeds) in the stream that interferes with the block net deployment immediately downstream of the sample reach. Do not remove in-stream habitat features (i.e. large wood) to install block nets. Instead, move the net up or down stream of structure.
   d. Pull the net across the stream ensuring enough slack in the lead line (bottom of net) to reach the stream substrate.
   e. Anchor lead line to substratum using large rocks or block net anchor stakes.
3. Place up to 10 equally spaced 5-gallon buckets with battery-operated aerators on the bank along the stream reach for holding fish during electrofishing.
4. Locate an appropriate (e.g., flat ground, preferably in the shade) fish processing location along the stream bank near the targeted sampling reach. Place processing equipment (e.g., fish measuring boards, digital scale, plastic weighing tray, sample bottles, preservative) at this location.

D.2 Backpack Electrofishing Field Set-up

Test settings on the backpack electrofisher before sampling begins. After settings are determined, they will be used for the remainder of the sampling bout. Electrofishing activities must take place at least 5 m from any in-stream electronics (e.g., sensor sets).

1. **VERY IMPORTANT:** All technicians MUST wear necessary personal protective equipment before stepping into the water, including waterproof chest waders with lug-soled boots (felt waders are not allowed at NEON sites), rubber lineman gloves to insulate the wearer from electrical shock, and polarized sunglasses to increase the efficiency of fish capture.
2. Assemble anode pole (Figure 1).
3. Measure and record water temperature and conductivity using the handheld conductivity meter. Record on Field Data Sheet (RD[11]).

4. Connect the cathode and anode to the backpack electrofishing unit (Figure 5).

Figure 5. Cathode and anode connections on backpack electrofishing unit

5. Connect the battery to backpack electrofishing unit and secure the batteries with the strap to the backpack frame (Figure 6).

Figure 6. Battery location and secure placement in the backpack electrofishing frame

6. Test the backpack electrofisher in the 20 m section that was left between the downstream permit boundary and the closest sampling reach.
7. Wade into the stream ensuring that the cathode (i.e., rattail) is submerged and anode ring is submerged.
    a. Begin electrofishing in shallow water (e.g., < 50 cm).

8. While the electrofisher operator is standing in the stream, set the frequency to 30 Hz, the duty cycle to 10%, and output voltage to 100 V and turn the electrofishing unit on. Backpack electrofisher settings should be based on stream conductivity, see Table 7 for additional settings information.

Table 7. Guidelines for initial and maximum settings for backpack electrofishing (NOAA NMFS 2000)

Settings	Initial Settings	Maximum Settings	
Voltage	100 V	Conductivity (µS/cm) < 100 100-300 > 300	Max Voltage 1,100 V 800 V 400 V
Pulse Width	500 µs	5 ms	
Pulse Rate (Frequency)	30 Hz	70 Hz	

9. Pause to verbally confirm settings on electrofisher and that the unit is turned on. Also confirm that all technicians are ready to proceed before pressing the activation switch on the anode pole.
    a. The anode ring must always be submerged before depressing the activation switch. When removed from the water, the unit will automatically turn off.
10. Press and hold the activation switch down, and observe the behavior of fish. If fish do not appear to be affected by electrofishing (e.g., are not momentarily stunned), release the activation switch on the anode pole and increase voltage by 100 V (e.g., from 100 V to 200 V) and repeat Steps 9-10.
    a. The goal is to immobilize fish using the lowest settings possible at the site to avoid harming sampled fish.
11. If 1,100 V is reached and fish are still not responding to electrofishing proceed decrease voltage to 250 V and increase the frequency by 10 Hz (e.g., from 30 Hz to 40 Hz) and repeat Steps 9-10.
    a. If 70 Hz and 1,100 V is reached and fish are present but not immobilized, stop electrofishing and contact the NEON Aquatic Ecologist.
    b. If fish are immobilized during testing, use dip nets to capture individuals and place in a bucket ½ - ¾ full of stream water carried by one of the netters and continue with Step 12.
12. Continue electrofishing until up to 20 individuals spanning a variety of sizes are netted.
13. Place netted fish in a bucket with fresh stream water and a battery operated aerator.
    a. If other top predators are captured, identify (if possible) and record species on field data sheet (RD[11]) and immediately release >20 m away from electrofishing activity.
14. Examine captured fish for signs of injury (e.g., bent backs, dark bruising, hemorrhaging of the gills). Record injury rate on Field Data Sheet (RD[11]). Less than 1% of the captured fish should be injured.
    a. If > 1% of captured fish are injured, suspend sampling and contact the domain manager and submit a trouble ticket through the NEON problem resolution system (JIRA).

b. Contact with the cathode or prolonged exposure to electricity due to failure to remove fish from the dip net quickly will increase injury rates.
c. If fish are injured, allow them to recuperate in a separate bucket with an aerator before releasing.
d. For any fish that do not recover, proceed to euthanization (SOP D.6).

