AOS PROTOCOL AND PROCEDURE: FISH SAMPLING IN LAKES

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Change Record

REVISION	DATE	ECO #	DESCRIPTION OF CHANGE
A	02/18/2014	ECO-01394	Initial release
B	01/22/2015	ECO-02632	Migration to new protocol template
C	11/18/2015	ECO-03328	Major updates to include IACUC requirements and input from technicians, removed datasheets from appendices and created NEON.DOC.003106 Datasheets for AOS Protocol and Procedure: Fish Sampling in Lakes
D	05/02/2017	ECO-04507	CM updated with new template and changes based on feedback from FOPS. Fish tissue DNA sampling included. Fish euthanasia with AQUI-S20E. Minimized sample size per species and included bulk processing steps.

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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 particularly useful indicators of ecological integrity because they are influenced by a variety of processes and regimes (i.e., resource availability, anthropogenic physiochemical disturbances), have the ability to alter aquatic ecosystems as top consumers, and are relatively long-lived species. 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 ecosystem health because they are a diverse taxonomic group with a broad range of habitat requirements and life history strategies: fish assemblages represent 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.

Assessing fish assemblages in lakes and impoundments is challenging due to numerous sampling biases (e.g., gear, season, location) that affect accurate characterization of populations and assemblages (Hayes et al. 1996). The quantitative assessment of fish assemblages is often limited by the cost associated with sampling because multiple sampling methods conducted across large temporal and spatial scales are required. Most research identifying appropriate gears for sampling fishes in lakes has focused on sport fish populations (Hubert 1996; Reynolds 1996). Although sport fishes are important from ecological and social perspectives, non-game fishes may be fundamental to ecosystem function and provide a better reflection of ecological integrity (e.g., Simon 1998). Consequently, little information is available on the appropriate methods to accurately and precisely estimate fish assemblage

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 lake fish sampling methods was based on the guidance from the NEON Fish Sampling Workshop. Specifically, 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 were instrumental in providing recommendations for the site-level fish sampling design at NEON lake sites. Additionally, the sampling protocols herein followed the guidelines recommended by the American Fisheries Society (Bonar et al. 2009) and were chosen to align with those of United States Environmental Protection Agency (USEPA 2007).

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.001204	AOS Protocol and Procedure: Macroinvertebrate Sampling in Lakes and Non-Wadeable Streams
RD[09]	NEON.DOC.001197	AOS Protocol and Procedure: Bathymetry and Morphology of Lakes and Non-Wadeable Streams
RD[10]	NEON.DOC.001195	AOS Protocol and Procedure: Riparian Habitat Assessment in Lakes and Non-Wadeable Streams
RD[11]	NEON.DOC.002494	Datasheets for AOS Sample Shipping Inventory
RD[12]	NEON.DOC.003106	Datasheets for Fish Sampling in Lakes

2.3 External References

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

2.4 Acronyms

Acronym	Definition
A	Ampere or amp
AFS	American Fisheries Society
AQUI-S20E	10% eugenol; fish anesthetic
Cm	Centimeter
DC	Direct current
DNA	Deoxyribonucleic acid
Hz	Hertz
M	Meter
mL	Milliliter
Mm	Millimeter
MS-222	Tricaine methanesulfonate
PFD	Personal flotation device
SL	Standard length
TL	Total length
USEPA	United States Environmental Protection Agency
USGS	United States 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. Electrode pole (anode) for backpack electrofishing unit (photo: store.smith-root.com)

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 true 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 which is commonly a stainless steel cable that is dragged behind the operator for backpack electrofishing.

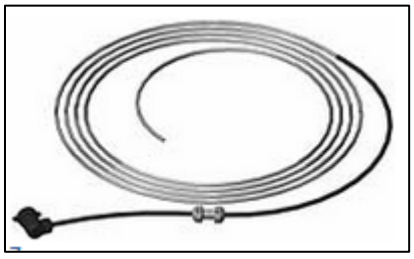

Figure 2. Cathode for backpack electrofishing unit (photo: store.smith-root.com)

Crepuscular: Of or relating to twilight, both dawn and dusk.

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 commonly have been associated with increased injuries to fish.

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

Lentic: Of or relating to still waters, e.g., lakes. Opposite of lotic, e.g. brooks, streams, and creeks.

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

Pulsed DC: Direct electrical current that is interrupted rapidly.

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

Thermocline: A distinct layer in a body of water where the change in temperature is more rapid than increasing depth - usually a change of more than 1^{o}C per meter. The denser and cooler layer below the thermocline is the hypolimnion. The warmer upper layer is termed the epilimnion.

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 of this document is to outline the sampling protocol and procedures used for sampling fish in lakes at NEON sites. Unlike rivers and streams where relatively few sampling methods (e.g., electrofishing, seines) are commonly used to characterize fish assemblages (Guy et al. 2009; Rabeni et al. 2009), numerous methods (gears; e.g., electrofishing, seines, fyke nets, gill nets, trawling) are used to sample fish assemblages in lakes and impoundments (Miranda and Boxrucker 2009; Murphy and Willis 1996). Multiple methods are typically required because lakes and impoundments have two distinct zones (i.e., pelagic and littoral) that differ in physicochemical characteristics and fish assemblage structure. Substantial differences in physical characteristics (e.g., depth, water clarity, vegetation) and the selectivity of species and sizes of fish affect the efficiency of sampling methods in differing zones. For example, multiple gears are often necessary to sample both juvenile and adult fish of the same species because of differing habitat use and size biases associated with various equipment (Boxrucker et al. 1995). Although a few studies have evaluated multiple sampling equipment types, most studies have focused on a limited number of species (e.g., gizzard shad *Dorosoma cepedianum*, black crappie *Poxomis nigromaculatus*) at small spatial scales (e.g., Boxrucker et al. 1995; Guy et al. 1996; Allen et. al 1999). Herein we describe a sampling method that attempts to overcome traditional problems associated with characterizing fish assemblages in lentic habitats, by using multiple active and passive methods (i.e., backpack electrofishing, mini-fyke nets, and gill nets) at different times throughout the year to capture fish of a variety of size classes and habitat preferences.

In this protocol, up to 10 segments will be established within the lake for studying nearshore and offshore fish populations (Figure 3). Three permanent “fixed” segments should be established which include habitat features most representative of the entire lake. The fixed segments will be sampled twice per year with a backpack electrofisher using multiple pass depletion, one mini-fyke net set, and one gill net set (Figure 3; Baker et al. 1997). The remaining seven lake segments will be established as “random” segments and sampled following a random stratified design to ensure that the variety of habitat types are sampled equally over time. A rotating sampling design with initial random selection of shoreline segments ensures appropriate spatial coverage of habitat types within the lake (Baker et al. 1997). The same random segments that were systematically chosen for additional sampling should be sampled in spring and fall. Random segments will be sampled with a single electrofishing pass (without block nets), one mini-fyke net set, and one gill net set. Sampling and net placement should be located far enough apart to minimize interactions from each effort.

