AOS PROTOCOL AND PROCEDURE: FISH SAMPLING IN WADEABLE STREAMS

PREPARED BY	ORGANIZATION	DATE
Stephanie Parker	AQU	09/05/2013
Jesse R. Fischer	AQU	07/09/2013

APPROVALS	ORGANIZATION	APPROVAL DATE
Dave Tazik	CCB PROJ SCI	01/17/2014
Mike Stewart	SE	02/11/2014

RELEASED BY	ORGANIZATION	RELEASE DATE
Stephen Craft	SE	02/18/2014

See configuration management system for approval history.

The National Ecological Observatory Network is a project solely funded by the National Science Foundation and managed under cooperative agreement by NEON, Inc. Any opinions, findings, and conclusions or recommendations expressed in this material are those of the author(s) and do not necessarily reflect the views of the National Science Foundation.

Change Record

REVISION	DATE	ECO #	DESCRIPTION OF CHANGE
A	02/18/2014	ECO-01394	Initial release

TABLE OF CONTENTS

LIST OF TABLES AND FIGURES

1 DESCRIPTION

1.1 Purpose

The primary purpose of this document is to provide a change-controlled version of Observatory protocols and procedures. This document provides the content for training and field-based materials for NEON staff and contractors. 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.

This document is a detailed description of the field data collection, relevant pre- and post-field tasks, and safety issues as they relate to this procedure and protocol.

1.2 Scope

This document relates the tasks for a specific field sampling or laboratory processing activity and directly associated activities and safety practices. This document does not describe:

- General safety practices
- Site-specific safety practices
- General equipment maintenance

It does identify procedure-specific safety hazards and associated safety requirements such as safe handling of small mammals or safe use of required chemicals and reagents.

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 information that shall be applied in the current document. Examples are higher level requirements documents, standards, rules and regulations.

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.001155	NEON Training Plan
AD [05]	NEON.DOC.050005	Field Operations Job Instruction Training Plan

2.2 Reference Documents

Reference documents contain information complementing, explaining, detailing, or otherwise supporting the information included in the current document.

RD [01]	NEON.DOC.000008	NEON Acronym List
RD [02]	NEON.DOC.000243	NEON Glossary of Terms
RD [03]		NEON DOORS Requirements
RD [04]	NEON.DOC.005003	NEON Scientific Data Products Catalog
RD [05]	NEON.DOC.014051	Field Audit Plan
RD [06]	NEON.DOC.000824	Data and Data Product Quality Assurance and Control Plan
RD [07]	NEON.DOC.001152	NEON Aquatic Sample Strategy Document
RD [08]	NEON.DOC.000690	NEON Protocol: Macroinvertebrate Sampling in Wadeable Streams
RD [09]	NEON.DOC.001154	NEON Aquatic Decontamination Protocol
RD [10]	NEON.DOC.001153	NEON Aquatics Wadeable Stream Morphology Mapping Protocol

2.3 Acronyms

A	Ampere or Amp
AFS	American Fisheries Society
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

2.4 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).

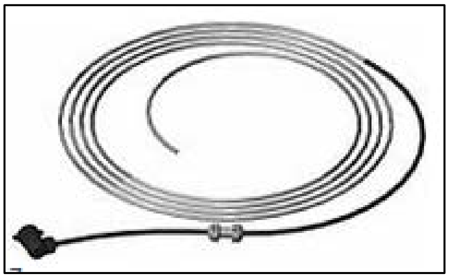

Figure 2. Cathode for backpack electrofishing unit (photo: store.smith-root.com)

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 temporary 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 water body 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 BACKGROUND AND OBJECTIVES

3.1 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.

3.2 NEON Science Requirements

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.

3.3 NEON Data Products

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, which are documented in the NEON Scientific Data Products Catalog (RD [04]), available on the NEON website.

4 PROTOCOL

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.

5 QUALITY ASSURANCE AND CONTROL

The procedures associated with this protocol will be audited according to the NEON Field Audit Plan (RD [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 (RD [06]).

When unexpected field conditions require deviations from this protocol, the following field implementation guidance must be followed to ensure quality standards are met:

1) 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.
   a) 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.
2) A minimum of 2 weeks between sample periods shall be observed.
3) Fish sampling shall occur only during daylight hours for safety and consistency of capture efficiency.
4) All three-passes in a fixed reach must occur within the same day, with at least 30 minutes between passes.

5.1 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.

6 SAFETY

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.

