AOS PROTOCOL AND PROCEDURE: FISH SAMPLING IN WADEABLE STREAMS

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Change Record

REVISION	DATE	ECO #	DESCRIPTION OF CHANGE
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
D	05/16/2017	ECO-04493	CM updated with new template and changes based on feedback from FOPS. Fish tissue DNA sampling included. Fish euthanasia with AQUI-S20E.
E	04/03/2017	ECO-05577	Major updates include revised sample contingency timing. An updated equipment list for voucher containers, working gloves, and mobile field device. Modified wader requirements. Added a step for recording water quality before every electrofishing pass, measuring each fish sampling reach, option for using two backpack electrofisher units, and net tending procedures for by-catch and handling birds or small mammals. Procedures for establishing fish sampling reaches where the permitted reach is less than 500 m. Included additional resources for using the mobile field device. Fish voucher photos shall be collected and recorded for specimens associated with tissue samples only. Revised sample ID and added barcode instructions. Removed all references to JIRA. Field datasheets have been updated following these protocol revisions and the development of the fish mobile device application. Removed the photo log from the datasheets as photos shall only be recorded using the mobile device and application. Included net integrity components. Fish euthanasia with AQUI-S20E now 100 mg/L.
F	01/29/2019	ECO-05978	Clarified training and safety sections. Added endangered species handling guidance and reporting procedures. Once electrofisher settings have been established, the same setting can be used on subsequent sampling days so long as the conductivity stays with (+/-) 50 µS/cm. Updated the reach condition function in the Fulcrum application to allow “heavy vegetation” as a constraint. Revised the AQUI-S20E dose with recommendations from the USFDA for salmonids and non-salmonids. Stressed the importance for using the morphospecies ID format. Emphasized the requirement to use anesthesia when collecting fish tissue for DNA where permitted.

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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 Level 1, Level 2 and Level 3 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. 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.004104	NEON Science Performance QA/QC Plan
AD[06]	NEON.DOC.002979	NEON Animal Care and Use Program: Training Plan for Personnel Working with Live Vertebrate Animals

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.002652	NEON Level 1, Level 2 and Level 3 Data Products Catalog
RD[04]	NEON.DOC.001271	AOS/TOS Protocol and Procedure: Data Management
RD[05]	NEON.DOC.001646	General AQU Field Metadata Sheet
RD[06]	NEON.DOC.001152	NEON Aquatic Sample Strategy Document
RD[07]	NEON.DOC.004257	NEON Standard Operating Procedure: Decontamination of Sensors, Field Equipment, and Field Vehicles
RD[08]	NEON.DOC.003046	AOS Protocol and Procedure: Aquatic Macroinvertebrate Sampling
RD[09]	NEON.DOC.003162	AOS Protocol and Procedure: Wadeable Stream Morphology
RD[10]	NEON.DOC.002494	Datasheets for AOS Sample Shipping Inventory
RD[11]	NEON.DOC.003107	Datasheets for Fish Sampling in Wadeable Streams

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-S®20E	10% eugenol; fish anesthetic
Cm	Centimeter
DC	Direct Current
DNA	Deoxyribonucleic acid
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	U.S. Environmental Protection Agency
USGS	U.S. 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)

Base flow: 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).

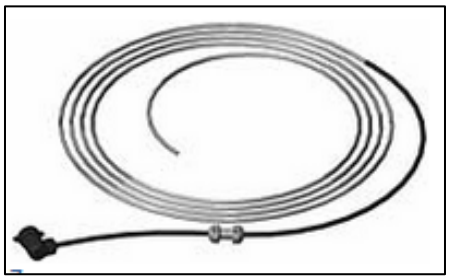

Figure 2. Cathode for backpack electrofishing unit (photo: store.smith-root.com)

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 bycatch. 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 are often unfeasibly large (e.g., >1,000 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).

Up to ten replicate non-overlapping ~100 m reaches are established to estimate species composition, species diversity, relative abundance, and an indication of the distribution of species within the NEON wadeable stream reach. Three permanent “fixed” reaches will be established and sampled twice every year using a three-pass electrofishing depletion approach. Fixed reaches should include representative habitat features that are found throughout the entire 1 km reach. The remaining seven reaches will be established as “random” reaches and are sampled following a stratified random design to ensure that the variety of habitat types are sampled equally over time. Three random reaches are sampled twice per year then a new set of three random reaches are sampled in subsequent years. Random reaches will be sampled via a single-pass depletion. Length and weight data will be collected as well as observations for

deformities, eroded fins, lesions, tumors, and parasites. Data collected from fixed three-pass depletion reaches can be used to estimate population sizes of fish within the random single-pass reaches.

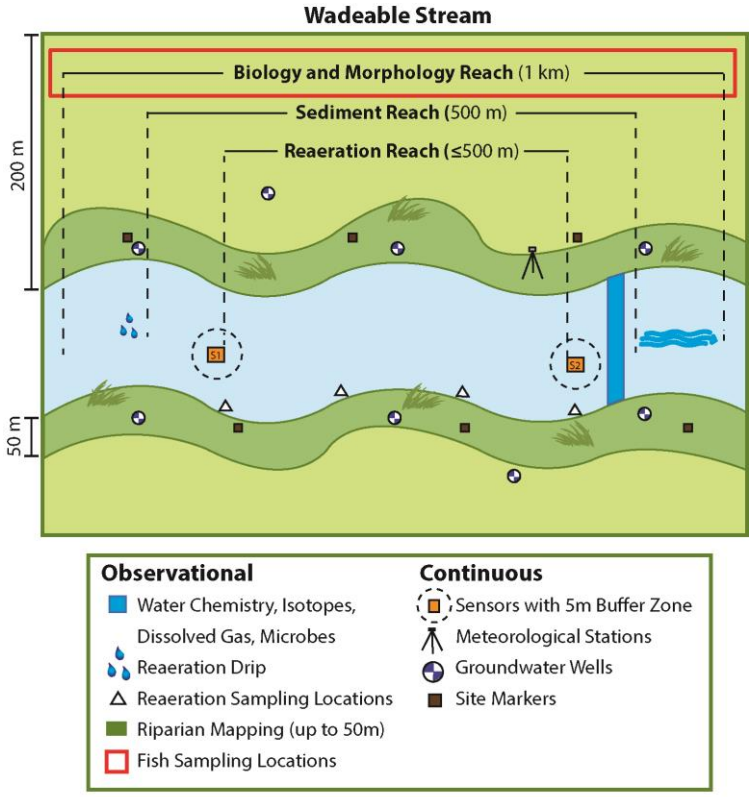

Figure 3. A generic wadeable stream site layout. Fish sampling will occur throughout entire biology and morphology reach.

This protocol also includes non-destructive methods for collecting fish tissues from live specimens in the field. A subset of captured fish will have tissue removed (fin clip) for DNA analysis. The DNA analysis will serve a number of purposes, including verification of taxonomy of specimens, clarification of the taxonomy of rare or cryptic species, and characterization of diversity using molecular markers. The goal of sampling tissues from fish is to investigate the DNA of targeted species without mortally damaging the specimen. Tissue samples collected in the field will be prepared for shipping to an external lab for genomic DNA extraction and purification, target DNA amplification of a marker region using polymerase chain reaction (PCR), and sequencing of the resulting PCR product. Any remaining extracted DNA will be archived at the external lab for future studies.

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 scientists **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 field scientists document the problem and enter it in NEON's problem tracking system.

Quality assurance will be performed on data collected via these procedures according to the NEON Science Performance QA/QC Plan (AD[05]).

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. The initial sample timing will be determined for each stream site using historical data including ice-out, water temperature (or accumulated degree-days), weather, and riparian peak greenness. Sample timing will be refined 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 bout windows for Macroinvertebrate Sampling in Wadeable Streams (RD[08]). Fish sampling must be scheduled within the specific bout window. Weather conditions at the site may push sampling outside of the bout window (RD[06]). Fish sampling should be scheduled as the last sampling activity of the bout to minimize impacts on other sampling objectives. Should fish sampling be scheduled before other biological sampling events, a minimum of 14 days is needed before periphyton or macroinvertebrate sample collection. If conditions do not allow for fish sampling to occur during bout 1, then sampling shall occur when safe conditions allow, up to 2 weeks before the start of bout 2. If conditions do not allow for fish sampling to occur during bout 3, then sampling shall occur when safe conditions allow up to 30 days beyond the end of bout 3.

A minimum of 2 weeks between sampling bouts shall be observed. Sampling bouts should not be longer than 5 days long assuming no weather or other unexpected schedule delays. All three passes in a fixed sampling reach must be sampled within a 24-hour period, 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

Fish and non-target voucher specimens may be stored for 1 month or longer following the preservation guidelines in SOP F.6. For storage and shipping timelines see SOP H. Fish fin clips may be taken from a maximum of 5 individuals per species per year for DNA analysis. In some cases, FOPS may need to store fin clip samples at the Domain Support Facility until instructed to ship for processing. The tissue samples will be kept in the labeled sample vials with ethanol (70-95%) and refrigerated or frozen until directed to ship the samples. DNA tissue samples can be stored in these conditions for up to 12 months. In addition, individual domain facilities will temporarily store preserved voucher specimens of fish (target species) as well as any amphibians or reptiles (non-target specimens) inadvertently injured and euthanized or killed during fish sampling activities. Vouchered specimens will be shipped to a designated external facility for long-term storage.

4.4 Sampling Timing Contingencies

Fish sampling in wadeable streams 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 (Table 1).

If sampling is impractical as a result of severe drought (dry), the stream is frozen, or that access to portions of the reach are blocked then indicate this for any affected reach on the mobile device or the *Wadeable Streams Fish Sampling Field Datasheet* (RD[11]). Should environmental conditions or resource availability impact the ability to fully execute the protocol for an entire bout, at a minimum, fully sample one fixed stream reach (one 3-pass electrofisher survey). Note the cause of the minimized sampling effort on the "Reach Condition" section in the mobile field device or on the *Wadeable Streams Fish Sampling Field Datasheet* (RD[11]). Also, as stated in Section 4.1, if conditions do not allow for fish sampling to occur during bout 1, then sampling shall occur when safe conditions allow up to 2 weeks before the start of bout 2. If conditions do not allow for fish sampling to occur during bout 3, then sampling shall occur when conditions allow up to 30 days beyond the end of bout 3.

Table 1. Contingent decisions

Delay/ Situation	Action	Outcome for Data Products
Hours	If flooding or other weather event occurs during electrofishing activities which necessitates premature cessation of sampling then 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 biological bout sampling window by more than three days, submit a trouble ticket through the NEON problem resolution system.
3-7 Days	If heavy rain or snowfall affects visibility, air temperatures drop to unsafe conditions, or flooding/high water occurs on or prior to the targeted sampling date, wait a minimum of 3 days to allow for safe conditions to return. This will also allow the fish community to recolonize habitats following a flooding/high water event.	None as long as samples are collected within the pre-determined sampling window. If waiting for favorable conditions causes sampling to occur outside of the biological bout sampling window by more than three days (or beyond the extended contingency windows; see above), submit a trouble ticket through the NEON problem resolution system.

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. Under ideal conditions fish sampling shall 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 conditions are safe to sample and when the entire sampling bout can be completed in 5 days. If fish sampling is interrupted and the resumption of fish sampling is expected to occur three days past the biological bout, (or beyond the extended contingency windows; see above) submit a trouble ticket (Table 1).
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 limiting the collection of voucher specimens.
4. Fish sampling-related injuries resulting from electrofishing, netting, and processing should affect < 3% of fish captured cumulatively at the reach-scale. If this number is exceeded at the site, stop sampling, release fish, and contact the NEON Aquatic Ecologist, Domain Manager, and submit a trouble ticket using the NEON problem tracking system.
    a. Please include the following information when submitting a trouble ticket for reporting fish injuries and mortality that exceeds 3%:
        1) Electrofisher settings: voltage, duty cycle, and frequency

2) Water quality: conductivity, dissolved oxygen, and temperature
3) Field observations and most likely root cause of injury or mortality
4) Description of species affected
5) Include any additional information that could help identify the root cause and for developing a solution (e.g. anode ring diameter and shape)

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 Scientist 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. Field scientists 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. Field scientists 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 (93 L/s; Lane and Fay 1997).
5. Safety Datasheet information shall be readily available to field scientists working with chemicals included in this protocol. Field scientists must also be trained in safe handling of formalin (AD[03]).
6. Field scientists must take the Hand and Power Tool Training for Field Operations prior to using fence post drivers and pullers and must use impact-resistant gloves for this task.

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

1. One member of the fish sampling crew must be fully trained by the equipment manufacturer 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 field scientists when electrofishing equipment is in operation.
3. Chest waders and heavy-duty rubber lineman gloves (rated to 1,000V AC/1,500V DC) must be worn while working near an electrofishing unit.

a. The requirements for wader and boot selection has been adapted by the recommendations of the American Fisheries Society Professional Safety Committee (2008).
    1) Non-breathable materials provide the most insulation against electrical shock and include PVC, neoprene, or silicon. Non-breathable waders with built-in lug-sole boots are the preferred wader style.
    2) Breathable materials including Gore-Tex® provide less insulation against electrical shock, but they may be more comfortable in warmer conditions and in environments with a lot of scrambling over boulders. The use of breathable waders with stocking feet is acceptable as long as the operator also wears dry clothing that covers any exposed skin while in the waders. Stocking foot style waders with a separate boot may be used but only at a single designated site.
    3) Studded-sole boots are allowed if they help secure footing in slippery conditions.
    4) Stocking foot waders with the built-in gaiter ankle cuff are difficult to decontaminate. This style of wader may only be used if dedicated to a single site and not transferred across sites.
    5) Felt-soles are prohibited.

b. At stream sites where the water level is less than knee deep at the maximum depth, rubber boots or hip waders are allowed. Follow the wader material guidance above.