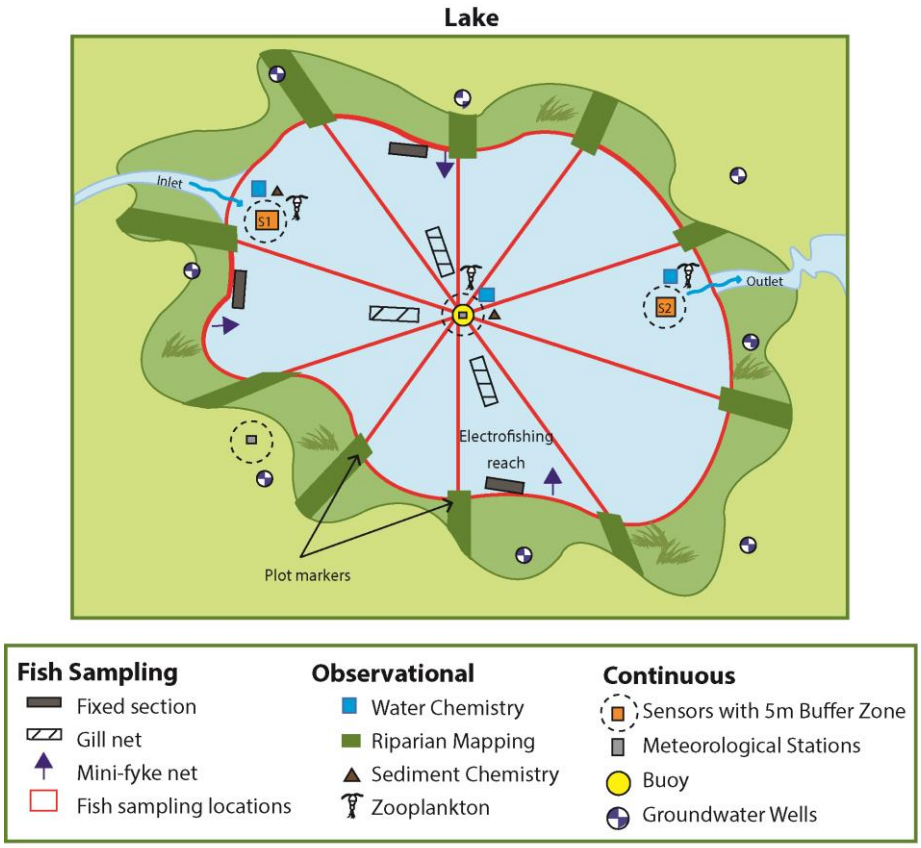

Figure 3. A generic lake site layout with fish sampling locations

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]). Sampling Schedule

3.1 Sampling Frequency and Timing

Lake fish sampling will occur two times per year during the growing season at each lake 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), 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 Lakes and Non-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[08]).

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 segment must be sampled within the same day, with at least 30 minutes between passes to allow fish to resettle in the reach.

Lake fish assemblage characterization requires multiple sampling methods that are optimal for sampling fish at different times of the day. Electrofishing will occur at night, starting 30 minutes after sunset and cease 30 minutes before sunrise (or during lowest-light hours at Arctic sites). Gill nets will be set and sampled during daylight hours, with a preferred set time of up to 1 hour and maximum set time of 2 hours. Gill nets should be set in the morning or early afternoon to allow for processing time. Mini-fyke nets will be set before sunset and allowed to remain in the water until after sunrise the following morning (Table 1). Mini-fyke nets should be set before sunset and retrieved the following morning. Within each fixed fish sampling location (Figure 3) three electrofishing passes will occur along with one mini-fyke and one gill net set. Random segments will include a single pass with an electrofisher, one mini-fyke, and one gill net. These sampling efforts may require additional time if needed within the sampling week requiring additional time and is indicated as "if needed" in the propose sampling schedule (Table 1).

Table 1. Proposed lake sampling activities for a crew of 3 during a 5 day period

	Day 1	Day 2	Day 3	Day 4	Day 5
Day	AM/PM: Set electrofishing block nets PM: Set mini-fyke nets	AM: Pull mini-fyke nets AM/PM: Run gill nets PM: Set mini-fyke nets	AM: Pull mini-fyke nets AM/PM: Run gill nets PM: Set mini-fyke nets	AM: Pull mini-fyke nets AM/PM: Run gill nets PM: Set mini-fyke nets (*if needed*)	AM: Pull mini-fyke nets (*if needed*) AM/PM: Run gill nets (*if needed*)
Night	Allow electrofishing segments to recolonize	Electrofish in fixed segments	Electrofish in fixed or random segments	Electrofish random segments (*if needed*)	

3.2 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 ice-out, water temperature (or accumulated degree days), weather, and riparian greenness.

3.3 Timing for Laboratory Processing and Analysis

Samples may be stored for up to 1 month following the preservation guidelines in SOP D.9. For storage and shipping timelines see SOP F. 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. 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.

3.4 Sampling Timing Contingencies

The setting of electrofishing block nets (at fixed segments), mini-fyke nets, and gill nets shall be set during the day; the mini-fyke and gill nets shall be pulled during the day. Electrofishing sample activities shall occur only after daylight hours and before sunrise to maximize capture efficiency. For additional safety requirements regarding nighttime electrofishing, refer to AD[02] Operations Field Safety and Security Plan. All three-passes in a fixed segment must occur within the same evening, with at least 30 minutes and no more than 2 hours between passes. Two to three segments will be sampled during one nighttime electrofishing event. A minimum of 2 weeks between sample periods shall be observed (Table 2).

Table 2. Contingent decisions

Delay/ Situation	Action	Outcome for Data Products
Hours	If weather conditions deteriorate and the lake becomes too windy (>9 km hr^{-1}) to hold the boat stationary over a sampling point, return to shore and wait in a safe location for 30 minutes. If wind subsides, resume sampling, if not, return to the Domain Support Facility and sample at another time.	None as long as samples are collected within the pre-determined sampling window. If waiting for favorable conditions causes sampling to occur outside of the biological bout sampling window by more than three days, submit a trouble ticket through the NEON problem resolution system (JIRA).
	If electrofishing activities are interrupted due to unsafe field conditions, captured fish should be released and sampling discontinued. If an entire segment cannot be completed, recollect all data on the next appropriate day.	
	Do not begin sample collection unless there is enough time to complete an entire sampling segment (i.e., all passes of an electrofishing segment, or a 1-hour gill net set).	
3 or More Days	If heavy rainfall affects visibility or flooding/high water occurs on or prior to the targeted sampling date, wait a minimum of 3 days to allow the fish community to recolonize habitats.	None as long as samples are collected within the pre-determined sampling window. If waiting for favorable conditions causes sampling to occur outside of the biological bout sampling window by more than three days, submit a trouble ticket through the NEON problem resolution system (JIRA).

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 *Lake Fish Sampling Field Datasheet* (RD[12]). 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 *Lake Fish Sampling Field Datasheet* (RD[12]).

3.5 Sampling Specific Concerns

1. Fish sampling should not occur while other aquatic sampling activities that could disturb sediments or hydrology (e.g., macroinvertebrate sampling) are occurring in the lake.
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 lake 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).

4 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 fishing 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. Electrofishing will not be conducted at these sites.
2. All employees shall have access to a form of communication for constant contact 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 should only be performed when flow conditions are safe. Wading is not allowed when the depth times velocity is equal to or greater than 10 ft^2/sec. Do not attempt to wade in a lake past waist-deep.

5. Safety Data Sheets for chemicals used in this protocol shall be reviewed and shall be readily available to technicians while the chemicals are in use.
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. Operator must be fully trained by manufacturer of equipment and all other associated personnel shall be trained on safe operations through established and approved training program (see Section 6.2 Training Requirements).
2. Audible signals must be used to alert technicians when electrofishing equipment is in operation.
3. Chest waders and heavy-duty rubber 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.
9. It is recommended that cold weather and waterproof clothing accompany each person actively participating in the fish sampling events. Chemical hand warmers and warm drinks are also recommended particularly during fall sampling activities.

5 PERSONNEL AND EQUIPMENT

5.1 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 3. Equipment list – Field preparation

Item No.	R/S	Description	Purpose	Quantity	Special Handling
Durable items					
	R	HDPE bottle, amber, 1 L	Stock solution (MS-222) container	2	N
	R	Lab safety glasses	Safe handling of chemicals	1 pair	N
	R	Battery charger (electrofishing batteries)	Charging the electrofisher	1	N
Consumable items					
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	AQUI-S20E	Anesthetizing specimens	50 mL	N
	R	Nitrile gloves (latex-free; pair)	Safe handling of chemicals and fish	1	N

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

R/S=Required/Suggested

Table 4. Equipment list – Segment establishment

Item No.	R/S	Description	Purpose	Quantity	Special Handling
Durable items					
RD[09]	R	Site-specific bathymetry map	Navigating to sampling segments	1	N
RD[10]	R	Site-specific riparian map	Navigating to sampling segments	1	N
	R	Plot survey markers (aluminum, site-specific)	Establishing sampling segments	10	N
	R	Meter tape (50 m)	Establishing sampling segments	2	N
	R	Handheld GPS (with batteries, ± 1 m accuracy)	Navigating to sampling segments	1	N

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

R/S=Required/Suggested

Table 5. Equipment list – Electrofishing

Item No.	R/S	Description	Purpose	Quantity	Special Handling
Durable items					
	R	Rubber mallet	Hammering PVC posts for random shore reaches	1	N
	R	PVC pipe 5 feet long	For setting random reach boundaries	24	N
	R	T-post insulator or caps with clips	Used to hang the block net on the T-posts	30	N
	R	Carabineers	Attaching weights to the block net	30	N
	R	Duck decoy weights (non-lead) 8 oz.	Anchoring the block net	36	N

