AOS PROTOCOL AND PROCEDURE: FISH SAMPLING IN WADEABLE STREAMS

PREPARED BY	ORGANIZATION	DATE
Brandon Jensen	AQU	10/01/2016
Stephanie Parker	AQU	09/05/2013
Jesse R. Fischer	AQU	07/09/2013

APPROVALS	ORGANIZATION	APPROVAL DATE
Dave Tazik	SCI	05/15/2017
Mike Stewart	SE	05/09/2017

RELEASED BY	ORGANIZATION	RELEASE DATE
Jennifer DeNicholas	CM	05/16/2017

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Change Record

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

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1 OVERVIEW

1.1 Background

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

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

1.2 Scope

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

1.2.1 NEON Science Requirements and Data Products

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

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

1.3 Acknowledgments

The design and implementation of stream fish sampling methods was based on the guidance from the NEON Fish Sampling Workshop (6-8 March, 2013, Boulder, CO) and attendees D. C. Dauwalter, A. J. Davis, E. A. Frimpong, G. D. Grossman, K. G. Gerow, R. M. Hughes, C. P. Paukert, and D. M. Walters. 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	NEON Protocol and Procedure: Manual Data Transcription
RD[05]	NEON.DOC.001646	General AQU Field Metadata Sheet
RD[06]	NEON.DOC.001152	NEON Aquatic Sampling Strategy
RD[07]	NEON.DOC.004257	All Systems Standard Operating Procedure: Decontamination of Sensors, Field Equipment, and Field Vehicles
RD[08]	NEON.DOC.00690	AOS Protocol and Procedure: Macroinvertebrate Sampling in Wadeable Streams
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	US Environmental Protection Agency
USGS	US Geological Survey
V	Volt
W	Watt

2.5 Definitions

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

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

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

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

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

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

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

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

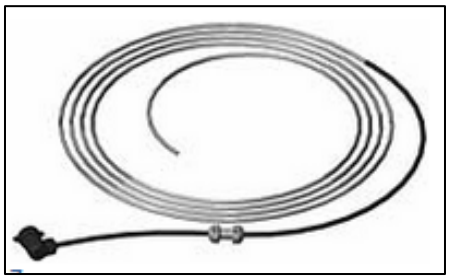

Figure 2. Cathode for backpack electrofishing unit (photo: store.smith-root.com)

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

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

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

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

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

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

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

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

Pulsed DC: Direct electrical current that is interrupted rapidly.

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

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

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

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

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

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

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

3 METHOD

The objective for this document is to outline the sampling protocol and procedures for sampling fish and other top predators at NEON wadeable stream sites. Other top predators (e.g., salamanders, crayfish), although not targeted in this sampling protocol, will be identified when collected as 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.

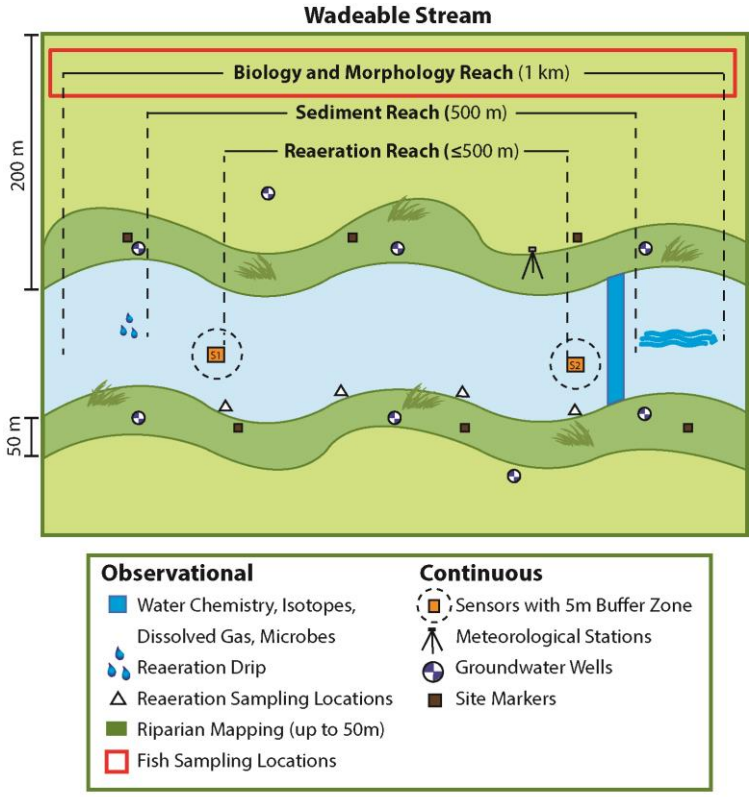

Figure 3. A generic wadeable stream site layout. Fish sampling will occur throughout entire biology and morphology reach.

This protocol also includes non-destructive methods for collecting fish tissues from live specimens in the field. A subset of collected fish will receive DNA analysis, which will serve a number of purposes, including verification of taxonomy of specimens that do not receive expert identification, 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 technicians **must** follow the protocol and associated SOPs. Use NEON's problem reporting system to resolve any field issues associated with implementing this protocol.

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

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

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

4.2 Criteria for Determining Onset and Cessation of Sampling

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

4.3 Timing for Laboratory Processing and Analysis

Samples may be stored for up to 1 month following the preservation guidelines in SOP D.2. For storage and shipping timelines see SOP F. Adipose fin clips may be taken from a maximum of 10 individuals per species per sampling bout for DNA analysis. Adipose fin clips will be collected using scissors that are large enough to clip the fin in one quick motion. The cut should be made perpendicularly to the fin rays and remove half of the fin or less. If the fish does not have an adipose fin, a clip of the left pelvic fin may be collected. Other fins maybe clipped if the adipose fin or left pelvic fin are not suitable. Fin clips will

be placed in labeled collection vials and returned to the laboratory for storage. In addition, individual domain facilities will store and maintain 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.

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. If sampling is impractical as a result of severe drought (dry) or that the lake is frozen then indicate this for any affected reach on the *Wadeable Streams Fish Sampling Field Datasheet* (RD[11]). Should environmental conditions impact the ability to fully sample a lake reach, commence with sampling but note the cause on the "Reach Condition" section in the mobile field device or on the *Wadeable Streams Fish Sampling Field Datasheet* (RD[11]).