15. Monitor captured fish for signs of normal respiration (gills open and close steadily) and swimming (upright, not listing) behavior for 10 minutes. If, after 10 minutes, fish are still on their side, upside-down, or injured, return to lower electrofishing settings. For moribund or injured fish, see E.5, fish processing step 10.
    a. It is important to note that some fish species (e.g., blacknose dace) are sensitive to electrofishing and may exhibit higher injury or mortality rates.
16. Once fish are swimming normally release back into the stream near where they were caught. Preferably immediately downstream of the current sample reach.
17. Maintain electrofisher settings at the lowest level that allows for the effective capture of fish. Record frequency, duty cycle, and voltage settings on the Field Data Sheet (RD[11]) and reset the timer on the electrofishing unit. These settings will be used for the entire sampling bout.

D.3 Backpack Electrofishing

1. Proceed to the downstream block net of the first sampling reach.
2. Record start time on Field Data Sheet (RD[11]) so that conductivity, turbidity, and other water quality measurements from the in-stream sensor sets can be coupled with the fish sampling bout.
3. Walk into the stream, ensuring that the cathode (i.e., rattail) is submerged as much as possible, while holding the anode pole in one hand (anode ring submerged).
    a. The electrofisher operator may, but is not required to, hold a dip net in the other hand if he/she feels comfortable.
4. The other crewmembers will enter the stream behind the electrofisher operator.
    a. The primary netter will stay close to the electrofisher operator to net fish.
    b. The secondary netter will carry the bucket and net any other stunned fish that are missed by the electrofisher operator or the primary netter.
5. Ensure that the electrofishing unit settings (frequency, duty cycle, and output voltage) are those determined in SOP D.2 and that the timer (“EF time”) has been reset to 0.
6. Turn the electrofishing unit on and notify the other technicians. Confirm that all technicians are ready to begin.
7. Depress and hold the activation switch on anode pole to begin electrofishing.
    a. The anode ring must always be submerged before depressing the activation switch and should never be taken out of the water with the switch depressed. The unit will automatically turn off if the anode is removed from the water or if the operator bends over forward.

8. Slowly sweep the anode inside (i.e., upstream) of block net side within the sample reach to target any fish that may be seeking cover in the net.
9. After sweeping the block net, the electrofisher operator should then turn upstream and slowly sweep the anode across the stream channel to expose all available habitats to electricity.
    a. This may require slowly walking from bank to bank in streams wider than 2 m.
10. As the anode is moved across the stream, the netters will capture drifting, immobilized fish.
    a. Dip nets should be held as close to the stream substrate as possible without picking up excessive substrate or debris.
    b. There should always be one net behind the anode.
    c. Fish are often attracted to the cathode (rattail). Netters should periodically check this area for stunned fish.
    d. Netters should be aware that immobilized fish may not always be visible, particularly benthic species (e.g., darters, sculpin), and netters should frequently inspect their nets to minimize injury to fish by continuous exposure to electricity.
    e. Crewmembers with dip nets should CALMLY net immobilized fish without excessive disturbance.
    f. Never put hands in the water to capture fish while activation switch is depressed (i.e., while electrical current is pulsing through the water). If the netter cannot capture a fish using the net (e.g., sculpin, young-of-year), notify the backpack electrofisher to stop shocking. The backpack electrofisher must release the activation switch and remove the anode from the water to ensure no pulse is being conducted, then verbally confirm that it is safe for the netter to put his/her hand (or use the small dip net) in the water. After capturing the fish, the netter removes his/her hands from the water and verbally confirms that he/she has done so. Only then may the backpack electrofisher place the anode in the water and depress the activation switch to continue fishing, notifying other technicians that the unit is on.
    g. If any endangered species (technicians will be notified of likelihood before sampling) or other vertebrates (e.g., salamanders, turtles) are caught, identify, photograph if possible, and release immediately downstream from electrofishing activities within the permitted reach.
11. Frequently remove fish from dip nets and place in buckets to minimize injury to the fish.
12. Sampling will continue upstream in a zig-zag pattern across the channel with attention to sampling all complex instream cover (e.g., overhanging or aquatic vegetation, woody debris, undercut banks).
    a. The electrofisher operator may take advantage of the response of fish to pulsed DC current (i.e., attraction of immobilized individuals towards the anode) in complex cover by:
        1) Releasing the activation switch on the anode pole.
        2) Inserting the anode into cover from the downstream direction and holding the anode temporarily still.

