AOS PROTOCOL AND PROCEDURE: FISH SAMPLING IN LAKES

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See configuration management system for approval history.

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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.
E	04/03/2018	ECO-05577	Major updates include revised sample contingency timing. An updated equipment list includes voucher containers, working gloves, and the mobile field device. Modified wader requirements. Record water quality before every pass for each gear type. Added net tending procedures for reduced by-catch and handling of birds or small mammals. Included additional guidance for using the mobile field device. Fish voucher photos shall be collected and recorded for specimens associated with tissue samples only. Revised sample ID and added barcode label instructions. Removed all references to JIRA. Field datasheets have been updated following these protocol revisions and the development of the fish mobile device application. Removed the photo log from the datasheets as photos shall only be recorded using the mobile device and application. Included net integrity components. Fish euthanasia with AQUI-S20E now 100 mg/L.
F	01/29/2019	ECO-05978	Clarified training and safety sections. Added endangered species handling guidance and reporting procedures. Once electrofisher settings have been established, the same setting can be used on subsequent sampling days so long as the conductivity stays with (+/-) 50 µS/cm. Revised the AQUI-S20E dose with recommendations from the USFDA for salmonids and non-salmonids. Stressed importance for using the morphospecies ID format. Electrofishing block net area must be 4 x 25 m; removed ability to change size. Emphasized the requirement to use anesthesia when collecting fish tissue for DNA where permitted.

.

TABLE OF CONTENTS

LIST OF TABLES AND FIGURES

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 readily 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, few methodologies have been developed with the appropriate methods to accurately and precisely estimate complete fish assemblages in lakes.

1.2 Scope

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

1.2.1 NEON Science Requirements and Data Products

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

Execution of this protocol procures samples and/or generates raw data satisfying NEON Observatory scientific requirements. These data and samples are used to create NEON data products, and are documented in the NEON Level 1, Level 2 and Level 3 Data Products Catalog (RD[03]).

1.3 Acknowledgments

The design and implementation of 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	AOS/TOS Protocol and Procedure: Data Management
RD[05]	NEON.DOC.001646	General AQU Field Metadata Sheet
RD[06]	NEON.DOC.001152	NEON Aquatic Sample Strategy Document
RD[07]	NEON.DOC.004257	NEON Standard Operating Procedure: Decontamination of Sensors, Field Equipment, and Field Vehicles
RD[08]	NEON.DOC.003046	AOS Protocol and Procedure: Aquatic Macroinvertebrate Sampling
RD[09]	NEON.DOC.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.

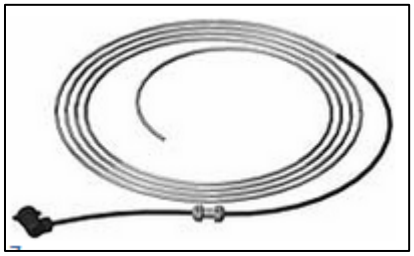

Figure 2. Cathode for backpack electrofishing unit (photo: store.smith-root.com)

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 a three-pass electrofishing depletion approach, 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.

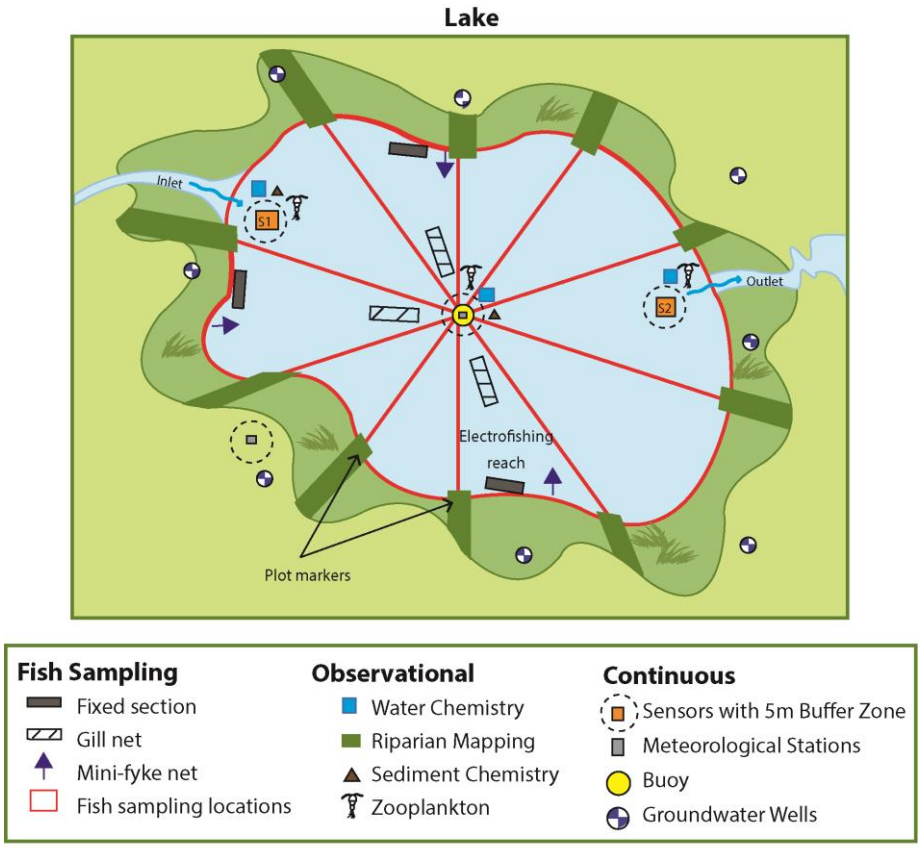

Figure 3. A generic lake site layout with fish sampling locations

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

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

The value of NEON data hinges on consistent implementation of this protocol across all NEON domains, for the life of the project. It is therefore essential that field personnel carry out this protocol as outlined

in this document. In the event that local conditions create uncertainty about carrying out these steps, it is critical that field scientists document the problem and enter it in NEON's problem tracking system.

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

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. 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 bout windows at lake sites (RD[06]). Fish sampling must be scheduled within the site-specific bout window. Fish sampling should be scheduled as the last sampling activity of the bout to minimize impacts on other sampling objectives. Weather conditions at the site may push sampling outside of the bout window. If conditions do not allow for fish sampling to occur during bout 1, then sampling shall occur when safe conditions allow up to 2 weeks before the start of bout 2. If conditions do not allow for fish sampling to occur during bout 3, then sampling shall occur when conditions allow up to 30 days beyond the end of bout 3.

A minimum of 2 weeks between sampling bouts shall be observed. Sampling bouts should not be longer than 5 days long assuming no weather or other unexpected schedule delays. All three passes in a fixed sampling segment must be sampled within a 24-hour period, 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 at least 30 minutes after sunset and ceasing at least 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. Other factors may dictate shorter gill net set times such as permitting requirements. 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) for easier fish processing in daylight. Mini-fyke net set times will not exceed 30 hours. A shortened mini-fyke net set may be applied at NEON lake sites as required by the collection permit or to limit excessive fish handling to avoid possible injuries. 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 within the sampling week; this is indicated as "if needed" in the proposed 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*)	

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

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

4.4 Sampling Timing Contingencies

The setting of electrofishing block nets (at fixed segments), mini-fyke nets, and gill nets shall be set during the day; it is preferred that the mini-fyke and gill nets are 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. A minimum of 2 weeks between sample periods shall be observed (Table 2).

If sampling is impractical as a result of severe drought (dry), the lake is frozen, or that access to portions of the lake are blocked, then indicate this for any affected reach on the mobile device or *Lake Fish Sampling Field Datasheet* (RD[12]). Should environmental conditions or resource availability impact the ability to fully execute the protocol for an entire bout, at a minimum, fully sample one fixed lake fish sampling segment. This shall include one 3-pass electrofisher survey, one mini-fyke net set, and one gill net set. Note the cause of the minimized sampling effort in the "Reach Condition" section of the mobile field device or on the *Lake Fish Sampling Field Datasheet* (RD[12]). In addition, as stated above in Section 4.1, if conditions do not allow fish sampling to occur during bout 1, then sampling shall occur when safe conditions allow up to 2 weeks before the start of bout 2. If conditions do not allow for fish sampling to occur during bout 3, then sampling shall occur when conditions allow up to 30 days beyond the end of bout 3.

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

4.5 Sampling Specific Concerns

1. Fish sampling should not occur while other aquatic sampling activities that could disturb sediments or hydrology (e.g., macroinvertebrate sampling) are occurring in the lake.
2. Under ideal conditions fish sampling shall be completed within a 5-day period per site. If field conditions appear unfavorable (e.g., prolonged thunderstorms, tropical storms, expected flooding) during the proposed sampling bout, postpone sampling until conditions are safe to sample and when the entire sampling bout can be completed in 5 days. If fish sampling is interrupted and the resumption of fish sampling is expected to occur three days past the biological bout, (or beyond the extended contingency windows; see above) submit a trouble ticket (Table 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 limiting the collection of voucher specimens.
4. Fish sampling-related injuries resulting from electrofishing, netting, and processing should affect < 3% of fish captured cumulatively at the reach-scale. If this number is exceeded at the site, stop sampling, release fish, and contact the NEON Aquatic Ecologist, Domain Manager, and submit a trouble ticket using the NEON problem tracking system.
    a. Please include the following information when submitting a trouble ticket for reporting fish injuries and mortality that exceeds 3%:
        1) Electrofisher settings: voltage, duty cycle, and frequency
        2) Water quality: conductivity, dissolved oxygen, and temperature
        3) Field observations and most likely root cause of injury or mortality
        4) Description of species affected
        5) Include any additional information that could help identify the root cause and for developing a solution (e.g. anode ring diameter and shape)

5 SAFETY

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

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

1. Field scientists are required not to put hands and feet in waters where alligators are present and to make sure a safe distance from hazards is maintained. 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. Field scientists should be aware of any site-specific hazards and to the waters of that particular location (i.e. current status, tidal charts, etc.).
4. Activities should only be performed when flow conditions are safe. Wading is not allowed when the depth multiplied by the velocity is ≥ 0.93 m^2/s (93 L/s). 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 field scientists while the chemicals are in use.
6. Field scientists must take the Hand and Power Tool Training for Field Operations prior to using fence post drivers and pullers and must use impact-resistant gloves for this task.
7. All personnel must be wearing a personal flotation device (PFD) prior to entering and while in the boat.