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 (JIRA).
3-7 Days	If heavy rainfall that affects visibility or flooding occur on or prior to the targeted sampling date (>1.5x above baseflow), or unsafe wading conditions occur (Lane and Fay 1997), wait a minimum of 3-7 days after water level drops to near-baseflow conditions (within 25% of baseflow as determined by sensor data) to allow the fish assemblage to redistribute	None as long as samples are collected within the pre-determined sampling window. If waiting for flooding to diminish causes sampling to occur outside of the biological bout sampling window by more than three days, submit a trouble ticket through the NEON problem resolution system (JIRA).

4.5 Sampling Specific Concerns

1. Fish sampling should not occur while other sampling activities are occurring upstream in the NEON reach that may disturb sediments or otherwise affect hydrology of the system.
2. Fish sampling must be completed within a 5-day period per site. If field conditions appear unfavorable (e.g., prolonged thunderstorms, tropical storms, expected flooding) during the

proposed sampling bout, postpone sampling until conditions are safe to sample and when the entire sampling bout can be completed in 5 days. If the resumption of fish sampling is expected to occur three days past the biological bout, submit a trouble ticket (Table 2).

3. Reasonable efforts should be made to minimize mortality to fish during sampling. This includes the use of best fish handling practices (e.g., frequent changes of stream water in buckets, aerators) and limited use of collected specimens.
4. Electrofishing-related injuries should affect < 3% of fish captured cumulatively at the reach-scale. If this number is exceeded at the site, stop sampling and contact the NEON Aquatic Ecologist, Domain Manager, and submit a trouble ticket using the NEON problem tracking system (JIRA).

5 SAFETY

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

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

1. Technicians are required not to put hands and feet in waters where alligators are present and to make sure a safe distance from hazards is maintained.
2. All employees shall have access to a form of communication with other team members such as a two-way radio.
3. Technicians should be aware of any site-specific hazards and to the waters of that particular location (i.e. current status, tidal charts, etc.).
4. Activities in streams should only be performed when flow conditions are safe. Do not attempt to wade a stream where velocity x depth is ≥ 0.93 m^2/s (10 ft/s; Lane and Fay 1997).
5. Safety Datasheet information shall be readily available to technicians working with chemicals included in this protocol. Technicians must also be trained in safe handling of formalin (AD[03]).
6. Technicians 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 technicians 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 requirement for wader selection follows the recommendations of the American Fisheries Society Professional Safety Committee (2008). Non-breathable materials provide the most insulation against electrical shock and include PVC, neoprene, or silicon. Although breathable materials including Gore-Tex® provide less insulation against electrical shock, it may be preferred and more comfortable in warmer conditions and environments. The use of breathable waders is acceptable as long as the operator also wears dry clothing that covers any exposed skin while in the waders.
4. Before sampling, inspect waders 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.
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 1. Equipment list – Field preparation

Item No.	R/S	Description	Purpose	Quantity	Special Handling
Durable items					
MX100345	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
Consumable items					
MX106819	R	Tricaine methanesulfonate (MS-222)	Euthanizing specimens	20 g	Y
MX106431	R	$NaHCO_3$	Buffering agent for MS-222	50 g	N
MX110318	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
	R	Field datasheets (print on waterproof paper, write in pencil)	Recording data	10	N

Item No.	R/S	Description	Purpose	Quantity	Special Handling
	R	Specimen labels (waterproof paper)	Labeling specimens	2 sheets	N

R/S=Required/Suggested

Table 2. Equipment list – Reach establishment

Item No.	R/S	Description	Purpose	Quantity	Special Handling
Durable items					
	R	Site-specific morphology map	Navigating to sampling reaches	1	N
	R	Plot survey markers (aluminum, site-specific)	Establishing sampling reaches	12	N
MX100318	R	Meter tape (50 or 100 m)	Establishing sampling reaches	1	N
MX110075	R	Handheld GPS (with batteries, ± 1 m accuracy)	Navigating to sampling reaches	1	N
Consumable items					
MX105556 MX105417 MX105419	R	Flagging tape	Establishing sampling reaches	1 roll	N

R/S=Required/Suggested

Table 3. Equipment list – Electrofishing

Item No.	R/S	Description	Purpose	Quantity	Special Handling
Durable items					
MX107115	R	Steel studded fence posts (i.e., T-post)	Securing block net at reach boundary	8	N
MX104756	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
	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
	R	Net repair kit: • needle • string • butane lighter • zip ties	Repairing nets	1	N
MX106855	R	Battery-powered backpack electrofishing unit	Electrofishing	1	N

Item No.	R/S	Description	Purpose	Quantity	Special Handling
MX106846	R	Anode pole (1.5-2.0 m) with attached anode ring	Electrofishing	1	N
MX106848	R	Cathode (rattail type; 1-2 m and 10-15 mm in diameter)	Electrofishing	1	N
MX110472 or MX106849	R	Electrofisher batteries (rechargeable)	Electrofishing	3	N
MX106854	R	Battery charger (electrofishing batteries)	Charging the electrofisher	1	N
	R	Abrasive pad/steel wool to clean anode rings	Electrofishing	1	N
	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	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	R	5 gallon buckets	Storing specimens	10	N
	R	Hand held conductivity/temperature meter (YSI)	Measuring conductivity and temperature	1	N

Item No.	R/S	Description	Purpose	Quantity	Special Handling
	R	Chest waders (approved for electrofishing)	Safe wading	1 pair per person	N
MX106824	R	Chest wader repair kit (e.g., Aquaseal) or extra waders	Safe wading	1	N
MX100479	R	Polarized sunglasses (amber or brown lenses recommended)	Increasing efficiency of fish capture	1 pair per person	N
Consumable items					
MX100338	R	Fish viewer	Viewing individual fish and taking photos	1	N
	R	Plastic weighing boat	For weighing fish under 75 mm	1	N