See Section 10 in the NEON Operations Field Safety and Security Plan (AD 01]). 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).

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

1) Audible signals must be used to alert technicians when electrofishing equipment is in operation.
2) Chest waders and heavy-duty rubber gloves must be worn while working near an electrofishing unit. Leave the water immediately if waders or gloves develop leaks.
3) Avoid operating near bystanders, pets, or livestock that are in or near the water.
4) Electrofishing must be suspended if anyone feels a shock, however minor, for investigation and repair of equipment.
5) Avoid operative an electrofishing unit in heavy rain (light rain is acceptable) as this can increase the probability of electrical shock.

7 PERSONNEL REQUIREMENTS

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.

8 TRAINING REQUIREMENTS

All technicians must complete required safety training as defined in the NEON Training Plan (AD [04]). Additionally technicians complete protocol specific training for safety and implementation of protocol as required in Field Operations Job Instruction Training Plan (AD [05]).

Personnel will be trained in fish sampling in wadeable streams safe working practices for streams field work. Specific training for wadeable stream fish sampling must also include electrofishing training for all technicians.

9 FIELD STANDARD OPERATING PROCEDURE

9.1 Sampling Frequency and Timing

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 [07]).

9.1.1 Criteria for Determining Sampling Dates

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.

9.1.2 Sampling Frequency

Wadeable stream fish sampling will occur two times per year during the growing season at each site, roughly spring and autumn.

9.1.3 Sample Timing Parameters

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 [07]).

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.

9.2 Equipment and Materials

Table 1. Field equipment list

Maximo Item No.	Item Description	Quantity	Habitat-Specific	Special Handling
	Field preparation			
	Tricaine methanesulfonate (MS-222)	20 g		Y
	$NaNCO_3$ (buffering agent for MS-222)	50 g		
	Clove oil (is not using MS-222)	10 mL		
	HDPE bottle, amber, 1 L	2		
	Nitrile gloves (pair)	1		
	Lab safety glasses (pair)	1		
	Field data sheets (print on waterproof paper, write in pencil)	10		

Maximo Item No.	Item Description	Quantity	Habitat-Specific	Special Handling
	Specimen labels (waterproof paper)	2 sheets		
	Battery charger (electrofishing batteries)	1		
	Reach establishment			
	Site-specific morphology map	1		
	Plot survey markers (aluminum, site-specific)	12		
	Flagging tape	1 roll		
	Meter tape (50 or 100 m)	1		
	Handheld GPS (with batteries, ± 1 m accuracy)	1		
	Electrofishing			
	Steel studded fence posts (i.e., T-post)	8		
	Fence post driver or small sledge	1		
	Fence post puller	1		
	3 mm mesh block nets with lead lines and top lines with floats	4		
	L-type block net stakes (e.g., ~45 cm long, 10 cm handle, 1 cm diameter stainless rod)	15		
	Net repair kit: • needle • string • butane lighter • zip ties	1		
	Battery-powered backpack electrofishing unit	1		
	Anode pole (1.5-2.0 m) with attached anode ring	1		
	Cathode (rattail type; 1-2 m and 10-15 mm in diameter)	1		
	Electrofisher batteries (rechargeable)	3		
	Abrasive pad to clean anode rings	1		
	6.4 mm mesh dip nets with fiberglass handles	4		
	Rubber lineman gloves (Class 0, rated for max use voltage 1,000V AC/ 1,500V DC)	1pair per person		
	5 gallon buckets	15		
	Hand held conductivity/temperature meter	1		
	Chest waders (approved for electrofishing)	1 pair per person		
	Chest wader repair kit (e.g., Aquaseal) or extra waders	1		
	Polarized sunglasses	1 pair per person		
	Minnow traps (use only if directed to do so by NEON HQ)			
	Gee minnow traps, galvanized steel, 6 cm mesh	40		

Maximo Item No.	Item Description	Quantity	Habitat-Specific	Special Handling
	Aquamax fish food (container)	1		
	Nylon rope, 1/2 inch diameter	200 m		
	Whirl-pak® sample bags, 24 oz.	40		
Fish processing				
	Fish and top predator taxonomic ID key specific to location or region (denotes endangered species)	1		
	Portable aerators (batteries, diffusion stone)	15		
	Small dip net (3.2 mm mesh)	5		
	Fish measuring boards (50 cm)	2		
	Portable digital scale (batteries, charger)	1		
	Plastic tray (for weighing fish)	2		
	Nitrile gloves (pair)	10 pairs		
	Digital camera (batteries, memory card)	1		
	Pencils	5		
	Clipboard	1		
	HDPE wide mouth specimen jars (1 L)	50		
	25-50 mL graduated cylinder, plastic	1		
	Nitrile gloves (pair)	10		
	MS-222 or clove oil stock solution	1 L		Y
	10% buffered formalin (37-40% formaldehyde)	20 L		Y