4. Before sampling, inspect waders, boots, and gloves for leaks. Leave the water immediately if waders or gloves develop leaks.
5. A portable automated external defibrillator (AED) must be available in the field for all electrofishing work. The AED and First Aid Kit can be stored in a work vehicle, boat, or other known location as long as it is within a 6-minute walk from the active sampling location.
6. Avoid operating near bystanders, pets, or livestock that are in or near the water.
7. Electrofishing must be suspended if anyone feels a shock, however minor, for investigation and repair of equipment.
8. 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 2. Equipment list – Field preparation

Item No.	Supplier	Supplier ID	R/S	Description	Purpose	Quantity	Special Handling
Durable items							
MX100345	Thomas Scientific, Inc.	1185B56	R	HDPE bottle, amber, 1 L	Stock solution ofMS-222 container	2	N
MX106819			R	Lab safety glasses	Safe handling of chemicals	1 pair	N
MX111388	CDW-G	4452963	R	Mobile field data recording device (Tablet)	Recording data	1	N
MX100514	Thomas Scientific, Inc. Fisher Scientific Company	1185K52 15177622	R	Multisonde	Measuring % DO, temperature and salinity	1	N
Consumable items							
MX106819	Fisher Scientific Company	AC118000500	R	Tricaine methanesulfonate (MS-222)	Euthanizing specimens	20 g	Y

Item No.	Supplier	Supplier ID	R/S	Description	Purpose	Quantity	Special Handling
MX106431	Grainger, W.W. Amazon Capital Services Inc.	33X679 B00BXOQK6K	R	$NaHCO_3$ (baking soda)	Buffering agent for MS-222	50 g	N
MX110318	AquaTactics Fish Health	AQUIS20E	R	10% eugenol (AQUI-S®20E)	Anesthetizing specimens	50 mL	Y
			R	Nitrile gloves (latex-free and powder-free; pair)	Safe handling of chemicals and fish	1	N
MX103942	Ben Meadows Co., Inc. Forestry Suppliers, Inc.	010510-1 49247	R	Field datasheets (print on waterproof paper, write in pencil)	Recording data	10	N
MX103942	Ben Meadows Co., Inc. Forestry Suppliers, Inc.	010510-1 49247	R	Specimen labels (waterproof paper)	Labeling specimens	2 sheets	N
			S	Adhesive barcode labels	Labeling sample bottles with barcode-readable labels	1 sheet	N
			R	Collection permit documents		1	N

R/S=Required/Suggested

Table 3. Equipment list – Reach establishment

Item No.	Supplier	Supplier ID	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
MX104369 MX100318	Ben Meadows Co., Inc. Forestry Suppliers, Inc.	213379 or 122733 37184 or 39986	R	Meter tape (50 or 100 m)	Establishing sampling reaches	1	N
MX110075 MX102739	Forestry Suppliers, Inc. Cabela's Inc. Recreational Equipment Inc.	39481 IK-270217 895022	R	Handheld GPS (with batteries, ± 1 m accuracy)	Navigating to sampling reaches	1	N
Consumable items							
MX103940	Grainger, W.W. Forestry Suppliers, Inc.	9WKP4 57880	R	Flagging tape	Establishing sampling reaches	1 roll	N

R/S=Required/Suggested

Table 4. Equipment list – Electrofishing

Item No.			R/S	Description	Purpose	Quantity	Special Handling
Durable items							
MX107115	Grainger, W.W.	4LVG6	R	Steel studded fence posts (i.e., T-post)	Securing block net at reach boundary	8	N
MX104756	Forestry Suppliers, Inc.	67251	R	Fence post driver or small sledge	Securing block net at reach boundary	1	N
MX110250			R	Fence post puller	Removing block net fence posts at reach boundary	1	N
	The Fish Net Company, LLC Duluth Nets		R	3 mm mesh block nets with lead lines and top lines with floats (custom-built for each site)	Catching drifting specimens	3	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
	Amazon Capital Services Inc.	B00XBFARBI	R	Net repair kit: • needle • net string • butane lighter	Repairing nets	1	N

Item No.			R/S	Description	Purpose	Quantity	Special Handling
				• zip ties			
			R	Gloves, Nitrile coated, Textured, Knitted cuff, Abrasion resistant, PIP or Equivalent	Handling gill nets and removing entangled birds or mammals	1	N
MX106855	Smith-Root	9632	R	Battery-powered backpack electrofishing unit	Electrofishing	1	N
MX106846	Smith-Root	7575	R	Anode pole (1.5-2.0 m) with attached anode ring	Electrofishing	1	N
MX106848	Smith-Root	6821	R	Cathode (rattail type; 1-2 m and 10-15 mm in diameter)	Electrofishing	1	N
MX110472 or MX106849	Smith-Root	06682 10765	R	Electrofisher batteries (rechargeable)	Electrofishing	3	N
MX106854	Smith-Root	10167	R	Battery charger (electrofishing batteries)	Charging the electrofisher	1	N

Item No.			R/S	Description	Purpose	Quantity	Special Handling
	Amazon Capital Services Inc.	B001KYQBX0	R	Abrasive pad/steel wool to clean anode rings	Electrofishing	1	N
	N&K Dip Nets	various	R	Mesh dip nets with fiberglass handles (a variety of sizes are available, select an appropriate size net for the specific sampling conditions)	Catching immobilized specimens	4	N
MX110609 - MX110613	Smith-Root	3106	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
MX100526	Grainger, W.W.	34A216	R	5 gallon buckets	Storing specimens	10	N
			R	Wrench (9/16th)	Used to tighten the anode ring to the fiberglass pole	1	N
MX100491 MX100494 MX107505	Ben Meadows Co., Inc. Grainger, W.W. Forestry Suppliers, Inc. Cabela's		R	Chest waders (approved for electrofishing)	Safe wading	1 pair per person	N

Item No.			R/S	Description	Purpose	Quantity	Special Handling
MX106824	Amazon Capital Services Inc.	B0000DCVYK	R	Chest wader repair kit (e.g., Aquaseal) or extra waders	Safe wading	1	N
MX100479	Grainger, W.W.	21V915	R	Polarized sunglasses (amber or brown lenses recommended)	Increasing efficiency of fish capture	1 pair per person	N
Consumable items							
MX110621	BioQuip Products Inc. Fisher Scientific Company	1426B NC0095946	R	Fish viewer	Viewing individual fish and taking photos	1	N
MX100689	Fisher Scientific Company, LLC	8732115	R	Plastic weighing boat	For weighing fish under 75 mm	1	N

R/S=Required/Suggested

Table 5. Equipment list – Fish processing

Item No.	Supplier	Supplier ID	R/S	Description	Purpose	Quantity	Special Handling
Durable items							

Item No.	Supplier	Supplier ID	R/S	Description	Purpose	Quantity	Special Handling
			R	Fish and top predator taxonomic ID key specific to location or region (denotes endangered species)	Identifying specimens	1	N
MX106815	Ben Meadows Co., Inc.	227438	R	Portable aerators (batteries, diffusion stone)	Aerating buckets	15	N
MX106832	Amazon Capital Services Inc.	B00H51AIYK	R	Small dip net (3.2 mm mesh)	Handling specimens	5	N
MX100481	Forestry Suppliers	77310	R	Fish measuring boards (60 cm)	Measuring specimen length	2	N
MX106902	Amazon Capital Services Inc.	B0051W81P8	R	Portable digital scale (batteries, charger)	Weighing specimens	1	N
MX100338	BioQuip Products Inc. Fisher Scientific Company	1426B NC0095946	R	Plastic tray	Weighing specimens	2	N
MX103527	B&H Photo Corp	TICSGS KOCSGS	R	Color separation guide	Photographing specimens	1	N
MX100379 MX100380	Fisher Scientific Company Thomas Scientific, Inc.	0300739 3571A20	R	25-50 mL graduated cylinder, plastic	Mixing anesthetic and euthanizing solution	1	N
MX107197	Fisher Scientific Company	S17337	R	Fin clipping scissors	Cutting fish fins for DNA barcode samples	1	N

Item No.	Supplier	Supplier ID	R/S	Description	Purpose	Quantity	Special Handling
MX103202	Fisher Scientific Company	S65023	R	Refillable butane lighter	Flame sterilization of tissue sampling equipment	1	Y
			R	Dish pan, plastic	Disinfecting tools with iodophor or betadine	1-12 qt.	N
Consumable items							
			R	Nitrile gloves (pair)	Safe handling of chemicals and fish	10	N
MX101218 MX100574 MX100652 MX100665	Fisher Scientific Company	033134A 033134B 033134D 033134E 033134F	R	HDPE wide mouth specimen jars (25 mL, 60 mL, 250 mL, 500 mL, and 1 L)	Specimen preservation containers	50	N
MX106819	Fisher Scientific Company	AC118000500	R	Tricaine methanesulfonate (MS-222) stock solution	Euthanizing specimens	1 L	Y
MX110318	AquaTactics Fish Health	AQUIS20E	R	AQUI-S®20E stock solution	Anesthetizing specimens	1 L	Y
MX106257	Thomas Scientific, Inc.	C998K62	R	10% buffered formalin (3.7-4.0% formaldehyde)	Preserving specimens	20 L	Y
MX101221	Fisher Scientific Company	BD305125	R	Needle, disposable 1 inch, 25 gauge, sterile	Injecting reptiles with MS-222	1 pack	Y

Item No.	Supplier	Supplier ID	R/S	Description	Purpose	Quantity	Special Handling
MX106200	Thomas Scientific, Inc. Fisher Scientific Company	8956A70 1482648	R	Needle, disposable 1 inch, 27 gauge, sterile	Injecting reptiles with MS-222	1 pack	Y
MX106261	Thomas Scientific, Inc. Fisher Scientific Company	1227H00 14823220	R	Syringe, 5mL luer lock syringe	Measuring and injecting euthanasia fluids	1 pack	Y
MX100213 MX100202	Fisher Scientific Company Thomas Scientific, Inc.	4355601 C954K61	R	Ethanol (70 -95%)	For preserving fin clips for DNA and sterilizing DNA equipment	250 mL	Y
MX100213 MX100202	Fisher Scientific Company Thomas Scientific, Inc.	4355601 C954K61	R	Ethanol (95%)	Euthanizing invertebrates	250 mL	Y
	Amazon Capital Services Inc.	B007R64URO	R	Iodophor or betadine disinfectant	Required by some States for decontaminating equipment	1 L	N
MX100549	VWR	82018048	R	50mL Centrifuge tube	Store syringe and needle, measure injection fluids	1	Y
MX101218	Fisher Scientific Company	033134B	R	60mL Clear bottle HDPE	Store dry MS-222 and baking soda for reptile euthanasia stage 1 solution	1	Y
MX101278	Thomas Scientific, Inc. Fisher Scientific Company	9718J20 0333723C	R	20mL HDPE Scintillation vial	Store dry MS-222 for reptile euthanasia stage 2 solution	1	Y

Item No.	Supplier	Supplier ID	R/S	Description	Purpose	Quantity	Special Handling
MX103240	Grainger, W.W.	2TUW7	S	Sharps Container, Portable, Slip Top	Receptacle for disposal of needles	1	N
	Thomas Scientific, Inc.	1236C14	R	Tissue containers (e.g. 1.5 mL microcentrifuge tubes)	For storing fin clips for DNA barcoding	100	N

R/S=Required/Suggested

6.2 Training Requirements

All field scientists must complete protocol-specific training for safety and implementation of this protocol as required in Field Operations Job Instruction Training Plan (AD[04]). Also, refer to the NEON Animal Care and Use Program: Training Plan for Personnel Working with Live Vertebrate Animals (AD[06]).

All personnel participating in fish sampling for NEON are to be trained in fish sampling safety for water-based fieldwork. Specific training for fish sampling must also include electrofishing training for all field scientists. All lead aquatic field scientists and those designated by their manager shall be required to receive an electrofishing safety training prior to operating a backpack electrofisher. The backpack electrofisher manufacturer (Smith-Root) will provide this training. Additionally, all field scientists shall complete the U.S. 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. Field scientists must pass the Electrofishing Safety Exam with a score >80%. Additional training resources include Wader Safety training which is available through the NCTC Electrofishing Safety training resource videos. Lastly, all field scientists shall complete the Collaborative Institute Training Initiative's (CITI) Institutional Animal Care and Use Committee (IACUC) fish and amphibian training. Field scientists must pass the final tests for each training (fish and amphibian) with an 80% or better. The CITI IACUC trainings are good for up to three years. Provide your manager with a copy of each applicable certificate documenting the successful completion of each required training. See the AFS Fisheries Safety Handbook for additional fish sampling safety information.

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 possess a current CPR/AED/First Aid training.

External Training Resources:

USFWS NCTC CSP2202-OLT Electrofishing Safety Course: Resources include presentation (PowerPoint and video) and the final exam (free; registration is not required):
http://nctc.fws.gov/courses/csp/csp2202/resources/index.html

USFWS NCTC Wader Safety Video: Produced by Utah State University
https://fws.rev.vbrick.com/#/videos/3acea0c2-b819-43e8-896b-5c75067d221c

CITI IACUC Fish and Amphibian training. Register through the National Ecological Observatory Network organization affiliation (affiliation "National Ecological Observatory Network, Inc.")

https://www.citiprogram.org/index.cfm?pageID=154&icat=0&clear=1

American Fisheries Society Fisheries Safety Handbook. AFS 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 three-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 a minimum of three qualified field scientists for up to 5 consecutive field days. There is no lab processing at the Domain Support Facility associated with this protocol. Fish tissue samples will be shipped to a central Domain Support Facility (D09) for processing annually. The processing Domain will ship processed tissues 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 shipped to external facilities by domain staff. Sample preservation and archival may require up to 10 minutes of processing per specimen for domain staff.