R/S=Required/Suggested

Table 4. Equipment list – Fish processing

Item No.	R/S	Description	Purpose	Quantity	Special Handling
Durable items					
	R	Fish and top predator taxonomic ID key specific to location or region (denotes endangered species)	Identifying specimens	1	N
MX106815	R	Portable aerators (batteries, diffusion stone)	Aerating buckets	15	N
MX106832	R	Small dip net (3.2 mm mesh)	Handling specimens	5	N
	R	Fish measuring boards (50 cm)	Measuring specimen length	2	N
MX106902	R	Portable digital scale (batteries, charger)	Weighing specimens	1	N
MX100338	R	Plastic tray	Weighing specimens	2	N
	R	Digital camera (batteries, memory card)	Photographing specimens	1	N
MX100379 or MX100380	R	25-50 mL graduated cylinder, plastic	Mixing anesthetic and euthanizing solution	1	N
	R	Fin clipping scissors	Cutting fish fins for DNA barcode samples	1	N

Item No.	R/S	Description	Purpose	Quantity	Special Handling
	R	Tissue containers	For preserving fin clips for DNA barcoding; maybe provided by an external lab	10	N
Consumable items					
	R	Nitrile gloves (pair)	Safe handling of chemicals and fish	10	N
MX100345	R	HDPE wide mouth specimen jars (1 L)	Specimen preservation containers	50	N
MX106819	R	Tricaine methanesulfonate (MS-222) stock solution	Euthanizing specimens	1 L	Y
MX110318	R	AQUI-S®20E stock solution	Anesthetizing specimens	1 L	Y
MX106257	R	10% buffered formalin (37-40% formaldehyde)	Preserving specimens	20 L	Y
MX101221	R	Needle, disposable 1 inch, 25 gauge, sterile	Injecting reptiles with MS-222	1 pack	Y
MX106200	R	Needle, disposable 1 inch, 27 gauge, sterile	Injecting reptiles with MS-222	1 pack	Y
MX106261	R	Syringe, 5mL luer lock syringe	Measuring and injecting euthanasia fluids	1 pack	Y
	R	70% (or greater) ethanol	Flame sterilization of tissue sampling equipment	500 mL	Y

Item No.	R/S	Description	Purpose	Quantity	Special Handling
	R	Refillable butane lighter	Flame sterilization of tissue sampling equipment	1	Y
MX100549	R	50mL Centrifuge tube	Store syringe and needle, measure injection fluids	1	Y
MX 101218	R	60mL Clear bottle HDPE	Store dry MS-222 and baking soda for reptile euthanasia stage 1 solution	1	Y
MX101278	R	20mL HDPE Scintillation vial	Store dry MS-222 for reptile euthanasia stage 2 solution	1	Y
MX103240	S	Sharps Container, Portable, Slip Top	Receptacle for disposal of needles	1	N

R/S=Required/Suggested

6.2 Training Requirements

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

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

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

External Training References:

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

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

CITI IACUC Fish and Amphibian training. Register through the National Ecological Observatory Network organization affiliation:
https://www.citiprogram.org/index.cfm?pageID=154&icat=0&clear=1

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

6.3 Specialized Skills

N/A

6.4 Estimated Time

The time required to implement a protocol will vary depending on a number of factors, such as skill level, system diversity, environmental conditions, and distance between sample plots. The timeframe provided below is an estimate based on completion of a task by a skilled 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 three qualified technicians for 5 consecutive field days. There is no lab processing at the Domain Support Facility associated with this protocol. Fish tissue samples may require shipping to a NEON-approved laboratory for DNA barcoding. Additionally, tissue samples and/or voucher specimens (target and non-target species) may be preserved, archived, and maintained by domain staff. Sample preservation and archival may require up to 10 minutes of processing per specimen for domain staff.

7 STANDARD OPERATING PROCEDURES

Preparing for Sampling

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, 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 the anode ring and cathode for corrosion. Remove corrosion using an abrasive pad or steel wool to gently scrub the surface.
3. Inspect lineman gloves and waders for holes and tears, repair if necessary.
4. Inspect dip nets and block nets for rips, tears, and holes. Repair, if necessary.
5. Inspect portable aquarium pumps, diffusion stones, and batteries.
6. Inspect buckets to ensure handles are present and functioning.
7. Ensure that all equipment has been decontaminated since last use (see RD[07]).
8. Print datasheets and specimen labels (RD[11]) on waterproof paper. Verify that the mobile data entry device is charged and synced prior to use.
9. Select random sampling reaches if this is the first sampling date for the year (SOP B).
10. 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. 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 A Establishing Sampling Reaches

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

1. Complete the *Wadeable Stream Fish Sampling Reach Establishment Field Datasheet*.
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 allows space for electrofisher testing and releasing processed fish.
    b. Due to heterogeneous stream morphology and habitat characteristics, sampling reaches may deviate from 100 m in stream length to allow reaches to end at the natural boundaries of channel units (e.g., riffle, run, pool). A minimum length of 80 m and maximum of 120 m sampling reach lengths are acceptable. If natural channel units are longer than 120 m, then end the reach at 100 m.
    c. If the permitted reach is < 1 km, a minimum of eight reaches must be present at the site.
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 location of each marker on the handheld GPS unit and on the *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 in the *Wadeable Stream Fish Sampling Reach Establishment Field Datasheet* (RD[11]). The right bank is preferred for consistency across sites.