Item No.	R/S	Description	Purpose	Quantity	Special Handling
	R	Wrench (9/16th)	Used to tighten the anode wring to the fiberglass pole	1	N
	R	Steel studded fence posts (i.e., T-post)	Securing block net at segment boundary	30	N
	R	Fence post driver or small sledge	Securing block net at segment boundary	1	N
	R	Fence post puller	Securing block net at segment boundary	1	N
	R	3 mm mesh block nets with lead lines and top lines with floats (35 m x 1.5 m)	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 segment boundary	15	N
	R	Net repair kit: • needle • string • butane lighter • zip ties	Repairing nets	1	N

Item No.	R/S	Description	Purpose	Quantity	Special Handling
	R	Battery-powered backpack electrofishing unit	Electrofishing	1	N
	R	Anode pole (1.5-2.0 m) with attached anode ring	Electrofishing	1	N
	R	Cathode (rattail type; 1-2 m and 10-15 mm in diameter)	Electrofishing	1	N
	R	Electrofisher batteries (rechargeable)	Electrofishing	3	N
	R	Abrasive pad to clean anode rings	Electrofishing	1	N
	R	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
	R	Rubber lineman gloves (Class 0, rated for max use voltage 1,000V AC/ 1,500V DC)	Safe handling of electrofishing equipment	1 pair per person	N
	R	5 gallon buckets	Storing specimens	10	N
	R	Hand held conductivity/temperature meter	Measuring conductivity and temperature	1	N
	R	Chest waders (approved for electrofishing)	Safe wading	1 pair per person	N

Item No.	R/S	Description	Purpose	Quantity	Special Handling
	R	Chest wader repair kit (e.g., Aquaseal) or extra waders	Safe wading	1	N
	R	Head lamps (with batteries)	Increasing efficiency of fish capture	1 per person	N
Consumable items					
	R	Zip ties (multiple sizes)	Taking up slack on the block net	A lot	N

R/S=Required/Suggested

Table 6. Equipment list – Gill nets

Item No.	R/S	Description	Purpose	Quantity	Special Handling
Durable items					
	R	Gill net tubs	Storing gill nets	6	N
	R	Gill net hooks	Securing gill nets	6	N
	R	Depth finder	Navigating to sampling locations	1	N

Item No.	R/S	Description	Purpose	Quantity	Special Handling
	R	Experimental monofilament sinking gill nets Panel dimensions – 3.1 m long × 1.8 m deep Mesh bar size – 19, 25, 32, 38, 44, 51, 57, 64 mm Mesh order – 38, 57, 25, 44, 19, 64, 32, 51 mm Hanging ratio – 0.5	For catching specimens	3	N
	R	Net floats	Securing gill nets	18	N
	R	12.7 mm diameter rope (3-10 m in length) for floats	Securing gill nets	18	N
	R	Net anchors	Securing gill nets	18	N
	R	Live well	For storing live fish on the boat for processing	1	N
Consumable items					
		(none)			

R/S=Required/Suggested

Table 7. Equipment list – Mini-fyke nets

Item No.	R/S	Description	Purpose	Quantity	Special Handling
Durable items					
	R	Mini modified fyke nets Mesh – 6.35 mm bar knot-less with asphalt coating Lead – One, 7.6 m long × 0.6 m deep Trap – Two 0.6 m × 1.2 m rectangular frames, two 0.6 m diameter hoops with one funnel, cod end with purse string closure.	For catching specimens	3	N
	R	Reusable nylon cable ties (46 cm)	Securing block nets	50	N
	R	T-type block net stakes (e.g., ~45 cm long, 20 cm handle, 1 cm diameter stainless rod)	Securing mini-fyke nets	6	N
	R	Waterproof blinking LED light	Marking mini-fyke net locations	6	N
Consumable items					
		(none)			

R/S=Required/Suggested

Table 8. Equipment list – Fish processing

Item No.	R/S	Description	Purpose	Quantity	Special Handling
Durable items					
	R	Fish and top predator taxonomic ID key specific to location or region (denotes endangered species)	Identifying specimens	1	N
	R	Portable aerators (batteries, diffusion stone)	Aerating buckets	15	N
	R	Small dip net (3.2 mm mesh)	Handling specimens	5	N
	R	Fish measuring boards (50 cm)	Measuring specimen length	2	N
	R	Portable digital scale (batteries, charger)	Weighing specimens	1	N
	R	Plastic tray	Weighing specimens	2	N
	R	Digital camera (batteries, memory card)	Photographing specimens	1	N
	R	25-50 mL graduated cylinder, plastic	Mixing anesthetic and euthanizing solution	1	N
Consumable items					
	R	Nitrile gloves (pair)	Safe handling of chemicals and fish	20	N

Item No.	R/S	Description	Purpose	Quantity	Special Handling
	R	HDPE wide mouth specimen jars (1 L)	Specimen preservation containers	50	N
	R	MS-222 stock solution	Euthanizing specimens	1 L	Y
	R	AQUI-S®20E (10% Eugenol)	Anesthetizing specimens	1 L	Y
	R	10% buffered formalin (37-40% formaldehyde)	Preserving specimens	20 L	Y
	R	Fin clipping scissors	Cutting fish fins for DNA barcode samples	1	N
	R	Tissue containers	For preserving fin clips for DNA barcoding; maybe provided by an external lab	10	N
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
MX100549	R	50mL Centrifuge tube	Store syringe and needle, measure injection fluids	1	Y

Item No.	R/S	Description	Purpose	Quantity	Special Handling
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

Table 9. Equipment list – General boating equipment

Item No.	R/S	Description	Purpose	Quantity	Special Handling
Durable items					
	R	Boat		1	Y
	R	Anchor with rope		1	N
	R	Oars		2	N
	R	Trolling Electric Motor		1	Y

Item No.	R/S	Description	Purpose	Quantity	Special Handling
	R	Battery (12 volt)		1	Y
	R	Safety kit for boat (e.g., flares, bailer, float with rope)		1	Y
	R	Personal Flotation Devices (PFDs)		1 per person	N
Consumable items					
		(none)			

R/S=Required/Suggested

5.2 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 lakes and safe working practices for boat-based fieldwork. Specific training for lake 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 that will be participating in electroshocking sampling activities 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 negative health impacts on 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 electrofishing activities shall have one member that has received the manufacturer safety training; all crew members that participate in electrofishing activities 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

5.3 Specialized Skills

N/A

5.4 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.

6 STANDARD OPERATING PROCEDURES

SOP A 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 is 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, boat motor, GPS unit, camera, portable scale, temperature/conductivity meter, portable aerators, and headlamps batteries overnight or longer.
2. Inspect electrofishing unit for normal operation (e.g., no frayed cathode or broken anode, no error message when turned ON, functioning activation switch).
3. Inspect boat, trailer, and motor for normal operation.
4. Inspect lineman gloves and waders for holes and tears, repair if necessary.
5. Inspect dip nets, block nets, gill nets, and mini-fyke nets for rips, tears, and holes. Repair if necessary.
6. Inspect portable aquarium pumps, diffusion stones, and batteries.
7. Inspect buckets to ensure handles are present and functioning.
8. Ensure that all equipment has been decontaminated since last use (see RD[07]).
9. Print data sheets and specimen labels (RD[12])) on waterproof paper. Verify that the mobile data entry device is charged and synced prior to use.
10. Select random sampling segments if this is the first sampling date for the year (SOP C).
11. Fish will be anesthetized and euthanized with 10% eugenol (AQUI-S®20E). Non-target species will not be anesthetized; however, mortally injured non-target species shall be euthanized with Tricaine methanesulfonate (MS-222).
    a. **10% eugenol (AQUI-S®20E):** This drug is currently available under the USFWS Investigational New Animal Drug (INAD) program; Study Protocol 11-741. As such, NEON must comply with data reporting requirements including drug acquisition and handling as well as fish treatment and disposition. Be sure to bring along the *INAD Reporting Datasheets* in the field when conducting fish sampling activities. 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 B Establishing Sampling Reaches

Establish sampling segments during the first year of sampling. Segments may need to be reestablished if significant morphological changes have occurred since the last sampling bout including water depth reductions from drought or morphological changes from flooding, landslides, or shoreline erosion. Use the pre-determined 10 riparian habitat sections (see Lake Riparian Habitat Assessment Protocol, RD[10]). Lake fish sampling does not need to occur within the same exact location every time. Instead, the electrofishing reaches (fixed and random), mini-fyke nets, and experimental gill nets should be placed within the 10 riparian segments (Figure 3).