9.3 Preparation

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

1) Charge or replace batteries for backpack electrofishing unit, GPS unit, camera, portable scale, temperature/conductivity meter, and portable aerators overnight or longer r.
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 [09]).
8) Print data sheets (Appendix A, Appendix B) and specimen labels (Appendix C) on waterproof paper.
9) Select random sampling reaches if this is the first sampling date for the year (Section 9.5).
10) Mix MS-222 or clove oil stock solution (site-specific, depends on EHS permits) in the Domain Support Facility.
    a) MS-222
        i) Wear nitrile gloves and eye protection, as MS-222 is a hazardous substance.
        ii) Weigh 20 g of MS-222 powder and 50 g $NaNCO_3$.
        iii) Mix 20 g MS-222 + 50 g $NaNCO_3$ in a bucket with 1 liter tap water.

iv) Pour the stock solution into two 1 L amber HDPE bottles.
v) Label bottles "MS-222 stock solution".

b) Clove oil
   i) Clove oil is low in toxicity and not carcinogenic, however nitrile gloves and eye protection are recommended.
   ii) Mix 10 mL clove oil with 1 L of tap water.
   iii) Pour stock solution into 1 L amber HDPE bottles.
   iv) Label bottles "clove oil stock solution".
c) Stock solutions must be stored in dark bottles in a room-temperature (~70 ^{o}F) environment. Stock solution may be reused over sampling bouts.

9.4 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 3).
   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, 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) with a minimum length of 80 m and maximum of 120 m. 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 an aluminum plot survey marker on the bank at river right at each reach boundary (including the permit boundaries; Figure 3). Record the location of each marker on the handheld GPS unit and on the Reach Delineation Data Sheet (Appendix A).
   a) GPS points should be added to the site-specific stream morphology map (RD [10]) 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 (Appendix A). The right bank is preferred for consistency across sites.

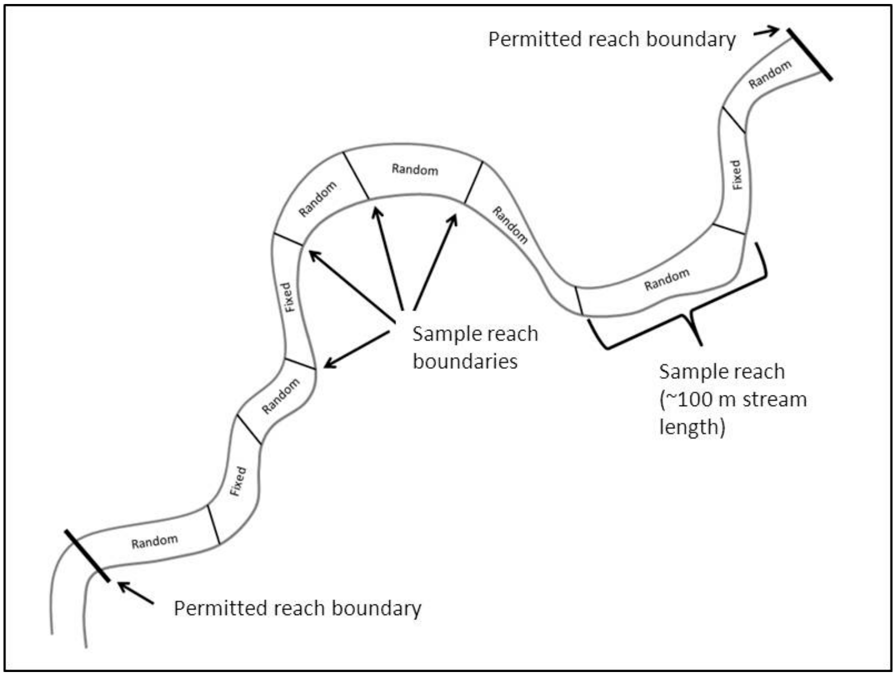

Figure 3. Schematic of 1 km permitted NEON stream site delineated into ten 100 m reaches: 3 fixed and 7 random sampling reaches. Three random reaches will be chosen each year for sampling

9.5 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 each establishment occurs.