3) The electrofisher operator then depresses the activation switch as netters hold dip nets immediately downstream of the anode and cover.
4) The activation switch should continue to be depressed until the abundance of observable immobilized fish decreases.
5) Continue electrofishing by moving the anode around the cover to immobilize additional fish, before continuing electrofishing.
6) Netters should make a few sweeps through any cover that was shocked to pull out immobilized individuals that are stuck in the cover or didn't follow the anode out.

13. While electrofishing, monitor the abundance of fish in the buckets to prevent escape or accidental spills.
    a. Be aware of fish overcrowding in the buckets. If fish appear to be gasping at the water surface, they are likely short on oxygen due to water temperature or overcrowding. Place fewer fish in buckets and supplement with cooler water and aerators.
        1) If a lot of predatory fish and prey species are collected they may need to be placed in separate buckets to reduce predator consumption of prey species.
    b. Bucket replacement and moving fish is easier for the netters to do as they will need to step out of the stream.
    c. Place buckets of fish out of direct sunlight if possible.
14. When the crew reaches the upstream block net, the electrofisher operator should slowly sweep the anode downstream of the block net as fish may have moved upstream to avoid the electrical field.
15. Once the entire sampling reach has been sampled, read and record the time (EF time) in seconds from the back of the electrofishing unit on the Field Data Sheet (RD[11]).

    a. Electrofisher time is critical for calculating sampling effort.
16. Turn the electrofisher off, remove and place on the bank with anode and cathode still attached.
17. Proceed to fish processing (SOP D.5).
    a. If this is a fixed reach, repeat Steps 1-16 until three passes have been completed.
    b. Observe a minimum of 30 minutes between passes to allow fish that were not captured to recover.
    c. Depletion is determined when two consecutive passes result in sequential decreases in total number of individuals sampled after the first pass (e.g., 1000 fish on first pass, 200 fish on second pass, 50 fish on third pass).
        1) If the number of sampled fish increase with each pass do the following:
            a) Re-check that the settings on the backpack electrofisher have not changed
            b) Inspect the block net for holes and that the lead line is laying across the stream bed
            c) Ensure that your anode sweeping technique is even and consistent
            d) Make sure the netters are alert during each pass

2) If this issue continues in a subsequent sample reach, contact the domain manager and submit a trouble ticket through the NEON problem resolution system (JIRA).

d. If this is a random reach, fish are sampled using only one pass.

18. Remove the downstream block net after Pass 1 and processing (random reaches) or Pass 3 and processing (fixed reaches) have been completed.
19. Remove the upstream block net if it is not needed for the next reach (e.g., this net may serve as the downstream block net for the next reach if reaches are contiguous) or there is not enough daylight to continue with the next reach.
20. Break down the backpack electrofishing unit if the crew cannot complete another reach during daylight hours:
    a. Disconnect the cathode and anode from the backpack electrofishing unit.
    b. Disconnect the battery from the backpack electrofishing unit and remove battery from the backpack frame.
    c. Place backpack electrofishing unit in case.
    d. Disassemble anode pole and store with backpack electrofishing unit.
    e. Place recently used battery separate from charged batteries where it can be easily distinguished for charging.

D.4 Minnow Traps

Minnow traps should only be used at sites where electrofishing has been shown to be inefficient. Contact NEON HQ if minnow traps are necessary.

1. Bait traps only if predatory fish are likely to be in the habitat (NEON HQ will provide this information on a site-specific basis before sampling).
    a. Poke several holes in a 24 oz. Whirl-pak® bag using a pencil.
    b. Add a small handful of Aquamax fish food to the bag and seal.
    c. Place bag in one end of the minnow trap.
2. Close the two halves of the minnow trap by fitting the tabs together on one side, and the clip on the other (Figure 7)