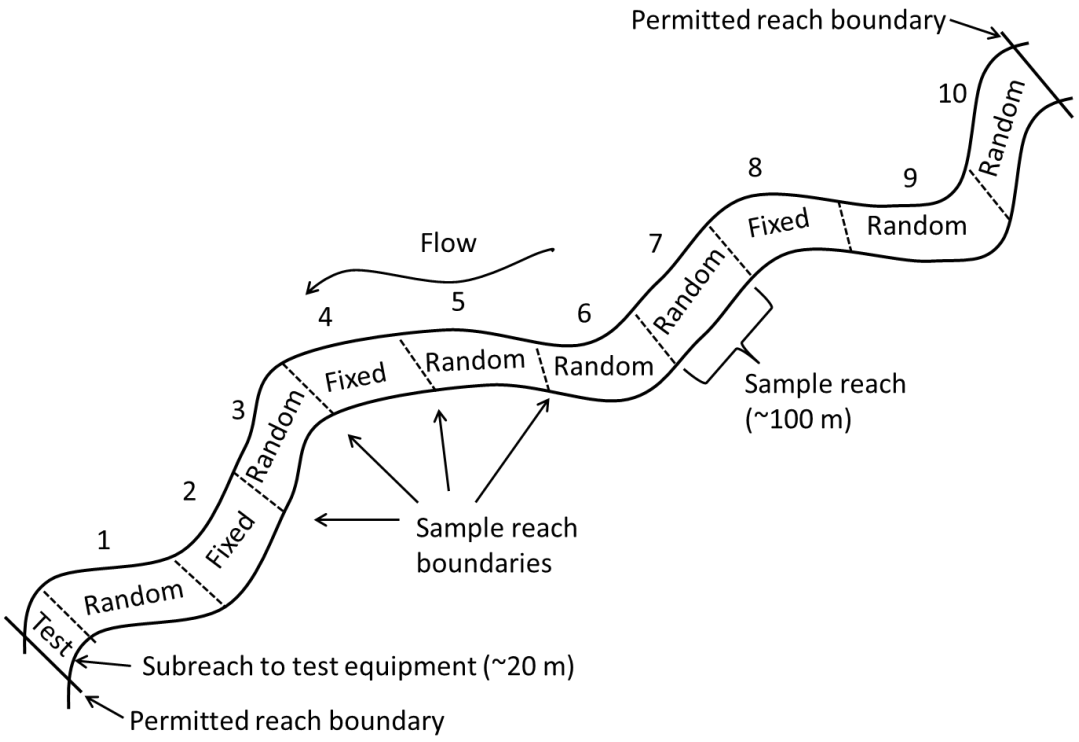

Figure 4. Schematic of 1 km permitted NEON stream site delineated into ten 100 m reaches: 3 fixed and 7 random sampling reaches. Three random reaches will be chosen each year for sampling

SOP B Fixed and Random Sampling Reach Selection

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

1. Up to six 100 m (± 20 m) reaches (three fixed and three random) will be sampled during each sampling bout (Table 5).
2. Select three of the 10 reaches to be the "fixed" reaches. Fixed reaches will be sampled two times per year throughout the duration of NEON measurements.

   a. The three fixed 100 m (+/- 20 m) reaches should be chosen to best represent the habitat variability throughout the 1 km permitted reach. For example, the inclusion of primary habitat (e.g., pools) and distinctive (e.g., waterfall and plunge pool) habitat features should be a priority when selecting permanent reaches. Fixed reaches will be selected by the NEON Aquatic Ecologist or Domain Aquatic Technician.

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

3. Select three of the remaining seven random reaches to be sampled annually. Refer to Appendix E for a randomized order of reaches for each wadeable stream site.
4. Use the same three random reaches for the two sampling dates (bouts) within one year (Table 5).
5. For each year of sampling, continue down the list of randomized reaches not sampled previously. In year three (if the stream contains 10 reaches), there should only be one reach that has not yet been sampled. Return to the first random reach on the list when all reaches have been sampled.
6. Follow this pattern for the remainder of the study.

Should low stream flow result in a partially dried reach, commence with sampling but note the shortened reach length on the *General AQU Field Metadata Sheet.* If fixed or random reaches are completely dry, make a note on the *Field Metadata Sheet* and proceed with sampling the next available sampling reach.

Table 5. Example of fixed and rotating reach design for one site over 10 years. Gray boxes denote when a reach is sampled. Randomized reach order for each site is presented in Appendix E.

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

SOP C Field Sampling

C.1 Site Set-up

1. Navigate to the most downstream sampling reach selected for this sampling bout using GPS points, the morphology map, or the plot survey markers.
   a. Sampling must begin at the downstream sampling reach and proceed upstream to minimize disturbance.
2. Set up fence posts and block nets at downstream and upstream boundaries of the sampling reach.

a. Always secure the downstream block net first, followed by the upstream net.
b. Secure a 3 mm mesh block net at the reach boundary using steel fence posts or existing structure (e.g., tree).
c. Relocate any debris (i.e., tumble weeds) in the stream that interferes with the block net deployment immediately downstream of the sample reach. Do not remove in-stream habitat features (i.e. large wood) to install block nets. Instead, move the net up or down stream of structure.
d. Pull the net across the stream ensuring enough slack in the lead line (bottom of net) to reach the stream substrate.
e. Anchor lead line to substratum using large rocks or block net anchor stakes.
f. 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.

C.2 Backpack Electrofishing Field Set-up

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

1. **VERY IMPORTANT:** All technicians MUST wear necessary personal protective equipment before stepping into the water, including waterproof chest waders with lug-soled boots (felt waders are not allowed at NEON sites), rubber lineman gloves to insulate the wearer from electrical shock, and polarized sunglasses to increase the efficiency of fish capture.
2. Assemble anode pole (Figure 1).
3. Measure and record water temperature and conductivity using the handheld conductivity meter at the test reach. Record on *Wadeable Stream Fish Sampling Field Datasheet* (RD[11]).
4. Connect the cathode and anode to the backpack electrofishing unit (Figure 5).

Figure 5. Cathode and anode connections on backpack electrofishing unit

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

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

6. Test the backpack electrofisher in the 20 m section that was left between the downstream permit boundary and the closest sampling reach.
7. Wade into the stream ensuring that the cathode (i.e., rattail) is submerged and anode ring is submerged.
   a. Begin electrofishing in shallow water (e.g., < 50 cm).
8. While the electrofisher operator is standing in the stream, set the frequency to 30 Hz, the duty cycle to 10%, and output voltage to 100 V and turn the electrofishing unit on. Backpack

electrofisher settings should be based on stream conductivity, see Table 6 for additional settings information.

Table 6. 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 - 700 V.

9. Pause to verbally confirm settings on electrofisher and that the unit is turned on. Also confirm that all technicians are ready to proceed before pressing the activation switch on the anode pole.
   a. The anode ring must always be submerged before depressing the activation switch. When removed from the water, the unit will automatically turn off.
10. Press and hold the activation switch down, and observe the behavior of fish. If fish do not appear to be affected by electrofishing (e.g., are not momentarily stunned), release the activation switch on the anode pole and increase voltage by 100 V (e.g., from 100 V to 200 V) and repeat Steps 8.a-10.