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 field scientists when electrofishing equipment is in operation.
3. Chest waders and heavy-duty rubber lineman gloves (rated to 1,000V AC/1,500V DC) must be worn while working near an electrofishing unit.
    a. The requirements for wader and boot selection has been adapted by the recommendations of the American Fisheries Society Professional Safety Committee (2008).
        1) Non-breathable materials provide the most insulation against electrical shock and include PVC, neoprene, or silicon. Non-breathable waders with built-in lug-sole boots are the preferred wader style.
        2) Breathable materials including Gore-Tex® provide less insulation against electrical shock, but they may be more comfortable in warmer conditions and in environments with a lot of scrambling over boulders. The use of breathable waders with stocking feet is acceptable as long as the operator also wears dry clothing that covers any exposed skin while in the waders. Stocking foot style waders with a separate boot may be used but only at a single designated site.
        3) Studded-sole boots are allowed if they help secure footing in slippery conditions.
        4) Stocking foot waders with the built-in gaiter ankle cuff are difficult to decontaminate. This style of wader may only be used if dedicated to a single site and not transferred across sites. .

5) Felt-soles are prohibited.

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

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.	Supplier	Supplier ID	R/S	Description	Purpose	Quantity	Special Handling
Durable items							
MX100345	Thomas Scientific, Inc.	1185B56	R	HDPE bottle, amber, 1 L	Stock solution (MS-222) container	2	N
MX106819			R	Lab safety glasses	Safe handling of chemicals	1 pair	N
MX106854	Smith-Root	10167	R	Battery charger (electrofishing batteries)	Charging the electrofisher	1	N
MX111388	CDW-G	4452963	R	Mobile field data recording device (Tablet)	Recording data	1	N
MX100514	Thomas Scientific, Inc. Fisher Scientific Company	1185K52 15177622	R	Multisonde	Measuring % DO, temperature and salinity	1	N
Consumable items							

Item No.	Supplier	Supplier ID	R/S	Description	Purpose	Quantity	Special Handling
MX106819	Fisher Scientific Company	AC118000500	R	Tricaine methanesulfonate (MS-222)	Euthanizing specimens	20 g	Y
MX106431	Grainger, W.W. Amazon Capital Services Inc.	33X679 B00BXOQK6K	R	$NaHCO_3$ (baking soda)	Buffering agent for MS-222	50 g	N
MX110318	AquaTactics Fish Health	AQUIS20E	R	AQUI-S20E	Anesthetizing specimens	50 mL	N
			R	Nitrile gloves (latex-free; pair)	Safe handling of chemicals and fish	1	N
MX103942	Ben Meadows Co., Inc. Forestry Suppliers, Inc.	010510-1 49247	R	Field data sheets (print on waterproof paper, write in pencil)	Recording data	10	N
MX103942	Ben Meadows Co., Inc. Forestry Suppliers, Inc.	010510-1 49247	R	Specimen labels (waterproof paper)	Labeling specimens	2 sheets	N
			S	Adhesive barcode labels	Labeling sample bottles with barcode-readable labels	1 sheet	N
			R	Collection permit documents		1	N

R/S=Required/Suggested

Table 4. Equipment list – Segment establishment

Item No.	Supplier	Supplier ID	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
MX104369	Ben Meadows Co., Inc. Forestry Suppliers, Inc.	213379 37184	R	Meter tape (50 m)	Establishing sampling segments	2	N
MX110075 MX102739	Forestry Suppliers, Inc. Cabela's Inc. Recreational Equipment Inc.	39481 IK-270217 895022	R	Handheld GPS (with batteries, ± 1 m accuracy)	Navigating to sampling segments	1	N
Consumable items							
MX103940	Grainger, W.W. Forestry Suppliers, Inc.	9WKP4 57880	R	Flagging tape	Establishing sampling segments	1 roll	N

R/S=Required/Suggested

Table 5. Equipment list – Electrofishing

Item No.	Supplier	Supplier ID	R/S	Description	Purpose	Quantity	Special Handling
Durable items							
MX101709	Grainger, W.W.	6R335	R	Rubber mallet	Hammering PVC posts for random shore reaches	1	N
	Home Depot U.S.A., INC.	530048	R	PVC pipe 5 feet long	For setting random reach boundaries	24	N
	Amazon Capital Services Inc.	ITCPW-Z ITTY-RS	R	T-post insulator or caps with clips	Used to hang the block net on the T-posts	30	N
	Amazon Capital Services Inc.	13031	R	Carabineers	Attaching weights to the block net	30	N
	Cabelas	422345	R	Duck decoy weights (non-lead) 8 oz.	Anchoring the block net	36	N
			R	Wrench (9/16th)	Used to tighten the anode ring to the fiberglass pole	1	N
MX107115	Grainger, W.W.	4LVG6	R	Steel studded fence posts (i.e., T-post)	Securing block net at segment boundary	30	N
MX104756	Forestry Suppliers, Inc.	67251	R	Fence post driver or small sledge	Securing block net at segment boundary	1	N

Item No.	Supplier	Supplier ID	R/S	Description	Purpose	Quantity	Special Handling
			R	Fence post puller	Securing block net at segment boundary	1	N
	The Fish Net Company, LLC Duluth Nets		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
	Amazon Capital Services Inc.	B00XBFARBI	R	Net repair kit: • needle • string • butane lighter • zip ties	Repairing nets	1	N
			R	Gloves, Nitrile coated, Textured, Knitted cuff, Abrasion resistant, PIP or Equivalent	Handling T-posts, nets, and removing entangled birds or mammals	1	N
MX106855	Smith-Root	9632	R	Battery-powered backpack electrofishing unit	Electrofishing	1	N

Item No.	Supplier	Supplier ID	R/S	Description	Purpose	Quantity	Special Handling
MX106846	Smith-Root	7575	R	Anode pole (1.5-2.0 m) with attached anode ring	Electrofishing	1	N
MX106848	Smith-Root	6821	R	Cathode (rattail type; 1-2 m and 10-15 mm in diameter)	Electrofishing	1	N
MX110472 MX106849	Smith-Root	06682 10765	R	Electrofisher batteries (rechargeable)	Electrofishing	3	N
	Amazon Capital Services Inc.	B001KYQBX0	R	Abrasive pad to clean anode rings	Electrofishing	1	N
	N&K Dip Nets	various	R	Mesh dip nets with fiberglass handles (a variety of sizes are available, select an appropriate size net for the specific sampling conditions)	Catching immobilized specimens	4	N
MX110609 MX110613	Smith-Root	3106	R	Rubber lineman gloves (Class 0, rated for max use voltage 1,000V AC/ 1,500V DC)	Safe handling of electrofishing equipment	1 pair per person	N
MX100526	Grainger, W.W.	34A216	R	5 gallon buckets	Storing specimens	10	N

Item No.	Supplier	Supplier ID	R/S	Description	Purpose	Quantity	Special Handling
MX100491 MX100494 MX107505	Ben Meadows Co., Inc. Grainger, W.W. Forestry Suppliers, Inc. Cabela's		R	Chest waders (approved for electrofishing)	Safe wading	1 pair per person	N
MX106824	Amazon Capital Services Inc.	B0000DCVYK	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							
MX102192	Grainger, W.W.	36J149	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.	Supplier	Supplier ID	R/S	Description	Purpose	Quantity	Special Handling
Durable items							

Item No.	Supplier	Supplier ID	R/S	Description	Purpose	Quantity	Special Handling
MX100526	Grainger, W.W.	34A216	R	Gill net tubs (i.e., 5 gallon bucket)	Storing gill nets	6	N
			R	Gill net hooks	Securing gill nets	6	N
MX109276	Amazon Capital Services Inc.	B00X0WT8MQ	R	Depth finder	Navigating to sampling locations	1	N
MX108919	Duluth Nets		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
	Amazon Capital Services Inc.	Lfloat-OPK	R	Net floats	Securing gill nets	18	N
	Amazon Capital Services Inc.	B077DVFT9J	R	12.7 mm diameter rope (3-10 m in length) for floats	Securing gill nets	18	N
	Amazon Capital Services Inc.	B003ZZG592	R	Net anchors	Securing gill nets	18	N

Item No.	Supplier	Supplier ID	R/S	Description	Purpose	Quantity	Special Handling
			R	Live well (site-specific, i.e., 5 gallon bucket or tote)	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.	Supplier	Supplier ID	R/S	Description	Purpose	Quantity	Special Handling
Durable items							
MX108918	Duluth Nets		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	For catching specimens	3	N

Item No.	Supplier	Supplier ID	R/S	Description	Purpose	Quantity	Special Handling
				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.			
	Amazon Capital Services Inc.	B076C6WKR9	R	Reusable nylon cable ties, natural or white	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.	Supplier	Supplier ID	R/S	Description	Purpose	Quantity	Special Handling
Durable items							
MX106815	Ben Meadows Co., Inc.	227438	R	Fish and top predator taxonomic ID key specific to location or region (denotes endangered species)	Identifying specimens	1	N
MX106832	Amazon Capital Services Inc.	B00H51AIYK	R	Portable aerators (batteries, diffusion stone)	Aerating buckets	15	N
MX100481	Forestry Suppliers	77310	R	Small dip net (3.2 mm mesh)	Handling specimens	5	N
MX106902	Amazon Capital Services Inc.	B0051W81P8	R	Fish measuring boards (60 cm)	Measuring specimen length	2	N
MX100338	BioQuip Products Inc. Fisher Scientific Company	1426B NC0095946	R	Portable digital scale (batteries, charger)	Weighing specimens	1	N
MX107144	B&H Photo Corp	PADMCTS30BL	R	Plastic tray	Weighing specimens	2	N
MX106815	Ben Meadows Co., Inc.	227438	R	Color separation guide	Photographing specimens	1	N
MX100379 MX100380	Fisher Scientific Company Thomas Scientific, Inc.	0300739 3571A20	R	25-50 mL graduated cylinder, plastic	Mixing anesthetic and euthanizing solution	1	N
MX107197	Fisher Scientific Company	S17337	R	Fin clipping scissors	Cutting fish fins for DNA barcode samples	1	N