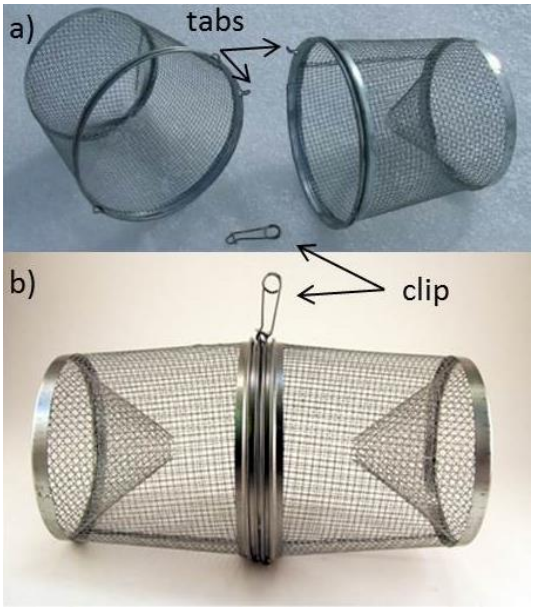

Figure 7. Gee minnow trap. a) The trap is split into two halves that are held together in the center by two tabs on one side and a clip on the other. b) Assembled minnow trap.

3. Tie a length or rope to the end of the clip. This will be tied off on the bank when the trap is set, so ensure that is it long enough to reach from the trap to a stable feature on the stream bank.
4. Minnow traps should be set approximately every 10 m along the sampling reach.
    a. Traps should set be a minimum of 2 m apart.
5. Set traps in pools or other slow-moving areas that are likely to harbor the most fish. Set traps only in locations that appear to be good fish habitat, including:
    a. Pools
    b. Complex habitats (downed trees, coarse woody debris)
    c. Under snags
    d. Bends in the channel
6. Lower trap to the stream bottom at the chosen location. Ensure that the trap is oriented so that the trap is parallel to stream flow so fish are more likely to swim through the openings, and that the trap openings are underwater.
7. Tie the trap off to riparian vegetation so it can easily be recovered.
8. Record start time on field data sheet (RD[11]).
9. Set 20 traps in each reach.
    a. Depending on the number of traps available, set traps in two reaches per day for 3 days.
10. Allow traps to stay in the water for 24 hours.
11. After approximately 24 hours, carefully retrieve the traps without tipping them so that no fish swim out and record the end time on the field data sheet (RD[11]).
12. Open the trap and place any fish captured in a 5-gallon bucket of stream water.

13. Continue until all traps have been retrieved and proceed to fish processing (SOP D.5).

D.5 Fish Processing

1. Ensure that all technicians handling fish keep hands wet with stream water and free of chemicals (e.g., insect repellent, sunscreen) while processing fish.
2. Designate one technician to identify fish throughout the sampling bout for taxonomic consistency.
3. For any non-fish top predators (e.g., salamanders) collected, identify and record species to lowest practical taxon on the Field Data Sheet (RD[11]) and release.
   a. Photograph the specimen before releasing if possible.
4. Ensure that electrofishing time and electrofisher settings have been recorded, and record pass number on the Field Data Sheet (RD[11]).
5. Setup the digital scale and a measuring board on a flat surface.
6. Place plastic measuring tray on scale pan and tare.
7. Mix anesthetic in one 5-gallon bucket.
   a. Fill the bucket approximately half full with native water (2.5 US gallons or ~10 L).
   b. A dosage treatment of 20-30 mg/L eugenol (AQUI-S20E is 10% eugenol) is recommended to sedate all fish species to handleable in most situations. Refer to Table 12 for calculated eugenol concentrations. Additionally, recommended concentrations can be calculated for different water treatment volumes using this formula:

$$AQUI - S20E\ (mL) = \mathrm{A} \times \mathrm{B}\ \times \mathrm{C}\ \div D$$

Where: A = target concentration eugenol (mg/L)
B = treatment water volume (gal)
C = 0.00378 (conversion factor for grams per gallon)
D = 0.1 (To account for the fact that AQUI-S20E is 10% eugenol)

   c. Using the 10 mL graduated cylinder, add 1.9 mL of AQUI-S20E 2.5 US gallons (~10 L) native water for an initial concentration of 20 mg/L. Mix well (the small dip-net makes a good mixer).

Table 8. Matrix for determining the amount (mL) of AQUI-S20E to add to treatment water for a specific concentration of eugenol.

Target Concentration of AQUI-S20E (10% eugenol)	Volume of Treatment Water (gal)					
	2.5	5	10	15	20	25
20 mg/L	1.9 mL	3.8 mL	7.6 mL	11.3 mL	15.1 mL	18.9 mL
25 mg/L	2.4 mL	4.7 mL	9.5 mL	14.2 mL	18.9 mL	23.6 mL
30 mg/L	2.8 mL	5.7 mL	11.3 mL	17.0 mL	22.7 mL	28.4 mL

d. Label bucket so all technicians know it is anesthetic.

