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

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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.014051	Field Audit Plan
AD[06]	NEON.DOC.000824	Data and Data Product Quality Assurance and Control Plan

2.2 Reference Documents

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

RD[01]	NEON.DOC.000008	NEON Acronym List
RD[02]	NEON.DOC.000243	NEON Glossary of Terms
RD[03]	NEON.DOC.005003	NEON Scientific Data Products Catalog
RD[04]	NEON.DOC.001271	NEON Protocol and Procedure: Manual Data Transcription
RD[05]	NEON.DOC.001646	General AQU Field Metadata Sheet
RD[06]	NEON.DOC.001152	NEON Aquatic Sample Strategy Document
RD[07]	NEON.DOC.001154	AOS Protocol and Procedure: Aquatic Decontamination
RD[08]	NEON.DOC.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 Mapping 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

RD[13]	NEON.DOC.001151	AOS Protocol and Procedure: Aquatic DNA Barcode

2.3 External References

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

2.4 Acronyms

Acronym	Definition
A	Ampere or amp
AFS	American Fisheries Society
AQUI-S20E	10% eugenol; fish anesthetic
Cm	Centimeter
DC	Direct current
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.

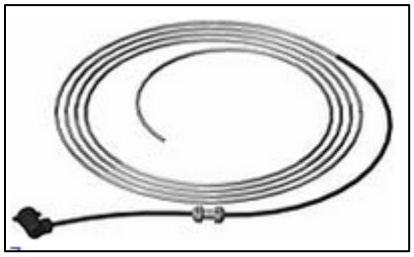

Figure 2. Cathode for backpack electrofishing unit (photo: store.smith-root.com)

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 (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, gill nets) at different times throughout the year to capture fish of a variety of size classes and habitat preferences.

In this protocol, permanent segments should be sampled biannually with a backpack electrofisher using multiple pass depletion, mini-fyke nets, and short-set gill nets at reaches within each segment (**Error! Reference source not found.**; Baker et al. 1997). Sampling and net placement should be located far enough apart to minimize interactions. The number of random segments selected for sampling should be positively related to lake size, but should not cover the entire shoreline. In any given sampling year, up to 40% of the shoreline should remain unsampled. 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.