Item No.	Supplier	Supplier ID	R/S	Description	Purpose	Quantity	Special Handling
			R	Dish pan, plastic	Disinfecting tools with iodophor or betadine	1-12 Qt.	N
Consumable items							
			R	Nitrile gloves (pair)	Safe handling of chemicals and fish	20	N
MX101218 MX100574 MX100652 MX100665	Fisher Scientific Company	033134A 033134B 033134D 033134E 033134F	R	HDPE wide mouth specimen jars (25 mL, 60 mL, 250 mL, 500 mL, and 1 L)	Specimen preservation containers	50	N
MX106819	Fisher Scientific Company	AC118000500	R	MS-222 stock solution	Euthanizing specimens	1 L	Y
MX110318	AquaTactics Fish Health	AQUIS20E	R	AQUI-S®20E (10% Eugenol)	Anesthetizing specimens	1 L	Y
MX106257	Thomas Scientific, Inc.	C998K62	R	10% buffered formalin (3.7-4.0% formaldehyde)	Preserving specimens	20 L	Y
	Thomas Scientific, Inc.	1236C14	R	Tissue containers (e.g. 1.5 mL cryo vials)	For storing fin clips for DNA barcoding	100	N

Item No.	Supplier	Supplier ID	R/S	Description	Purpose	Quantity	Special Handling
MX100213 MX100202	Fisher Scientific Company Thomas Scientific, Inc.	4355601 C954K61	R	Ethanol (70 -95%)	For preserving fin clips for DNA and sterilizing DNA equipment	250 mL	Y
MX100213 MX100202	Fisher Scientific Company Thomas Scientific, Inc.	4355601 C954K61	R	Ethanol (95%)	Euthanizing invertebrates	250 mL	Y
	Amazon Capital Services Inc.	B007R64URO	R	Iodophor or betadine disinfectant	Required by some States for decontaminating equipment	1 L	N
MX101221	Fisher Scientific Company	BD305125	R	Needle, disposable 1 inch, 25 gauge, sterile	Injecting reptiles with MS-222	1 pack	Y
MX106200	Thomas Scientific, Inc. Fisher Scientific Company, LLC	8956A70 1482648	R	Needle, disposable 1 inch, 27 gauge, sterile	Injecting reptiles with MS-222	1 pack	Y
MX106261	Thomas Scientific, Inc. Fisher Scientific Company, LLC	1227H00 14823220	R	Syringe, 5mL luer lock syringe	Measuring and injecting euthanasia fluids	1 pack	Y
MX100549	VWR	82018048	R	50mL Centrifuge tube	Store syringe and needle, measure injection fluids	1	Y
MX 101218	Fisher Scientific Company	033134B	R	60mL Clear bottle HDPE	Store dry MS-222 and baking soda for reptile euthanasia stage 1 solution	1	Y

Item No.	Supplier	Supplier ID	R/S	Description	Purpose	Quantity	Special Handling
MX101278	Thomas Scientific, Inc. Fisher Scientific Company	9718J20 0333723C	R	20mL HDPE Scintillation vial	Store dry MS-222 for reptile euthanasia stage 2 solution	1	Y
MX103240	Grainger, W.W.	2TUW7	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.	Supplier	Supplier ID	R/S	Description	Purpose	Quantity	Special Handling
Durable items							
			R	Boat		1	Y
MX107097	Amazon Capital Services Inc.	B003ZZG5EM	R	Anchor with rope		1	N
MX100457			R	Oars		2	N
MX100458	West Marine Products, Inc.	13487178	R	Trolling Electric Motor		1	Y
MX100899	Grainger, W.W.	2UKJ5	R	Battery (12 volt)		1	Y

Item No.	Supplier	Supplier ID	R/S	Description	Purpose	Quantity	Special Handling
MX100435	Amazon Capital Services Inc.	B003QKQ4V0	R	Safety kit for boat (navigation lights, fire extinguisher, whistle, 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 field scientists must complete protocol-specific training for safety and implementation of this protocol as required in Field Operations Job Instruction Training Plan (AD[04]). Also, refer to the NEON Animal Care and Use Program: Training Plan for Personnel Working with Live Vertebrate Animals (AD[06]).

All personnel participating in fish sampling for NEON are to be trained in fish sampling safety for water-based fieldwork. Specific training for fish sampling must also include electrofishing training for all field scientists. All lead aquatic field scientists and those designated by their manager shall be required to receive an electrofishing safety training prior to operating a backpack electrofisher. The backpack electrofisher manufacturer (Smith-Root) will provide this training. Additionally, all field scientists shall complete the U.S. Fish and Wildlife Service (USFWS) online Electrofishing Safety training course (National Conservation Training Center [NCTC] CSP 2202-OLT) once per season before the first sampling bout. Field scientists must pass the Electrofishing Safety Exam with a score >80%. Additional training resources include Wader Safety training which is available through the NCTC Electrofishing Safety training resource videos. Lastly, all field scientists shall complete the Collaborative Institute Training Initiative's (CITI) Institutional Animal Care and Use Committee (IACUC) fish and amphibian training. Field scientists must pass the final tests for each training (fish and amphibian) with an 80% or better. The CITI IACUC trainings are good for up to three years. Provide your manager with a copy of each applicable certificate documenting the successful completion of each required training. See the AFS Fisheries Safety Handbook for additional fish sampling safety information.

The overall goal for participating in and successfully completing these training courses is to enhance the safety of all NEON staff and public bystanders in the fish sampling area. Additionally, it is of great importance to limit the 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 possess a current CPR/AED/First Aid training.

External Training Resources:

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

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

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

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

6.3 Specialized Skills

N/A

6.4 Estimated Time

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

This protocol requires a minimum of four qualified technicians for up to 5 consecutive field days. There is no lab processing at the Domain Support Facility associated with this protocol. Fish tissue samples will be shipped to a central Domain Support Facility (D09) for processing annually. The processing Domain will ship processed tissues to a NEON-approved laboratory for DNA barcoding. Additionally, tissue samples and/or voucher specimens (target and non-target species) may be preserved, archived, and shipped to external facilities by domain staff. Sample preservation and archival may require up to 10 minutes of processing per specimen for domain staff.

7 STANDARD OPERATING PROCEDURES

SOP A Preparing for Data Capture

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

SOP B Preparing for Sampling

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

Begin preparations at least two days before going to the field to allow batteries to fully charge. It 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, mobile field data recording device with 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). Carefully inspect the metal surfaces of the anode ring and cathode for corrosion. Remove corrosion using an abrasive pad or steel wool to gently scrub the surface.
3. Inspect boat, trailer, and motor for normal operation.
4. Inspect waders for holes and tears. Repair waders if necessary.
5. Inspect lineman gloves DO NOT repair torn lineman gloves. Discard them and order a replacement pair.
6. Inspect dip nets, block nets, gill nets, and mini-fyke nets for rips, tears, and holes. Repair if necessary.
7. Inspect portable aquarium pumps, diffusion stones, and batteries.
8. Inspect buckets to ensure handles are present and functioning.
9. Ensure that all equipment has been decontaminated since last use (see RD[07]).
10. 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.
11. Select random sampling segments if this is the first sampling date for the year (SOP D).

12. Fish will be anesthetized and euthanized with 10% eugenol (AQUI-S®20E). Non-target species will not be anesthetized; however, mortally injured non-target species shall be euthanized with Tricaine methanesulfonate (MS-222).
    a. **10% eugenol (AQUI-S®20E):** This drug is currently available under the USFWS Investigational New Animal Drug (INAD) program, Study Protocol 11-741. As such, NEON must comply with data reporting requirements including drug acquisition and handling as well as fish treatment and disposition. Be sure to bring along the *INAD Reporting Datasheets* in the field when conducting fish sampling activities. Also, make sure that the batch of AQUI-S®20E has not expired. Any questions regarding this program or how to complete the field datasheets should be directed to the study monitor or the investigator responsible for reporting the study results.
        1) 10% eugenol should be added directly to the treatment water. Do not make stock solutions or other dilute solutions of 10% eugenol.
    b. **Tricaine methanesulfonate (MS-222)**

        1) Mix stock solution of MS-222 (site-specific, depends on EHS permits) in the Domain Support Facility.
        2) Wear nitrile gloves and eye protection, as MS-222 is a skin and eye irritant.
        3) Weigh 20 g of MS-222 powder and 50 g $NaHCO_3$.
        4) Mix 20 g MS-222 + 50 g $NaHCO_3$ in a bucket with 1-liter tap water.
        5) Pour the stock solution into two 1 L amber HDPE bottles.
        6) Label bottles “MS-222 stock solution”.
        7) MS-222 stock solution must be stored in dark bottles away from light and refrigerated at 4°C. Stock solution may be stored and used in one month from the date of preparation.
        8) Discard unused solution in the lab following the NEON EHS chemical hygiene guidelines (AD[03]).

SOP C Establishing Sampling Reaches

Establish sampling 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 D to establish the electrofishing reach and the deployment locations for the mini-fyke net and experimental gill nets.

SOP D 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. Electrofishing in sections with sensors must occur ≥5 m away from all in-lake electronics.
3. 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). The NEON Aquatic Ecologist or Domain Aquatic Technician will select fixed reaches.
4. 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.
5. Use the same three random sections for all sampling dates within one year (Table 10).
6. 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.
7. 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 E Field Sampling

E.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 field scientists MUST be trained in the use of fence post drivers/pullers prior to deploying with the Lake Fish Sampling Team. See the Post Driver safety training on the NEON Safety SharePoint Page.
    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.
    f. Note in the Pass remarks if the block net area is less than 4 x 25 m and an estimate of its size.
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 (if necessary to anchor the bottom of the net in the substrate) 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).