8. Remove fish from the first bucket using the small handheld dip net.
   a. Larger fish may need to be removed carefully by hand.
9. Place one fish at a time in the anesthetic bucket. Carefully monitor respiration, spontaneous movements, and swimming behavior to gauge the state of sedation and movements to determine when fish is anesthetized. If the fish can be easily handled without flipping its tail back and forth, it is sufficiently anesthetized. Sedation should be achieved in 1 - 5 minutes following emersion in the anesthetic solution. Fish will be able to be handled within 3-5 minutes. The required sedation time should be <5 minutes.
   a. If this dose of anesthetic is insufficient, add 0.5 mL of AQUI-S20E to increase the concentration of 25 mg/L until anesthetization is achieved. Do not exceed an AQUI-S20E concentration of 30 mg/L.

   b. Do not exceed 5 fish in the anesthetization bucket at one time.
   c. Leaving fish in the anesthetization bucket for too long can cause mortality. Monitor respiration and gill movement constantly.
   d. Be sure to include required information within the datasheets for the INAD.

10. Identify fish to species using the 4-letter species code (e.g., *Cottus cognatus* = COCO) and record on Field Data Sheet (RD[11]).
    a. If the species cannot be identified or identification is uncertain, weigh and measure following Steps 11-13, and euthanize the specimen.

       1) Do not collect more than 5 specimens of the same unknown species. Rather, morphotype and label with a unique identifier on the Field Data Sheets (RD[11]).
       2) Do not euthanize endangered species (site specific lists will be provided before sampling). Additionally, fish > 200 mm standard length should not be euthanized for vouchering, however, should fish > 200 mm be injured during sampling and require euthanization proceed to step 10.4 below.
       3) Photograph fish and record the camera image number on the Field Data Sheet along with the relevant weight and length information about the fish (RD[11]) before reviving and releasing.
       4) Euthanize fish using a lethal dose of MS-222,200 mg/L of stream waterin the field.
       5) Add 1 L of stream water and 10 mL of MS-222 stock solution to a new 5 gallon bucket. Mix thoroughly.
       6) Transfer fish from the holding bucket to the bucket containing the anesthetic solution with the small handheld dip net.
       7) Monitor fish until respiration ceases.
       8) Place fish into appropriate sample container (e.g., wide mouth HPDE bottles) with completed specimen label (**Error! Reference source not found.**) and add 10% formalin preservative. One taxon per specimen bottle.

b. Amphibians that are injured as a result of fish sampling will be euthanized using a lethal dose of MS-222 following the protocol for fish euthanasia above.
c. For reptiles, a two-stage method of euthanasia is recommended. This procedure includes an intracoelomic injection of 250 to 500 mg/kg of a pH-neutralized solution (0.7% to 1.0%) of MS-222. This will produce a loss of consciousness in less than five minutes (AVMA 2013). Following loss of consciousness, a second intracoelomic injection of unbuffered 50% MS-222 is administered. Any euthanized or dead animals will be collected, preserved in formalin in a collection jar, and deposited at a fish collections facility.
d. Aquatic invertebrate species, including arthropods and molluscs, that are injured while performing fish sampling tasks will be euthanized. Methods for euthanizing aquatic invertebrates will follow the U.S. Environmental Protection Agency Rapid Bioassessment Protocols for Use in Streams and Wadeable Rivers (Barbour et al 1999). Injured aquatic invertebrates will be euthanized by completely submerging specimens in 95% ethanol. Injured or dead aquatic invertebrates will be preserved for vouchering in 70% ethanol (Hauer and Resh 2006).
e. In the event that a federal or state listed threatened or endangered species is morbidly injured, the organism will be euthanized following the procedures identified above. NEON will contact the regional U.S. Fish and Wildlife Service office and the local state fish and wildlife department to report the incident. Specimens will also be preserved following the methods described above. If the inadvertent death of a protected species is discovered once the specimen has been shipped to a taxonomic specialist or the curation facility, NEON will immediately contact the federal and state fish and wildlife authorities within the region where the specimen was collected.

f. Photo voucher 1 representative specimen from each taxon. Include non-target specimens (e.g. amphibians and reptiles).
    1) Include metric ruler for scale using the measuring board.
    2) Photograph 1: Lateral photo with fish's head facing to the left.
    3) Photograph 2: Ventral photo that includes the mouth (mouth position, lip structure, and barbels can be important distinguishing features.