1) Up to six 100 m (± 20 m) reaches (three fixed and three random) will be sampled during each sampling bout (Table 2).
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 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 or STREON baskets 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 D for a randomized order of reaches for each wadeable stream site.
4) Use the same three random reaches for the two sampling dates within one year (Table 2).

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 2. 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 D

	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										

9.6 Sample Collection in the Field

9.6.1 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 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) Pull the net across the stream ensuring enough slack in the lead line (bottom of net) to reach the stream substrate.
   d) Anchor lead line to substratum using large rocks or block net anchor stakes.
3) Place 5 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.

9.6.2 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, STREON baskets).

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

Figure 4. 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 5).

Figure 5. 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 12% (i.e., 4 ms pulse width), and output voltage to 250 V and turn the electrofishing unit on.
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 250 V to 350 V) and repeat Steps 9-10.
    a) The goal is to immobilize fish using the lowest settings possible at the site.
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 60 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 approximately 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 (Appendix B) 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 (Appendix B). Less than 1% of the captured fish should be injured.
    a) If > 1% of captured fish are injured, suspend sampling and submit a problem ticket.
    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 (Section 9.7).
15) Monitor captured fish for signs of normal respiration and swimming behavior for 10 minutes. If, after 10 minutes, fish are still on their side, upside-down, or injured, return to lower electrofishing settings.
    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.
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 (Appendix B) and reset the timer on the electrofishing unit. These settings will be used for the entire sampling bout.

9.6.3 Backpack Electrofishing

1) Proceed to the downstream blocknet of the first sampling reach.
2) Record start time on Field Data Sheet (Appendix B) so that conductivity and turbidity can be determined from the in-stream sensor sets.
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 Section 9.6.2 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.
8) Slowly sweep the anode inside (i.e., upstream) of block net 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 away from electrofishing activities.

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:
        i) Releasing the activation switch on the anode pole.
        ii) Inserting the anode into cover from the downstream direction and holding the anode temporarily still.
        iii) The electrofisher operator then depresses the activation switch as netters hold dip nets immediately downstream of the anode and cover.
        iv) The activation switch should continue to be depressed until the abundance of observable immobilized fish decreases.
        v) Continue electrofishing by moving the anode around the cover to immobilize additional fish, before continuing electrofishing.
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.
    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 (Appendix B).
    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 (Section 9.6.4).
    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).
    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.

9.6.4 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 6)

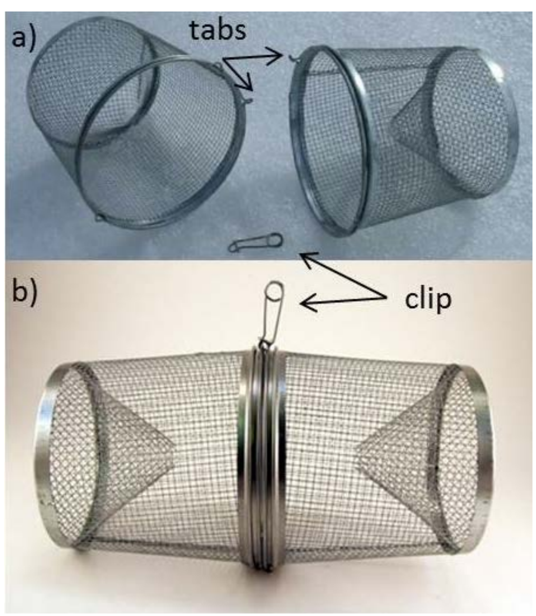

Figure 6. Gee minnow trap. a) The trap is split into two halves that are held together in the center by two tabs on one side and a clip on the other.
b) Assembled minnow trap.

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 (Appendix B).
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.
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 (Section 9.6.5).