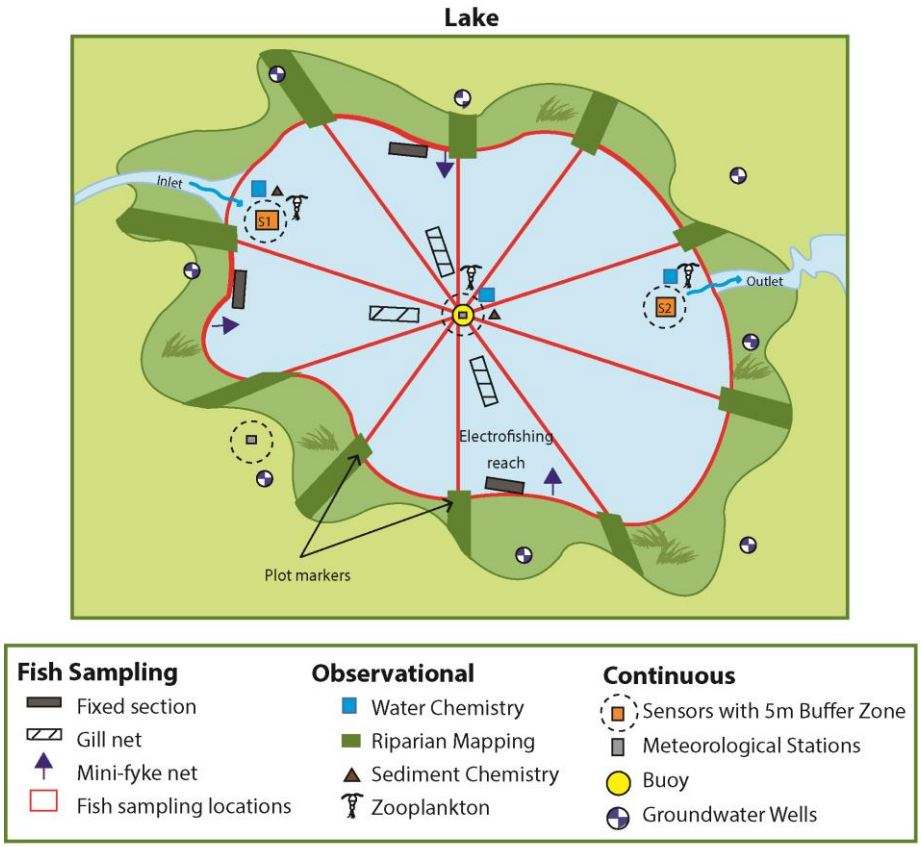

Figure 3. A generic lake site layout with fish sampling locations

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

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

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

4 SAMPLING SCHEDULE

4.1 Sampling Frequency and Timing

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

Sampling corresponds with the first and third sampling windows for Macroinvertebrate Sampling in 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 after sunset and 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 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). Fyke nets should be set in late afternoon, ensuring that all nets will be set at least 1 hour before sunset.

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*)	

4.2 Criteria for Determining Onset and Cessation of Sampling

A range of dates for each site will be determined *a priori*, based on historical data including ice-out, water temperature (or accumulated degree days), weather, and riparian greenness.

4.3 Timing for Laboratory Processing and Analysis

Samples may be stored for up to 1 month following the preservation guidelines in SOP D.7 For storage and shipping timelines see **Error! Reference source not found.**. Adipose fin clips may be taken from a maximum of 10 individuals per species per sampling bout for isotope and/or DNA analysis. Fin clips will be collected using scissors that are large enough to clip the fin in one quick motion. The cut should be made perpendicularly to the fin rays and remove half of the fin or less. If the fish does not have an adipose fin, a clip of the left pelvic fin may be collected. Fin clips will be placed in labeled collection vials and returned to the laboratory for storage. 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. Refer to RD[13] AOS Protocol and Procedure: Aquatic DNA Barcode Detailed procedures for collecting fish tissue samples.

4.4 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 day, 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. 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 sampling window, data must be flagged.
	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 sampling window, data must be flagged.

4.5 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 the next appropriate time when the entire sampling bout can be completed in 5 days.
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 < 1% of fish captured. If this number is exceeded at the site, stop sampling and contact the Domain Manager and submit a trouble ticket using the NEON problem tracking system (JIRA).

5 SAFETY

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

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

In addition the following general 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. 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.

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

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

6 PERSONNEL AND EQUIPMENT

6.1 Equipment

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

Table 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					
	R	Tricaine methanesulfonate (MS-222)	Euthanizing specimens	20 g	Y
	R	$NaHCO_3$	Buffering agent for MS-222	50 g	N
	R	AQUI-S20E	Anesthetizing specimens	50 mL	N
	R	Nitrile gloves (pair)	Safe handling of chemicals	1	N
	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

Item No.	R/S	Description	Purpose	Quantity	Special Handling
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 or 100 m)	Establishing sampling segments	1	N
	R	Handheld GPS (with batteries, ± 1 m accuracy)	Navigating to sampling segments	1	N
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	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

Item No.	R/S	Description	Purpose	Quantity	Special Handling
	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
	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	6.4 mm mesh dip nets with fiberglass handles	Catching immobilized specimens	4	N
	R	Rubber lineman gloves (Class 0, rated for max use voltage 1,000V AC/ 1,500V DC)	Safe handling of electrofishing equipment	1 pair per person	N
	R	5 gallon buckets	Storing specimens	10	N
	R	Hand held conductivity/temperature meter	Measuring conductivity and temperature	1	N
	R	Chest waders (approved for	Safe wading	1 pair per	N

Item No.	R/S	Description	Purpose	Quantity	Special Handling
		electrofishing)		person	
	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					
		(none)			

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
	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	For catching specimens	6	N

Item No.	R/S	Description	Purpose	Quantity	Special Handling
		Hanging ratio – 0.5			
	R	Net floats	Securing gill nets	18	N
	R	12.7 mm diameter rope (3-4 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	6	N
	R	Reusable nylon cable ties (46 cm)	Securing fyke nets	50	N
	R	T-type block net stakes (e.g., ~45 cm long, 20 cm handle, 1 cm diameter stainless rod)	Securing fyke nets	6	N
	R	Waterpoof blinking LED light	Marking 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					