E.2 Backpack Electrofishing Field Set-Up

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

1. **VERY IMPORTANT:** All field scientists 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. Headlamps must also be worn during nighttime sampling.

2. Assemble anode pole (Figure 1).
3. Measure the water temperature and specific conductivity using the handheld conductivity meter.
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). There is a notch that the strap goes through at the battery handle. If using the lithium ion battery, be sure to include the adapter.

Figure 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. At sites where conductivity exceeds 500 µS/cm, initial setting should be as follows: 50 V, 15 Hz, and 30% duty cycle.

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 field scientists are ready to proceed before pressing the activation switch on the anode pole.
    a. The anode ring must always be submerged before depressing the activation switch. When removed from the water, the unit will 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. **Note:** Be sure to review the collection permit requirements and stay within the approved electrofisher settings.
    b. 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 E.3.
13. Place fish in a bucket or live well with fresh lake water and a battery operated aerator.
    a. If other top predators are captured, identify (if possible) and record species on the mobile device or the *Lake Fish Sampling Field Datasheet* (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 the injury rate on the mobile device or the *Lake Fish Sampling Field Datasheet* (RD[12]). Less than 3% of the captured fish should be injured.

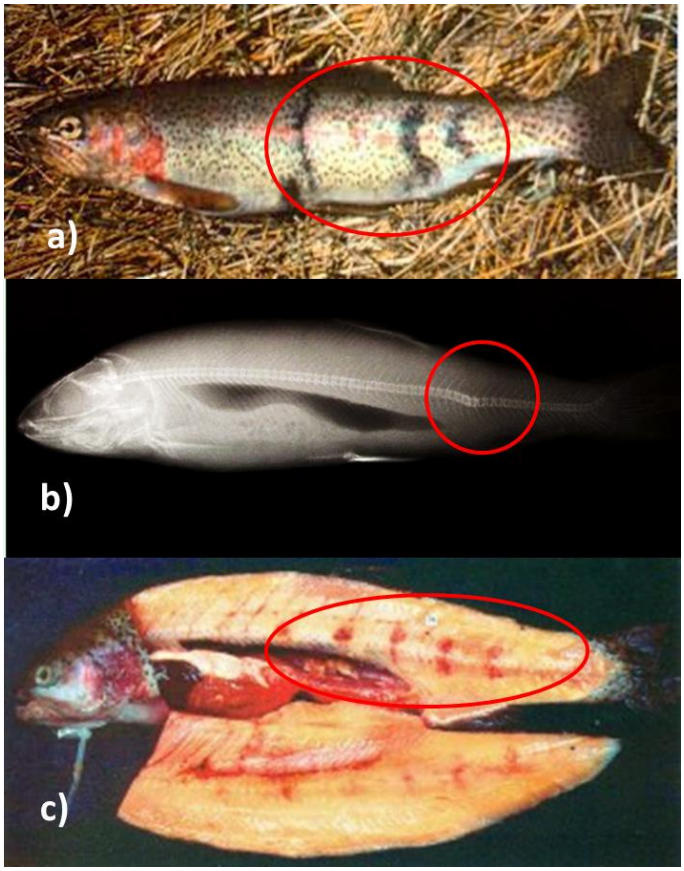

Figure 6. Examples of electrofishing injuries to fish, a) bruising or "burn" marks, b) spinal injury, and c) internal hemorrhaging. Photos from Snyder 2003.

    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.
    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 Euthanizing Fish and Non-Target Species (SOP F.4).SOP F.4).SOP F.4).SOP F.4).

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 mobile device or the *Lake Fish Sampling Field Datasheet* (RD[12]) and reset the timer on the electrofishing unit.
    a. **Note:** The electrofisher settings established on the first day of sampling can be used on subsequent days so long as the conductivity stays within (+/-) 50 µS/cm.

E.3 Backpack Electrofishing

1. At every fish sampling segment, there are several details to record on the mobile device or the *Lake Fish Sampling Field Datasheet* (RD[12]).
    a. Record the dominant habitat type (littoral or pelagic).
    b. Indicate if the reach is inaccessible by recording sampling impractical (dry, frozen, snow, other) and document if the reach condition could affect the data collection (normal flow, segmented pools, disconnected side channel, other low flow <100 m sampled, high flow < 100% of reach sampled, calm, windy, below stage, bankfull stage, flood stage, heavy vegetation, skipped reach). If multiple reach conditions affect data collection, document the most impactful condition.
2. Measure and record the water temperature, conductivity, and dissolved oxygen within the lake shore study area using the handheld water quality meter before the start of every pass on the mobile device or the *Lake Fish Sampling Field Datasheet* (RD[12]). The water quality measurements may be copied for each additional pass within one fish segment (i.e. if sampling at a fixed reach the water quality data collected before the first pass can be applied to the second and third pass).
3. 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 sampled with in a 4 x 25 m area delineated with T-posts, rebar, or PVC pipe. .

4. Record the pass start date and time on the mobile device or the Lake Fish Sampling Field Datasheet (RD[12]) so that conductivity, turbidity, and other water quality measurements from the in-lake sensors can be coupled with the fish sampling bout. The pass start and pass end times are for recording the full length of time for sampling each reach. This is different from the Electrofisher (EF) time which is the timer on the backpack electrofisher records the amount of time (in seconds) that the unit is actively shocking. The EF time is reset before every pass.
5. 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.
6. 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 many fish are typically captured, it is useful to add a fourth crew member to help distribute the work effort.
7. Ensure that the electrofishing unit settings (frequency, duty cycle, and output voltage) are those determined in SOP E.2 and that the timer (“EF time”) has been reset to “0”.
8. Turn the electrofishing unit on and notify the other field scientists. Confirm that all field scientists are ready to begin.
9. Depress and hold the activation switch on anode pole to begin electrofishing.
    a. The anode ring must always be submerged before depressing the activation switch and should never be taken out of the water with the switch depressed. The unit will automatically turn off if the anode is removed from the water.
10. Slowly sweep the anode inside the end of the block net to target any fish that may be seeking cover in the net. Inspect the folds of the block net closely and carefully remove any fish or non-target species that may have been trapped while immobilized.
11. After sweeping the block net perpendicular to the shore, 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.

12. 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 field scientists that the unit is on.
    g. If any endangered species (review collection permit before sampling) or other vertebrates (e.g., salamanders, turtles) are caught, identify, take a photo and release immediately. Stop all fish sampling activities. Follow the guidance of the collection permit for specimen vouchering and reporting procedures.
13. Frequently remove fish from dip nets and place in buckets or a live well to minimize injury to the fish.
14. 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).

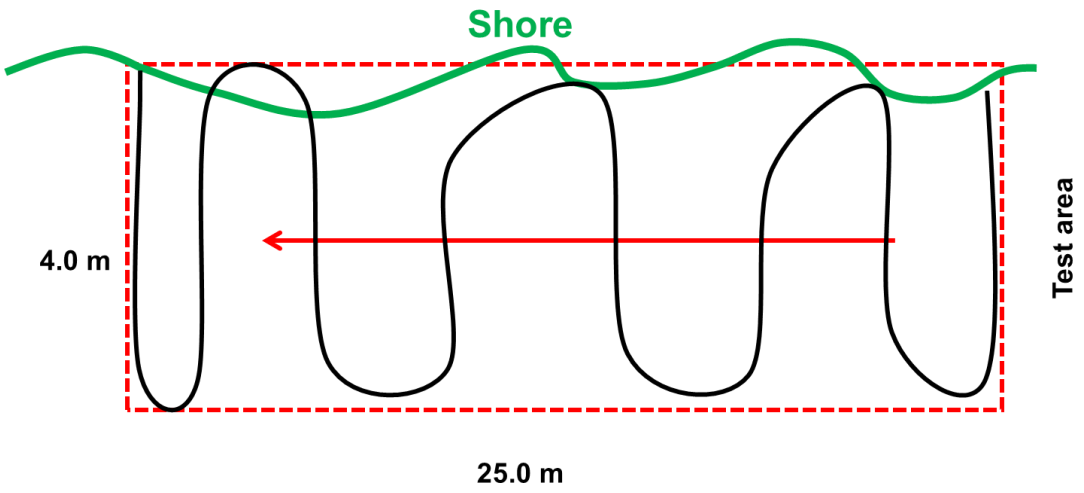

Figure 7. Lake electrofishing sampling pattern

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 did not follow the anode out.

15. If sampling should occur during periods of high winds and waves it will be necessary to regularly inspect the block nets at fixed study sites while sampling. The lead field scientist should monitor the net to prevent net failure, which could allow fish to enter or exit the fixed study area. The top of the net should be out of the water and fixed to the T-posts at regular intervals. The bottom on the block net needs to be secured to the lake bed without any openings. Remove any debris that accumulates on the block net. Inspect for and remove any fish or non-target species that may have been trapped in the net.
    a. Should the block net effectiveness become compromised (collapses or a hole develops) while actively sampling, indicate the degree to which the net integrity is affected. This could be a single point of failure or the cumulative total area of several failures. Using the mobile device or the *Lake Fish Sampling Field Datasheet* (RD[12]), record if the Net Integrity as:
       1) No compromise – no noticeable gap that would allow fish to enter or exit the study area.

2) <10% compromise – Approximately 10% of the net area (or less) has a gap, is collapsed, sagging, or has a hole through which a few fish may enter or exit the study area.
3) >10% compromise – More than 10% of the net area has a gap, is collapsed, sagging, or has a hole through which fish may freely enter or exit the study area.