9.6.5 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 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 (Appendix B).
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 bucket approximately half full with stream water (~10 L).
    b) For both MS-222 and clove oil, add 25 mL of anesthetic stock solution to 10 L stream water. Mix well (the small dip net makes a good mixer).
    c) 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 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.
   a) If this dose of anesthetic is insufficient, you may add stock solution in 5 mL increments until anesthetization is achieved.
   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.
10) Identify fish to species using the 4-letter species code (e.g., *Cottus cognatus* = COCO) and record on Field Data Sheet (Appendix B).
   a) If the species cannot be identified or identification is uncertain, weigh and measure following Steps 11-13, and euthanize the specimen.
      i) 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 (Appendix B).
      ii) Do not euthanize endangered species (site specific lists will be provided before sampling) or fish > 200 mm standard length. Photograph fish and record the camera image number on the Field Data Sheet along with the relevant weight and length information about the fish (Appendix B) before reviving and releasing.
      iii) Euthanize fish <200 mm standard length using a lethal dose of anesthetic (10 mL stock solution of either MS-222 or clove oil/L stream water) in the field.
      iv) Add 1 L of stream water and 10 mL of MS-222 or clove oil stock solution to a new 5 gallon bucket. Mix thoroughly.
      v) Transfer fish from the holding bucket to the bucket containing the anesthetic solution with the small handheld dip net.
      vi) Monitor fish until respiration ceases.
      vii) Place fish into appropriate sample container (e.g., wide mouth HPDE bottles) with completed specimen label (Appendix C). One taxon per specimen bottle.
   b) Photo voucher 1 representative specimen from each taxon.
      i) Include metric ruler for scale using the measuring board.
      ii) Photograph 1: Lateral photo with fish's head facing to the left.
      iii) 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 (Appendix B).
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 7) to the nearest mm and record on Field Data Sheet (Appendix B).

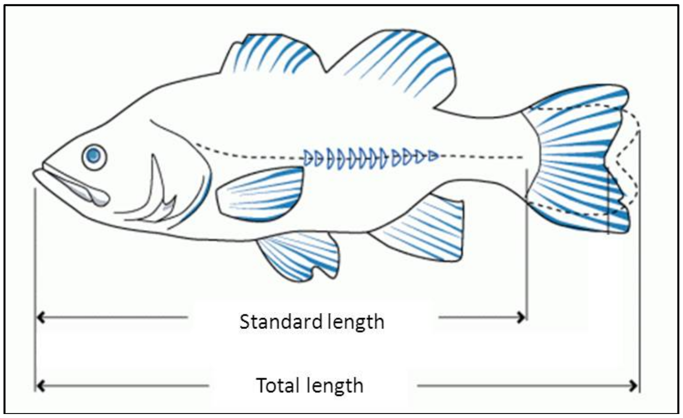

Figure 7. Measurement of standard and total length. Total length is measured by pinching the fork together.

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 (Appendix B).
14) Place processed fish in a bucket containing fresh stream water and a battery powered aerator for later release.
    a) Do not overcrowd fish in the reviving buckets, they need as much aerated water as possible.
15) 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.
16) Release the processed, revived fish back into the stream downstream of the blocknet.
    a) If mortality occurs during processing, save individuals for collections and note on Field Data Sheet (see Sample Preservation, Section 9.7).

9.7 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 in the field according to NEON EHS chemical hygiene guidelines (AD [03]).

9.8 Sample Shipping

1) Ship samples within 1 month of the end of the sampling bout.
2) 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.
3) Follow shipping and Hazmat procedures for formalin.
4) Place package inside appropriately-sized cooler or other sturdy shipping container. Add packing material, as necessary, to take up excess space in container.
5) Tape and label container for shipping.
6) Ground ship to Fish Taxonomist (*to be determined pending lab contracts*) for identification and long-term preservation.

9.9 Data Handling

1) Download all images from the camera and save in folder named "SiteCode_YYYYMMDD_SpecimenID".
2) Enter all data from the Field Data Sheets (Appendix A, Appendix B) within 14 days.
3) Archive/file all paper field sheets at the Domain Support Facility.
4) Upload data to the NEON Cyber-Infrastructure database.

9.10 Refreshing the Sampling Kit

1) Replace batteries for all battery operated equipment (e.g., GPS unit, portable aerators).
2) Refill/restock preservative and anesthetic stock solution containers.

9.11 Equipment Maintenance, Cleaning, and Storage

1) Wash all equipment that has come in contact with stream water according to the NEON Aquatic Decontamination Protocol (RD [09]).
2) Dry all equipment thoroughly between sites and before storage.
3) Check all nets for holes and patch if necessary.

10 REFERENCES

Bayley, P. B. and D. C. Dowling. 1990. Gear efficiency calibrations for stream and river sampling. Aquatic Ecology Technical Report 90/08, Illinois Natural History Survey, Champaign.

Bayley, P. B. and J. T. Peterson. 2001. An approach to estimate probability of presence and richness of fish species. Transactions of the American Fisheries Society 130:620-633.