Item No.	R/S	Description	Purpose	Quantity	Special Handling
	R	Fish and top predator taxonomic ID key specific to location or region (denotes endangered species)	Identifying specimens	1	N
	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	10	N
	R	HDPE wide mouth specimen jars (1 L)	Specimen preservation containers	50	N
	R	MS-222 stock solution	Euthanizing specimens	1 L	Y
	R	AQUI-S20E (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	10	N

Item No.	R/S	Description	Purpose	Quantity	Special Handling
			provided by an external lab		

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
	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

6.2 Training Requirements

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

Personnel are to be trained in fish sampling in 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 shall complete the US Fish and Wildlife Service (USFWS) online Electrofishing Safety training course (National Conservation Training Center [NCTC] CSP 2202-OLT) once per season before the first sampling bout. Technicians must pass the Electrofishing Safety Exam with a score >80%. Additional training resources include Wader Safety training which is available through the NCTC.

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

External Training References:

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

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

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

6.3 Specialized Skills

N/A

6.4 Estimated Time

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

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

7 STANDARD OPERATING PROCEDURES

SOP A Preparing for Sampling

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

1. **VERY IMPORTANT:** Charge or replace batteries for backpack electrofishing unit, 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 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.
10. Select random sampling segments if this is the first sampling date for the year (SOP C).
11. Preparing fish anesthetic (AQUI-S20E; 10% eugenol) and euthanizing agent (MS-222) in the Domain Support Facility.
    a. **Anesthetic** (AQUI-S20E): This drug is currently available under the USFWS Investigational New Animal Drug (INAD) program; Study Protocol 11-741. As such, NEON must comply with data reporting requirements including drug acquisition and handling as well as fish treatment and disposition. Be sure to bring along the INAD reporting datasheets in the field when conducting fish sampling activities.

1) AQUI-S20E should be added directly to the treatment water. Do not make stock solutions or other dilute solutions of AQUI-S20E.

b. **Euthanizing Agent** (MS-222)

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

SOP 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 Mapping Protocol, RD[10]).

1. Using the site-specific Riparian Map (RD[10]), determine the length of shoreline contained in each of the 10 riparian segments.
   a. Fish sections ideally include at least 300 m of shoreline so that sampling sets (i.e., fyke nets, gill nets, electrofishing segments) are not too close together. If sections are <300 m, combine riparian sections.
2. Install a permanent aluminum plot survey marker on the shoreline at each section boundary (if not already present; **Error! Reference source not found.**). Record the location of each marker on the handheld GPS unit and on the Reach Delineation Data Sheet (RD[12]).
   a. Add GPS points to the lake bathymetry map (RD [09]) at the Domain Support Facility.
   b. If you are unable to install plot survey markers due to permitting restrictions, record GPS data so you can return to the location.

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 reaches should be chosen to best represent the habitat variability throughout the lake (e.g., presence or absence of vegetation, substratum type; **Error! Reference source not found.**). Fixed reaches will be selected by the NEON Aquatic Ecologist or Domain Aquatic Technician.
   b. Avoid having sensor sets within electrofishing reaches. Electrofishing must occur ≥5 m away from all in-lake electronics.
3. 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 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. Do not disturb the area inside the 4 x 25 m sampling area.
   d. Space fence posts a maximum of 2 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 the top of the block net to each fence post in the fixed reaches with a reusable nylon cable tie approximately 30 cm above the water line.
   a. Fence posts 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.
   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 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.).

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 lake, set the frequency to 30 Hz, the duty cycle to 12% and output voltage to 100 V and turn the electrofishing unit on. For additional information regarding backpack electrofisher settings see Table 11.

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	

9. Pause to verbally confirm settings on electrofisher and that the unit is turned on. Also confirm that all technicians are ready to proceed before pressing the activation switch on the anode pole.
    a. The anode ring must always be submerged before depressing the activation switch. When removed from the water, the unit will automatically turn off.
10. Press and hold the activation switch down, and observe the behavior of fish. If fish do not appear to be affected by electrofishing (e.g., are not momentarily stunned), release the activation switch on the anode pole and increase voltage by 100 V (e.g., from 100 V to 200 V) and repeat Steps 9-10.
    a. The goal is to immobilize fish using the lowest settings possible at the site.
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 9-10
    a. If 70 Hz and 1,100 V is reached and fish are present but not immobilized, stop electrofishing and contact the NEON Aquatic Ecologist.
    b. If fish are immobilized during testing, use dip nets to capture individuals and place in a bucket ½ - ¾ full of 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.
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). Record injury rate on Field Data Sheet (RD[12]). Less than 1% of the captured fish should be injured.
    a. If > 1% 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 D.6).