7 STANDARD OPERATING PROCEDURES

SOP A Preparing for Data Capture

Mobile applications are the preferred mechanism for data entry. Data should be entered into the protocol-specific application as they are being collected, whenever possible, to minimize data transcription errors and improve data quality. For detailed instructions on protocol-specific data entry into mobile devices, see the NEON Internal Sampling Support Library (SSL). Mobile devices should be synced at the end of each field day, where possible; alternatively, devices should be synced immediately upon return to the Domain Support Facility. However, given the potential for mobile devices to fail under field conditions, it is imperative that paper datasheets are always available to record data. Paper datasheets should be carried along with the mobile devices to sampling locations at all times.

SOP B Preparing for Sampling

1. Review the federal and/or state collection permit thoroughly.
2. Be sure to notify the site host of the dates and times of the fish sampling activities.
3. Retain a copy of the collection permit during the sampling activities.

Begin preparations at least two days before going to the field to allow batteries to fully charge. It also good practice to field test the equipment several days to one week ahead of the sampling date.

1. **VERY IMPORTANT:** Charge or replace batteries for backpack electrofishing unit, GPS unit, mobile field data recording device with 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). Carefully inspect the metal surfaces of the anode ring and cathode for corrosion. Remove corrosion using an abrasive pad or steel wool to gently scrub the surface.
3. Inspect waders for holes and tears. Repair waders if necessary.
4. Inspect lineman gloves DO NOT repair torn lineman gloves. Discard them and order a replacement pair.
5. Inspect dip nets and block nets for rips, tears, and holes. Repair, if necessary.
6. Inspect portable aquarium pumps, diffusion stones, and batteries.
7. Inspect buckets to ensure handles are present and functioning.
8. Ensure that all equipment has been decontaminated since last use (see RD[07]).
9. Print datasheets and specimen labels (RD[11]) on waterproof paper. Verify that the mobile data entry device is charged and synced prior to use.
10. Select random sampling reaches if this is the first sampling date for the year (SOP D).
11. Fish will be anesthetized and euthanized with 10% eugenol (AQUI-S®20E). Non-target species will not be anesthetized; however, mortally injured non-target species shall be euthanized with Tricaine methanesulfonate (MS-222).

a. **10% eugenol (AQUI-S®20E):** 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. Also, make sure that the batch of AQUI-S®20E has not expired. Any questions regarding this program or how to complete the field datasheets should be directed to the study monitor or the investigator responsible for reporting the study results.
   1) 10% eugenol should be added directly to the treatment water. Do not make stock solutions or other dilute solutions of 10% eugenol.

b. **Tricaine methanesulfonate** (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 away from light and refrigerated at 4°C. Stock solution may be stored and used in one month from the date of preparation.
   8) Discard unused solution in the lab following the NEON EHS chemical hygiene guidelines (AD[03]).

SOP C 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. Complete the data entry on the mobile device or *Wadeable Stream Fish Sampling Reach Establishment Field Datasheet* (RD[11]).
2. Ensure the *General AQU Field Metadata Sheet* (RD[05]) is completed per field site visit.
3. Using a field measuring tape, measure out ten, non-overlapping, 100 m (± 20 m) reaches, starting ~20 m above the downstream permit boundary (Figure 4). This measurement is best taken by following the thalweg of the main channel.
   a. Leave 20 m at the downstream boundary to allow for space to test the electrofisher and to release 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. See Appendix G, Site-Specific Information: Shortened Sampling Reach for a fish sampling study design with a reach that is less than 500 m.

4. Install a permanent aluminum plot survey marker on the bank at river right at each reach boundary (including the permit boundaries; Figure 4). Each fish sampling reach is numbered sequentially beginning with "1" at the bottom (downstream) but just above the test reach (Figure 4). Record the coordinate at the plot marker location as well as the coordinate uncertainty using the Trimble data dictionary for AOS locations. If the Trimble is not available at the time of transect selection, record on the Reach Establishment Datasheet (RD[11]) and return at a later date to record locations using the Trimble.
   a. Refer to the Morphology Mapping (RD[09]) protocol for further descriptions and examples of stream habitats.
   b. If you are unable to install plot survey markers on the right bank (river right), use the left bank and note on the AOS Trimble data dictionary. If the Trimble is not available at the time of transect selection, record on the Reach Establishment Datasheet (RD[11]) and return at a later date to record locations using the Trimble. The right bank is preferred for consistency across sites.

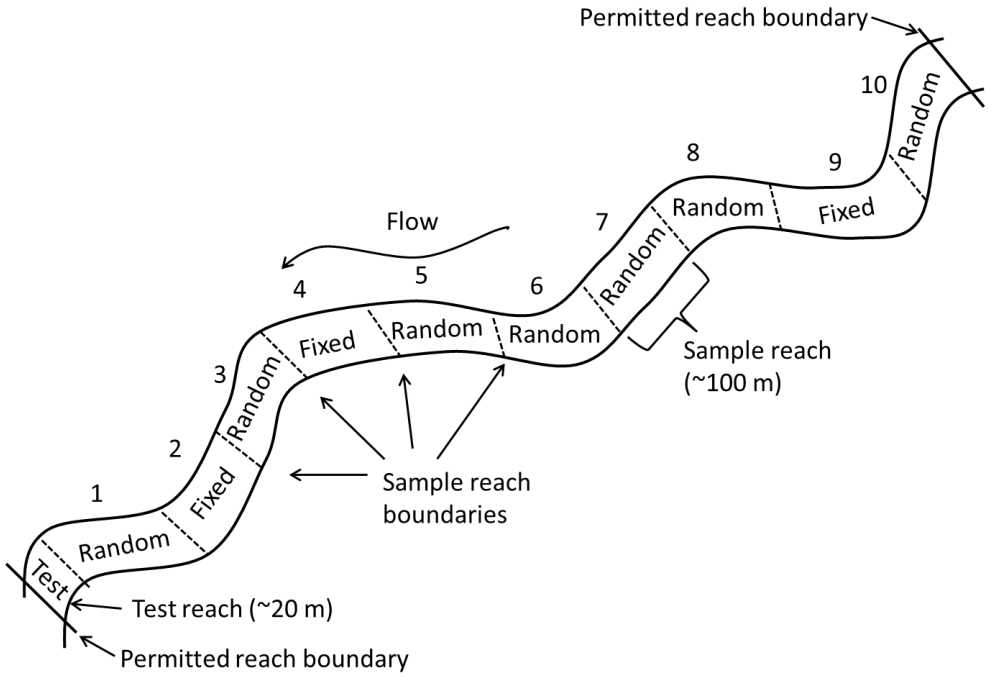

Figure 4. Schematic of a 1 km permitted NEON stream site delineated into ten 100 m reaches: 3 fixed and 7 random sampling reaches. Three random reaches will be chosen each year for sampling.

SOP D 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 the stream morphology changes significantly, as detected by the results of the Wadeable Stream Morphology Protocol, then it may be necessary to establish new reaches or the entire fish sampling reach. Submit a trouble ticket through the NEON problem resolution system.

1. Up to six 100 m (± 20 m) reaches (three fixed and three random) will be sampled during each sampling bout (Table 6).
2. Electrofishing in reaches with sensors (S1 or S2) must occur ≥5 m away from all in-stream electronics.
3. 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 Field scientist.
4. 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.
5. Use the same three random reaches for the two sampling dates (bouts) within one year (Table 6).
6. 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.
7. 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 E Field Sampling

E.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. Locate fish reach boundaries at narrow sections of the reach if possible.
   b. Always secure the downstream block net first, followed by the upstream net.
   c. Secure a 3 mm mesh block net at the reach boundary using steel fence posts or existing vegetation (e.g., tree).
   d. 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.
   e. Pull the net across the stream ensuring enough slack in the lead line (bottom of net) to reach the stream substrate.
   f. Anchor lead line to substratum using large rocks or block net anchor stakes. Sand bags may be used where native sand, gravel, or rocky material is available. Fill the sand bags with native material collected from the shore and place along the anchor line to secure the block net.
   g. Minimize the length of time and physical disturbance of the habitat (suspension of sediments) while establishing the sampling reach.
3. Stage 5-gallon buckets with battery-operated aerators on the bank along the stream reach for holding fish during electrofishing. For stream sites where hundreds of fish are typically captured, use several buckets staged every 15-25 m though out the sampling reach. Be sure to have one crew member rinse buckets with native water and monitor captured fish.
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, and sample bottles, preservative) at this location.

E.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 field scientists 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. Determine if the intended sampling site requires the use of two backpack electrofishers. It is recommended that two backpack electrofisher units are used to sample wadeable stream reaches with a wetted width of more than 7 m (Johnson et al 2007). See Appendix F, Site-Specific Information: Two Backpack Electrofishers Approach.
3. Assemble anode pole (Figure 1).
4. Measure the water temperature and conductivity using the handheld conductivity meter at the test reach.
5. Connect the cathode and anode to the backpack electrofishing unit (Figure 5).

Figure 5. Cathode and anode connections on backpack electrofishing unit

6. Connect the battery to backpack electrofishing unit and secure the batteries with the strap to the backpack frame (Figure 6). There is a notch that the strap goes through at the battery handle. If using the lithium ion battery, be sure to include the adapter.

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

7. Test the backpack electrofisher in the 20 m section that was left between the downstream permit boundary and the closest sampling reach.
8. 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).
9. 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. At sites where conductivity exceeds 500 µS/cm, initial setting should be as follows: 50 V, 15 Hz, and 30% duty cycle.

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	

    a. When electrofishing in low conductivity water (<100 µS/cm) the following settings have been used to successfully immobilize fish: frequency 30 Hz, duty cycle to 50%, and output voltage between 500 V to 700 V.

10. Pause to verbally confirm settings on electrofisher and that the unit is turned on. Also, confirm that all field scientists 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.
11. Press and hold the activation switch down, and observe the behavior of fish.
    a. **Note:** Be sure to review the collection permit requirements and stay within the approved electrofisher settings.
12. 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 8-11.
    a. The goal is to immobilize fish using the lowest settings possible at the site to avoid harming sampled fish.
    b. Signs that fish are responding to the electrofisher settings include swimming toward the anode ring and flashing of the ventral (belly) portion of the fish. Be sure to check for immobilized fish near the cathode.
13. If 1,100 V is reached and fish are still not responding to electrofishing decrease voltage to 250 V and increase the frequency by 10 Hz (e.g., from 30 Hz to 40 Hz) and repeat Steps 9.a-11.
    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 14.
14. Continue electrofishing until up to 20 individuals spanning a variety of sizes are netted.
    a. It is possible that no fish are captured within the test reach. If this occurs, use the most conservative settings on the electrofisher and commence to sampling as described in SOP E.2.
15. 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 to the lowest taxonomic level on the mobile device or the *Wadeable Stream Fish Sampling Field Datasheet* (RD[11]) and immediately release >20 m away and downstream from electrofishing activity.
16. Examine captured fish for signs of injury (e.g., bent backs, dark bruising, hemorrhaging of the gills; Figure 7). Record injury rate on the mobile device or the *Wadeable Stream Fish Sampling Field Datasheet* (RD[11]). No more than 3% of the captured fish should be injured.

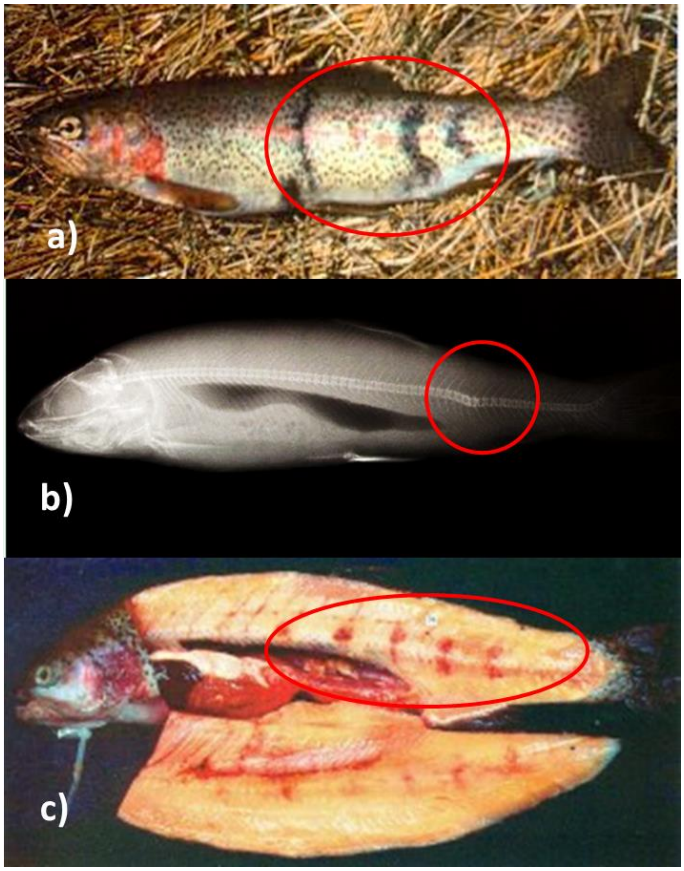

Figure 7. Examples of electrofishing injuries to fish, a) bruising or "burn" marks, b) spinal injury, and c) internal hemorrhaging. Photos from Snyder 2003.

a. If > 3% of captured fish are injured, suspend sampling and contact the domain manager and submit a trouble ticket through the NEON problem resolution system.
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 Euthanizing Fish and Non-Target Species (SOP F.4).