1. Using the site-specific Riparian Habitat Assessment locations (RD[10]), fish sampling will occur within each of the 10 riparian segments.
2. Navigate to the permanent aluminum plot survey markers on the shoreline at each section boundary if present. If the plot markers have not been installed, navigate to the location following the GPS coordinates.
3. Follow the steps in SOP C to establish the electrofishing reach and the deployment locations for the mini-fyke net and experimental gill nets.

SOP C Fixed and Random Sampling Section Selection

Section selection occurs during the first year of sampling. Sections will be revisited over the following years.

1. Up to six sections (three fixed and three random) will be sampled during each sampling bout (Table 10) depending on the size of the lake (Appendix E).
2. Select three of the 10 riparian sections to be the "fixed" sections. Fixed sections will be sampled two times per year throughout the duration of NEON measurements.

   a. The three fixed 100 m^2 reaches should be chosen to best represent the habitat variability throughout the lake (e.g., presence or absence of vegetation, substratum type). Fixed reaches will be selected by the NEON Aquatic Ecologist or Domain Aquatic Technician.

3. Electrofishing in sections with sensors must occur ≥5 m away from all in-lake electronics. Select three of the remaining seven random sections to be sampled annually. Refer to Table 10 for randomized order of sections for each lake site.
4. Use the same three random sections for all sampling dates within one year (Table 10).
5. For each year of sampling, continue down the list of randomized sections not sampled previously. In year three (if the lake contains 10 sections), there should only be one section that has not yet been sampled. Return to the first random section when all sections have been sampled.
6. Follow this pattern for the remainder of the study.

Table 10. Example of rotating section design for sampling one lake site over 10 years. Gray boxes denote when a section is sampled. Randomized 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 D Field Sampling

D.1 Electrofishing Segment Set-Up

1. Navigate to the first riparian section selected for sampling using GPS points, the morphology map, or plot survey markers.
2. Setup fence posts and block nets for electrofishing in fixed segments. For random segments, setup fence posts or PVC pipe to mark random segments but do not setup block nets.
   a. Electrofishing shall only be conducted the night following block net and/or fence post setup or later to allow fish to acclimate after disturbing the area.
3. Drive fence posts using the fence post driver into the lake substrate outlining the 4 m × 25 m electrofishing reach, with the long axis parallel to the shoreline.

   a. **VERY IMPORTANT:** All technicians MUST be trained in the use of fence post drivers/pullers prior to deploying with the Lake Fish Sampling Team.
   b. Start at the shoreline.
   c. Minimize the disturbance to the sampling area inside the 4 x 25 m electrofishing reach.
   d. Space fence posts a maximum of 4 m apart.

e. Ensure that the deep edge is ≤ 1 m deep to allow for safe electrofishing. It is ok if the reach is less than 4 m x 25 m as long as the size has been measured and recorded.

4. Attach PVC caps or electric fence insulators to the top of the T-posts to hang block net in the fixed reaches. Use reusable nylon cable ties (zip ties) to bundle loose portions of the net approximately 30 cm above the water line.
   a. Fence posts or PVC pipes in random segments are used to delineate the electrofishing area, but will not have block nets attached to them.
5. Bundle the remaining net and secure to the fence post with a reusable nylon cable tie, keeping the unused portion of the block net above the water line. Clip decoy weights on the bottom portion of the block net between the posts to anchor the block net.
   a. The random segments (up to 3) will be sampled via non-enclosure electrofishing (i.e., no block nets).

D.2 Backpack Electrofishing Field Set-Up

Test settings on the backpack electrofisher before sampling begins. After settings are determined, they will be used for the remainder of the sampling bout. Electrofisher settings should be adjusted however, should injury or mortality to sampled fish occur after the initial settings are determined.

1. **VERY IMPORTANT:** All technicians MUST wear necessary personal protective equipment before stepping in the water, including waterproof chest waders with appropriate fitting rubber lugged-soled boots, rubber lineman gloves to insulate the wearer from electrical shock. Head lamps must also be worn during nighttime sampling.
2. Assemble anode pole (Figure 1).
3. Measure and record water temperature and specific conductivity using the handheld conductivity meter. Record on Field Data Sheet (RD[12]).
4. Connect the cathode (Figure 2) and anode to the backpack electrofishing unit (Figure 4).

Figure 4. Cathode and anode connections on backpack electrofishing unit

5. Connect the battery to backpack electrofishing unit and secure the batteries with the strap to the backpack frame (Figure 5.). If using the lithium ion battery, be sure to include the adapter.

Figure 5. Battery location and secure placement in the backpack electrofishing frame.

6. Test the backpack electrofishing unit >50 m away from the designated electrofishing reach.
   a. Select an area of the lake shoreline that has characteristics similar to that of the sampling reach (e.g., similar depth or vegetation).
7. Wade into the lake 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 water, 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 the conductivity, see Table 11 for additional settings information.

Table 11. 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 not deliver a shock.
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.
11. If 1,100 V is reached and fish are still not responding to electrofishing decrease voltage to 250 V and increase the frequency by 10 Hz (e.g., from 30 Hz to 40 Hz) and repeat Steps 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 lake water carried by one of the netters and continue with Step 12.
12. Continue electrofishing until approximately 20 individuals spanning a variety of sizes are netted. 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 D.3.
13. Place fish in a bucket with fresh lake water and a battery operated aerator.
    a. If other top predators are captured, identify (if possible) and record species on field data sheet (RD[12]) and immediately release >50 m away from electrofishing activity.
14. Examine captured fish for signs of injury (e.g., bent backs, dark bruising, hemorrhaging of the gills; Figure 6). Record injury rate on Field Data Sheet (RD[12]). Less than 3% of the captured fish should be injured.

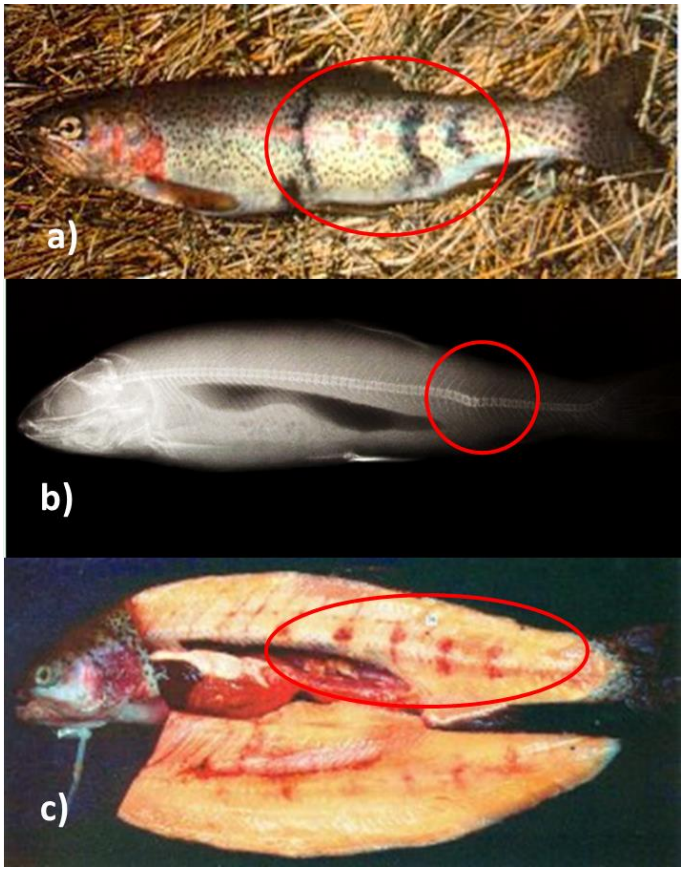

Figure 6. Examples of electrofishing injuries to fish, a) bruising or "burn" marks, b) spinal injury, and c) internal hemorrhaging

a. If > 3% of captured fish are injured, stop 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 in timely fashion will increase injury rates.
c. If fish are injured, allow them to recuperate in a separate bucket or live well with an aerator before releasing.
d. For any fish that do not recover, proceed to euthanization (SOP SOP E).