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.
    c. In random reaches, no block-net is necessary. Fish will be electrofished along a ~4 x 25 m area qualitatively.
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).
    a. The electrofisher operator may, but is not required to, hold a dip net in the other hand if he/she feels comfortable.
4. The other crewmembers 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.
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.
11. Frequently remove fish from dip nets and place in buckets to minimize injury to the fish.
12. Sampling will continue along the shoreline in a zig-zag pattern in a single pass with attention to sampling all complex cover (e.g., overhanging or aquatic vegetation, woody debris, undercut banks).
    a. The electrofisher operator may take advantage of the response of fish to pulsed DC current (i.e., attraction of immobilized individuals towards the anode) in complex cover by:

1) Releasing the activation switch on the anode pole.
2) Inserting the anode into cover from the 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. Place buckets of fish out of direct sunlight if possible.
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 Field Data Sheet (RD[12]).

    a. Electrofisher time is critical for calculating sampling effort.
16. Turn the electrofisher off, remove and place on the bank with anode and cathode still attached.
17. Proceed to fish processing (SOP D.6).
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 1 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, 50 fish on third pass).
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, depth finder).
2. Locate pre-selected riparian segment using GPS (**Error! Reference source not found.**).
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 6.).
   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.

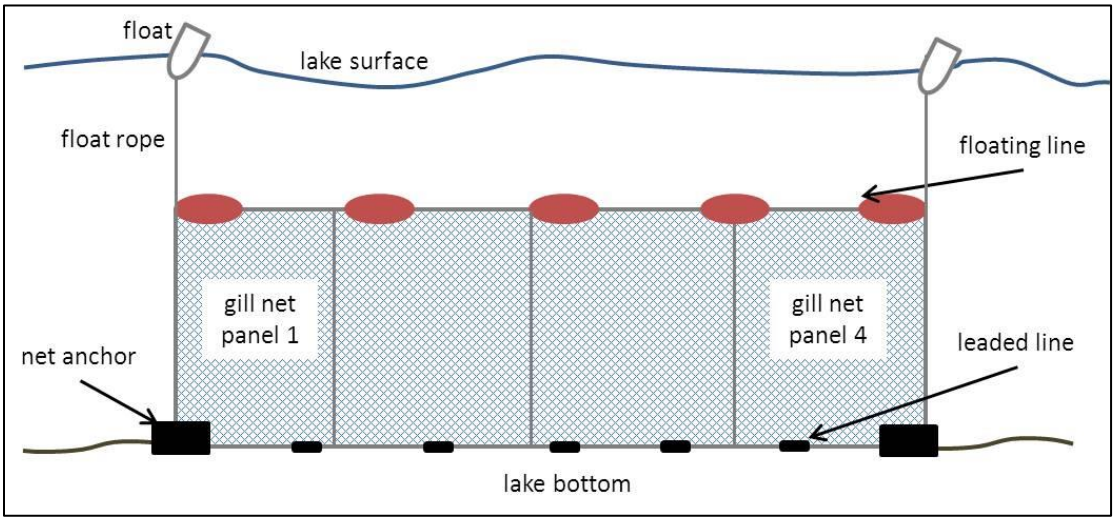

Figure 6. Example of gill net setup. The dimensions of this net are approximately 3.1 m long and 1.8 m deep..

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 GPS location, depth from the depth finder, and start time (24-hour time plus time zone, e.g., 13:30 MDT) on the Field Data Sheet (RD[12]).
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 (**Error! Reference source not found.**) while the gill net is being deployed.