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

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

16. While electrofishing, monitor the abundance of fish in the buckets to prevent escape or accidental spills.
    a. Be aware of fish overcrowding in the buckets. If fish appear to be gasping at the water surface, they are likely short on oxygen due to water temperature or overcrowding. Place fewer fish in buckets and supplement with cooler water and aerators. Water temperature should not rise more than 4°C above the ambient water temperature.
        1) If a lot of predatory fish and prey species are collected, they may need to be placed in separate buckets to reduce predator consumption of prey species.
        2) Separate different age classes to prevent larger fish from harming small individuals.
    b. Bucket replacement and moving fish is easier for the netters to do, as they will need to step out of the water.
    c. Place buckets of fish out of direct sunlight if possible. Placing some floating vegetation in the bucket can also provide cover or shade for captured fish and reduce stress. Secure a mesh netting across the top of the bucket if fish are able to jump out or when transporting them.
    d. The duration that fish are retained in the holding buckets should be kept to less than one hour. This may require that fish captured in the beginning of the reach are processed before the reach is fully sampled.
17. When the crew reaches the 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.
18. Once the entire sampling reach has been sampled, read and record the time (EF time) in seconds from the back of the electrofishing unit on the mobile device or the Lake Fish Sampling Field Datasheet (RD[12]).
    a. Electrofisher time is critical for calculating sampling effort.
    b. Record the final electrofisher settings on the mobile device or the *Lake Fish Sampling Field Datasheet* at the end of each pass as the settings may have changed.
19. Turn the electrofisher off, remove and place on the bank with anode and cathode still attached.
20. Proceed to fish processing (SOP F). Record the pass end date and time on the mobile device or the Lake Fish Sampling Field Datasheet (RD[12]) once fish have been processed.
21. If this is a fixed reach, repeat Steps 4-17 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).
        1) If the number of sampled fish increase with each pass do the following:
            a) Re-check that the settings on the backpack electrofisher have not changed
            b) Inspect the block net for holes and that the lead line is laying across the bottom
            c) Ensure that your anode sweeping technique is even and consistent
            d) Make sure the netters are alert during each pass

2) If this issue continues in a subsequent sample reach, contact the domain manager and submit a trouble ticket through the NEON problem resolution system.

c. If this is a random reach, fish are sampled using only one pass without block nets.

22. Remove block nets and fence posts if all passes are complete.
23. 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.

E.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 (**Error! Reference source not found.**.
    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.

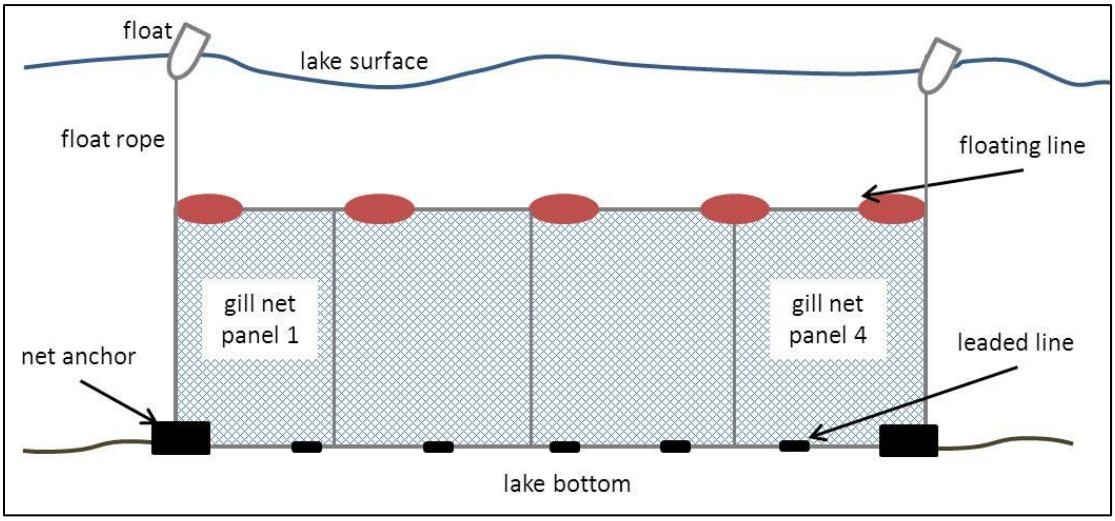

Figure 8. Example of gill net setup. The dimensions of this net are approximately 3.1 m long and 1.8 m deep.

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 pass start date and time (24-hour time plus time zone, e.g., 13:30 MDT) on the mobile device or the *Lake Fish Sampling Field Datasheet* (RD[12]). The pass start and pass end times are for recording the full length of time for sampling each reach with the gill net. This is different from the net deployment (set) time which is the amount of time that the net was actively set and sampling in the water
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.

Note: 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. Once the gill net is fully deployed, record the net deployment set date and time as well as the maximum net set depth (check in multiple locations and record the deepest reading). Also, record the surface water quality conditions (conductivity, dissolved oxygen, and temperature) from the midpoint of the set net on the mobile device or the *Lake Fish Sampling Field Datasheet* (RD[12]).
    c. Target gill net set time is 1 hour (maximum 2 hours or as directed by collection permit requirements) 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.
7. While the gill net is deployed, monitor the area for waterfowl, diving birds, or aquatic mammals that could become entangled in the deployed gill nets.
    a. If any of these animals appear to be congregating around the deployed gill nets it may be necessary to only deploy one net at a time. Remain in the boat at the deployment site to keep birds and aquatic mammals away from the site.
    b. If it is suspected that a bird or aquatic mammal may have become entangled in the net immediately pull up the net.
        1) Before removing entangled animals, protect yourself first. Protect your eyes with sunglasses or safety glasses. Use gloves to handle the animal and to prevent getting lacerations from the monofilament line. Cut away the line with a sharp knife and always cut away from yourself and others.

2) If the animal is injured or killed then stop sampling. Immediately report diving bird or aquatic mammal entanglements by contacting the NEON Aquatic Ecologist, Domain Manager, and submit a trouble ticket using the NEON problem tracking system.
3) Record the non-target species information on the mobile device or *Lake Fish Sampling Field Datasheet* (RD[12]).

8. After 1 hour (or other set time depending on permit requirements), proceed to the first net set and begin pulling in the net.
9. Untie the float line and net anchor and set aside.
10. Record the net deployment end date and time on the mobile device or *Lake Fish Sampling Field Datasheet* (RD[12]).
11. Gently remove captured fish from each mesh panel. Take care to close fish operculum (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. If while retrieving the gill net, it appears that the net effectiveness has been compromised (collapsed, tangled, holes developed) while deployed, indicate the degree to which the net integrity is affected. This could be a single point of failure or the cumulative total area of several failures. Using the mobile device or *Lake Fish Sampling Field Datasheet* (RD[12]), record if the gill Net Integrity has:
    1) No compromise – no noticeable opening (hole) or restriction (tangled or collapsed) that would allow fish to pass through the net without being captured.
    2) <10% compromise – Approximately 10% (~0.5 m^2) of the net area (or less) has a hole, is collapsed, sagging, or has a tangle for which a few fish may pass through the net without being caught.
    3) >10% compromise – More than 10% (~0.5 m^2) of the net area has a hole, is collapsed, sagging, or has a tangle for which the effectiveness of the net to capture fish is significantly impacted.

    b. Following the Net Integrity tracking described in the previous step, the following actions are recommended:
    1) If <10% of the gill net integrity is compromised, process and record captured fish. Repair the net before deploying it at other locations.
    2) If >10% of the gill net is compromised, release captured fish, repair the net, and re-deploy the net.
    3) Do not discard any data until the re-deployment is complete and new data have been recorded.
14. Process all fish (SOP F) from one net before pulling the next net. Record pass end date and time on the mobile device or on the *Lake Fish Sampling Field Datasheet* (RD[12]) once all fish have been processed.

15. Gill netting-related injuries (net lacerations, damaged operculum, and listlessness) should affect < 3% of fish captured cumulatively at each deployment location. 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.
    a. Please include the following information when submitting a trouble ticket for reporting fish injuries and mortality that exceeds 3%:
        1) Gill net set time
        2) Water quality: conductivity, dissolved oxygen, and temperature
        3) Field observations and most likely root cause of injury or mortality
        4) Description of species affected
        5) Include any additional information that could help identify the root cause and for developing a solution

E.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. Record the pass start date and time on the mobile device or the Lake Fish Sampling Field Datasheet (RD[12]). The pass start and pass end times are for recording the full length of time for sampling each reach with the mini-fyke net. This is different from the net deployment (set) time, which is the amount of time that the net was actively set and sampling in the water.
4. Set the mini-fyke net in the appropriate segment and avoid setting the mini-fyke net directly within the electrofishing area.
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 (**Error! Reference source not found.**
    a. Leave enough slack in the lead line so that the bottom fully contacts the substrate (e.g., so that fish cannot swim underneath). This may require the addition of weights to keep the lead line flush with the bottom.
    b. Ensure that the float line is long enough to prevent the float from sinking.

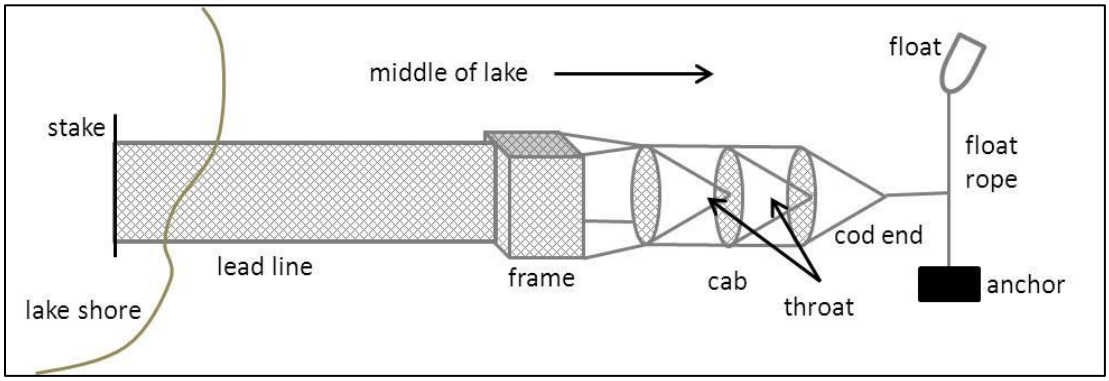

Figure 9. Example of a mini modified fyke net. The entire net from stake to cod end is approximately 10.5 m long with a net depth of 1.2 m.