11. Place the fish in the plastic tray on the tared digital scale. Determine weight to nearest 0.1 g and record on Field Data Sheet (RD[11]).
12. With wet, clean hands, remove the fish from the plastic tray and place the fish on the measuring board with mouth at the "0" end of the board. Measure total length to the tip of the pinched-together tail (Figure 8) to the nearest mm and record on Field Data Sheet (RD[11]).

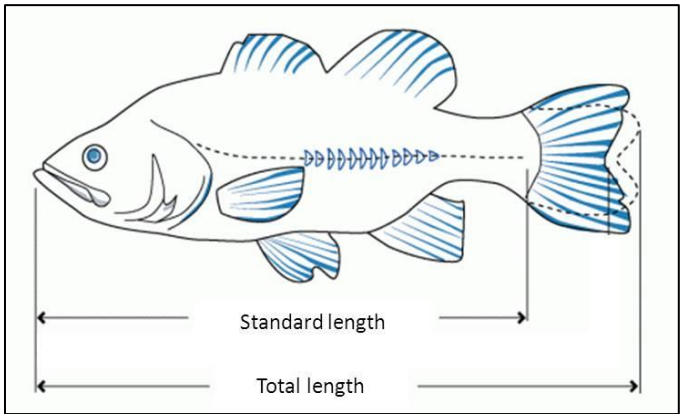

Figure 8. Measurement of standard and total length. Total length is measured by pinching the fork together.

13. Inspect the fish for deformities, including eroded fins, external lesions, parasites, and tumors (DELTS) and electrofishing injuries (burn marks, bent spine, hemorrhage) and record on Field Data Sheet (RD[11]).
14. If collecting fin clips for DNA barcoding samples, refer to RD[13] AOS Protocol and Procedure: Aquatic DNA Barcode.
15. Place processed fish in a bucket containing fresh stream water and a battery powered aerator for later release. Monitor fish for respiration and swimming behavior.
    a. Do not overcrowd fish in the reviving buckets, they need as much aerated water as possible.
16. Repeat Steps 8-14 until ≤100 fish per species are identified, weighed, measured, and inspected for deformities.
    a. If more than 100 individuals in one species are captured, anesthetize, weigh, and measure the first 100 and simply count the remaining fish (no anesthetization) to speed processing time and alleviate stress to fish.
17. Release the processed, revived fish back into the stream downstream of the block net. If sampling at the furthest downstream reach, place prcessed fish within the initial 20 m reach within the permitted boundary.
    a. With the use of 10% eugenol (AQUI-S®20E), treated freshwater fish species may be immediately released following recovery; no withdrawal time is required.
    b. If mortality occurs during processing, save individuals for collections and note on Field Data Sheet (see Sample Preservation, SOP D.6).
18. The anesthetic solution will be disposed of in accordance with the terms and conditions of applicable permits. Disposal in the field (away from the stream) will only occur where permitted. Field disposal of anesthetic will take place at least 10 m from the bank away from sensor sets or groundwater wells so as not to impact any other water chemistry measurements or sample collection. Anesthetic solutions are not expected to affect any other aquatic measurements if disposed of away from the stream. If field disposal is not permitted, waste anesthetic solution will be carried out and disposed of at the Domain Support Facility.

D.6 Sample Preservation

1. Fill jar with a 10% buffered formalin solution to fix specimens within one hour of euthanizing.
2. Secure lid tightly and store upright at room temperature (~70 ^{o}F).
3. Discard used anesthetic solution according to NEON EHS chemical hygiene guidelines (AD[03]).

D.7 Ending the Sampling Day

1. Refreshing the sampling kit
   a. Replace batteries for all battery operated equipment (e.g., GPS unit, portable aerators).
   b. Refill/restock preservative and anesthetic stock solution containers.
2. Equipment maintenance, cleaning and storage
   a. Wash all equipment that has come in contact with stream water according to the NEON Aquatic Decontamination Protocol (RD[07]).
   b. Dry all equipment thoroughly between sites and before storage.
   c. Check all nets for holes and patch if necessary using the net repair kit. Mending fish nets takes practice and patience. There are several resources available online. The following link is a resource provided by Oregon State University: http://ir.library.oregonstate.edu/xmlui/bitstream/handle/1957/25084/SGNO831989.pdf

SOP E Data Entry and Verification

As a best practice, field data collected on paper datasheets should be digitally transcribed within 7 days of collection or the end of a sampling bout (where applicable). However, given logistical constraints, the maximum timeline for entering data is within 14 days of collection or the end of a sampling bout (where applicable). See RD[04] for complete instructions regarding manual data transcription.