7. After the net is completely deployed, record the stop GPS location, stop time and lake depth on the Field Data Sheet (RD[12]).
   a. 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. Subsequent gill nets may be set and sampled the following day.
   b. 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.
   c. Target gill net set time is 1 hour (maximum 2 hours) to minimize mortality.
   d. Gill netting must occur during daylight hours, so nets should be set in the morning or early afternoon to allow for processing time.
8. After at least 1 hour, proceed to the first net set and begin pulling.
9. Untie the float line and net anchor and set aside.
10. Record stop time and gill net mesh panel number of the first panel pulled (RD[12]).
11. 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.
12. Place net anchors and float lines in appropriate buckets or tubs.
13. Process all fish (SOP D.6) from one net before pulling the next net.

D.5 Mini-fyke Netting

1. Begin setting mini-fyke nets in late afternoon, ensuring that all nets will be set at least 1 hour before sunset.
2. Load boat with necessary fish processing equipment (e.g., fyke nets, live well, measuring board, digital scale).
3. Locate pre-selected riparian segment using GPS (**Error! Reference source not found.**).
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 the t-bar stake and push the stake into the shore above the waterline (Figure 7.).
   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.

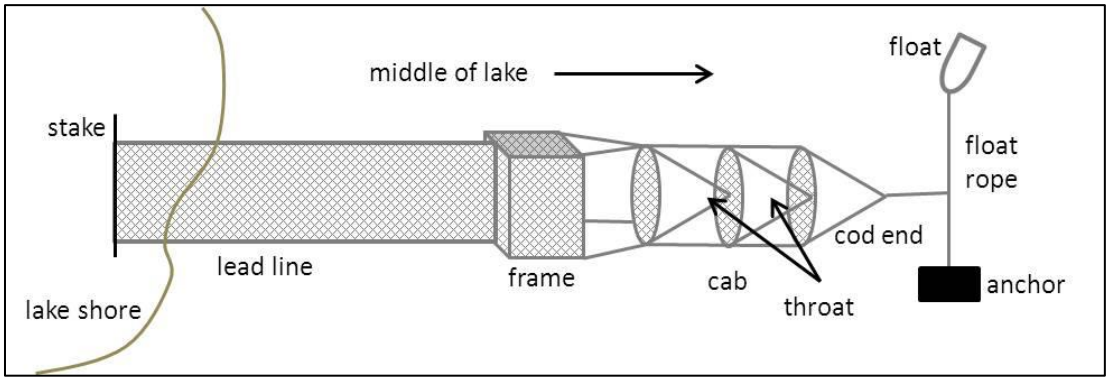

Figure 7. Example of a mini modified fyke net. The entire net from stake to cod end is approximately 10.5 m long with a net depth of 1.2 m.

6. Attach the net anchor to the cod (narrow) end and float line (with float attached) to the net anchor.
7. Begin slowly maneuvering the boat in reverse while deploying the net from the front of the boat.
    a. 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 start GPS location from the stake, as well as the start time and depth on the Field Data Sheet (RD[12]).
9. Repeat Steps 2-8 until all mini-fyke nets have been set (one net 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).
    a. This may be done from shore with waders or with the boat depending on conditions and permitting considerations.
    b. Fyke nets must not be pulled earlier than 30 minutes after sunrise and no later than 30 minutes before sunset.
    c. Fyke nets must be set for 2 crepuscular periods (i.e., dusk and dawn).
11. Record stop time on Field Data Sheet (RD[12]) .
12. Remove the net anchor and float line and set aside.
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 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 D.6) from each net before pulling the next net.

D.6 Fish Processing

1. Ensure that all technicians handling fish keep hands wet with lake water and free of chemicals (e.g., sunscreen, insect repellent) while processing fish.
2. Designate one technician to identify fish throughout the sampling bout for taxonomic consistency.
3. For any non-fish top predators (e.g., salamanders) collected, identify and record species to lowest practical taxon on the Field Data Sheet and release.
    a. Photograph the specimen before releasing if possible.
4. Ensure that electrofishing time, electrofisher settings, and pass time, or stop time of nets, as appropriate, had been recorded on the Field Data Sheet (**Error! Reference source not found.**).
    a. For gill nets, record which panels were pulled first.
5. Setup the digital scale and a measuring board on a flat surface.
6. Place plastic measuring tray on scale pan and tare scale.
7. Mix anesthetic in one 5-gallon bucket.
    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-S20E is 10% eugenol) is recommended to sedate all fish species to handleable in most situations. Refer to Table 12 for calculated eugenol concentrations. Additionally, recommended concentrations can be calculated for different water treatment volumes using this formula:

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

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

    c. Using the 10 mL graduated cylinder, add 1.9 mL of AQUI-S20E 2.5 US gallons (~10 L) 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-S20E to add to treatment water for a specific concentration of eugenol.