15. Monitor captured fish for signs of normal respiration and swimming behavior for 10 minutes. If, after 10 minutes, fish are still on their side, upside-down, or injured, return to lower electrofishing settings.
    a. It is important to note that some fish species (e.g., blacknose dace) are sensitive to electrofishing and may exhibit higher injury or mortality rates.
16. Once fish are swimming normally release fish back into the lake outside of the block net area (fixed segments) and at least 50 m away from where they were caught in random segments.
17. Maintain electrofisher settings at the lowest level that allows for the effective capture of fish. Record frequency, duty cycle, and voltage settings on the Field Data Sheet (RD[12]) and reset the timer on the electrofishing unit. These settings will be used for the entire sampling bout.

D.3 Backpack Electrofishing

1. Slowly enter the lake (so as not to disturb fish) and begin lowering the block net by releasing the reusable nylon cable ties.
   a. This activity is best accomplished by the two netters immediately after backpack electrofishing setting testing. Each netter can start on the shoreline and work towards each other while trying to minimize disturbance to the area.
   b. If necessary (e.g., excessive vegetation) secure the bottom of the block net with stainless block net stakes or added weights (decoy lead weight).
   c. In random segments, no block-net is necessary. Fish will be electrofished along a ~4 x 25 m area delineated with T-posts of PVC pipe. .
2. Record the start time on the Field Data Sheet (RD[12]) so that conductivity, turbidity, and other water quality measurements from the in-lake sensors can be coupled with the fish sampling bout.
3. Beginning at one end of the sampling reach, walk into the lake ensuring that the cathode (i.e., rattail) is submerged as much as possible, while holding the anode pole in one hand (anode submerged). It is good practice to have the same person operate the backpack electrofisher when sampling a complete reach (three passes for fixed reaches; one complete pass for random reaches).
   a. The electrofisher operator (crewmember 1) may, but is not required to, hold a dip net in the other hand if he/she feels comfortable.
   b. A minimum of three netters are recommended when electrofishing.
4. The other crewmembers with dip nets will enter the lake behind the electrofisher operator.
   a. The primary netter will stay close to the electrofisher operator to net fish.
   b. The secondary netter will carry the bucket and net any other stunned fish that are missed by the electrofisher operator or the primary netter.
   c. The crew leader 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. If the electrofisher operator does not choose to also hold a net, then the crew leader will also need to act as a netter.
   d. At some sites where a lot of fish are typically captured, it is useful to add a fourth crew member to help distribute the work effort.
5. Ensure that the electrofishing unit settings (frequency, duty cycle, and output voltage) are those determined in SOP D.2 and that the timer (“EF time”) has been reset to 0.
6. Turn the electrofishing unit on and notify the other technicians. Confirm that all technicians are ready to begin.
7. Depress and hold the activation switch on anode pole to begin electrofishing.

a. The anode ring must always be submerged before depressing the activation switch and should never be taken out of the water with the switch depressed. The unit will automatically turn off if the anode is removed from the water.

8. Slowly sweep the anode inside the end of the block net to target any fish that may be seeking cover in the net.
9. After sweeping the block net, the electrofisher operator should turn parallel to the shoreline and slowly sweep the anode from the shore to the block net to expose all available habitats to electricity.
    a. This may require walking slowly from side to side.
10. As the anode is moved from side to side, the netters will capture drifting, immobilized fish.
    a. Dip nets should be held as close to the lake substrate as possible without picking up excessive substrate or debris.
    b. There should always be one net behind the anode.
    c. Fish are often attracted to the cathode (rattail). Netters should periodically check this area for stunned fish.
    d. Netters should be aware that immobilized fish may not always be visible, particularly benthic species (e.g., darters, sculpins), and netters should frequently inspect their nets to minimize injury to fish by continuous exposure to electricity.
    e. Crewmembers with dip nets should CALMLY net immobilized fish without excessive disturbance.
    f. Never put hands in the water to capture fish while activation switch is depressed (i.e., while electrical current is pulsing through the water). If the netter cannot capture a fish using the net (e.g., sculpin, young-of-year), notify the backpack electrofisher to stop shocking. The backpack electrofisher must release the activation switch and remove the anode from the water to ensure no pulse is being conducted, then verbally confirm that it is safe for the netter to put his/her hand (or use the small dip net) in the water. After capturing the fish, the netter removes his/her hands from the water and verbally confirms that he/she has done so. Only then may the backpack electrofisher place the anode in the water and depress the activation switch to continue fishing, notifying other technicians that the unit is on.
    g. If any endangered species (technicians will be notified of likelihood before sampling) or other vertebrates (e.g., salamanders, turtles) are caught, identify, photograph if possible, and release immediately away from electrofishing activities. Record the photoID (camera file name) on the mobile data device or photo log datasheet.
11. Frequently remove fish from dip nets and place in buckets or a live well to minimize injury to the fish.
12. Sampling will continue along the shoreline in a zig-zag pattern (Figure 7) in a single pass with attention to sampling all complex cover (e.g., overhanging or aquatic vegetation, woody debris, undercut banks).

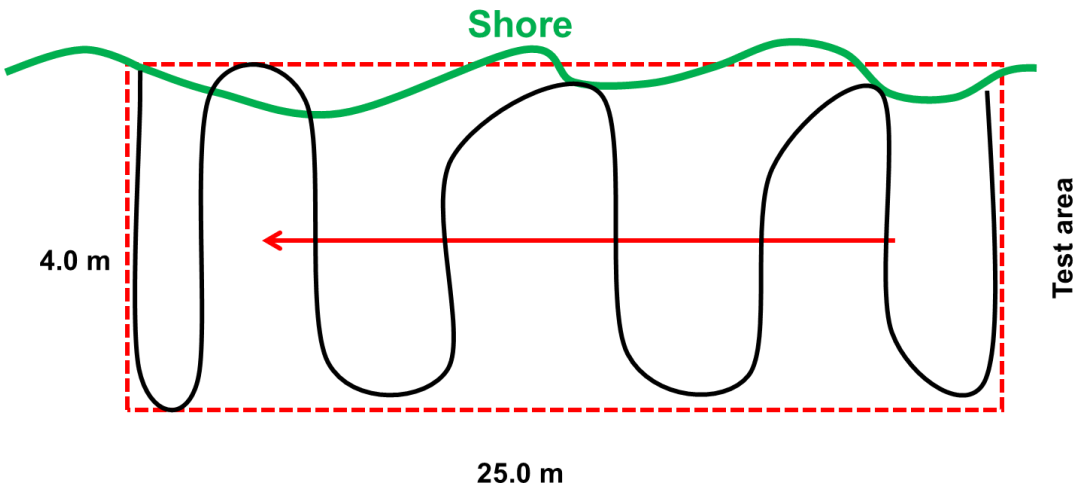

Figure 7. Lake electrofishing sampling pattern

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

13. While electrofishing, monitor the abundance of fish in the buckets to prevent escape or accidental spills.
   a. Be aware of fish overcrowding in the buckets. If fish appear to be gasping at the water surface, they are likely short on oxygen due to water temperature or overcrowding. Place fewer fish in buckets and supplement with cooler water and aerators.
      1) If a lot of predatory fish and prey species are collected they may need to be placed in separate buckets to reduce 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 lake.
   c. 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 sampling activity are processed before the segment is fully sampled.

14. When the crew reaches the end of the block net, the electrofisher operator should slowly sweep the anode inside the block net as fish may have moved to avoid the electrical field.
15. Once the entire sampling reach has been sampled, read and record the time (EF time) in seconds from the back of the electrofishing unit on the *Wadeable Stream Fish Sampling Field Datasheet* (RD[12]).
    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.
    c.
16. Turn the electrofisher off, remove and place on the bank with anode and cathode still attached.
17. Proceed to fish processing (SOP SOP E).
18. If this is a fixed reach, repeat Steps 2-17 until three passes have been completed. If this is a random reach, complete only one pass.
    a. Observe a minimum of 30 minutes between passes to allow fish that were not captured to recover.
    b. Depletion is determined when two consecutive passes result in sequential decreases in total number of individuals sampled after the first pass (e.g., 1000 fish on first pass, 200 fish on second pass, and 50 fish on third pass). In some cases, it may be necessary to do more than three passes for a depletion. If the number of fish appear to increase with each pass, check that the block net is anchored along the bottom or that a whole hasn't developed allowing fish to move into the sample area.
19. Remove block nets and fence posts if all passes are complete.
20. Break down the backpack electrofishing unit if the crew cannot complete another reach during dark hours:
    a. Disconnect the cathode and anode from the backpack electrofishing unit.
    b. Disconnect the battery from the backpack electrofishing unit and remove battery from the backpack frame.
    c. Place backpack electrofishing unit in case.
    d. Disassemble anode pole and store with backpack electrofishing unit.
    e. Place recently used battery separate from charged batteries where it can be easily distinguished for charging.