Bonar, S., W. Hubert, and D. Willis (editors). 2009. Standard methods for sampling North American freshwater fishes. American Fisheries Society, Bethesda, Maryland.

Dunham, J. B., A. E. Rosenberger, R. F. Thurow, C. A. Dolloff, and P. J. Howell. 2009. Coldwater fish in wadeable streams. Pages 119-138 *in* S. A. Bonar, W. A. Hubert, and D. W. Willis, editors. Standard sampling methods for North American freshwater fishes. American Fisheries Society, Bethesda, Maryland.

Fischer, J. R. and C. P. Paukert. 2009. Effects of sampling effort, assemblage similarity, and habitat heterogeneity on estimates of species richness and relative abundance of stream fishes. Canadian Journal of Fisheries and Aquatic Sciences 66:277-290.

Goodchild, G. A. 1990. Electric fishing and safety. Pages 157-175 *in* I. G. Cowx and P. Lamarque, editors. Fishing with electricity, applications in freshwater fisheries management. Fishing News Books, Oxford, UK.

Lane, S. L. and R. G. Fay. 1997. Safety in field activities: U.S. Geological Survey techniques of water-resources investigations, Book 9, Chapter A9. Accessed 11 April 2013, at http://pubs.water.usgs.gov/twri9A9/.

Li, H. W. and J. L. Li. 2006. Role of fish assemblages in stream communities. Pages 489-533 *in* F. R. Hauer and G. A. Lamberti, editors. Methods in Stream Ecology, Second Edition. Academic Press, Boston, MA.

Meador, M.R., T.F. Cuffney, and M.E. Gurtz. 1993. Methods for sampling fish communities as part of the National Water Quality Assessment Program. USGS Open File Report 93-104. U.S. Geological Survey, Raleigh, North Carolina.

Meador, M. R. 2012. Effectiveness of seining after electrofishing to characterize stream fish communities. North American Journal of Fisheries Management 32:177-185.

Mercado-Silva, N. and D. S. Escandon-Sandoval. 2008. A comparison of seining and electrofishing for fish community bioassessment in a Mexican Atlantic Slope montane river. North American Journal of Fisheries Management 28:1725-1732.

Moulton, S. R., J. G. Kennen, R. M. Goldstein, and J. A. Hambrook. 2002. Revised protocols for sampling algal, invertebrate, and fish communities as part of the national water-quality assessment program. Open-file report 02-150, Reston, Virginia.

Onorato, D. P., R. A. Angus, and K. R. Marion. 1998. Comparison of a small-mesh seine and a backpack electroshocker for evaluating fish populations in a north-central Alabama stream. North American Journal of Fisheries Management 18:361-373.

Peck, D.V., A.T. Herlihy, B.H. Hill, R.M. Hughes, P.R. Kaufmann, D.J. Klemm, J. M. Lazorchak, F. H. McCormick, S.A. Peterson, P.L. Ringold, T. Magee, and M.R. Cappaert. 2006. Environmental Monitoring and Assessment Program-Surface Waters: Western Pilot Study field operations manual for wadeable streams. EPA/620/R-06/003. USEPA. Washington, DC.

Poos, M. S., N. E. Mandrak, and R. L. McLaughlin. 2007. The effectiveness of two common sampling methods for assessing imperiled freshwater fishes. Journal of Fish Biology 70:691-708.

Price, A. L. and J. T. Peterson. 2010. Estimation and modeling of electrofishing capture efficiency for fishes in wadeable warm water streams. North American Journal of Fisheries Management 30:481-498.

Quist, M. C., K. G. Gerow, M. R. Bower, and W. A. Hubert. 2006. Random versus fixed-site sampling when monitoring relative abundance of fishes in headwater streams of the Upper Colorado River Basin. North American Journal of Fisheries Management 26:1011-1019.

Rabeni, C. F., J. T. Peterson, J. Lyons, and N. Mercado-Silva. 2009. Warm water fish in wadeable streams. Pages 43-56 *in* S. A. Bonar, W. A. Hubert, and D. W. Willis, editors. Standard sampling methods for North American freshwater fishes. American Fisheries Society, Bethesda, Maryland.

Reynolds, J. B., and A. L. Kolz. 2013. Electrofishing. Pages 305-361 *in* A. V. Zale, D. L. Parrish, and T. M. Sutton, editors. Fisheries techniques, 3rd edition. American Fisheries Society, Bethesda, MD.