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. It may be more effective to deploy the entire net while wading so that the T-post does not become dislodged.
    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. Once the mini-fyke net is fully deployed, record the surface water quality conditions (conductivity, dissolved oxygen, and temperature) at the mouth of the set net on the mobile device or the Lake Fish Sampling Field Datasheet (RD[12]).
9. Record the net deployment start date and time as well as the length of lead line (float line) from the mouth of the mini-fyke net to the shore on the mobile device or the Lake Fish Sampling Site Establishment Field Datasheet (RD[12]).
10. One mini-fyke net shall be set in each electrofishing section.
11. 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).
12. Record net deployment end date and time on the mobile device or the Lake Fish Sampling Field Datasheet (RD[12]).

13. 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.
14. 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.
15. Remove T-stake and set aside.
16. Fold mini-fyke net lead over the frame while wrapping corners and set aside in boat.
17. Place net anchors, float lines, and T-stakes in appropriate buckets or tubs.
18. If while retrieving the mini-fyke net, it appears that the net effectiveness has been compromised (collapsed, tangled, holes developed) while deployed, indicate the degree to which the net integrity is affected. This could be a single point of failure or the cumulative total area of several failures. Using the mobile device or *Lake Fish Sampling Field Datasheet* (RD[12]), record if the gill Net Integrity has:
    1) No compromise – no noticeable hole or restriction (tangled or collapsed) that would allow fish to pass through the min-fyke net without being captured.
    2) <10% compromise – Approximately 10% (~0.5 m^2) of the lead line or the cab and cod end has a hole, is collapsed, sagging, or has a tangle for which a few fish may avoid being captured.
    3) >10% compromise – More than 10% (~0.5 m^2) of the lead line or the cab and cod end has a hole, is collapsed, sagging, or has a tangle for which the effectiveness of the net to capture fish is significantly impacted.

    b. Following the Net Integrity tracking described in the previous step, the following actions are recommended:
        1) If <10% of the mini-fyke net integrity is compromised, process and record captured fish. Repair the net before deploying it at other locations.
        2) If >10% of the mini-fyke net is compromised, release captured fish, repair the net, and re-deploy the net.
        3) Do not discard any data until the re-deployment is complete and new data have been recorded.
19. Process all fish (SOP F) from each net before pulling the next net. Record pass end date and time on the mobile device or the Lake Fish Sampling Field Datasheet (RD[12]) once all fish have been processed.
20. While the mini-fyke net is deployed, monitor the area for waterfowl, diving birds, or aquatic mammals that could become trapped in the deployed mini-fyke nets.
    a. It will not be possible to stay on site to monitor the mini-fyke net during its overnight deployment. To reduce the opportunity for birds or aquatic mammals to enter the net, make sure the top portion of the frame is at or just below the water surface.
    b. When retrieving the net, if a bird or aquatic mammal has become trapped in the net follow these steps:

1) Before removing entangled animals protect yourself first. Protect your eyes with sunglasses or safety glasses and use gloves to handle the animal.
2) First, attempt to work the animal out of the mini-fyke net through the frame opening instead of through the cod end to avoid harming the caught fish. If this is not possible, attempt to remove the animal from the cod end but pour caught fish into a processing bucket first. If none of these steps are effective it may be necessary to cut the side of the mini-fyke net open to retrieve the animal.
3) If the animal is injured or killed then stop sampling. Immediately report diving bird or aquatic mammal entanglements by contacting the NEON Aquatic Ecologist, Domain Manager, and submit a trouble ticket using the NEON problem tracking system.
4) Record the non-target species information on the mobile device or *Lake Fish Sampling Field Datasheet* (RD[12]).

21. Mini-fyke netting-related injuries should affect < 3% of fish captured cumulatively at each deployment location. 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.
    a. Please include the following information when submitting a trouble ticket for reporting fish injuries and mortality that exceeds 3%:
        1) Mini-fyke net set time
        2) Water quality: conductivity, dissolved oxygen, and temperature
        3) Field observations and most likely root cause of injury or mortality
        4) Description of species affected
        5) Include any additional information that could help identify the root cause and for developing a solution

SOP F Fish Handling

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

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

F.1 Processing Samples

1. If no fish are caught within a sampling reach, indicate “No” in the “Target Taxa Present?” box on the mobile device or the Lake Fish Sampling Field Datasheet (RD[12]).
2. Ensure that all field scientists handling fish keep hands wet with lake water and free of chemicals (e.g., sunscreen, insect repellent) while processing fish.
3. Field scientists shall confer with each other and the field guides when identifying fish species. Designate one field scientist to record fish throughout the sampling bout for taxonomic consistency
4. For any non-fish top predators (e.g., salamanders) collected, identify and record species to lowest practical taxon on the Lake Fish Sampling Field Datasheet (RD[12]) and release. ***Note:*** *do not weigh or measure non-target species.*
5. 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 (RD[12]).
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. **Preparing Anesthesia: AQUI-S®20E (10% eugenol)** reduces the stress experienced by the target species and minimizes the risk of injury to the field scientist (e.g. sharp spine pricks). Mix anesthetic in one 5-gallon bucket. The use of fish anesthetic is at the discretion of the field scientist but is highly encouraged. Anesthetic **MUST BE USED** (where permitted) when collecting fish tissue for DNA, see Section F.5.
    a. Fill the bucket approximately half-full with native water (2.5 U.S. gallons or ~10 L) or more as needed.
    b. The dosage treatment of AQUI-S®20E is as follows:
        1) 25 - 40 mg/L for freshwater salmonids treated no longer than 5 minutes.
        2) 40 - 100 mg/L for freshwater non-salmonids treated no longer than 5 minutes.

3) **Note:** The non-salmonid dose range is very large and the 100 mg/L concentration is the does used for euthanizing fish. Only use the minimum concentration which effectively renders fish species as handleable.

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

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

Where: A = target concentration eugenol (mg/L)
B = treatment water volume (gal)
C = 0.00378 (conversion factor for grams per gallon)
D = 0.1 (to account for the fact that AQUI-S®20E is 10% eugenol)
E = 1.124 (specific gravity of AQUI-S®20E)

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

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

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

9. Remove fish from the first bucket 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 within the described below.

        1) For salmonids, use 25 – 40 mg/L of AQUI-S® 20E, do **not** exceed a concentration of 40 mg/L.
        2) For non-salmonids, use 40 – 100 mg/L of AQUI-S® 20E, **do not** exceed a concentration of 100 mg/L.
    b. **Note:** In some cases, it may be necessary to create a second bucket of anesthesia to treat a subset of species that require a higher concentration of AQUI-S®20E to achieve sedation and limit sedation time to less than 5 minutes.
    c. Do not exceed 5 fish in the anesthetization bucket at one time.
    d. Leaving fish in the anesthetization bucket for too long can cause mortality. Monitor respiration and gill movement constantly.
    e. Be sure to include required information within the *INAD Field Datasheets*.
11. Identify fish to species using the mobile data device drop down species list for fish. If recording fish species on the datasheet it is recommended to use a 6-letter species code (e.g., *Cottus cognatus* = COTCOG). Also, include in the margin of the datasheet a decoder indicating the taxonomic definition (full species name) for each 6-letter code. Indicate capture method on the datasheet (i.e. electrofishing, gill net, or mini-fyke net).
    a. If the species cannot be identified or identification is uncertain and a voucher specimen is desired, weigh and measure following steps in SOP F.2 below. Only voucher mortalities (inadvertently killed fish) or specimens that require euthanasia due to injuries.

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

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 (Figure 10) (to the nearest millimeter and record on the mobile device or the *Lake Fish Sampling Field Datasheet* (RD[12]).

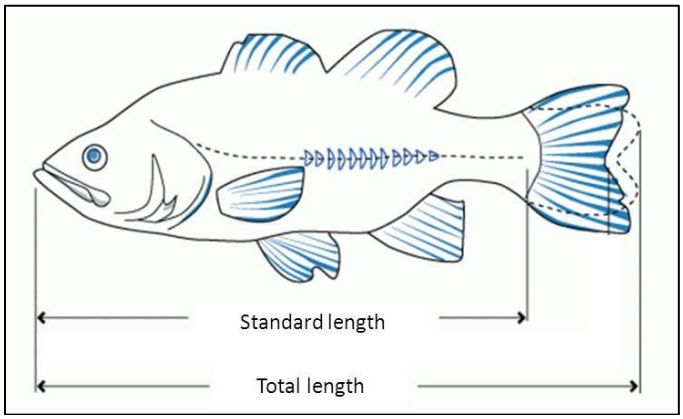

Figure 10. Measure the total length of each fish by pinching the fork together.

15. Inspect the fish for deformities, including eroded fins, external lesions, parasites, and tumors (DELTS; Figure 11). DELTS should be considered as a pre-existing health condition that an individual fish may have been experiencing before being sampled. If there are multiple DELTS, indicate the single most impactful to the specimen. Also, document if the fish was killed or injured because of capture and processing. These could include electrofishing injuries (burn marks, bent spine, hemorrhage; Figure 6) as well as gill or mini-fyke net related injuries (damage to skin, gills, or fins) sustained while captured. Mortality or injury may also include damage because of other captured species. Record DELTS and capture/processing-related injuries on the mobile device or the Lake Fish Sampling Field Datasheet (RD[12]).