D.4 Gill Nets

1. Load boat with necessary fish sampling equipment (e.g., gill nets in tubs, live wells, measuring board, digital scale, and depth finder).
2. Locate pre-selected riparian segment using GPS.
3. Prepare the gill net to be deployed by attaching net anchors to each end of leaded bottom line and attaching the float rope (with float attached) to the net anchor (Figure 8.).

a. Start with the end of the net that will be deployed first (i.e., net end that is towards the top of the gill net tub).
b. The net can remain in the gill net tub with float lines and anchors attached until it is deployed.
c. Ensure that the float line is long enough to float on the water surface.

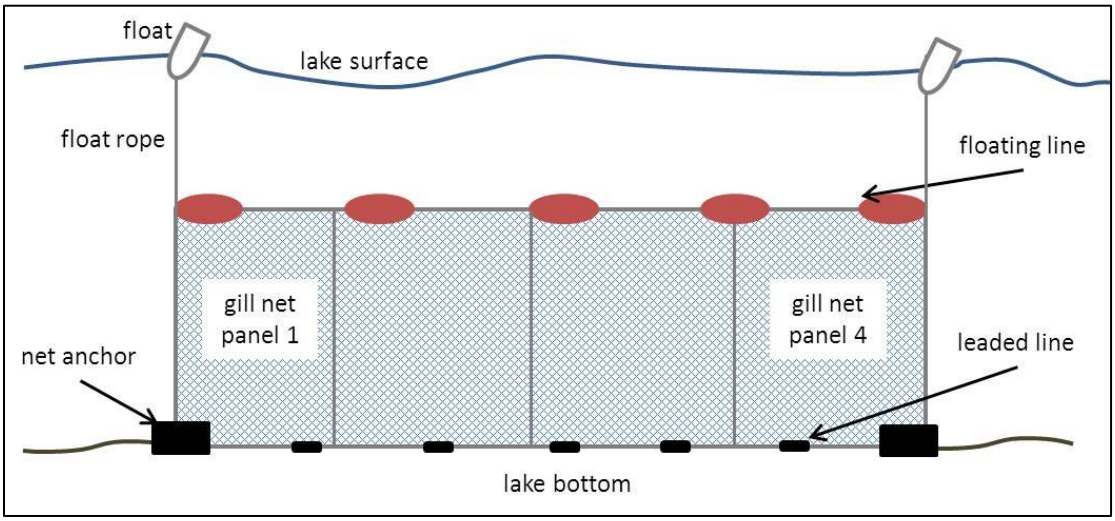

Figure 8. Example of gill net setup. The dimensions of this net are approximately 3.1 m long and 1.8 m deep.

4. Maneuver boat to the appropriate depth (> 2 m or as deep as possible in shallower lakes) using the depth finder within the riparian section boundaries and hold the boat in a still position (using the motor or oars) with the stern facing the approximate center of the lake.
5. Record the start time (24-hour time plus time zone, e.g., 13:30 MDT) on the *Lake Fish Sampling Field Datasheet.*
6. Begin slowly releasing the gill net into the water, ensuring that the net is not twisted.
   a. Start maneuvering the boat slowly in reverse, perpendicular to, and away from, the shore while the gill net is being deployed.
      1) Setup gill nets at additional sampling locations if fish density is relatively low at the site and fish processing will take < 1 hour per net. Stagger multiple gill net sets such that each net can be retrieved within 1-2 hours (or shorter if directed). Subsequent gill nets may be set and sampled the following day.
      2) If the site has high fish density such that fish processing cannot keep up with the number of fish caught, sample only 1-2 gill nets in one day.
   b. Target gill net set time is 1 hour (maximum 2 hours) to minimize mortality.
   c. Gill netting must occur during daylight hours, so nets should be set in the morning or early afternoon to allow for processing time.
7. After at least 1 hour, proceed to the first net set and begin pulling.
8. Untie the float line and net anchor and set aside.
9. Record the end time on the *Lake Fish Sampling Field Datasheet.*

10. Gently remove captured fish from each mesh panel. Take care to close fish operculi (gill plates) and untangle fins or spines before pulling fish from the net. Place specimens in a live well filled with fresh lake water with a battery powered aerator. Fold the net back into the gill net tub until all panels have been processed.
11. Place net anchors and float lines in appropriate buckets or tubs.
12. Process all fish (SOP SOP E) from one net before pulling the next net.

D.5 Mini-fyke Netting

1. Begin setting mini-fyke nets during the day and before sunset while there is still some daylight remaining to see the deployment. Keep the cab and cod end out of the water until the evening deployment. Ensure that all nets will be set at least 1 hour before sunset.
2. Load boat with necessary fish processing equipment (e.g., mini-fyke nets, live well, measuring board, digital scale).
3. Locate pre-selected riparian segment using GPS.
4. Maneuver the boat near shore while keeping the boat perpendicular to the shoreline.
5. Wrap the end of the mini-fyke lead line around a T-post stake and push the stake into the shore above the waterline (Figure 9.).
    a. Leave enough slack in the lead line so that the bottom fully contacts the substrate (e.g., so that fish cannot swim underneath).
    b. Ensure that the float line is long enough to prevent the float from sinking.

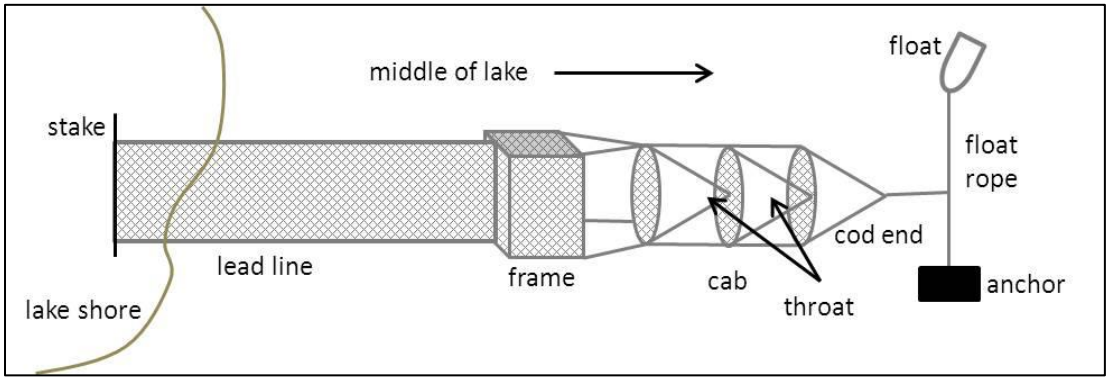

Figure 9. Example of a mini modified fyke net. The entire net from stake to cod end is approximately 10.5 m long with a net depth of 1.2 m.

6. Attach the net anchor to the cod (narrow) end and float line (with float attached) to the net anchor. Alternatively, a T-post can be used to stake the cod end as an anchoring method.
7. Begin slowly maneuvering the boat in reverse while deploying the net from the front of the boat.
    a. Mini-fyke nets must be set tightly to decrease the chance of the net collapsing on itself. Use the float line to pull the net as tight as possible before letting the float line go.
    b. The throat of the net must be underwater for fish to pass freely into the trap.

c. A portion of the cab should remain above the water line for turtles or other vertebrates to breathe.
d. The trap of the net must be above the thermocline (see site-specific bathymetric map).
e. Affix blinking LED light to the float at sites where watercraft are present.

8. Record the start time and the length of the lead line as deployed on the *Lake Fish Sampling Site Establishment Field Datasheet* (RD[12]).
9. One mini-fyke net shall be set in each electrofishing section.
10. The following morning at least one hour after sunrise, begin pulling the first mini-fyke net set the previous day (maximum set time = 30 hours). Note: the duration of the mini-fyke "soak" time should be modified based on the catches from previous bouts.
    a. This may be done from shore with waders or with the boat depending on conditions and permitting considerations.
    b. Mini-fyke nets must not be pulled earlier than 30 minutes after sunrise and no later than 30 minutes before sunset.
    c. Mini-fyke nets must be set for 2 crepuscular periods (i.e., dusk and dawn).
11. Record stop time on the *Lake Fish Sampling Field Datasheet.*
12. Remove the net anchor and float line and set aside. Note: if a large number of fish are captured (>1,000) it may be useful to use the cab portion of the mini-fyke net as a live well to hold some of this for processing. Be sure that no holes or openings are present.
13. Untie the cod end and empty the fish into a live well filled with fresh lake water with a battery powered aerator by lifting the rectangular frames of the mini-fyke net above the live well.
14. Remove T-stake and set aside.
15. Fold mini-fyke net lead over the frame while wrapping corners and set aside in boat.
16. Place net anchors, float lines, and T-stakes in appropriate buckets or tubs.
17. Process all fish (SOP SOP E) from each net before pulling the next net.