Download all images from the camera and save in folder named “SiteCode_YYYYMMDD_SpecimenID”.

SOP F Sample Shipment

Information included in this SOP conveys science-based packaging, shipping, and handling requirements, not lab-specific or logistical demands. For that information, reference the CLA shipping document on CLA's NEON intranet site.

Ground ship to Fish Taxonomist (*to be determined pending lab contracts*) for identification and long-term preservation.

F.1 Handling Hazardous Material

Follow shipping and Hazmat procedures for formalin.

F.2 Supplies/Containers

1. Place sealed specimen containers inside a heavy-duty trash bag. Wrap excess trash bag material around the samples and secure with duct or packing tape to prevent leaks.
2. Place package inside appropriately-sized cooler or other sturdy shipping container. Add packing material, as necessary, to take up excess space in container.
3. Tape and label container for shipping.

F.3 Timelines

Ship samples within 1 month of the end of the sampling bout.

F.4 Conditions

Samples will be shipped following the Hazmat procedures for formalin as described above.

F.5 Grouping/Splitting Samples

N/A

F.6 Return of Materials or Containers

N/A

F.7 Shipping Inventory

Include sample shipment inventory (RD[10]). Email shipping inventory to external lab contact and copy the NEON CLA contact.

F.8 Laboratory Contact Information and Shipping/Receipt Days

See the CLA shipping document on CLA's NEON intranet site.

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APPENDIX A DATASHEETS

The following datasheets are associated with this protocol:

Table 9. Datasheets associated with this protocol

NEON Doc. #	Title
NEON.DOC.001646	General AQU Field Metadata Sheet
NEON.DOC.002494	Datasheets for AOS Sample Shipping Inventory
NEON.DOC.003107	Datasheets for Fish Sampling in Wadeable Streams

These datasheets can be found in Agile or the NEON Document Warehouse.

APPENDIX B QUICK REFERENCES

Step 1 – Prepare equipment, data sheets and specimen labels, and ensure all batteries are fully charged.

Step 2 – For anesthetizing fish, AQUI-S20E should be added directly to the treatment water. Do not make stock solutions or other dilute solutions of AQUI-S20E. Use the table below to determine the amount of AQUI-S20E to add to the treatment water for specific concentrations of eugenol:

Target Concentration of AQUI-S20E (10% eugenol)	Volume of Treatment Water (gal)					
	2.5	5	10	15	20	25
20 mg/L	1.9 mL	3.8 mL	7.6 mL	11.3 mL	15.1 mL	18.9 mL
25 mg/L	2.4 mL	4.7 mL	9.5 mL	14.2 mL	18.9 mL	23.6 mL
30 mg/L	2.8 mL	5.7 mL	11.3 mL	17.0 mL	22.7 mL	28.4 mL

Mix stock solutions of MS-222 in the Domain Support Facility.

Step 3 –Ensure the General AQU Field Metadata Sheet (RD[05]) is completed per field site visit.

Step 4 – If this is your first sampling year, establish and select random sampling reaches.

Step 5 – Set up block nets at downstream and upstream boundaries of the sampling reach and place 5 to 10 equally spaced 5-gallon buckets with battery-operated aerators on the bank along the stream reach.

Step 6 – Assemble backpack electrofisher and test the settings by collecting, inspecting and releasing 20 fish.

Step 7 – Move the anode across the stream in a zigzag pattern and capture drifting, immobilized fish. Place immobilized fish in 5 gallon buckets.

Step 8 – If this is a random reach, sample fish using only one pass. If this is a fixed reach, sample fish using three passes.

Step 9 – If directed to use minnow traps by NEON HQ, set traps in pools or other slow-moving areas that are likely to harbor the most fish. Set traps only in locations that appear to be good fish habitat, including:

a. Pools
b. Complex habitats (downed trees, coarse woody debris)
c. Under snags
d. Bends in the channel

Step 10 – Anesthetize caught fish in a 5 gallon bucket with solutions of AQUI-S20E.

Step 11 – Identify fish to species using the 4-letter species code (e.g., *Cottus cognatus* = COCO) and record on Field Data Sheet (RD[11]). Euthanize the fish if it cannot be identified in the field.

Step 12 – Measure the weight and length of the specimen and inspect for deformities.

Step 13 – Place processed fish in a bucket containing fresh stream water and a battery powered aerator for later release. Once revived, release the fish downstream of the block net.

Step 14 – Preserve euthanized specimen in a jar with a 10% buffered formalin and ship to taxonomist.