USEPA (U.S. Environmental Protection Agency). 2013. National Rivers and Streams Assessment 2008-2009 a collaborative survey. EPA/841/D-13/001. Office of Wetlands, Oceans and Watersheds and Office of Research and Development, Washington, D.C.

USFWS (U.S. Fish and Wildlife Service). 1992 Occupational safety and health series, Part 241 safety operations, FW 6 electrofishing. USFWS Service Manual, Washington, D.C.

APPENDIX A REAECH ESTABLISHMENT DATA SHEETS

NEON Wadeable Stream Fish Sampling: Reach Establishment

Domain:
Site:
Date:
Time:

Technicians:

Reach ID	Downstream coordinate	Upstream coordinate	Plot marker ID	Left or Right bank (plot marker)	Reach length (m)	Habitat features
1						
2						
3						
4						
5						
6						
7						
8						
9						
10						

Notes:

APPENDIX B FIELD DATA SHEETS

NEON Wadeable Stream Fish Sampling

Domain: ____________________ **Weather:** ____________________

Site: ____________________ ____________________

Date: ____________________ **Technicians:** ____________________

Reach ID: ____________________ ____________________

Water temp (^{o}C): ____________________ **Frequency:** ____________________

Conductivity: ____________________ **Duty cycle:** ____________________

Start time: ____________________ **Voltage:** ____________________

End time: ____________________ **EF time:** ____________________

#	Pass	Species	Total length (mm)	Weight (g)	DELT, electrofish injury	Comment/Photo ID

NEON Wadeable Stream Fish Sampling

Domain: ______________________ **Page _____ of _____**

Site: ______________________

Date: ______________________

Reach ID: ______________________

#	Pass	Species	Total length (mm)	Weight (g)	DELT, electrofish injury	Comment/Photo ID

APPENDIX C SPECIMEN LABELS

Domain: _ _ _ _ _ _ _ _ Site: _ _ _ _ _ _ _ _ Date: _ _ _ _ _ _ _ _ Reach ID: _ _ _ _ _ _ _ _ Pass #: _ _ _ _ _ _ _ _ Specimen #: _ _ _ _ _ _ _ _ Collector: _ _ _ _ _ _ _ _	Domain: _ _ _ _ _ _ _ _ Site: _ _ _ _ _ _ _ _ Date: _ _ _ _ _ _ _ _ Reach ID: _ _ _ _ _ _ _ _ Pass #: _ _ _ _ _ _ _ _ Specimen #: _ _ _ _ _ _ _ _ Collector: _ _ _ _ _ _ _ _	Domain: _ _ _ _ _ _ _ _ Site: _ _ _ _ _ _ _ _ Date: _ _ _ _ _ _ _ _ Reach ID: _ _ _ _ _ _ _ _ Pass #: _ _ _ _ _ _ _ _ Specimen #: _ _ _ _ _ _ _ _ Collector: _ _ _ _ _ _ _ _
Domain: _ _ _ _ _ _ _ _ Site: _ _ _ _ _ _ _ _ Date: _ _ _ _ _ _ _ _ Reach ID: _ _ _ _ _ _ _ _ Pass #: _ _ _ _ _ _ _ _ Specimen #: _ _ _ _ _ _ _ _ Collector: _ _ _ _ _ _ _ _	Domain: _ _ _ _ _ _ _ _ Site: _ _ _ _ _ _ _ _ Date: _ _ _ _ _ _ _ _ Reach ID: _ _ _ _ _ _ _ _ Pass #: _ _ _ _ _ _ _ _ Specimen #: _ _ _ _ _ _ _ _ Collector: _ _ _ _ _ _ _ _	Domain: _ _ _ _ _ _ _ _ Site: _ _ _ _ _ _ _ _ Date: _ _ _ _ _ _ _ _ Reach ID: _ _ _ _ _ _ _ _ Pass #: _ _ _ _ _ _ _ _ Specimen #: _ _ _ _ _ _ _ _ Collector: _ _ _ _ _ _ _ _