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

30 mg/L	2.8 mL	5.7 mL	11.3 mL	17.0 mL	22.7 mL	28.4 mL

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

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

   b. Do not exceed 5 fish in the anesthetization bucket at one time.
   c. Leaving fish in the anesthetization bucket for too long can cause mortality. Monitor respiration and gill movement constantly.
   d. Be sure to include required information within the datasheets for the INAD.
10. Identify fish to species using the 4-letter species code (e.g., *Cottus cognatus* = COCO) and record on Field Data Sheet (**Error! Reference source not found.**). Indicate capture method on the datasheet (i.e. electrofishing, gill net, or fyke net).
    a. If the species cannot be identified or identification is uncertain and a voucher specimen is desired, weigh and measure following Steps 12-14 below. Then proceed to euthanize the specimen.

       1) Do not collect more than 5 specimens of the same unknown species. Rather, morphotype and label with a unique identifier on the Field Data Sheets (**Error! Reference source not found.**).
       2) Do not euthanize endangered species (site specific lists will be provided before sampling) or fish > 200 mm standard length.
       3) For all specimens >200 mm standard length and any fish not able to be euthanized (i.e., due to permitting concerns), photograph specimen carefully and record the camera image number on the Field Data Sheet along with the relevant weight and length information about the fish (**Error! Reference source not found.**).
       4) Euthanize fish < 200 mm standard length using a lethal dose of 10 mL stock solution of MS-222 /1 L of lake water in the field.
       5) Add 1 L of lake water and 10 mL of MS-222 stock solution to a new 5 gallon bucket. Mix thoroughly.
       6) Transfer fish from the holding live well or bucket to the bucket containing the euthanizing solution with the small handheld dip net.

7) Monitor fish until respiration ceases.
8) Place fish into appropriate sample container (e.g., wide mouth HPDE bottles) with completed specimen label (**Error! Reference source not found.**) and add 10% formalin preservative. One taxon per specimen bottle.

b. Amphibians that are injured as a result of fish sampling will be euthanized using a lethal dose of MS-222 following the protocol for fish euthanasia above.

c. For reptiles, a two-stage method of euthanasia is recommended. This procedure includes an intracoelomic injection of 250 to 500 mg/kg of a pH-neutralized solution (0.7% to 1.0%) of MS-222. This will produce a loss of consciousness in less than five minutes (AVMA 2013). Following loss of consciousness, a second intracoelomic injection of unbuffered 50% MS-222 is administered. Any euthanized or dead animals will be collected, preserved in formalin in a collection jar, and deposited at a fish collections facility.

d. Aquatic invertebrate species, including arthropods and molluscs, that are injured while performing fish sampling tasks will be euthanized. Methods for euthanizing aquatic invertebrates will follow the U.S. Environmental Protection Agency Rapid Bioassessment Protocols for Use in Streams and Wadeable Rivers (Barbour et al 1999). Injured aquatic invertebrates will be euthanized by completely submerging specimens in 95% ethanol. Injured or dead aquatic invertebrates will be preserved for vouchering in 70% ethanol (Hauer and Resh 2006).

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

11. Photo voucher 1 representative specimen from each taxon. Include non-target specimens (e.g. amphibians and reptiles).
    1) Include metric ruler for scale using the measuring board.
    2) Photograph 1: Lateral photo with fish's head facing to the left.
    3) Photograph 2: Ventral photo that includes the mouth (mouth position, lip structure, and barbels can be important distinguishing features).
12. Place the fish in the plastic tray on the tared digital scale. Determine weight to nearest 0.1 g and record on field data sheet (**Error! Reference source not found.**).
13. With wet, clean hands, remove the fish from the plastic tray and place the fish on the measuring board with mouth at the "0" end of the board. Measure total length to the tip of the pinched-together tail (Figure 8.) to the nearest mm and record on Field Data Sheet (**Error! Reference source not found.**).