SOP E 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).

E.1 Processing Samples

1. If no fish are caught within a sampling reach, indicate "No" in the "Target Taxa Present?" box on *Lake Fish Sampling Field Datasheet.*
2. Ensure that all technicians handling fish keep hands wet with lake water and free of chemicals (e.g., sunscreen, insect repellent) 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) collected, identify and record species to lowest practical taxon on the *Lake Fish Sampling Field Datasheet* and release.
    a. Photograph the specimen before releasing if possible.
5. Record the photoID (camera file name) on the mobile data device or photo log datasheet. Ensure that electrofishing time, electrofisher settings, and pass time, or stop time of nets, as appropriate, had been recorded on the *Lake Fish Sampling Field Datasheet.*
    a. For gill nets, record which panels were pulled first.
6. Setup the digital scale and a measuring board on a flat surface.
7. Place plastic measuring tray on scale pan and tare scale.
8. If anesthetic is to be used, 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 lake 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 12 for calculated eugenol concentrations. Additionally, recommended concentrations can be calculated for different water treatment volumes using this formula:

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

Where: A = target concentration eugenol (mg/L)
B = treatment water volume (gal)
C = 0.00378 (conversion factor for grams per gallon)
D = 0.1 (To account for the fact that AQUI-S®20E is 10% eugenol)

    c. Using the 10 mL graduated cylinder, add 1.7 mL of AQUI-S®20E 2.5 US gallons (~10 L) lake water for an initial concentration of 20 mg/L. Mix well (the small dip-net makes a good mixer).

Table 12. 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

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

9. Remove fish from the first bucket or live well using the small handheld dip net.
   a. Larger fish may need to be removed carefully by hand.
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 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 and a voucher specimen is desired, weigh and measure following Steps **Error! Reference source not found.**-**Error! Reference source not found.** below. Only voucher mortalities (inadvertently killed fish) or specimens that require euthanization due to injuries.

        1) For uncertain species follow the guidance provided in SOP E.2. For morphotype species and labeling with a unique identifier, refer to SOP E.3.
    b. Euthanize mortally wounded fish and non-target species following SOP E.4 below.

12. Photo voucher at least one representative specimen from each taxon and from different life history stages (young of year, juvenile, 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 .on the *Lake Fish Sampling Field Datasheet* along with the relevant weight and length information about the fish on the Field Datasheet before reviving and releasing.
    a. Include metric ruler for scale using the measuring board.
    b. Photograph 1: Lateral photo with fish's head facing to the left.
    c. 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 *Lake Fish Sampling Field Datasheet*.
    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 *Lake 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 *Lake Fish Sampling Field Datasheet*.

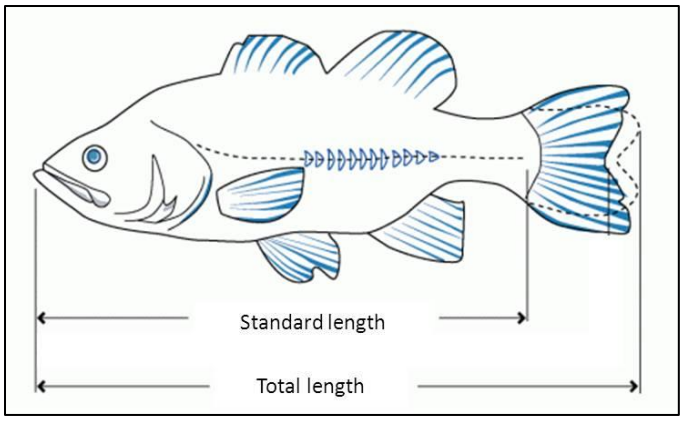

Figure 7. Measure the total length of each fish by pinching the fork together.

15. Inspect the fish for deformities, including eroded fins, external lesions, parasites, and tumors (DELTS; Figure 11) and electrofishing injuries (burn marks, bent spine, hemorrhage; Figure 6) and record the *Lake Fish Sampling Field Datasheet*.

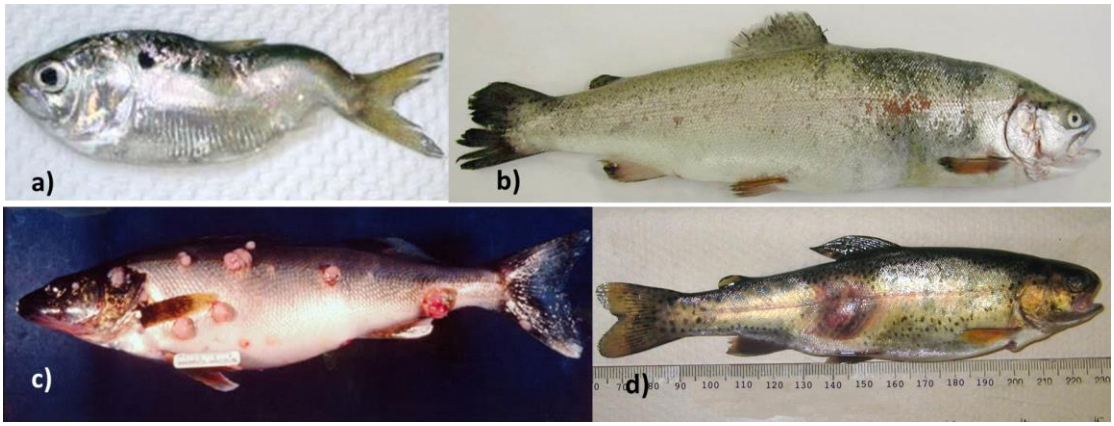

Figure 8. Example DELTS, a) deformity, b) eroded fins, c) tumors, and d) lesion

16. If collecting fin clips for DNA barcoding samples, refer to Fish Tissue Sampling for DNA Analysis (SOP E.5).
17. Indicate the life stage of the specimen (e.g. larval, young of the year, juvenile, adult, or gravid) on the *Lake Fish Sampling Field Datasheet*.
18. Place processed fish in a bucket labelled "recovery bucket" containing native 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 **Error! Reference source not found.**-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) 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.
        2) 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.
        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 specimens 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.

1) Using the mobile data device, enter the number of individuals counted per species. 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 lake.
    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 *Lake Fish Sampling Field Datasheet*; see Voucher Specimen Preservation, SOP **Error! Reference source not found.**).
21. The anesthetic solution 10% eugenol (AQUI-S®20E), will be disposed of in accordance with the terms and conditions of applicable permits. Disposal in the field (away from the lake) 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 lake. If field disposal is not permitted, waste anesthetic solution will be carried out and disposed of at the Domain Support Facility.

E.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 13). 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 13. 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.

E.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.

E.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 14 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 14. 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 12; **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.

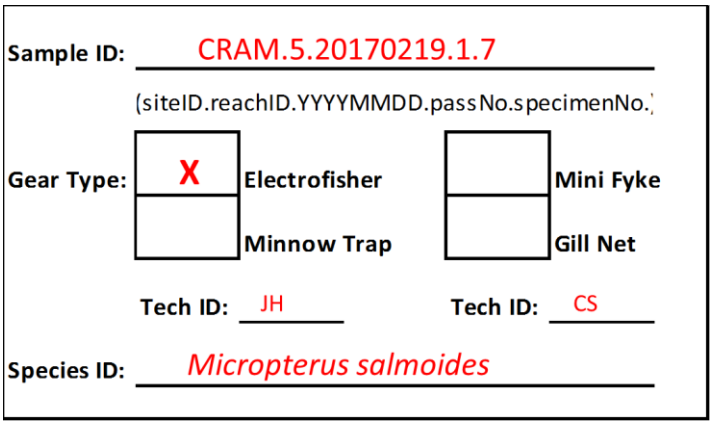

Figure 12. Example specimen label

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 12; 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 13) follow below.

Figure 9. Reptile Euthanasia kit with 50 mL centrifuge tube to store the 5 mL syringe and needle, 60 mL HDPE amber bottle is for 1st stage solution, 20 mL HDPE scintillation vial for 2nd stage solution.