Domain: _ _ _ _ _ _ _ _ Site: _ _ _ _ _ _ _ _ Date: _ _ _ _ _ _ _ _ Reach ID: _ _ _ _ _ _ _ _ Pass #: _ _ _ _ _ _ _ _ Specimen #: _ _ _ _ _ _ _ _ Collector: _ _ _ _ _ _ _ _	Domain: _ _ _ _ _ _ _ _ Site: _ _ _ _ _ _ _ _ Date: _ _ _ _ _ _ _ _ Reach ID: _ _ _ _ _ _ _ _ Pass #: _ _ _ _ _ _ _ _ Specimen #: _ _ _ _ _ _ _ _ Collector: _ _ _ _ _ _ _ _	Domain: _ _ _ _ _ _ _ _ Site: _ _ _ _ _ _ _ _ Date: _ _ _ _ _ _ _ _ Reach ID: _ _ _ _ _ _ _ _ Pass #: _ _ _ _ _ _ _ _ Specimen #: _ _ _ _ _ _ _ _ Collector: _ _ _ _ _ _ _ _
Domain: _ _ _ _ _ _ _ _ Site: _ _ _ _ _ _ _ _ Date: _ _ _ _ _ _ _ _ Reach ID: _ _ _ _ _ _ _ _ Pass #: _ _ _ _ _ _ _ _ Specimen #: _ _ _ _ _ _ _ _ Collector: _ _ _ _ _ _ _ _	Domain: _ _ _ _ _ _ _ _ Site: _ _ _ _ _ _ _ _ Date: _ _ _ _ _ _ _ _ Reach ID: _ _ _ _ _ _ _ _ Pass #: _ _ _ _ _ _ _ _ Specimen #: _ _ _ _ _ _ _ _ Collector: _ _ _ _ _ _ _ _	Domain: _ _ _ _ _ _ _ _ Site: _ _ _ _ _ _ _ _ Date: _ _ _ _ _ _ _ _ Reach ID: _ _ _ _ _ _ _ _ Pass #: _ _ _ _ _ _ _ _ Specimen #: _ _ _ _ _ _ _ _ Collector: _ _ _ _ _ _ _ _
Domain: _ _ _ _ _ _ _ _ Site: _ _ _ _ _ _ _ _ Date: _ _ _ _ _ _ _ _ Reach ID: _ _ _ _ _ _ _ _ Pass #: _ _ _ _ _ _ _ _ Specimen #: _ _ _ _ _ _ _ _ Collector: _ _ _ _ _ _ _ _	Domain: _ _ _ _ _ _ _ _ Site: _ _ _ _ _ _ _ _ Date: _ _ _ _ _ _ _ _ Reach ID: _ _ _ _ _ _ _ _ Pass #: _ _ _ _ _ _ _ _ Specimen #: _ _ _ _ _ _ _ _ Collector: _ _ _ _ _ _ _ _	Domain: _ _ _ _ _ _ _ _ Site: _ _ _ _ _ _ _ _ Date: _ _ _ _ _ _ _ _ Reach ID: _ _ _ _ _ _ _ _ Pass #: _ _ _ _ _ _ _ _ Specimen #: _ _ _ _ _ _ _ _ Collector: _ _ _ _ _ _ _ _
Domain: _ _ _ _ _ _ _ _ Site: _ _ _ _ _ _ _ _ Date: _ _ _ _ _ _ _ _ Reach ID: _ _ _ _ _ _ _ _ Pass #: _ _ _ _ _ _ _ _ Specimen #: _ _ _ _ _ _ _ _ Collector: _ _ _ _ _ _ _ _	Domain: _ _ _ _ _ _ _ _ Site: _ _ _ _ _ _ _ _ Date: _ _ _ _ _ _ _ _ Reach ID: _ _ _ _ _ _ _ _ Pass #: _ _ _ _ _ _ _ _ Specimen #: _ _ _ _ _ _ _ _ Collector: _ _ _ _ _ _ _ _	Domain: _ _ _ _ _ _ _ _ Site: _ _ _ _ _ _ _ _ Date: _ _ _ _ _ _ _ _ Reach ID: _ _ _ _ _ _ _ _ Pass #: _ _ _ _ _ _ _ _ Specimen #: _ _ _ _ _ _ _ _ Collector: _ _ _ _ _ _ _ _

APPENDIX D 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	West Branch Bigelow Brook	9, 5, 3, 6, 8, 1, 2, 7, 4, 10
D01	Sawmill Brook	7, 9, 1, 5, 2, 3, 8, 10, 4, 6
D02	*Site to be determined*	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	McDowell Creek	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	Bozeman Creek	9, 7, 8, 10, 2, 5, 3, 6, 4, 1
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	Little Vasquez 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	Planting Creek	5, 6, 7, 9, 1, 2, 4, 3, 10, 8
D17	Convict Creek	9, 7, 3, 6, 5, 4, 8, 10, 1, 2
D17	Providence Creek	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