17. 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.
18. Once fish are swimming normally, release back into the stream near where they were caught and within the permitted boundary.

19. Maintain electrofisher settings at the lowest level that allows for the effective capture of fish. Record frequency, duty cycle, and voltage settings on the mobile device or the *Wadeable Stream Fish Sampling Field Datasheet* (RD[11]) and reset the timer on the electrofishing unit.
    a. **Note:** The electrofisher settings established on the first day of sampling can be used on subsequent days so long as the conductivity stays with (+/-) 50 µS/cm.

E.3 Backpack Electrofishing

1. Proceed to the downstream block net of the first sampling reach.
    a. It is good practice to begin fish sampling at the furthest downstream reach and work upstream throughout the sampling bout. Sampling schedule and conditions may require that upstream reaches are sampled before a downstream reach. Minimize impacts to downstream reaches that have not been sampled when accessing upstream reaches.
2. At every fish sampling reach, there are several details to record on the mobile device or the *Wadeable Stream Fish Sampling Field Datasheet* (RD[11]).
    a. Record the dominant habitat type (riffle, run, pool, step pool). Also, if there is a mix of habitats, record the subdominant habitat type.
    b. Indicate if the reach is inaccessible by recording sampling impractical (dry, frozen, snow, other) and document if the reach condition could affect the data collection (normal flow, segmented pools, disconnected side channel, other low flow <100 m sampled, high flow < 100% of reach sampled, heavy vegetation, skipped reach). If multiple reach conditions affect data collection, document the most impactful condition.
3. Measure and record the water temperature, conductivity, and dissolved oxygen within the fish sampling reach using the handheld water quality meter before the start of every pass on the mobile device or the *Wadeable Stream Fish Sampling Field Datasheet* (RD[11]). The water quality measurements may be copied for each additional pass within one fish reach (i.e. if sampling at a fixed reach the water quality data collected before the first pass can be applied to the second and third pass).
4. Record the pass start date and time on the mobile device or the *Wadeable Stream Fish Sampling Field Datasheet* (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. The pass start and pass end times are for recording the full length of time for sampling each reach. This is different from the Electrofisher (EF) time which is the timer on the backpack electrofisher records the amount of time (in seconds) that the unit is actively shocking. The EF time is reset before every pass.
5. 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 (crew member 1) may, but is not required to, hold a dip net in the other hand if he/she feels comfortable.
6. The two other crewmembers will enter the stream behind the electrofisher operator.
    a. The primary netter (crew member 2) will stay close to the electrofisher operator to net fish.

b. The third crew member serves as the lead and as the secondary netter. As such, the lead crew member will carry the bucket and net any other stunned fish that are missed by the electrofisher operator or the primary netter. This crew member will monitor the electrofishing operation; ensure that fish are effectively immobilized, that captured fish in the buckets are remaining healthy, and that the crew is operating safely. The lead will also insure that any potential bystanders are not entering the water.
c. At some sites where the stream is relatively large or where many fish are typically captured, it is useful to add a fourth crew member to help distribute the work effort. It is recommended that two backpack electrofisher units are used to sample wadeable stream reaches with a wetted width of more than 7 m (Johnson et al 2007; Appendix F).

7. Ensure that the electrofishing unit settings (frequency, duty cycle, and output voltage) are those determined in SOP E.2 and that the timer ("EF time") has been reset to "0".
   a. Record the initial electrofishing settings at the beginning of each pass.
8. Turn the electrofishing unit on and notify the other field scientists. Confirm that all field scientists are ready to begin.
9. 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.
10. Slowly sweep the anode inside (i.e., upstream) of the block net side within the sample reach to target any fish that may be seeking cover in the net. Inspect the folds of the block net closely and carefully remove any fish or non-target species that may have been trapped while immobilized.
11. 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.
12. 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. Be sure that the appropriate net size is used depending on the stream size or conditions. Generally, smaller nets are used in smaller streams or shallow conditions. Larger nets perform better in larger streams that are relatively deep with steady flow.
    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 field scientists that the unit is on.

g. If any endangered species (review collection permit before sampling) or other vertebrates (e.g., salamanders, turtles) are caught, identify, take a photo, and release immediately. Stop all fish sampling activities. Follow the guidance of the collection permit for specimen vouchering and reporting procedures.

13. Always remove fish from dip nets and place in buckets to minimize injury to the fish.
14. 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.
            a) If unable to net immobilized fish (e.g. they are tangled in the complex cover), cease using this method to prevent prolonged exposure to electrical current.
        7) Record on the mobile device or *Wadeable Stream Fish Sampling Reach Establishment Field Datasheet* (RD[11]) the total number of times the battery was changed and the total number of times the electrofisher settings were changed.
15. If sampling should occur during periods of high debris flow, it will be necessary to regularly inspect the block nets while sampling. One field scientist should return to the downstream net

and remove large wood pieces, sticks, and leaf litter. This material may increase the drag on the net resulting in a partial or total collapse of the block net allowing fish to enter or exit the study reach. Inspect for and remove any fish or non-target species that may have been trapped in the net.

a. Should the block net effectiveness become compromised (collapses or a hole develops) while actively sampling, indicate the degree to which the net integrity is affected. This could be a single point of failure or the cumulative total area of several failures. Using the mobile device or *Wadeable Stream Fish Sampling Reach Establishment Field Datasheet* (RD[11]), record if the Net Integrity has:
   1) No compromise – no noticeable gap that would allow fish to enter or exit the study area.
   2) <10% compromise – Approximately 10% of the net area (or less) has a gap, is collapsed, sagging, or has a hole through which a few fish may enter or exit the study area.
   3) >10% compromise – More than 10% of the net area has a gap, is collapsed, sagging, or has a hole through which fish may freely enter or exit the study area.

b. Following the Net Integrity tracking described in the previous step, if <10% of the net integrity is compromised, fix the net, and continue to sample to completions. (**Note:** *it is extremely important that three electrofishing passes occur at the fixed sampling reaches and that they are securely blocked as these data support a variety of fish population calculations.*) If >10% of the block net is compromised, the following actions are recommended:
   1) Electrofishing at fixed reaches
      a) The first pass is partially or completely sampled but >10% of the net becomes compromised.
         (1) Return fish to the sampling reach and discard any collected data.
         (2) Re-establish and secure the block nets a minimum of 12 hours after the initial sampling attempt or when safe to do so. Then, re-initiate sampling at that reach.
      b) The second pass is partially or completely sampled but >10% of the net becomes compromised.
         (1) Stop sampling, release fish to the sampling reach, and discard any data collected for the second pass.
         (2) Re-establish and secure the block nets at least 12 hours after the initial sampling attempt or when safe to do so. Then, re-initiate sampling at that reach beginning with the first pass.
         (3) Do not discard any data collected for the first pass as long as the net integrity was <10% compromised. If the reach is re-established and sampling is re-initiated, discard the data collected from the first pass.
      c) The third pass is partially or completely sampled but >10% of the net becomes compromised.

(1) Stop sampling, release fish to the sampling reach, and discard any data collected for the third pass.
(2) Re-establish and secure the block nets 12 hours after the initial sampling attempt or when safe to do so. Then, re-initiate sampling at that reach beginning with the first pass.
(3) Do not discard any data collected for the first or second pass as long as the net integrity was <10% compromised. If the reach is re-established and sampling is re-initiated, discard the data collected from the first and second pass.

2) Electrofishing at random reaches
   a) The pass is partially or completely sampled but >10% of the net becomes compromised.
      (1) Return fish to the sampling reach and discard any collected data.
      (2) Re-establish and secure the block nets 12 hours after the initial sampling attempt or when safe to do so. Then, re-initiate sampling at that reach.

Note: *to avoid having to re-initiate fish sampling at a particular location, be sure that the block nets are setup in conditions and within locations that minimize the opportunity for the net to fail. Monitor the net integrity often!*

16. 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. Water temperature should not rise more than 4°C above the ambient stream temperature.
       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.
       2) Separate different age classes to prevent larger fish from harming small individuals.
    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. Placing some floating vegetation in the bucket can also provide cover or shade for captured fish and reduce stress. Secure a mesh netting across the top of the bucket if fish are able to jump out or when transporting them.
    d. The duration that fish are retained in the holding buckets should be kept to less than one hour. This may require that fish captured in the beginning of the reach are processed before the reach is fully sampled.
17. 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.

a. Make sure the netters are in position. Then the backpack operator will sweep the anode along the base of the block net. For nets set in water deeper than a meter, be sure to sweep the block net along the mid water column.

18. 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 mobile device or *Wadeable Stream Fish Sampling Field Datasheet* (RD[11]).
    a. Electrofisher time is critical for calculating sampling effort.
    b. Record the final electrofisher settings as they may be changed while sampling in the mobile device *Wadeable Stream Fish Sampling Field Datasheet* (RD[11]) at the end of each pass.
19. Turn the electrofisher off, remove and place on the bank with anode and cathode still attached.
20. Proceed to fish processing (SOP F.1). Record the pass end date and time on the mobile device or the Wadeable Stream Fish Sampling Field Datasheet (RD[12]) once fish have been processed.
21. If this is a fixed reach, repeat Steps 1-19 until three passes have been completed.
    a. Observe a minimum of 30 minutes between the end of the previous pass and the second or third pass within a fixed reach. This allows for fish that were not captured to recover.
    b. 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, and 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.
    c. If this is a random reach, fish are sampled using only one pass.
22. Once the reach has been completely sampled, measure the reach length from the downstream block net to the upstream block net following the thalweg. Record the length on the mobile device or *Wadeable Stream Fish Sampling Reach Establishment Field Datasheet* (RD[11]).
23. Remove the downstream block net after Pass 1 and processing (random reaches) or Pass 3 and processing (fixed reaches) have been completed.
24. 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.
25. 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. Inspect the anode and cathode for corrosion and clean as described in SOP Preparing for Sampling section above.
f. Place recently used battery separate from charged batteries where it can be easily distinguished for charging.

SOP F Fish Handling

The NEON master taxon list of fish species codes can be found on the NEON sampling support library within the Fish Sampling Protocol tab. Field scientists must use ONLY the NEON master code on all datasheets for fish. The NEON master taxon lists also include codes for instances when identification below a given taxonomic rank (e.g., family, genus) cannot be made. These are indicated by a 'sp.' or 'spp.' in the scientific name, where the former is used when only one unknown species is involved and the latter when the group of individuals in question might belong to more than one species. When one of these taxa is selected, an identification qualifier is not needed, unless the lowest taxonomic rank indicated (e.g., family, genus) is uncertain. There is not a master taxon list for non-target species. These should be recorded to lowest taxonomic level.

Read the section on identification and taxonomic uncertainty for more information about the use of identification qualifiers, identification codes and morphospecies designations (Handling uncertainty in species identifications).

F.1 Processing Samples

1. If no fish are caught within a sampling reach, indicate “No” in the “Target Taxa Present?” box on the mobile device or *Wadeable Stream Fish Sampling Reach Establishment Field Datasheet* (RD[11]).
2. Ensure that all field scientists handling fish keep hands wet with stream water and free of chemicals (e.g., insect repellent, sunscreen) while processing fish.
3. Field scientists shall confer with each other and the field guides when identifying fish species. Designate one field scientist to record fish throughout the sampling bout for taxonomic consistency.
4. For any non-fish top predators (e.g., salamanders, turtles, frogs) collected, identify and record species to lowest practical taxon on the mobile device or *Wadeable Stream Fish Sampling Field Datasheet* (RD[11]) and release. ***Note:*** *do not weigh or measure non-target species.*
5. Ensure that electrofishing time and electrofisher settings have been recorded and record pass number on the mobile device or *Wadeable Stream Fish Sampling Field Datasheet* (RD[11]).
6. Setup the digital scale and a measuring board on a flat surface.
7. Place plastic measuring tray on scale pan and tare.
8. **Preparing Anesthesia: AQUI-S®20E (10% eugenol)** reduces the stress experienced by the target species and minimizes the risk of injury to the field scientist (e.g. sharp spine pricks). Mix anesthetic in one 5-gallon bucket. The use of fish anesthetic is at the discretion of the field

scientist but is highly encouraged. Anesthetic **MUST BE USED** (where permitted) when collecting fish tissue for DNA, see Section F.5.

a. Fill the bucket approximately half-full with native water (2.5 U.S. gallons or ~10 L) or more as needed.

b. The dosage treatment of AQUI-S®20E is as follows:
   1) 25 - 40 mg/L for freshwater salmonids treated no longer than 5 minutes.
   2) 40 - 100 mg/L for freshwater non-salmonids treated no longer than 5 minutes.
      a) Only use the minimum concentration which effectively renders fish species as handleable.

c. Refer to Table 8 for calculated eugenol concentrations. Additionally, recommended concentrations can be calculated for different water treatment volumes using this formula:

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

   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-S®20E is 10% eugenol)
   E = 1.124 (specific gravity of AQUI-S®20E)

d. Example. Using the 10 mL graduated cylinder, add 2.1 mL of AQUI-S®20E to 2.5 U.S. gallons (~10 L) native water for an initial concentration of 25 mg/L. Mix well (the small dip-net makes a good mixer).

e. Label the bucket so the crew knows it contains anesthetic.

Table 8. Matrix for determining the amount (mL) of AQUI-S®20E to add to treatment water for a specific concentration of eugenol.