APPENDIX C REMINDERS

Before heading into the field:

- ☑ Collect and prepare all equipment including labels.
- ☑ Pre-print labels on waterproof paper.
- ☑ Ensure all batteries are fully charged.
- ☑ When mixing the stock solution, wear nitrile gloves and eye protection, as MS-222 is a skin and eye irritant.

Sample collection:

- ☑ All technicians MUST wear necessary personal protective equipment before stepping into the water, including waterproof chest waders with lug-soled boots (felt waders are not allowed at NEON sites), rubber lineman gloves to insulate the wearer from electrical shock, and polarized sunglasses to increase the efficiency of fish capture.
- ☑ While electrofishing, monitor the abundance of fish in the buckets to prevent escape or accidental spills.
- ☑ Sample all complex instream cover types (e.g., overhanging or aquatic vegetation, woody debris, undercut banks).
- ☑ Observe a minimum of 30 minutes between passes to allow fish that were not captured to recover.
- ☑ Netters should frequently inspect their nets to minimize injury to fish by continuous exposure to electricity.
- ☑ Never put hands in the water to capture fish while activation switch is depressed.
- ☑ If endangered species are caught, identify and photograph and release immediately away from electrofishing activities.
- ☑ Release the processed, revived fish back into the stream downstream of the block net.

Sample processing:

- ☑ Do not collect more than 5 specimens of the same unknown species.
- ☑ Do not euthanize endangered species.
- ☑ If more than 100 individuals in one species are captured, anesthetize, weigh and measure the first 100 and simply count the remaining fish (no anesthetization).
- ☑ Do not exceed 5 fish in the anesthetization bucket at one time.

APPENDIX D ESTIMATED DATES FOR ONSET AND CESSATION OF SAMPLING

See the Site Specific Sampling Strategy Document on AQU's NEON intranet site.

APPENDIX E SITE-SPECIFIC INFORMATION: RANDOMIZED REACH SELECTION PER SITE

Randomized reach order is shown for each site below. Skip numbers that have either been chosen as a fixed reach, or do not exist at the site (i.e., sites that are < 1 km may have fewer than 10 reaches).

Domain	Site	Randomized reach order
D01	Hop Brook	9, 5, 3, 6, 8, 1, 2, 7, 4, 10
D02	*TBD*	6, 5, 7, 9, 3, 2, 4, 8, 1, 10
D02	Posey Creek	6, 10, 4, 9, 1, 5, 2, 8, 7, 3
D04	Rio Guilarte	2, 8, 7, 4, 6, 9, 5, 1, 3, 10
D04	Rio Cupeyes	8, 3, 5, 10, 4, 7, 1, 6, 2, 9
D06	Kings Creek	9, 8, 10, 3, 2, 4, 6, 1, 5, 7
D06	*TBD*	4, 9, 3, 7, 2, 10, 8, 6, 1, 5
D07	Leconte Creek	3, 5, 9, 4, 2, 6, 10, 8, 1, 7
D07	Walker Branch	9, 1, 4, 3, 2, 6, 5, 10, 7, 8
D08	Mayfield Creek	4, 6, 8, 7, 1, 2, 3, 10, 5, 9
D10	Arikaree River	7, 5, 2, 3, 4, 8, 9, 10, 1, 6
D11	Pringle Creek	8, 6, 2, 10, 5, 9, 4, 1, 3, 7
D12	Blacktail Deer Creek	7, 8, 1, 2, 10, 4, 9, 3, 5, 6
D13	Como Creek	10, 5, 9, 7, 3, 2, 4, 1, 8, 6
D13	West St. Louis Creek	2, 9, 8, 10, 5, 3, 6, 4, 7, 1
D14	Sycamore Creek	4, 7, 9, 1, 8, 2, 5, 10, 6, 3
D15	Red Butte Creek	3, 4, 1, 9, 7, 10, 6, 2, 5, 8
D16	McRae Creek	7, 6, 9, 1, 2, 4, 10, 5, 8, 3
D16	Martha Creek	5, 6, 7, 9, 1, 2, 4, 3, 10, 8
D17	*TBD*	9, 7, 3, 6, 5, 4, 8, 10, 1, 2
D17	*TBD*	9, 8, 10, 2, 1, 6, 5, 7, 3, 4
D18	Oksrukuyik Creek	6, 4, 5, 8, 10, 1, 3, 2, 9, 7
D19	Caribou Creek	1, 5, 9, 8, 2, 3, 10, 6, 4, 7