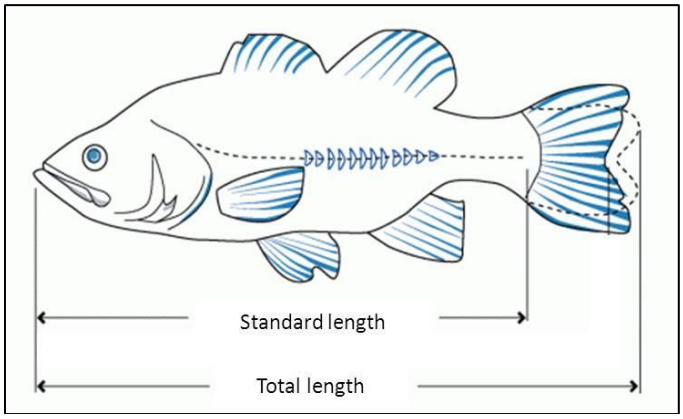

Figure 8. Measurement of standard and total length. Total length is measured by pinching the fork together.

14. Inspect the fish for deformities, including eroded fins, external lesions, parasites, and tumors and electrofishing injuries (burn marks, bent spine, hemorrhage) and record on Field Data Sheet (**Error! Reference source not found.**).
15. If collecting fin clips for DNA barcoding samples, refer to RD[13] AOS Protocol and Procedure: Aquatic DNA Barcode.
16. Place processed fish in a bucket containing fresh lake 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.
17. Repeat Steps 8-16 until ≤100 fish per species are identified, weighed, measured, and inspected for deformities.
    a. If more than 100 individuals in one species are captured, anesthetize, weigh, and measure the first 100 and simply count the remaining fish (no anesthetization) to speed processing time and alleviate stress to fish.
18. Release the processed, revived fish back into the lake outside of the blocknet or near the collection location. With the use of 10% eugenol (AQUI-S®20E), treated freshwater fish species may be immediately released following recovery; no withdrawal time is required.
    a. If mortality occurs during processing, save individuals for collections (see Sample Preservation, SOP D.7).
19. The anesthetic solution will be disposed of in accordance with the terms and conditions of applicable permits. At present, field disposal is not allowed. Waste anesthetic and euthanizing solutions will be carried out of the field site and disposed of at the Domain Support Facility.

D.7 Sample Preservation

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

D.8 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. The following link is a resource provided by Oregon State University: http://ir.library.oregonstate.edu/xmlui/bitstream/handle/1957/25084/SGNO831989.pdf

SOP E Data Entry and Verification

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

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

SOP F Sample Shipment

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

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

F.1 Handling Hazardous Material

Follow shipping and Hazmat procedures for formalin.

F.2 Supplies/Containers

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

F.3 Timelines

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

F.4 Conditions

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

F.5 Grouping/Splitting Samples

N/A

F.6 Return of Materials or Containers

N/A

F.7 Shipping Inventory

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

F.8 Laboratory Contact Information and Shipping/Receipt Days

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

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APPENDIX A DATASHEETS

The following datasheets are associated with this protocol:

Table 13. 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-S20E should be added directly to the treatment water. Do not make stock solutions or other dilute solutions of AQUI-S20E. Use the table below to determine the amount of AQUI-S20E to add to the treatment water for specific concentrations of eugenol:

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

For euthanizing fish or non-target species, 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 Field Data Sheet (**Error! Reference source not found.**). 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 and ship to taxonomist.

APPENDIX C REMINDERS

Before heading into the field:

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

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

APPENDIX D ESTIMATED DATES FOR ONSET AND CESSATION OF SAMPLING

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

APPENDIX E SITE-SPECIFIC INFORMATION: RANDOMIZED 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).

Domain	Site	Randomized reach order
D03	Lake Barco	10, 9, 2, 6, 8, 7, 4, 1, 5, 3
D03	Lake Suggs	9, 4, 5, 7, 2, 6, 3, 10, 8, 1
D05	Crampton Lake	3, 6, 1, 2, 4, 10, 5, 7, 9, 8
D05	Round 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
D11	South Pond at Klemme	7, 9, 1, 4, 5, 3, 3, 10, 2, 8
D18	Toolik Lake	2, 6, 5, 10, 7, 4, 1, 3, 8, 9