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

2) <u>1st stage injection solution - 1% MS-222</u>
   a) Prepare MS-222 and baking soda to be mixed with water in the field when needed)
   b) Measure out 0.25 g MS-222 plus 0.157 g baking soda using the lab balance; weigh out MS-222 and baking soda in a small container (amber 60 mL HDPE bottle) at the Domain Support Facility.

c) Bring along 1 L of tap water from the Domain Support Facility to mix in the field with the MS-222 and baking soda when the stage 1 euthanasia solution is needed. Add 25 mL of water while in the field and shake to mix well.

3) 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 14).

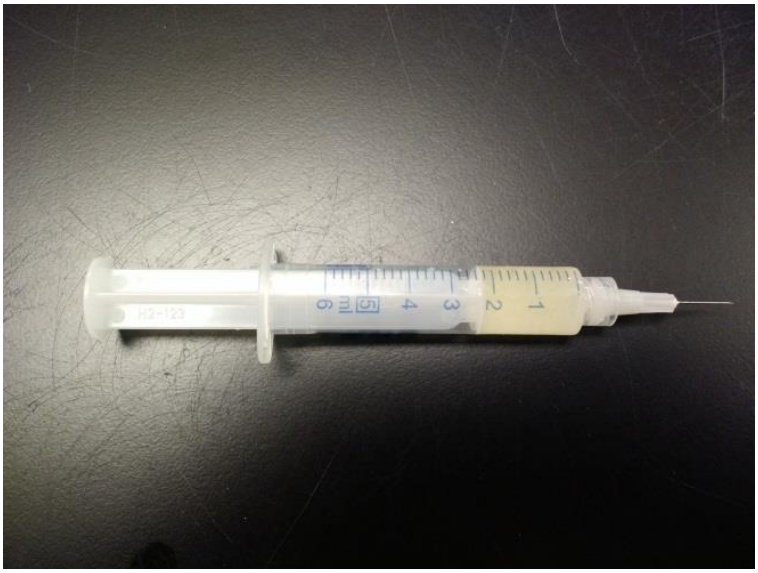

Figure 10. 2nd stage 50% (v/v) unbuffered MS-222 solution. Note yellow cloudy appearance of solution

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 15 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 15. 1st Stage dosage calculator using 1% MS-222 for reptiles by weight (g).

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

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

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

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

Figure 11. 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 10).

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 16).

Table 16. 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

b) Insert the needle through the skin of the reptile into the abdominal wall. In the case of delivering the dose into the body of a turtle, inject the stage one solution into the inguinal fossa (Figure 15).

c) 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.

E.5 Fish Tissue Sampling for DNA Analysis

1. Put on gloves (nitrile or latex free).
2. Anesthetize target fish following SOP E.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 D.6) steps.
6. Using the cutting tool, remove a piece of the adipose fin ray (Figure 16). 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 16). 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.

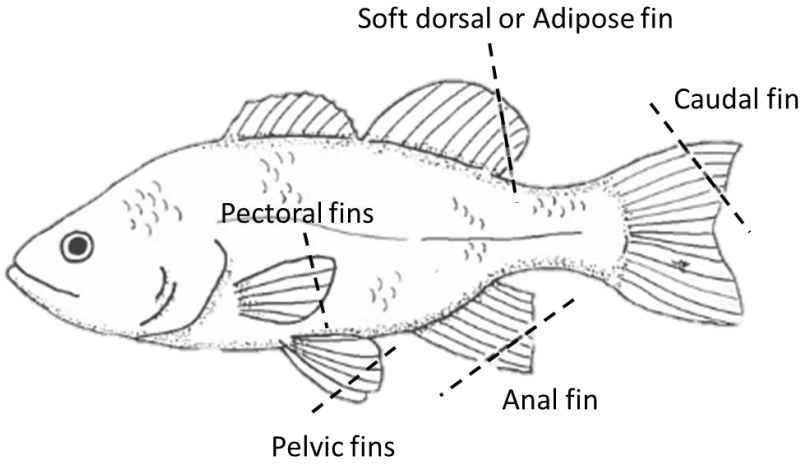

Figure 12. Optional fins to clip for tissue sampling

1. 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 for DNA format "siteID.reachID.YYYYMMDD.passNO.specimenNo.DNA".
2. Place fish that have had tissues samples collected into the recovery bucket.
3. Indicate on the *Lake Fish Sampling Field Datasheet* that a DNA tissue sample was taken and provide a sample ID for each specimen.
4. 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.
5. Repeat Steps 1-9 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.

E.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 12).
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).

E.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 lake 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 F 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 on the mobile data device or on the photo log datasheet."

SOP G 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.

G.1 Handling Hazardous Material

Follow shipping and Hazmat procedures for formalin.

G.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.

G.3 Timelines

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

G.4 Conditions

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

G.5 Grouping/Splitting Samples

N/A

G.6 Return of Materials or Containers

N/A

G.7 Shipping Inventory

Include sample shipment inventory (RD[11]). Email shipping inventory to external lab contact and copy the NEON CLA contact.

G.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 16. 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.003106	Datasheets for Fish Sampling in Lakes

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

APPENDIX B QUICK REFERENCES

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

Step 2 – For anesthetizing fish, AQUI-S®20E should be added directly to the treatment water. Do not make stock solutions or other dilute solutions of AQUI-S20E. Use the table below to determine the amount of AQUI-S®20E to add to the treatment water for specific concentrations of eugenol:

Target Concentration of AQUI-S®20E (10% eugenol)	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 segments.

Step 5 – Set electrofishing block nets, mini-fyke nets and gill nets according to the following timeline:

	Day 1	Day 2	Day 3	Day 4	Day 5
Day	AM/PM: Set electrofishing block nets PM: Set mini-fyke nets	AM: Pull mini-fyke nets AM/PM: Set electrofishing block nets AM/PM: Run gill nets PM: Set mini-fyke nets	AM: Pull mini-fyke nets AM/PM: Set electrofishing block nets AM/PM: Run gill nets PM: Set mini-fyke nets	AM: Pull mini-fyke nets AM/PM: Run gill nets PM: Set mini-fyke nets (*if needed*)	AM: Pull mini-fyke nets (*if needed*) AM/PM: Run gill nets (*if needed*)
Night	Allow electrofishing segments to recolonize	Electrofish in fixed segments	Electrofish in fixed or random segments	Electrofish random segments (*if needed*)	

Step 6 – Anesthetize caught fish in a 5 gallon bucket with solutions of AQUI-S20E.

Step 7 – Identify fish to species using the 4-letter species code (e.g., *Cottus cognatus* = COCO) and record on *Lake Fish Sampling Field Datasheet*. Euthanize the fish if it cannot be identified in the field.

Step 8 – Measure the weight and length of the specimen and inspect for deformities.

Step 9 – Place processed fish in a bucket containing fresh water and a battery powered aerator for later release. Once revived, release the fish outside of the designated segments.

Step 10 – Preserve euthanized specimen in a jar with a 10% buffered formalin.

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.
- ☑ Assemble and review all required Safety Data Sheets for chemicals used in this protocol.

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 habitat 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 lake outside of the block net.

Sample processing:

- ☑ If more than 50 individuals in 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

Sampling corresponds with the first and third sampling windows for Macroinvertebrate Sampling in Lakes and Non-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[08]).

Also, see the Site Specific Sampling Strategy Document on AQU's NEON intranet site.

APPENDIX E SITE-SPECIFIC INFORMATION: RANDOMIZED SECTION 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 *Lake Fish Sampling Field Datasheet* (RD[12]). 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 *Lake Fish Sampling Field Datasheet* (RD[12]).

Domain	Site	Randomized reach order
D03	Lake Barco – Not currently sampled	10, 9, 2, 6, 8, 7, 4, 1, 5, 3
D03	Lake Suggs – Not currently sampled	9, 4, 5, 7, 2, 6, 3, 10, 8, 1
D05	Crampton Lake	3, 6, 1, 2, 4, 10, 5, 7, 9, 8
D05	Little Rock Lake	5, 9, 7, 10, 4, 2, 1, 6, 3, 8
D09	Prairie Lake	8, 5, 3, 10, 9, 7, 2, 4, 6, 1
D09	Prairie Pothole	9, 2, 10, 6, 3, 5, 8, 7, 4, 1
D18	Toolik Lake	2, 6, 5, 10, 7, 4, 1, 3, 8, 9