   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.
11. If 1,100 V is reached and fish are still not responding to electrofishing proceed decrease voltage to 250 V and increase the frequency by 10 Hz (e.g., from 30 Hz to 40 Hz) and repeat Steps 8.a-10.
   a. If 70 Hz and 1,100 V is reached and fish are present but not immobilized, stop electrofishing and contact the NEON Aquatic Ecologist.
   b. If fish are immobilized during testing, use dip nets to capture individuals and place in a bucket ½ - ¾ full of stream water carried by one of the netters and continue with Step 12.
12. Continue electrofishing until up to 20 individuals spanning a variety of sizes are netted.
   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 C.3.

13. Place netted fish in a bucket with fresh stream water and a battery operated aerator.
    a. If other top predators are captured, identify (if possible) and record species to the lowest taxonomic level on the *Wadeable Stream Fish Sampling Field Datasheet* (RD[11]) and immediately release >20 m away and downstream from electrofishing activity.
14. Examine captured fish for signs of injury (e.g., bent backs, dark bruising, hemorrhaging of the gills; Figure 7). Record injury rate on the *Wadeable Stream Fish Sampling Field Datasheet* (RD[11]). Less than 3% of the captured fish should be injured.

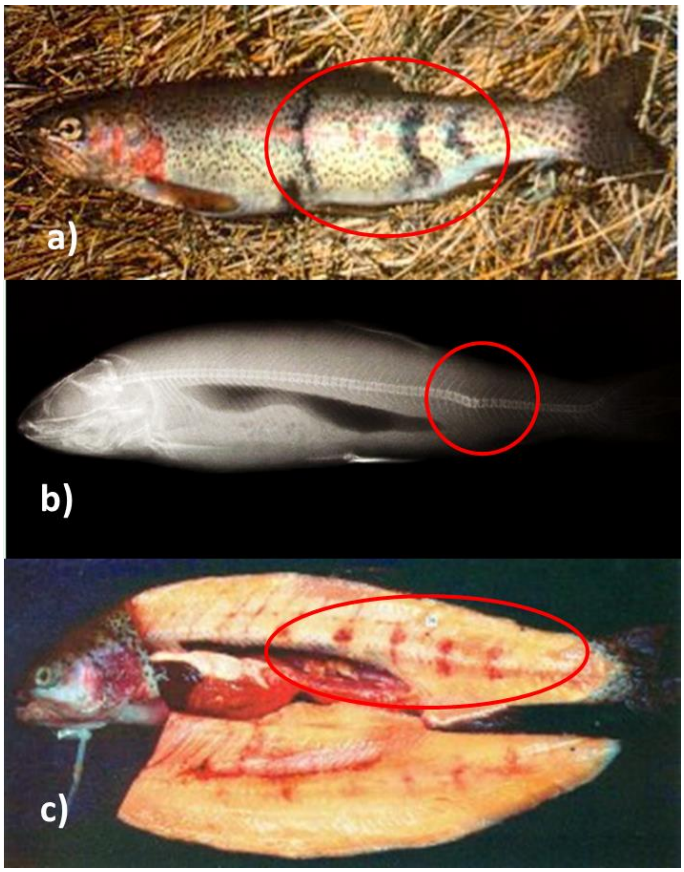

Figure 7. Examples of electrofishing injuries to fish, a) bruising or "burn" marks, b) spinal injury, and c) internal hemorrhaging

    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 (JIRA).
    b. Contact with the cathode or prolonged exposure to electricity due to failure to remove fish from the dip net quickly will increase injury rates.
    c. If fish are injured, allow them to recuperate in a separate bucket with an aerator before releasing.
    d. For any fish that do not recover, proceed to euthanization (SOP D.2).

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

C.3 Backpack Electrofishing

1. Proceed to the downstream block net of the first sampling reach.
    a. Always start fish sampling at the furthest downstream reach and work upstream.
2. Measure and record water temperature and conductivity using the handheld conductivity meter at each reach. Record on *Wadeable Stream Fish Sampling Field Datasheet* (RD[11]).
3. Record start time on 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.
4. 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.
5. 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 a lot of fish are typically captured, it is useful to add a fourth crew member to help distribute the work effort.
6. Ensure that the electrofishing unit settings (frequency, duty cycle, and output voltage) are those determined in SOP C.2 and that the timer (“EF time”) has been reset to 0.
    a. Record the initial electrofishing settings at the beginning of each pass.

7. Turn the electrofishing unit on and notify the other technicians. Confirm that all technicians are ready to begin.
8. 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.
9. 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.
10. 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.
11. 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 technicians that the unit is on.
    g. If any endangered species (technicians will be notified of likelihood before sampling) or other vertebrates (e.g., salamanders, turtles) are caught, identify, photograph if possible, and release immediately downstream from electrofishing activities within the permitted

reach. Record the photoID (camera file name) on the mobile data device or photo log datasheet.

12. Frequently remove fish from dip nets and place in buckets to minimize injury to the fish.
13. 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 *Wadeable Stream Fish Sampling Field Datasheet* the total number of times the battery was changed and the total number of times the electrofisher settings were changed.
14. 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.
    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.
    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.

15. 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.
16. 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 *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 *Wadeable Stream Fish Sampling Field Datasheet* at the end of each pass.
17. Turn the electrofisher off, remove and place on the bank with anode and cathode still attached.
18. Proceed to fish processing (SOP C.6).
    a. If this is a fixed reach, repeat Steps 1-17 until three passes have been completed.
    b. 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.
    c. Depletion is determined when two consecutive passes result in sequential decreases in total number of individuals sampled after the first pass (e.g., 1000 fish on first pass, 200 fish on second pass, 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 (JIRA).
    d. If this is a random reach, fish are sampled using only <u>one pass</u>.
19. Remove the downstream block net after Pass 1 and processing (random reaches) or Pass 3 and processing (fixed reaches) have been completed.
20. 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.
21. 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 D 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. Technicians 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).