Target Concentration of AQUI-S20E (10% eugenol) mg/L	Volume of Treatment Water (gal)					
	2.5	5	10	15	20	25
Dose for Salmonids (mL)						
25 mg/L	2.1	4.2	8.4	12.6	16.8	21.0
30 mg/L	2.5	5.0	10.1	15.1	20.2	25.2
35 mg/L	2.9	5.9	11.8	17.7	23.5	29.4
40 mg/L	3.4	6.7	13.5	20.2	26.9	33.6
Dose for Non-salmonids (mL)						
40 mg/L	3.4	6.7	13.5	20.2	26.9	33.6
45 mg/L	3.8	7.6	15.1	22.7	30.3	37.8
50 mg/L	4.2	8.4	16.8	25.2	33.6	42.0
55 mg/L	4.6	9.2	18.5	27.7	37.0	46.2
60 mg/L	5.0	10.1	20.2	30.3	40.4	50.4
65 mg/L	5.5	10.9	21.9	32.8	43.7	54.6
70 mg/L	5.9	11.8	23.5	35.3	47.1	58.9
75 mg/L	6.3	12.6	25.2	37.8	50.4	63.1
80 mg/L	6.7	13.5	26.9	40.4	53.8	67.3
85 mg/L	7.1	14.3	28.6	42.9	57.2	71.5
90 mg/L	7.6	15.1	30.3	45.4	60.5	75.7
95 mg/L	8.0	16.0	31.9	47.9	63.9	79.9
100 mg/L	8.4	16.8	33.6	50.4	67.3	84.1

9. Remove fish from the first bucket using the small handheld dip net.
    a. Larger fish may need to be removed carefully by hand. Be sure that your hands are clean and free of soap or sunscreen residue. You may also wear nitrile or latex-free gloves.
10. 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 increments of AQUI-S®20E to increase the concentration until anesthetization is achieved within the limits described below.

        1) For salmonids, use 25 – 40 mg/L of AQUI-S®20E, **do not** exceed a concentration of 40 mg/L.
        2) For non-salmonids, use 40 – 100 mg/L of AQUI-S®20E, **do not** exceed a concentration of 100 mg/L.

b. **Note:** In some cases, it may be necessary to create a second bucket of anesthesia to treat a subset of species that require a higher concentration of AQUI-S®20E to achieve sedation and limit sedation time to less than 5 minutes.
c. Do not exceed 5 fish in the anesthetization bucket at one time.
d. Leaving fish in the anesthetization bucket for too long can cause mortality. Monitor respiration and gill movement constantly.
e. Be sure to include required information within the *INAD Field Datasheets*.

11. Identify fish to species using the mobile data device drop down species list for fish. If recording fish species on the datasheet it is recommended to use a 6-letter species code (e.g., *Cottus cognatus* = COTCOG). Also, include in the margin of the datasheet a decoder indicating the taxonomic definition (full species name) for each 6-letter code. Indicate capture method on the datasheet (i.e. electrofishing, gill net, or mini-fyke net).
    a. If the species cannot be identified or identification is uncertain, weigh and measure following Steps 12-14. Only voucher mortalities (inadvertently killed fish) or specimens that required euthanasia due to injuries.

        1) For uncertain species, follow the guidance provided in SOP F.2. For morphotype species and labeling with a unique identifier, refer to SOP F.3.
    b. Euthanize mortally wounded fish and non-target species following SOP F.4 below.
12. Place the fish in the plastic tray on the tared digital scale. Determine weight to nearest 0.1 g and record on the mobile device or the *Wadeable Stream Fish Sampling Field Datasheet* (RD[11]).
    a. The sensitivity of the scale in the field is not accurate below 0.5 g. Therefore, when recording fish weights that are less than 0.5 g, enter the value 0.25 g on the mobile data device or the *Wadeable Stream Fish Sampling Field Datasheet.*
13. With gloved hands (dipped in native water), 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 millimeter and record on the mobile device or the *Wadeable Stream Fish Sampling Field Datasheet* (RD[11]).

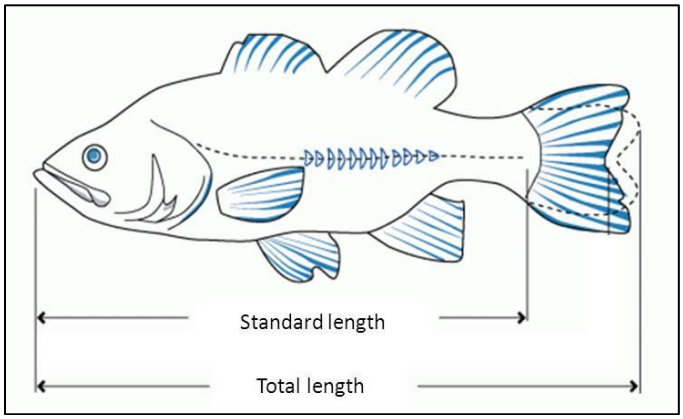

Figure 8. Measure the total length of each fish by pinching the fork together.

14. Inspect the fish for deformities, including eroded fins, external lesions, parasites, and tumors (DELTS; Figure 9). DELTS should be considered as a pre-existing health condition that an individual fish may have been experiencing before being sampled. If there are multiple DELTS, indicate the single most impactful to the specimen. Also, document if the fish was killed or injured as a result of capture and processing. These could include electrofishing injuries (burn marks, bent spine, hemorrhage; Figure 9) as well as gill or mini-fyke net related injuries (damage to skin, gills, or fins) sustained while captured. Mortality or injury may also include damage as a result of other captured species. Record DELTS and capture/processing-related injuries on the mobile device or the *Wadeable Stream Fish Sampling Field Datasheet* (RD[11]).

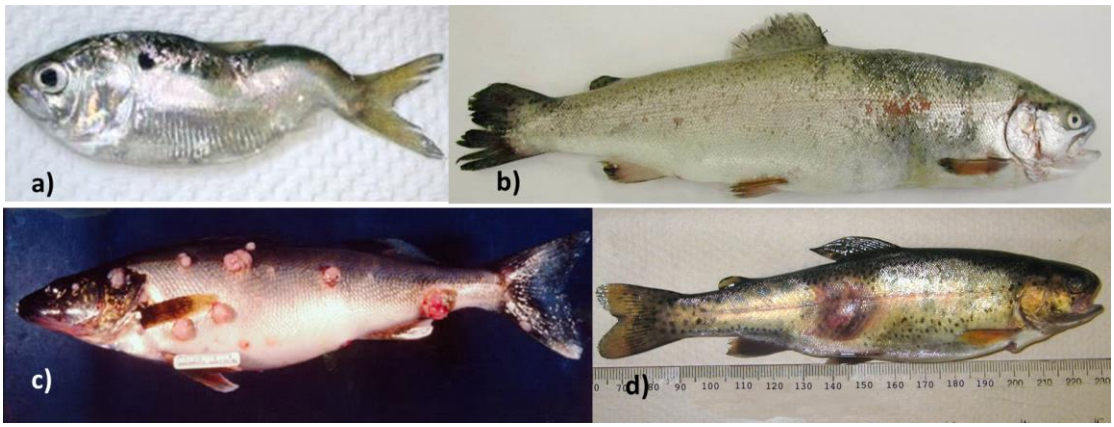

Figure 9. Example DELTS, a) deformity, b) eroded fins, c) tumors, and d) lesion. Photos by a) Kane 2005, b) Leander 2017, c) Cornell 2009, and d) Photo by Craig Banner 2004.

15. If collecting fin clips for DNA barcoding samples, refer to Fish Tissue Sampling for DNA Analysis (SOP F.5).
16. Indicate the life stage of the specimen (e.g. larval, young of the year, juvenile, adult, or gravid) on the mobile device or the *Wadeable Stream Fish Sampling Field Datasheet* (RD[11]).

17. Place processed fish in a bucket labelled “recovery 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. Use multiple buckets to reduce the concentration of captured fish.
18. Repeat Steps 10-15 until a minimum of 50 fish per species are identified, weighed, measured, and inspected for deformities. When less than 50 fish per species are identified, all of them shall be weighed, measured, and inspected for DELTS.
    a. If more than 50 individuals of one species are captured, anesthetize, weigh, and measure the first 50 and simply count the remaining fish (no anesthetization) to speed processing time and alleviate stress to fish.
        1) The 50 individuals that are fully processed should represent the average age class, length, and weight distribution of each species. Therefore, the 50 individuals weighed and measured should be randomly chosen.
        2) Using the mobile data device, enter the number of individuals counted per species. Record the total number of individuals counted on the datasheet in the bulk count section.
        3) Place the counted individuals in the recovery bucket with the other processed individuals.
    b. In cases where thousands or more fish are captured of one species, after processing a minimum of 50 fish, it may be helpful to bulk count the remaining fish. Gently scoop and count the total number of individuals in one dip net. Then count each scoop of fish and multiply that number by the total caught in the first net. This method assumes a homogenous composition of species. Bulk processing counts are added to the total fish count.
        1) Using the mobile data device, enter the estimated number of individuals counted per species and indicate that the bulk processing method was applied. Record the estimated number of individuals counted using bulk processing on the datasheet but do not include the 50 fish weighed and measured with the total.
        2) Place the counted individuals in the recovery bucket with the other processed individuals.
        3) If it is desired to record a weight and length measurement of an individual or to collect a tissue sample or a whole specimen voucher for an individual fish identified in the bulk count group, create an individual specimen record. This will allow for an appropriate sample ID to be generated. Be sure to subtract the number of specimens that were recorded individually from the bulk count number.
19. Release the processed, revived fish back into the stream immediately downstream of the block net. If sampling at the furthest downstream reach (fish reach 1), place processed fish within the initial 20 m test reach within the permitted boundary.
    a. If sampling disconnected pools, release fish in other suitable pools downstream or disconnected from the next upstream reach.

b. With the use of 10% eugenol (AQUI-S®20E), treated freshwater fish species may be immediately released following recovery; no withdrawal time is required.
c. If mortality occurs during processing, save individuals for collections and note on the mobile device or the *Wadeable Stream Fish Sampling Field Datasheet* (RD[11]; see Voucher Specimen Preservation, SOP F.6).

20. 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 affect 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.

F.2 Handling Uncertainty in Species Identifications

This section of the SOP is adapted from the TOS Protocol and Procedures: Ground Beetle Sampling (NEON.DOC.014050).

All specimens must have a taxonID associated with them. However, taxonomic identifications based on morphological features can involve uncertainty for a variety of reasons. When specimens are badly damaged such that key features or body parts missing, then taxonomic identification can be compromised such that species-level identifications are impossible and coarser taxonomic assignments are unavoidable. Identifications of damaged specimens should be as specific as possible using the features that are present without being inaccurate.

Specimens that are in good condition should be identified to the species-level, where possible. However, there will be instances where accurate identification to species is not feasible *even* for an intact specimen. Some features may be reliable morphological markers, but require high-powered microscopy, extensive dissection, or a decade's worth of experience to identify properly. In these cases, field scientists can indicate the finest known level of taxonomic information in one of two ways: 1) recording an identification qualifier and a taxonID with finer taxonomic resolution or 2) assigning a morphospecies and a taxonID with coarser resolution.

An identification qualifier contains information that indicates the taxonomic level at which there is uncertainty. If there is confidence about the genus of a specimen and uncertainty in the species identification, then 'cf. species' or 'aff. species' indicates that the provided species identification is possibly incorrect (Table 9). If a specimen is definitively of a particular tribe (i.e., Cyprinini) and the field scientist is uncertain in their assignment of genus (i.e., *Cyprinus*), then 'cf. genus' or 'aff. genus' could be used to indicate uncertainty in the genus-level assignment. If there is no uncertainty associated with lowest taxonomic rank specified, the identification qualifier field should be left blank. An inappropriate usage of the qualifier occurs when the level of the selected identification qualifier does not match the given identification of the specimen. For example, if the scientific name of a specimen is *Lepomis* sp.,

then only the genus is known; it is inappropriate to use the 'cf. species' identification qualifier because that is saying the *species* ID is uncertain without having provided species level information.

Identification qualifiers are preferred when the specimen's identity can be narrowed down to one of a few choices. If the genus or subgenus of a specimen is obvious and the specimen is one of a few species (3 species maximum), assign that specimen the taxonomic identification with which it most closely aligns and the identification qualifier at the appropriate level. For example, a domain collects 10 specimens that are either *Hybognathus hankinsoni* or *Hybognathus placitus*. Based on morphological features, the identifying field scientist feels that two seem more like *Hybognathus hankinsoni* and the other eight are more closely aligned with *Hybognathus placitus*. The field scientist would use the 'cf. species' identification qualifier, because these specimens are all definitely *Hybognathus* but the species identifications are uncertain. In the remarks, **briefly** indicate possible other species identifications and reason for rejecting them. In this example, the remarks might say "ID either *H. hankinsoni* or *placitus;* identification based on rounded tip of dorsal fin".

Table 9. Codes for identification qualifier entries

idQ Code	Identification Qualifier Description*
CS	cf.species
AS	aff.species
CG	cf.genus
AG	aff.genus
CF	cf.family
AF	aff.family
*cf. roughly equals "not sure"; aff. roughly equals "similar to, but is not"	

Morphospecies designations must be used when the specimen is in good condition, but field scientists cannot narrow the field of possible identifications to just two or three choices. Keep notes in the morphospecies tracking sheet that include descriptive information about the specimen plus any known taxonomic information. A good description might be: "similar to or is either *Etheostoma spectabile* or *Etheostoma exile*. Approximately 7 cm long. 10 dark, squarish blotches along their side. Cream colored throat. Second dorsal, and caudal fin have small dark spots." This example description contains taxonomic information and detailed morphology, which indicates that it is within the genus *Etheostoma*. Therefore, *Etheostoma spp.* would be included in the TaxonID. This still requires that a taxonID be assigned, but it can be of a coarser resolution than at the species level.