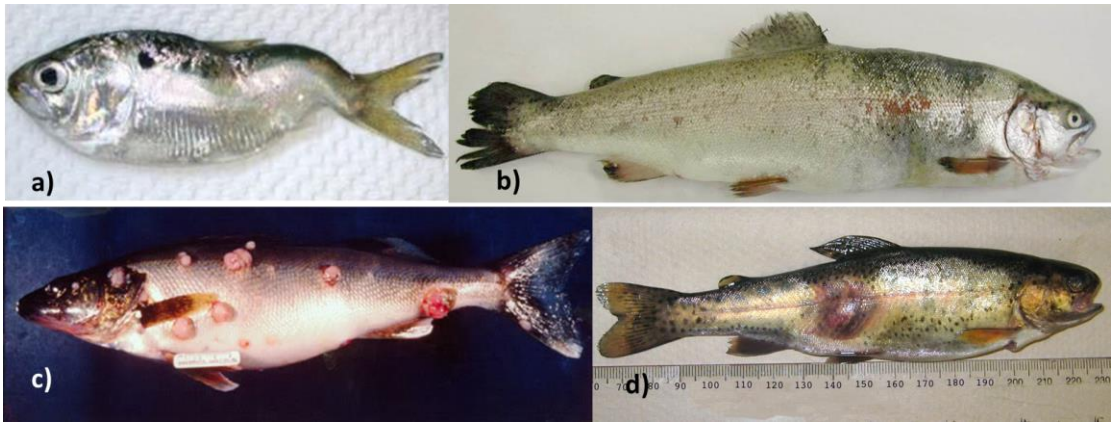

Figure 11. Example DELTS, a) deformity, b) eroded fins, c) tumors, and d) lesion. Photos from a) Kane 2005, b) Leander 2017, c) Cornell 2009, and d) Photo by Craig Banner 2004.

16. If collecting fin clips for DNA barcoding samples, refer to Fish Tissue Sampling for DNA Analysis (SOP F.5).
17. Indicate the life stage of the specimen (e.g. larval, young of the year, juvenile, adult, or gravid) on the mobile device or the Lake Fish Sampling Field Datasheet (RD[12]).
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 SOP E.1, 1-18 above until a minimum of 50 fish per species are identified, weighed, measured, and inspected for deformities. When less than 50 fish per species are identified, all of them shall be weighed, measured, and inspected for DELTS.
    a. If more than 50 individuals of one species are captured, anesthetize, weigh, and measure the first 50 and simply count the remaining fish (no anesthesia) 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.
        3) If it desired to record a weight and length measurement of an individual or to collect a tissue sample or a whole specimen voucher for an individual fish identified in the bulk count group, create an individual specimen record. This will allow for an appropriate sample ID to be generated. Be sure to subtract the number of specimens that were recorded individually from the bulk count number.
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 mobile device or the *Lake Fish Sampling Field Datasheet* (RD[12]); see Voucher Specimen Preservation, SOP F.6.

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 affect any other water chemistry measurements or sample collection. Anesthetic solutions are not expected to affect any other aquatic measurements if disposed of away from the lake. If field disposal is not permitted, waste anesthetic solution will be carried out and disposed of at the Domain Support Facility.

F.2 Handling Uncertainty in Species Identifications

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

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

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

An identification qualifier contains information that indicates the taxonomic level at which there is uncertainty. If there is confidence about the genus of a specimen and uncertainty in the species identification, then 'cf. species' or 'aff. species' indicates that the provided species identification is possibly incorrect (Table 13). If a specimen is definitively of a particular tribe (i.e., Cyprinini) and the field scientist is uncertain in their assignment of genus (i.e., *Cyprinus*), then 'cf. genus' or 'aff. genus' could be used to indicate uncertainty in the genus-level assignment. If there is no uncertainty associated with lowest taxonomic rank specified, the identification qualifier field should be left blank. An inappropriate usage of the qualifier occurs when the level of the selected identification qualifier does not match the given identification of the specimen. For example, if the scientific name of a specimen is *Lepomis* sp., then only the genus is known; it is inappropriate to use the 'cf. species' identification qualifier because that is saying the *species* ID is uncertain without having provided species level information.

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

reason for rejecting them. In this example, the remarks might say "ID either *H. hankinsoni* or *placitus;* identification based on rounded tip of dorsal fin".

Table 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 field scientists cannot narrow the field of possible identifications to just two or three choices. Keep notes in the morphospecies tracking sheet that include descriptive information about the specimen plus any known taxonomic information. A good description might be "similar to or is either *Etheostoma spectabile* or *Etheostoma exile*. Approximately 7 cm long. 10 dark, squarish blotches along their side. Cream colored throat. Second dorsal, and caudal fin have small dark spots." This example description contains taxonomic information (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 field scientists such that only species thought to be present in a domain are available for selection. With climate change and species introductions, it is likely that field scientists will observe species within their site that represent the first ever records of that species in that area. When this happens, the mobile application will not have the scientific name of that species available for selection. In that case, field scientists must use the taxon code "OTHE" for fish that are new to their domain. In the remarks field, write the scientific name of the identified fish. NEON will revise that record to reflect the correct scientific name after confirmation of identification. In addition, larval fish that are collected are difficult to identify in the field. Use the taxon code "LARV" for larval fish

that cannot be properly identified during fish processing. The taxon table will be updated for the subsequent year to make that species name available to field scientists within that domain.

F.3 About Morphospecies Designations

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

A morphospecies is a temporary designation for an individual or group of fish that are of the same group (ideally, that group is a species; morphospecies only requires that all individuals look the same). A subset of each field scientist-identified species or morphospecies will be sent for later taxonomic confirmation by DNA analysis. Sometimes morphospecies designations turn out to contain multiple species. While this is unavoidable for certain species complexes that are difficult to resolve, in most cases this can be avoided by designating multiple morphospecies for similar (but not identical) unknown specimens. Should these morphospecies be assigned the same species designation by 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 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.
- Generally, split groups that look similar but not identical into different morphospecies, focusing on features like size, color, presence of bars/par marks, and shape of the dorsal fin, length of the upper jaw, eye diameter, and lateral line features. It is easy to lump them together later, but difficult to later split them into multiple species.
- If it is unclear whether a newly captured specimen is the same as individuals from a previously assigned morphospecies, a new morphospecies ID should be assigned (it is better to have the same species designated as different morphospecies than to have multiple different species designated as the same morphospecies).
- The format of a morphospecies ID includes: the domainID where the specimen was captured, the year of capture, and the word “Morph” followed by one or more unique letters. For example, “D15.2014.MorphA” would be the first morphospecies from domain 15 that was captured in 2014. **Note:** it is important to use the morphospecies ID to document, track, and resolve uncertain fish taxa.
- The letter at the end of the morphospecies ID (e.g., “A”) should *never* be repeated for any other morphospecies than that for which it was originally designated, in a given year. If more than 26 morphospecies are encountered in a given domain in a given year, the 27th morphospecies ID should include two letters at the end (e.g., the 27th morphospecies in domain 15, for 2014, would be “D15.2014.MorphAA”). For every additional 26 morphospecies, a new letter will be added (i.e., the 54thmorphospecies would be “D15.2014.MorphAAB”).

F.4 Euthanizing Fish and Non-Target Species

1. Euthanize fish using a lethal dose of 10% eugenol at a concentration of 100 mg/L. Refer to Table 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
100	8.4	16.8	33.6	50.4	67.3	84.1

a) Add 16.8 mL of 10% eugenol to 5.0 U.S. gallons of native 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) and add 10% formalin preservative. One taxon per specimen bottle. See steps on specimen preservation in SOP F.6.

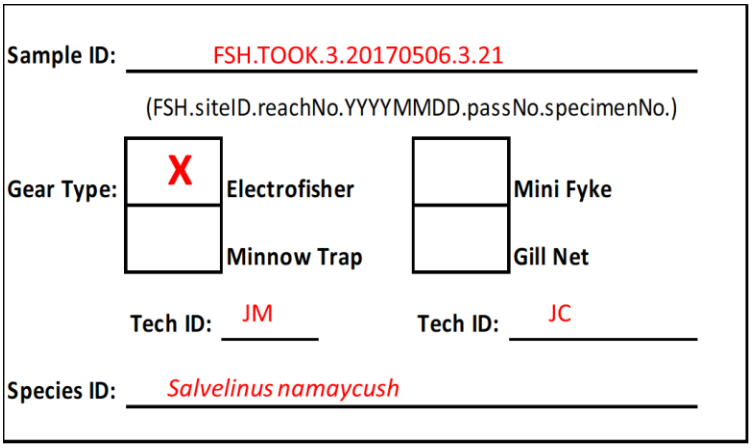

Figure 12. Example specimen label

(1) Adhesive barcode labels (**Error! Reference source not found.**3) will be added to the sample containers and scanned by the mobile app (**Error! Reference source not found.**4).
(2) Keep a human-readable label on each bottle with a minimum of the sample ID printed to assist with organization and shipping.

Figure 13. Example of an adhesive Type II barcode label.

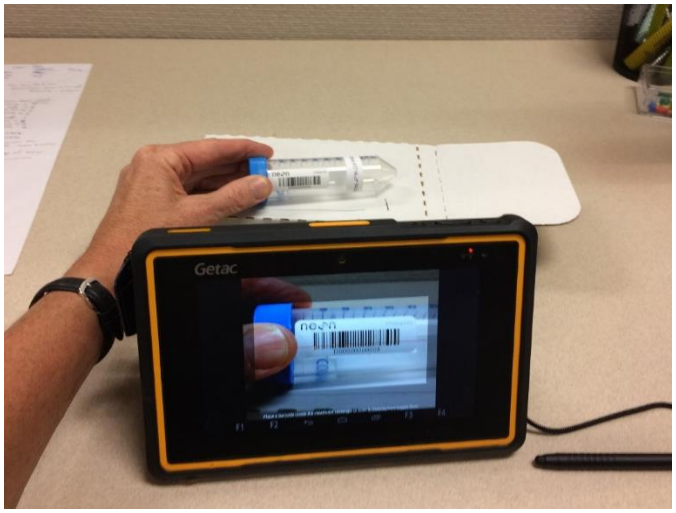

Figure 14. Barcode label scanning.

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

b. Amphibians that are injured because of fish sampling will be euthanized using a lethal dose of MS-222, 200 mg/L of native water in the field.
   1) Add 1 L of native 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 F.6.

a) When the system is available, adhesive barcode labels will be added to the sample containers and scanned by the mobile app (**Error! Reference source not found.**4). Figure 14). Figure 14). Figure 14).

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 15) follow below.