D.1 Processing Samples

1. If no fish are caught within a sampling reach, indicate “No” in the “Target Taxa Present?” box on the *Wadeable Streams Fish Sampling Field Datasheet.*
2. Ensure that all technicians handling fish keep hands wet with stream water and free of chemicals (e.g., insect repellent, sunscreen) while processing fish.
3. Designate one technician to identify 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 *Wadeable Stream Fish Sampling Field Datasheet* (RD[11]) and release.
    a. Photograph the specimen before releasing if possible. Record the photoID (camera file name) on the mobile data device or photo log datasheet.
5. Ensure that electrofishing time and electrofisher settings have been recorded, and record pass number on the *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. Mix anesthetic in one 5-gallon bucket. The use of fish anesthetic is at the discretion of the field scientist.

a. Fill the bucket approximately half full with native water (2.5 US gallons or ~10 L).
b. A dosage treatment of 20-30 mg/L eugenol (AQUI-S®20E is 10% eugenol) is recommended to sedate all fish species to "handleable" in most situations. This reduces the stress experienced by the target species and minimizes the risk if injury to the field scientist (e.g. sharp spine pricks). Refer to Table 7 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)

c. Using the 10 mL graduated cylinder, add 1.9 mL of AQUI-S®20E 2.5 US gallons (~10 L) native water for an initial concentration of 20 mg/L. Mix well (the small dip-net makes a good mixer).
d. Label the bucket so the crew knows it is the anesthetic.

Table 7. Matrix for determining the amount (mL) of AQUI-S®20E to add to treatment water for a specific concentration of eugenol.

Target Concentration of 10% eugenol (AQUI-S®20E)	Volume of Treatment Water (gal)					
	2.5	5	10	15	20	25
20 mg/L	1.7 mL	3.4 mL	6.7 mL	10.1 mL	13.5 mL	16.8 mL
25 mg/L	2.1 mL	4.2 mL	8.4 mL	12.6 mL	16.8 mL	21.0 mL
30 mg/L	2.5 mL	5.0 mL	10.1 mL	15.1 mL	20.2 mL	25.2 mL

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 of AQUI-S®20E to increase the concentration of 25 mg/L until anesthetization is achieved. Do not exceed an AQUI-S®20E concentration of 30 mg/L.

b. Do not exceed 5 fish in the anesthetization bucket at one time.
c. Leaving fish in the anesthetization bucket for too long can cause mortality. Monitor respiration and gill movement constantly.
d. Be sure to include required information within the *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 13-15, and photograph the specimen. Record the photoID (camera file name) on the mobile data device or photo log datasheet. Only voucher mortalities (inadvertently killed fish) or specimens that required euthanization due to injuries.

        1) For uncertain species follow the guidance provided in SOP D.2. For morphotype species and labeling with a unique identifier, refer to SOP D.3.
    b. Euthanize mortally wounded fish and non-target species following SOP D.4 below.
12. Photo voucher at least one representative specimen from each taxon and from different life history stages (young of year, juvenile, gravid, adult). Include non-target specimens (e.g. amphibians and reptiles). Record the photoID (camera file name) on the mobile data device or photo log datasheet.
        1) Include metric ruler for scale using the measuring board.
        2) Photograph 1: Lateral photo with fish's head facing to the left.
        3) Photograph 2: Ventral photo that includes the mouth (mouth position, lip structure, and barbels can be important distinguishing features.
13. Place the fish in the plastic tray on the tared digital scale. Determine weight to nearest 0.1 g and record on 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 in the mobile data device or the *Wadeable Stream Fish Sampling Field Datasheet.*
14. 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 (**Error! Reference source not found.**) to the nearest millimeter and record on the *Wadeable Stream Fish Sampling Field Datasheet* (RD[11]).

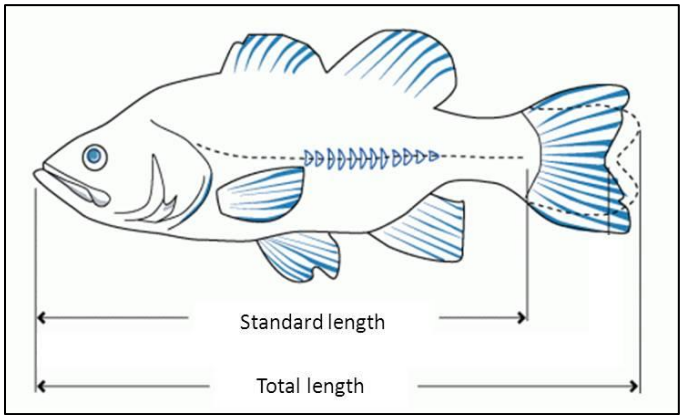

Figure 8. Measure the total length of each fish by pinching the fork together.

15. Inspect the fish for deformities, including eroded fins, external lesions, parasites, and tumors (DELTS) and electrofishing injuries (burn marks, bent spine, hemorrhage; Figure 9) and record these on the *Wadeable Stream Fish Sampling Field Datasheet* (RD[11]).

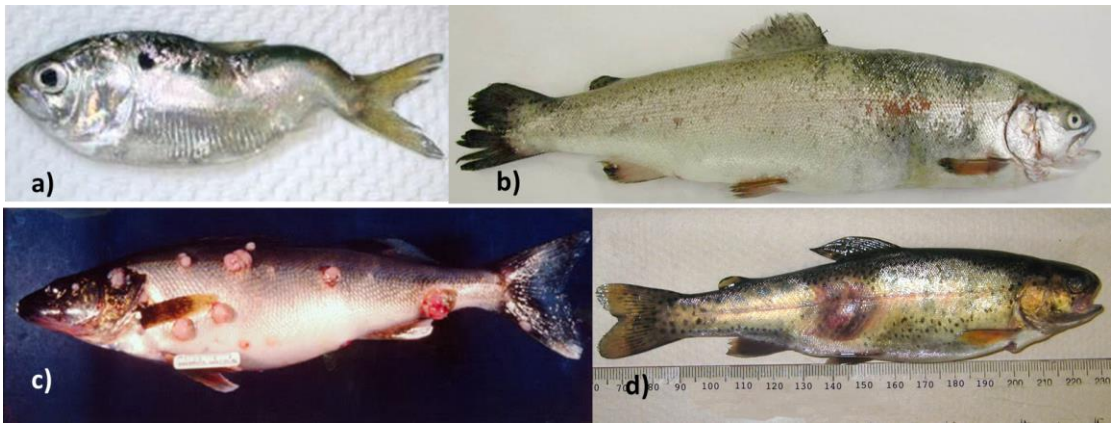

Figure 9. Example DELTS, a) deformity, b) eroded fins, c) tumors, and d) lesion

16. If collecting fin clips for DNA barcoding samples, refer to Fish Tissue Sampling for DNA Analysis (SOP D.5).
17. Indicate the life stage of the specimen (e.g. larval, young of the year, juvenile, adult, or gravid) on the *Wadeable Stream Fish Sampling Field Datasheet* (RD[11]).
18. 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.