If domain staff are able to subsequently identify the morphospecies, the full scientific name associated with that ID must be provided with the datasheet, either via annotation (see RD[04]) or by attaching a key to each datasheet. If domain staff are not able to identify a given morphospecies prior to data entry, the morphospecies ID and description from the morphospecies tracking datasheet must be transcribed into the appropriate spreadsheet on the NEON intranet on the fish-specific sampling support library.

Cryptic species issues arise when two species that are morphologically indistinguishable in the field co-occur (or might co-occur) at a site. NEON intends to add these species pairs to the master taxon lists to

account for this. If a cryptic species pair is not currently available in the master list, the proposed species pair must be entered in the crypticSpeciesGroups spreadsheet on the NEON intranet on the fish-specific sampling support library.

Finally, the mobile application currently limits field scientists such that only species thought to be present in a domain are available for selection. With climate change and species introductions, it is likely that field scientists will observe species within their site that represent the first ever records of that species in that area. When this happens, the mobile application will not have the scientific name of that species available for selection. In that case, field scientists must use the taxon code "OTHE" for fish that are new to their domain. In the remarks field, write the scientific name of the identified fish. NEON will revise that record to reflect the correct scientific name after confirmation of identification. Also, larval fish that are collected are difficult to identify in the field. Use the taxon code "LARV" for larval fish that cannot be properly identified during fish processing. The taxon table will be updated for the subsequent year to make that species name available to field scientists within that domain.

F.3 About Morphospecies Designations

This section of the SOP is adapted from the TOS Protocol and Procedures: Ground Beetle Sampling (NEON.DOC.014050).

A morphospecies is a temporary designation for an individual or group of fish that are of the same group (ideally, that group is a species; morphospecies only requires that all individuals look the same). A subset of each field scientist-identified species or morphospecies will be sent for later taxonomic confirmation by DNA analysis. Sometimes morphospecies designations turn out to contain multiple species. While this is unavoidable for certain species complexes that are difficult to resolve, in most cases this can be avoided by designating multiple morphospecies for similar (but not identical) unknown specimens. Should these morphospecies be assigned the same species designation by DNA analysis, the two morphospecies can later be merged into the same species designation.

- If a species assignment cannot be made based on the collection of other identification resources and identification qualifiers are not useful (could be one of more than 3 options), give a morphospecies name to that type of fish.
- As a general rule, split groups that look similar but not identical into different morphospecies, focusing on features like: size, color, presence of bars/par marks, and shape of the dorsal fin, length of the upper jaw, eye diameter, and lateral line features. It is easy to lump them together later, but difficult to later split them into multiple species.
- If it is unclear whether a newly captured specimen is the same as individuals from a previously assigned morphospecies, a new morphospecies ID should be assigned (it is better to have the same species designated as different morphospecies than to have multiple different species designated as the same morphospecies).
- The format of a morphospecies ID includes: the domainID where the specimen was captured, the year of capture, and the word "Morph" followed by one or more unique letters. For example, "D15.2014.MorphA" would be the first morphospecies from domain 15 that was

captured in 2014. **Note:** it is important to use the morphospecies ID to document, track, and resolve uncertain fish taxa.

- The letter at the end of the morphospecies ID (e.g., "A") should *never* be repeated for any other morphospecies than that for which it was originally designated, in a given year. If more than 26 morphospecies are encountered in a given domain in a given year, the 27th morphospecies ID should include two letters at the end (e.g., the 27th morphospecies in domain 15, for 2014, would be "D15.2014.MorphAA"). For every additional 26 morphospecies, a new letter will be added (i.e., the 54thmorphospecies would be "D15.2014.MorphAAB").

F.4 Euthanizing Fish and Non-Target Species

1. Euthanize fish using a lethal dose of 10% eugenol at a concentration of 100 mg/L. Refer to Table 10 for calculated lethal dose concentrations of 10% eugenol. The American Veterinary Medical Association (AVMA 2013) recommends the use of eugenol as an acceptable agent for euthanizing fish. Fish exposed to a lethal dose of 10% eugenol are considered unsafe for human consumption.

Table 10. Lethal dose of AQUI-S®20E for euthanizing fish

Target Concentration of AQUI-S20E (10% eugenol) mg/L	Volume of Treatment Water (gal)					
	2.5	5	10	15	20	25
100	8.4	16.8	33.6	50.4	67.3	84.1

a) Add 16.8 mL of 10% eugenol to 5.0 U.S. gallons of stream water. Mix thoroughly.
b) Transfer fish from the holding bucket to the bucket containing the euthanizing solution with the small handheld dip net.
c) Monitor fish until respiration ceases. Fish should be exposed to the lethal dose of 10% eugenol for a minimum of 10 minutes.
d) Place fish into appropriate sample container (e.g., wide mouth HPDE bottles) with completed specimen label (Figure 10) and add 10% formalin preservative. One taxon per specimen bottle. See steps on specimen preservation in SOP F.6.

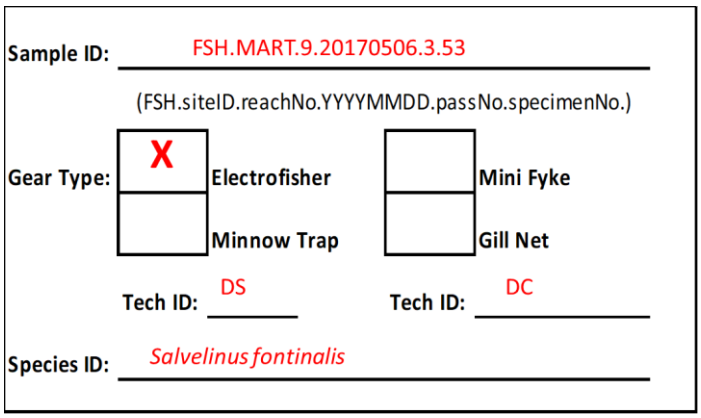

Figure 10. Example specimen label

(1) Adhesive barcode labels (**Error! Reference source not found.**1) will be added to the sample containers and scanned by the mobile app (**Error! Reference source not found.**2).

(2) Keep a human-readable label on each bottle with a minimum of the sample ID printed to assist with organization and shipping.

Figure 11. Example of an adhesive Type II barcode label.

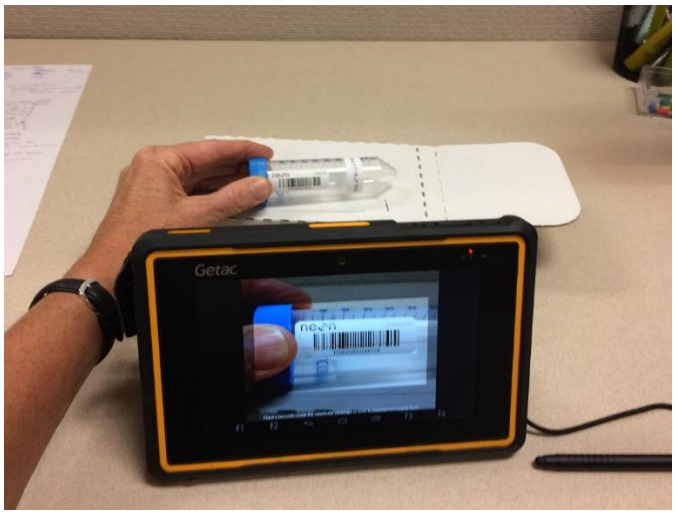

Figure 12. Barcode label scanning.

e) If the voucher specimen was pulled out of a bulk sample then there needs to be an individual record included on the datasheet and a sample ID generated. Subtract the number of individuals pulled from the bulk sample count for each individual entry. The specimen voucher sample ID format is FSH.siteID.reachNo.YYYYMMDD.passNo.specimenNo.

b. Amphibians that are injured as a result of fish sampling will be euthanized using a lethal dose of MS-222, 200 mg/L of stream water in the field.
   1) Add 1 L of stream water and 10 mL of MS-222 stock solution to a new 5-gallon bucket. Mix thoroughly. Label the bucket so the crew knows that it contains the MS-222 euthanizing solution.
   2) Transfer amphibians from the holding bucket to the bucket containing the euthanizing solution with the small handheld dip net.
   3) Monitor the amphibians until respiration ceases.
   4) Place the amphibian into an appropriate sample container (e.g., wide mouth HPDE bottles) with completed specimen label (Figure 10; Appendix A.) and add 10% formalin preservative. One taxon per specimen bottle. See steps on specimen preservation in SOP F.6.
      a) When the system is available, adhesive barcode labels will be added to the sample containers and scanned by the mobile app (**Error! Reference source not found.**1).

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).
   1) Following loss of consciousness, a second intracoelomic injection of unbuffered 50% MS-222 is administered. Directions for preparing the reptile euthanasia kit (Figure 13) follow below.

Figure 13. Reptile Euthanasia kit with 50 mL centrifuge tube to store the 5 mL syringe and needle, 60 mL HDPE amber bottle is for 1st stage solution, 20 mL HDPE scintillation vial for 2nd stage solution.

a) A fresh bottle of stage 1 and stage 2 reptile euthanasia solutions should be prepared for each day of sampling. Keep these solutions in dark containers, away from sunlight, and refrigerated when not in use. Discard unused solutions at the end of each fish sampling bout in the lab following the NEON EHS chemical hygiene guidelines (AD[03]).

2) <u>1st stage injection solution - 1% MS-222</u>
   a) Prepare MS-222 and baking soda to be mixed with water in the field when needed)
   b) Measure out 0.25 g MS-222 plus 0.157 g baking soda using the lab balance; weigh out MS-222 and baking soda in a small container (amber 60 mL HDPE bottle) at the Domain Support Facility.
   c) Bring along 1 L of tap water from the Domain Support Facility to mix in the field with the MS-222 and baking soda when the stage 1 euthanasia solution is needed. Add 25 mL of water while in the field and shake to mix well.
3) <u>2nd stage injection solution - 50% (v/v) unbuffered MS-222</u>
   a) Make a fresh solution in the field as needed.
   b) Add one part MS-222 (g) to 2 parts water (mL); no baking soda (unbuffered).
   c) Pre-weigh out 1 g of MS-222 at the Domain Support Facility into an appropriately sized container (ex. 20 mL scintillation vial covered in duct tape or foil to protect from light).

d) Add 1 g MS-222 in 2 mL tap water for 2 mL of 50% (v/v) MS-222 in the field when needed. A syringe can be used to measure out 2 mL of water. Shake the scintillation vial to mix the solution.

e) The solution will be thick with precipitates, cloudy, and pale yellow (Figure 14).

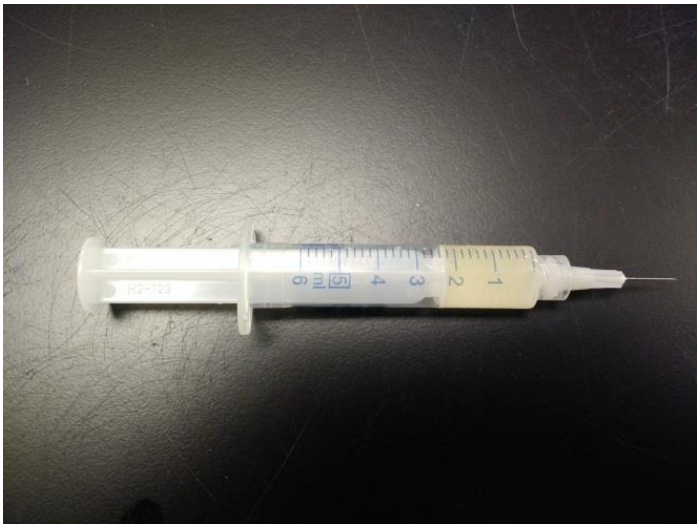

Figure 14. 2nd stage 50% (v/v) unbuffered MS-222 solution. Note yellow cloudy appearance of solution

4) 1st stage injection instructions:

a) To inject 250 mg /kg into reptile use following formula:

(1) Reptile weight (kg) x 250 mg/kg x 1 g/1000mg x 100 mL /1 g MS-222 = # mL of 1% MS-222 to inject into reptile. See Table 11 for the 1st stage dosage calculator based on reptile weight in grams. The dose can be adjusted by adding or subtracting the 1% MS-222 solution volume using the table. For example, euthanizing a specimen that weighs 150 g with the 250 mg/kg concentration of 1% MS-222, inject 2.5 mL plus 1.25 mL (total 3.75 mL) of 1% MS-222.

Table 11. 1st Stage dosage calculator using 1% MS-222 for reptiles by weight (g).

1st Stage	Dosage Calculator for Reptile Weights (g)						
1% MS-222	**10**	**50**	**100**	**200**	**300**	**400**	**500**
250 mg/kg	0.25	1.25	2.5	5	7.5	10	12.5
500 mg/kg	0.5	2.5	5	10	15	20	25

b) Use an appropriately sized syringe and needle (either a 5 mL syringe for smaller specimens or a 60 mL for larger specimens).

c) Draw up the appropriate volume of the stage 1 solution into the syringe.

d) Insert the needle through the skin of the reptile into the abdominal wall. In the case of delivering the dose into the body of a turtle, inject the stage one solution into the inguinal fossa, which is the soft spot anterior to the hind foot (Figure 15).

Figure 15. Intracoelomic injection into the inguinal fossa of a turtle. Photo by Kaufman 2017.

e) A 250 mg/kg dose will be sufficient to anesthetize reptiles. In the event that the 500 mg/kg dose is needed, double the dose (Table 12).

5) 2nd stage injection instructions:
   a) Using a 5 mL syringe, inject 0.1 mL to 1.0 mL of the 50% (v/v) MS-222 unbuffered solution into reptile, depending on the weight of the specimen (Table 12).