19. Repeat Steps 0-16 until a minimum of 50 fish per species are identified, weighed, measured, and inspected for deformities.
    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.
20. 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. With the use of 10% eugenol (AQUI-S®20E), treated freshwater fish species may be immediately released following recovery; no withdrawal time is required.
    b. If mortality occurs during processing, save individuals for collections and note on the *Wadeable Stream Fish Sampling Field Datasheet* (RD[11]; see Voucher Speciment Preservation, SOP D.2).
21. The anesthetic solution will be disposed of in accordance with the terms and conditions of applicable permits. Disposal in the field (away from the stream) will only occur where permitted. Field disposal of anesthetic will take place at least 10 m from the bank away from sensor sets or groundwater wells so as not to impact any other water chemistry measurements or sample collection. Anesthetic solutions are not expected to affect any other aquatic

measurements if disposed of away from the stream. If field disposal is not permitted, waste anesthetic solution will be carried out and disposed of at the Domain Support Facility.

D.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, technicians 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 8). If a specimen is definitively of a particular tribe (i.e., Cyprinini) and the technician 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 technician feels that two seem more like *Hybognathus hankinsoni* and the other eight are

more closely aligned with *Hybognathus placitus*. The technician 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 8. 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 technicians 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 (it is within the genus *Etheostoma*) and detailed morphological. 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 technicians 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 technicians 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, technicians 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. The taxon table will be updated for the subsequent year to make that species name available to technicians within that domain.

D.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 technician-identified species or morphospecies will be sent for later taxonomic confirmation by an expert taxonomist and/or 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 the taxonomist, the two morphospecies can later be merged into the same species designation.

- If a species assignment cannot be made based on the collection of NEON photo vouchers or 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, 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.
- 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”).
  Note: Because domain 13 is split across two support facilities, the MorphA assigned by the Boulder office will not be the same as the MorphA assigned by the support facility in Utah. To avoid confusion, the Utah domain support facility will put an extra letter (“Z”) between “Morph” and their unique letter combinations. Unique letters will be used as described above. The first

morphs would be called MorphZA, MorphZB, MorphZC, etc. The 27th morphospecies at the Utah facility will be MorphZAA.

D.4 Euthanizing Fish and Non-Target Species

1. Euthanize fish using a lethal dose of 10% eugenol at a concentration of 150 mg/L. Refer to Table 9 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 9. 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
150	12.6 mL	25.2 mL	50.4 mL	75.7 mL	100.9 mL	126.1 mL

a) Add 25.2 mL of 10% eugenol to 5.0 US 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; **Error! Reference source not found.**) and add 10% formalin preservative. One taxon per specimen bottle. See steps on specimen preservation in SOP C.7.
   (1) 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.

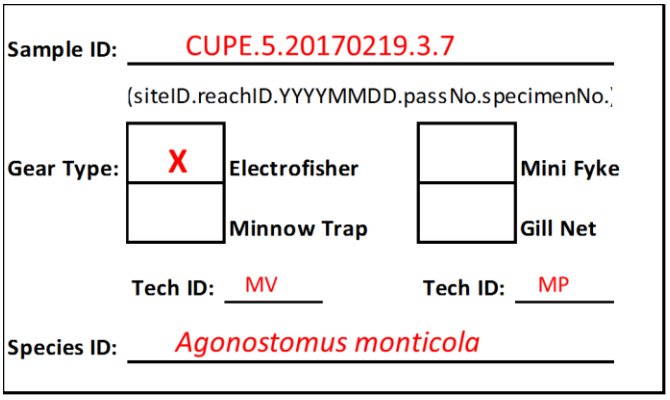

Figure 10. Example specimen label

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 C.7.

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 11) follow below.

Figure 11. 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) 1st stage injection solution - 1% MS-222
   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) 2nd stage injection solution - 50% (v/v) unbuffered MS-222
   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 12).

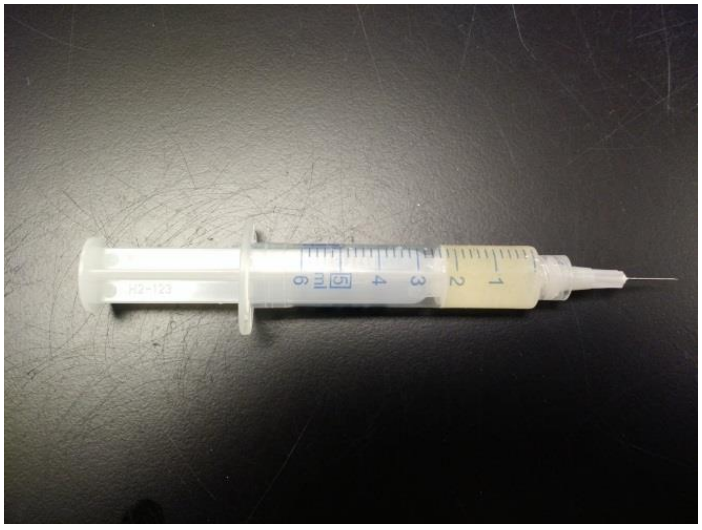

Figure 12. 2nd stage 50% (v/v) unbuffered MS-222 solution. Note yellow cloudy appearance of solution

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 10 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 10. 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 13).

Figure 13. Intracoelomic injection into the inguinal fossa of a turtle.

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 11).

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 11).

Table 11. 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 13).

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. NEON will contact the regional U.S. Fish and Wildlife Service office and the local state fish and wildlife department to report the incident. Specimens will also be preserved following the methods described above. If the inadvertent death of a protected species is discovered once the specimen has been shipped to a taxonomic specialist or the curation facility, NEON will immediately contact the federal and state fish and wildlife authorities within the region where the specimen was collected.