Table 12. 2nd stage dosage calculator using 50% MS-222 for reptiles by weight (g).

2nd Stage	Dosage Calculator for Reptile Weights (g)						
50% MS-222	**< 30**	**50**	**100**	**200**	**300**	**400**	**500**
0.1 mL/30 g	0.1	0.17	0.33	0.67	1.00	1.33	1.67

   a) Insert the needle through the skin of the reptile into the abdominal wall. In the case of delivering the dose into the body of a turtle, inject the stage one solution into the inguinal fossa (Figure 15).
   b) Inject 0.1ml for specimens weighing less than 30 g. Add an additional 0.1 mL for every additional 30 grams of reptile.
6) Any euthanized or dead animals will be collected, preserved in formalin in a collection jar, and deposited at a fish collections facility. Do not dispose of specimens euthanized with MS-222 in the field or stream. Do not dispose of the MS-222 solutions in the field or stream.

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 unless otherwise instructed via the collection permit. Follow the permit guidelines for the disposition of killed endangered species. Refer to the site-specific sampling strategy for additional guidance when handling listed species.
    1) Stop all fish sampling activities.
    2) Contact the Domain manager to report the incident. The manager will contact the regional U.S. Fish and Wildlife Service office and the local state fish and wildlife department to report the incident.
    3) Specimens will also be preserved following the methods described above.
    4) If the inadvertent death of a protected species is discovered once the specimen has been collected as a voucher or shipped to a curation facility, NEON will immediately contact the federal and state fish and wildlife authorities within the region where the specimen was collected.

F.5 Fish Tissue Sampling for DNA Analysis

1. Put on gloves (nitrile or latex free).
2. Anesthetize target fish with AQUI-S®20E following SOP F.1. Tissues **MUST BE COLLECTED** from anesthetized fish where allowed (check your collection permit; if unsure, contact the lead aquatic scientist).

3. Take a single photo voucher of the specimen (**before clipping any fins**) from which the tissue sample was collected. Orient the fish to capture a lateral view, with the head facing left, and place the fish on top of a monochromatic background. A white field sampling tray would be appropriate. Include a scale bar and color separation guide in the field of view to calibrate the image (Figure 16).
    a. Take the photo using the tablet and mobile application. Record the “photo view” which is auto-populated as lateral. If the photo is oriented as a dorsal or ventral view, select that from the photo view options.
    b. Be sure that the resulting image is in the “landscape” orientation. Include as much of the specimen in the field of view as possible. Minimize shadows and keep hands or other objects out of the image. See Figure 16 for an example.
    c. A caption can be entered but is not required. Click on the photo that was collected and enter a remark in the caption field.
    d. Delete any photos that are not of suitable quality or otherwise should not be associated with the fish data.

e. A photo ID will be generated and then joined with the DNA sample ID in the mobile application.

Note: *photos shall only be collected using the mobile application. If the mobile device is not available, record the sample ID for DNA samples on the* Wadeable Stream Fish Sampling Field Datasheet *but do not collect a photo with a separate camera as the images will not be joined with the record.*

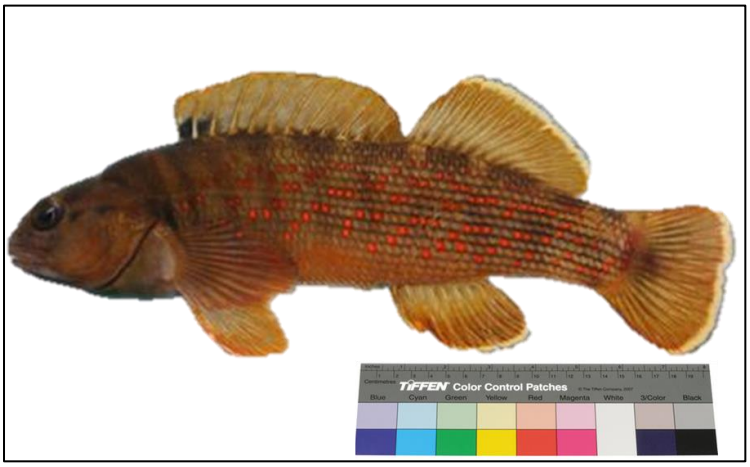

Figure 16 Example voucher photo. Lateral, head left, with color separation guide and scale bar. Photo from OSU 2017.

4. Dip the fin clipping scissors and forceps into high concentration ethanol (70% or greater) and ignite with lighter to flame sterilize the tissue sampling tools. If flame sterilization is unavailable, wet the instruments with native water after dipping in ethanol and wipe with a clean cloth.
5. Some State collection permits (AK) require that tools used to sample fish tissue be soaked in an iodophor or betadine disinfectant bath. Using a clean dishpan or other container, add a concentration of 1/100 iodine-based disinfectant and clean tap water (bottled water) solution. There should be enough disinfectant to completely submerge the tools. Soak tools for 10 minutes between tissue sampling. It may speed up the process to have several sets of fin clipping tools available to minimize the disinfection time.
6. **VERY IMPORTANT:** Fish tissues collected for DNA extractions cannot be exposed to or preserved in formalin.
7. Using the cutting tool, remove a piece of the target fin ray (Figure 17). Suitable fins for DNA include the left pelvic fin or the caudal fin. The adipose fin is not an ideal tissue to sample as it contains fat that reduces the effectiveness of the DNA extraction process. Also, note that some States (AK) prohibit the collection of adipose fins; check your collection permit stipulations. Tissue should be a minimum of 2 mm but no larger than 5 mm in diameter. Smaller tissue samples should be harvested from smaller fish.

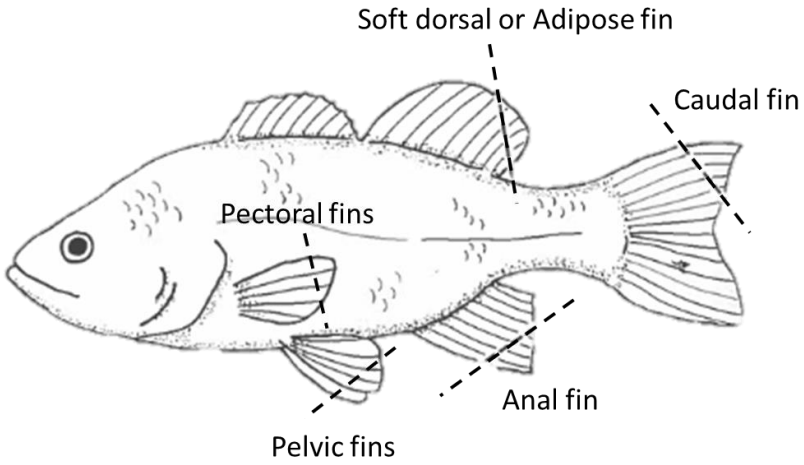

Figure 17. Optional fins to clip for tissue sampling

8. With the forceps, place the fin clip in the appropriate tissue container (1.5 mL cryo vial). Pre-fill the vial with ethanol (70% or greater) to keep the specimen wet. Be sure that the container is completely closed and labeled with the appropriate sample ID using the following DNA fish sample format "FSH.siteID.reachNo.YYYYMMDD.passNo.specimenNo.DNA".
9. Collecting DNA tissue samples for fish that weigh less than 0.5 g and removing sufficient amounts of fin tissue will likely reduce their survivorship. Instead, euthanize the specimen, remove an adequate portion of fin tissue, and place in the tissue collection vial. The remaining specimen can be collected as a voucher (see Section F.6 Whole Fish Voucher Specimen Preservation) or discarded following the guidance of the collection permit.
10. Place live fish that have had tissues samples collected into the recovery bucket.
11. Indicate on the mobile device or the *Wadeable Stream Fish Sampling Field Datasheet* that a DNA tissue sample was taken and provide a sample ID format "FSH.siteID.reachNo.YYYYMMDD.passNo.specimenNo.DNA" for each specimen.
12. Flame sterilize (or wipe) the cutting tool and forceps or wipe with an alcohol pad before collecting the next tissue sample to avoid cross-contamination. Be sure that no fish tissue, slime, or blood remains on the instruments.
13. Repeat Steps 1-8 until all targeted fish samples have had tissues collected. Up to 5 individuals per species per year are sampled for DNA analysis.
14. If a tissue sample was collected from a fish in a bulk sample then there needs to be an individual record included on the datasheet and a sample ID generated. Subtract the number of individuals used for tissue collection from the bulk sample count for each individual entry.
    a. For tissue storage procedures, refer to Section 4.3 Timing for Laboratory Processing and Analysis and SOP H for shipping guidance.

F.6 Whole Fish Voucher Specimen Preservation

1. Fill jar with a 10% buffered formalin solution to fix specimens within one hour of euthanizing.

a. If using concentrated formalin (37% formaldehyde), dilute 1 part concentrated formalin with nine parts water (can use native water).
b. In general, use a ratio of 10:1 formalin to fish tissue by weight (example: use 1,000 mL formalin: 100 g fish).
    1) Use an appropriate size HDPE wide mouth specimen jar for the preservation of an individual specimen in a single jar. Groups of individuals of one species may be included in a single jar only if the individual has the Sample ID label physically attached to the specimen.
c. For large fish greater than 200 mm total length, make a lateral 2-3 cm incision on the lower right side into the peritoneal. Open the body cavity, taking care not to damage bones or organs, will allow for better penetration of the fixative.
d. Formalin should be injected into the dorsal muscles of specimens with muscle wider the 14 mm.

2. Secure lid tightly and store upright at room temperature (~70 °F). Be sure that the specimen label is included with the specimen and that the jar is properly labeled with the Sample ID in the following format "FSH.siteID.reachNo.YYYYMMDD.passNo.specimenNo." (Figure 10).
3. Discard used anesthetic solution according to NEON EHS chemical hygiene guidelines (AD[03]).
4. For long-term preservation, allot the specimens to soak in 10% formalin for up to one week (up to four weeks for large specimens). Discard the used formalin accordingly (AD[03]). Transfer the specimens into an alcohol fixative; either 70%-95% ethanol or isopropanol, making sure that the specimen is fully submerged.
5. Secure lid tightly and store upright at room temperature (~70 °F) with appropriate specimen labels inside and outside of the container.
6. Should the fish sampling activities be suspended or cancelled due to a change in environmental conditions becoming unsafe, stop all specimen collection. If samples were collected, contact the domain manager and submit a trouble ticket through the NEON problem resolution system.

F.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 All Systems Standard Operating Procedure: Decontamination of Sensors, Field Equipment, and Field Vehicles (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. See the Oregon State University (OSU) 1989 reference for a resource to mend and patch fish nets.
    d. Inspect the anode ring on the electrofisher unit for corrosion. For light corrosion, use a steel wool pad to scrub the ring clean of rust. This will reduce unnecessary overloads on the

backpack electrofisher. If corrosion is heavy, which is more common in water with high conductivities, use fine grit sandpaper to remove rust.

SOP G Data Entry and Verification

The importance of thorough, accurate data transcription cannot be overstated; the value of the efforts in the field is only manifested once the data are properly entered for delivery to NEON's end users. Mobile applications are the preferred mechanism for data entry. Data should be entered into the protocol-specific application as they are being collected, whenever possible, to minimize data transcription and improve data quality. If available, adhesive barcode labels should be used and scanned into the mobile application. Mobile devices should be synced at the end of each field day, where possible; alternatively, devices should be synced immediately upon return to the Domain Support Facility.

However, given the potential for mobile devices to fail under field conditions, it is imperative that paper datasheets are always available to record data. Paper datasheets should be carried along with the mobile devices to sampling locations at all times. 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.

If paper datasheets are used, the procedure is as follows:

1. Enter data from field datasheets and the number of vials generated from sample processing into the pertinent Fish mobile application, according to instructions in the NEON Protocol and Procedure: Manual Data Transcription (RD[04]).
2. Scan datasheets and save in PDF file format.
3. Save paper copy of datasheets.
4. No fish photos will be collected if the mobile device and application are not available.

SOP H 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 specimen vouchers to the external facilities (*to be determined pending lab contracts*) for long-term preservation.

Ground ship fin clip tissues to the centralized Domain Support Facility (D09) for tissue processing and shipment to the external lab for sequencing (Canadian Centre for DNA Barcoding).

H.1 Handling Hazardous Material

Follow shipping and Hazmat procedures for formalin.

H.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.