D.5 Fish Tissue Sampling for DNA Analysis

1. Put on gloves (nitrile or latex free).
2. Anesthetize target fish following SOP D.1.
3. 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.
4. **VERY IMPORTANT:** Fish tissues collected for DNA extractions cannot be exposed to or preserved in formalin.
5. Tissues must be collected from anesthetized fish as part of the Fish Processing (SOP C.5) steps.
6. Using the cutting tool, remove a piece of the adipose fin ray (Figure 14). If the fish does not have an adipose fin, collect a piece of the left pelvic fin. Other fins are available for tissue sampling if the adipose fin or left pelvic fin are not suitable (Figure 14). Tissue should be a minimum of 7 mm (paper hole punch) but no larger than 20 mm (nickel) in diameter. Smaller tissue samples should be harvested from smaller fish.

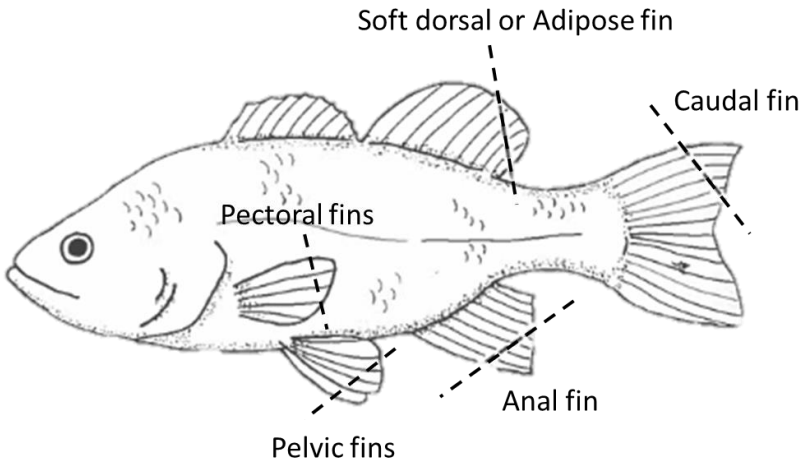

Figure 14. Optional fins to clip for tissue sampling

7. With the forceps, place the fin clip in the appropriate tissue container (envelop or vial provided by the lab). Be sure that the container is completely closed and labeled with the appropriate sample ID.
8. Place fish that have had tissues samples collected into the recovery bucket.
9. Indicate on the *Wadeable Stream Fish Sampling Field Datasheet* that a DNA tissue sample was taken and provide a sample ID format "siteID.reachID.YYYYMMDD.passNo.specimenNo.DNA" for each specimen.
10. Flame sterilize (or wipe) the cutting tool and forceps before collecting the next tissue sample to avoid cross-contamination. Be sure that no fish tissue, slime, or blood remains on the instruments.
11. Repeat Steps 1-8 until all targeted fish samples have had tissues collected.

a) 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.

D.6 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).
   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 “siteID.reachID.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% 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 (JIRA).

D.7 Ending the Sampling Day

1. Refreshing the sampling kit
   a. Replace batteries for all battery operated equipment (e.g., GPS unit, portable aerators).
   b. Refill/restock preservative and anesthetic stock solution containers.
2. Equipment maintenance, cleaning and storage
   a. Wash all equipment that has come in contact with stream water according to the NEON Aquatic Decontamination Protocol (RD[07]).
   b. Dry all equipment thoroughly between sites and before storage.

c. Check all nets for holes and patch if necessary using the net repair kit. Mending fish nets takes practice and patience. There are several resources available online. 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, this is more common when operating in water with high conductivities, use fine grit sandpaper to remove rust.

SOP E 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. 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). Vertebrate sorting takes place within 24 hours of trap collection, thus digital transcription should happen within 14 days of collection. Invertebrate bycatch may not be processed until the end of the field season; digital data transcription of invertebrate bycatch is therefore not required until 14 days after sorting of invertebrates occurs.

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.

Download all images from the camera and save in folder named "SiteID_YYYYMMDD_FishPhotos". Save individual photographs by the photoID recorded in the mobile data device or photo log datasheet.

SOP F Sample Shipment

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

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

F.1 Handling Hazardous Material

Follow shipping and Hazmat procedures for formalin.

F.2 Supplies/Containers

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

F.3 Timelines

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

F.4 Conditions

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

F.5 Grouping/Splitting Samples

N/A

F.6 Return of Materials or Containers

N/A

F.7 Shipping Inventory

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

F.8 Laboratory Contact Information and Shipping/Receipt Days

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

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APPENDIX A DATASHEETS

The following datasheets are associated with this protocol:

Table 12. Datasheets associated with this protocol

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

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

APPENDIX B QUICK REFERENCES

Step 1 – Prepare equipment, 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 10% eugenol (AQUI-S®20E)	Volume of Treatment Water (gal)					
	2.5	5	10	15	20	25
20 mg/L	1.7 mL	3.4 mL	6.7 mL	10.1 mL	13.5 mL	16.8 mL
25 mg/L	2.1 mL	4.2 mL	8.4 mL	12.6 mL	16.8 mL	21.0 mL
30 mg/L	2.5 mL	5.0 mL	10.1 mL	15.1 mL	20.2 mL	25.2 mL

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

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

Step 4 – If this is your first sampling year, establish and select random sampling reaches (see SOP B).

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 4-letter species code (e.g., *Cottus cognatus* = COCO) and record on the *Wadeable Stream Fish Sampling Field Datasheet* (RD[11]). Euthanize the fish if it cannot be identified in the field.

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

Step 12 – 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 13 – Preserve euthanized specimen in a jar with a 10% buffered formalin and ship to taxonomist.

APPENDIX C REMINDERS

Before heading into the field:

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

Sample collection:

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

Sample processing:

- ☑ Do not euthanize 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. Skip numbers that have either been chosen as a fixed reach, or do not exist at the site (i.e., sites that are < 1 km may have fewer than 10 reaches). If sampling is impractical as a result of severe drought (dry) or that the lake is frozen then indicate this for any affected reach on the *Wadeable Streams Fish Sampling Field Datasheet* (RD[11]). Should environmental conditions impact 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 *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