H.3 Timelines

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

H.4 Conditions

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

H.5 Grouping/Splitting Samples

N/A

H.6 Return of Materials or Containers

N/A

H.7 Shipping Inventory

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

H.8 Laboratory Contact Information and Shipping/Receipt Days

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

8 REFERENCES

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APPENDIX A DATASHEETS

The following datasheets and mobile device applications are associated with this protocol:

Table 13. Datasheets and mobile device applications associated with this protocol

NEON Doc. #	Title	Mobile Application
NEON.DOC.001646	General AQU Field Metadata Sheet	(AOS) Field Metadata [PROD]
NEON.DOC.002494	Datasheets for AOS Sample Shipping Inventory	Shipping App [PROD]
NEON.DOC.003107	Datasheets for Fish Sampling in Wadeable Streams	(AOS) Fish [PROD]

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

APPENDIX B QUICK REFERENCES

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

Step 2 – For anesthetizing fish, AQUI-S®20E should be added directly to the treatment water. Do not make stock solutions or other dilute solutions of AQUI-S®20E. Use the table below to determine the amount of AQUI-S®20E to add to the treatment water for specific concentrations of eugenol:

Target Concentration of AQUI-S20E (10% eugenol) mg/L	Volume of Treatment Water (gal)					
	2.5	5	10	15	20	25
Dose for Salmonids (mL)						
25 mg/L	2.1	4.2	8.4	12.6	16.8	21.0
30 mg/L	2.5	5.0	10.1	15.1	20.2	25.2
35 mg/L	2.9	5.9	11.8	17.7	23.5	29.4
40 mg/L	3.4	6.7	13.5	20.2	26.9	33.6
Dose for Non-salmonids (mL)						
40 mg/L	3.4	6.7	13.5	20.2	26.9	33.6
45 mg/L	3.8	7.6	15.1	22.7	30.3	37.8
50 mg/L	4.2	8.4	16.8	25.2	33.6	42.0
55 mg/L	4.6	9.2	18.5	27.7	37.0	46.2
60 mg/L	5.0	10.1	20.2	30.3	40.4	50.4
65 mg/L	5.5	10.9	21.9	32.8	43.7	54.6
70 mg/L	5.9	11.8	23.5	35.3	47.1	58.9
75 mg/L	6.3	12.6	25.2	37.8	50.4	63.1
80 mg/L	6.7	13.5	26.9	40.4	53.8	67.3
85 mg/L	7.1	14.3	28.6	42.9	57.2	71.5
90 mg/L	7.6	15.1	30.3	45.4	60.5	75.7
95 mg/L	8.0	16.0	31.9	47.9	63.9	79.9
100 mg/L	8.4	16.8	33.6	50.4	67.3	84.1

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 (see SOP C).

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. Take care to minimize habitat disturbances.

Step 6 – Assemble backpack electrofisher and test the settings by collecting, inspecting and releasing 20 fish. Check anode and cathode for corrosion; remove with steel wool or other abrasive pad.

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 – Anesthetize caught fish in a 5-gallon bucket with solutions of AQUI-S®20E.

Step 10 – Identify fish to species using the 6-letter species code (e.g., *Cottus cognatus* = COTCOG) and record on the mobile device or the *Wadeable Stream Fish Sampling Field Datasheet* (RD[11]). For uncertain species, follow the guidance provided in SOP F.2. For morphotype species and labeling with a unique identifier, refer to SOP F.3.

Step 11 – Measure the weight and length of the specimen and inspect for deformities.

Step 12 – If collecting fish tissues for DNA analysis, target specimens **MUST BE** anesthetized with AQUI-S®20E.

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 – If any endangered species (review collection permit before sampling) or other vertebrates (e.g., salamanders, turtles) are caught, identify, take a photo, and release immediately. **STOP** all fish sampling activities. Follow the guidance of the collection permit for reporting procedures.

Step 15 – Preserve euthanized specimen in a jar with a 10% buffered formalin (then ethanol for long-term storage. Ship to the external facility when directed to.

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 field scientists 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 release immediately. Stop all fish sampling activities. Follow the guidance of the collection permit for reporting procedures.
- ☑ Release the processed, revived fish back into the stream downstream of the block net.

Sample processing:

- ☑ If an endangered species is morbidly injured, stop all fish sampling activities, follow the permit guidance before applying euthanasia. Also, follow the permit guidelines for the disposition of killed endangered species.
- ☑ If more than 50 individuals of one species are captured, anesthetize, weigh and measure the first 50 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 for sites with 10 fish reaches. See Appendix G for sites with shortened reaches. Skip numbers that either have 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). If sampling is impractical as a result of severe drought (dry) or that the stream is frozen then indicate this for any affected reach on the mobile device or the *Wadeable Streams Fish Sampling Field Datasheet* (RD[11]). Should environmental conditions affect the ability to fully sample a stream reach, commence with sampling but note the cause on the "Reach Condition" section in the mobile field device or on the mobile device or the *Wadeable Streams Fish Sampling Field Datasheet* (RD[11]).

Domain	Site	Randomized reach order
D01	Hop Brook	9, 5, 3, 6, 8, 1, 2, 7, 4, 10
D02	Lewis Run	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	McDiffett 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
D11	Blue River	10, 5, 9, 7, 3, 2, 4, 1, 8, 6
D12	Blacktail Deer Creek	7, 8, 1, 2, 10, 4, 9, 3, 5, 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	Teakettle Creek	9, 7, 3, 6, 5, 4, 8, 10, 1, 2
D17	Upper Big 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

APPENDIX F SITE-SPECIFIC INFORMATION: TWO BACKPACK ELECTROFISHERS APPROACH

At wadeable stream sites where the median wetted width of an individual fish sampling reach or the entire biological reach is greater than 7 m, two backpack electrofishers can be used simultaneously to sample fish (Johnson et al 2007). The use of two backpack units improves the effectiveness and capture of fish in wider stream segments. Follow the electrofisher field set-up procedures described in SOP E.2. Test each unit individually then both together in the 20 m test reach. Be sure to avoid any anode-to-anode contact, this may result in damage to the backpack units. Also, if the electrical fields overlap, the backpack unit may produce an audible warning (or shut off). Should this happen, space out the anodes to minimize interference. Any changes should be made so that the initial settings are identical on both units. Maintain electrofisher settings at the lowest level that allows for the effective capture of fish. Record the initial frequency, duty cycle, and voltage settings on the mobile device or the *Wadeable Stream Fish Sampling Field Datasheet* (RD[11]) for each electrofishing unit and reset the timer on both units before each pass.

1. Proceed to the first sampling reach, starting from the furthest downstream reach and working upstream. Follow the electrofishing guidance in SOP E.3.
2. Measure and record water temperature and conductivity using the handheld conductivity meter at each reach before every pass. Record on the mobile device or the Wadeable Stream Fish Sampling Field Datasheet (RD[11]).
3. The electrofisher operators should work side by side, each working one-half of the wadeable stream. The operators need to work together to push fish upstream while covering a variety of habitat types. Do not let one get ahead of the other. If you are too close, the electrofisher backpack will beep or shut down; if you are too far from each other, fish will escape.
4. There should be a minimum of three netters (four is more ideal) to cover the two electrofisher operators. Each operator should have a designated netter with the third netter working between the groups to collect immobilized fish in a bucket. The third netter will serve as the crew lead and monitor the electrofishing operations. The lead will ensure that fish are effectively immobilized, that captured fish in the buckets are remaining healthy, and that the crew is operating safely. The lead will also insure that any potential bystanders are not entering the water.
5. If one operator is sampling along the shoreline, the second operator should be sampling at the mid-channel. Operators should not each be sampling the opposite shorelines simultaneously as fish will likely move downstream at the mid-channel. Maintain good communication between to two operators so that no habitat features are missed and to maximize spatial coverage while minimizing anode interference.
6. When encountering pool habitat or log jams, the two teams can work together. One operator should stay at the downstream edge of the pool or jam (acting like a fence) and the second operator can sample the perimeter of the pool or jam. At least one netter needs to be following the operator circumnavigating the pool or jam while the other netter(s) and the crew lead stay with the downstream operator.

7. Electrofisher settings may be changed for each unit independently while sampling. While the initial settings should be identical, the final settings may not be the same for both units. Record the final frequency, duty cycle, and voltage settings from each unit on the mobile device or the *Wadeable Stream Fish Sampling Field Datasheet* after each pass (RD[11]).
8. Once the entire reach has been sampled, read and record the time (EF time) in seconds from both of the electrofisher units on the mobile device or *Wadeable Stream Fish Sampling Field Datasheet* (RD[11]) after each pass.

9. Reset the timer, turn off the units, and proceed to fish processing (SOP F.1).

APPENDIX G SITE-SPECIFIC INFORMATION: SHORTENED SAMPLING REACH

Establishing Shortened Sampling Reaches

The NEON wadeable streams fish sampling design requires a minimum reach length of 1 km (SOP C). The following information provides guidance for establishing fish sampling reaches at NEON sites that are 500 m in length, or half of a typical NEON aquatic stream reach. The spatial sampling design was modified in order to collect comparable fish data with other NEON wadeable stream sites that are 1 km. Procedures provided below should be implemented at other NEON wadeable stream sites where only a 500 m reach can be established. It should be noted that a 500 m reach is the absolute minimum length for a NEON wadeable stream site.

1. Complete the data entry on the mobile device or *Wadeable Stream Fish Sampling Reach Establishment Field Datasheet* (RD[11]).
2. Ensure the *General AQU Field Metadata Sheet* (RD[05]) is completed per field site visit.
3. Using a field measuring tape measure out five, non-overlapping, 80 m (± 20 m) reaches, starting 10-20 m above the downstream permit boundary (Figure **4**18). This measurement is best taken by following the thalweg of the main channel.
    a. Leave 10-20 m at the downstream boundary to allow for space to test the electrofisher and to release processed fish.
    b. Due to heterogeneous stream morphology and habitat characteristics, sampling reaches may deviate from 80 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 60 m and maximum of 100 m sampling reach lengths are acceptable. If natural channel units are longer than 100 m, then end the reach at 80 m.
    c. A minimum of five reaches must be present at wadeable stream sites that are less than 500 m.
4. Install a permanent aluminum plot survey marker on the bank at river right at each reach boundary (including the permit boundaries; Figure 4). Each fish sampling reach is numbered sequentially beginning with “1” at the bottom (downstream) but just above the test reach (Figure 18). Record the coordinate at the plot marker location as well as the coordinate uncertainty using the Trimble data dictionary for AOS locations. If the Trimble is not available at the time of transect selection, record on the Reach Establishment Datasheet (RD[11]) and return at a later date to record locations using the Trimble.
5. Record the location of each marker on the handheld GPS unit and the mobile device or *Wadeable Stream Fish Sampling Reach Establishment Field Datasheet* (RD[11]).
    a. GPS points should be added to the site-specific stream morphology map (RD[09]) at the Domain Support Facility. Also, refer to the Morphology Mapping (RD[09]) protocol for further descriptions and examples of stream habitats.

b. If you are unable to install plot survey markers on the right bank (river right), use the left bank and note on the AOS Trimble data dictionary. The right bank is preferred for consistency across sites.

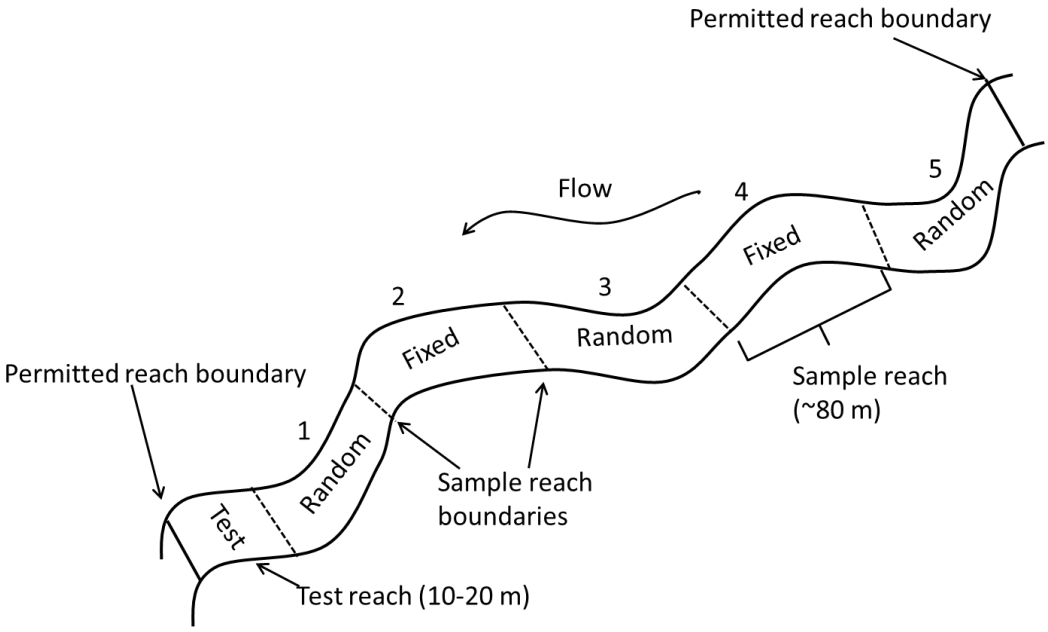

Figure 18. Schematic of a 500 m permitted NEON stream site delineated into five 80 m reaches: 2 fixed and 3 random sampling reaches. One random reach will be chosen each year for sampling.

Fixed and Random Shortened 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 the stream morphology changes significantly, as detected by the results of the Wadeable Stream Morphology Protocol, then it may be necessary to establish new reaches or the entire fish sampling reach. Submit a trouble ticket through the NEON problem resolution system.

6. Up to three 80 m (± 20 m) reaches (two fixed and one random) will be sampled during each sampling bout (see the tTable 6).
7. Select two of the five reaches to be the "fixed" reaches. Fixed reaches will be sampled two times per year throughout the duration of NEON measurements.

a. The two fixed 80 m (+/- 20 m) reaches should be chosen to best represent the habitat variability throughout the 500 m 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. The NEON Aquatic Ecologist or Domain Aquatic Field scientist will select the fixed reaches.

b. Electrofishing in reaches with sensors (S1 or S2) must occur ≥5 m away from all in-stream electronics.

8. Select one of the remaining three random reaches to be sampled annually. Refer to the table below for a randomized order of reaches for a 500 m wadeable stream site.
9. Use the same random reach for the two sampling dates (bouts) within one year (see the tTable 6).
10. For each year of sampling, continue down the list of randomized reaches not sampled previously. In year four, return to the first random reach on the list when all random reaches have been sampled (Table 14).
11. Follow this pattern for the remainder of the study.
12. Resume with the procedures in SOP E, Field Sampling above.

Table 14. Example of fixed and rotating design for one site less the 500 m 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
Year	Random	Fixed	Random	Fixed	Random
1					
2					
3					
4					
5					
6					
7					
8					
9					
10					