Figure 15. Reptile Euthanasia kit with 50 mL centrifuge tube to store the 5 mL syringe and needle, 60 mL HDPE amber bottle is for 1st stage solution, 20 mL HDPE scintillation vial for 2nd stage solution.

a) A fresh bottle of stage 1 and stage 2 reptile euthanasia solutions should be prepared for each day of sampling. Keep these solutions in dark containers, away from

sunlight, and refrigerated when not in use. Discard unused solutions at the end of each fish sampling bout in the lab following the NEON EHS chemical hygiene guidelines (AD[03]).

2) 1st stage injection solution - 1% MS-222
   a) Prepare MS-222 and baking soda to be mixed with water in the field when needed)
   b) Measure out 0.25 g MS-222 plus 0.157 g baking soda using the lab balance; weigh out MS-222 and baking soda in a small container (amber 60 mL HDPE bottle) at the Domain Support Facility.
   c) Bring along 1 L of tap water from the Domain Support Facility to mix in the field with the MS-222 and baking soda when the stage 1 euthanasia solution is needed. Add 25 mL of water while in the field and shake to mix well.
3) 2nd stage injection solution - 50% (v/v) unbuffered MS-222
   a) Make a fresh solution in the field as needed.
   b) Add one part MS-222 (g) to 2 parts water (mL); no baking soda (unbuffered).
   c) Pre-weigh out 1 g of MS-222 at the Domain Support Facility into an appropriately sized container (ex. 20 mL scintillation vial covered in duct tape or foil to protect from light).
   d) Add 1 g MS-222 in 2 mL tap water for 2 mL of 50% (v/v) MS-222 in the field when needed. A syringe can be used to measure out 2 mL of water. Shake the scintillation vial to mix the solution.
   e) The solution will be thick with precipitates, cloudy, and pale yellow (Figure 16).

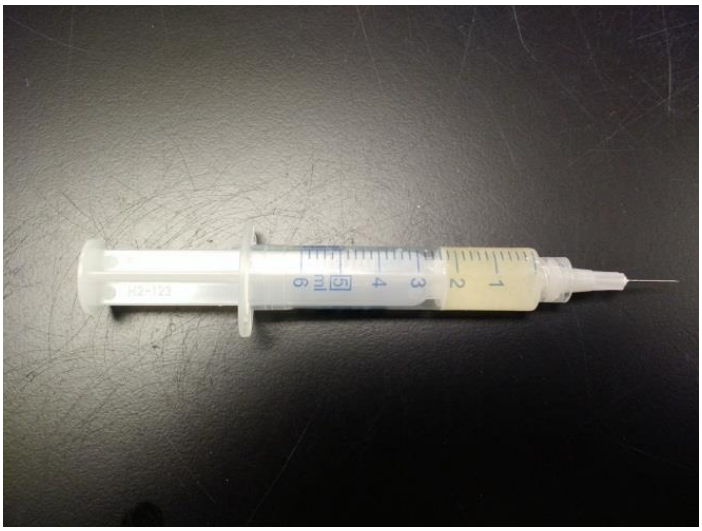

Figure 16. 2nd stage 50% (v/v) unbuffered MS-222 solution. Note yellow cloudy appearance of solution

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 17).

Figure 17. Intracoelomic injection into the inguinal fossa of a turtle. Photo by Kaufman 2017.

e) A 250 mg/kg dose will be sufficient to anesthetize reptiles. In the event that the 500 mg/kg dose is needed, double the dose (Table 15).

5) <u>2nd stage injection instructions:</u>
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 17).

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 lake. Do not dispose of the MS-222 solutions in the field or lake.

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

e. In the event that a federal or state listed threatened or endangered species is morbidly injured, the organism will be euthanized following the procedures identified above unless otherwise instructed via the collection permit. Follow the permit guidelines for the disposition of killed endangered species. Refer to the site-specific sampling strategy for additional guidance when handling listed species.

1) Stop all fish sampling activities.

2) Contact the Domain manager to report the incident. The manager will contact the regional U.S. Fish and Wildlife Service office and the local state fish and wildlife department to report the incident.

3) Specimens will also be preserved following the methods described above.

4) If the inadvertent death of a protected species is discovered once the specimen has been collected as a voucher or shipped to a curation facility, NEON will immediately contact the federal and state fish and wildlife authorities within the region where the specimen was collected.

F.5 Fish Tissue Sampling for DNA Analysis

1. Put on gloves (nitrile or latex free).

2. Anesthetize target fish with AQUI-S®20E following SOP F.1. Tissues **MUST BE COLLECTED** from anesthetized fish where allowed (check your collection permit; if unsure, contact the lead aquatic scientist).
3. Take a single photo voucher of the specimen (**before clipping any fins**) from which the tissue sample was collected. Orient the fish to capture a lateral view, with the head facing left, and place the fish on top of a monochromatic background. A white field sampling tray would be appropriate. Include a scale bar and color separation guide in the field of view to calibrate the image (Figure 18).
    a. Take the photo using the tablet and mobile application. Record the "photo view" which is auto-populated as lateral. If the photo is oriented as a dorsal or ventral view, select that from the photo view options.
    b. It may be helpful to provide additional lighting when taking photos of fish in low light situations. A headlamp or an electric camping lantern can provide additional lighting.
    c. Be sure that the resulting image is in the "landscape" orientation. Include as much of the specimen in the field of view as possible. Minimize shadows and keep hands or other objects out of the image. See Figure 18 for an example.
    d. A caption can be entered but is not required. Click on the photo that was collected and enter a remark in the caption field.
    e. Delete any photos that are not of suitable quality or otherwise should not be associated with the fish data.
    f. A photo ID will be generated and then joined with the DNA sample ID in the mobile application.

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

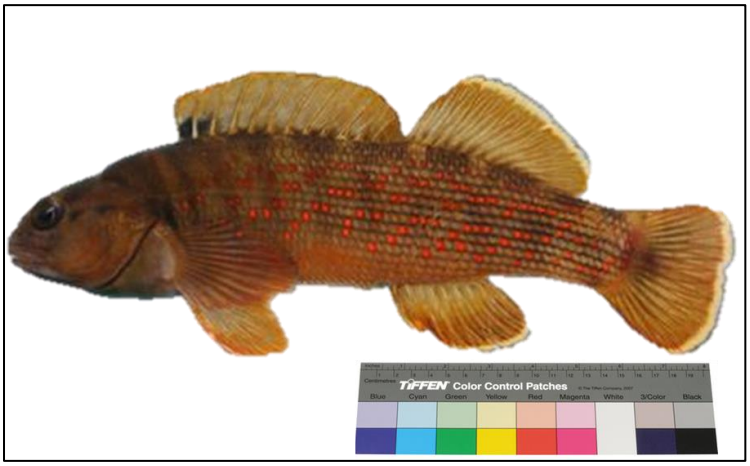

Figure 18. Example voucher photo. Lateral, head left, with color separation guide and scale bar. Photo from OSU 2017.

4. Dip the fin clipping scissors and forceps into high concentration ethanol (70% or greater) and ignite with lighter to flame sterilize the tissue sampling tools. If flame sterilization is unavailable, wet the instruments with native water after dipping in ethanol and wipe with a clean cloth.
5. Some State collection permits (AK) require that tools used to sample fish tissue be soaked in an iodophor or betadine disinfectant bath. Using a clean dishpan or other container, add a concentration of 1/100 iodine-based disinfectant and clean tap water (bottled water) solution. There should be enough disinfectant to completely submerge the tools. Soak tools for 10 minutes between tissue sampling. It may speed up the process to have several sets of fin clipping tools available to minimize the disinfection time.
6. **VERY IMPORTANT:** Fish tissues collected for DNA extractions cannot be exposed to or preserved in formalin.
7. Tissues must be collected from anesthetized fish as part of the Fish Processing (SOP F.1) steps.
8. Using the cutting tool, remove a piece of the target fin ray (Figure 19). Suitable fins for DNA include the left pelvic fin or the caudal fin. The adipose fin is not an ideal tissue to sample as it contains fat that reduces the effectiveness of the DNA extraction process. Also, note that some States (AK) prohibit the collection of adipose fins; check your collection permit stipulations. Tissue should be a minimum of 2 mm but no larger than 5 mm in diameter. Smaller tissue samples should be harvested from smaller fish.

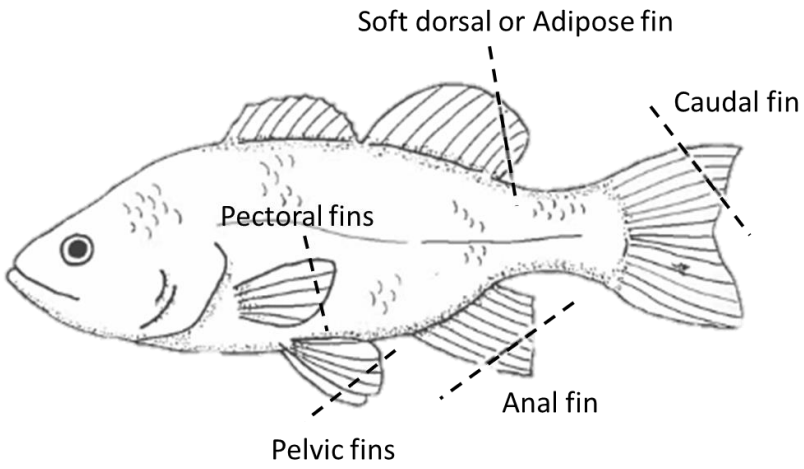

Figure 19. Optional fins to clip for tissue sampling

9. With the forceps, place the fin clip in the appropriate tissue container (1.5 mL cryo vial). Pre-fill the vial with ethanol (70% or greater) to keep the specimen wet. Be sure that the container is completely closed and labeled with the appropriate sample ID using the following DNA fish sample format “FSH.siteID.reachNo.YYYYMMDD.passNo.specimenNo.DNA”.
10. Collecting DNA tissue samples for fish that weigh less than 0.5 g, removing sufficient amounts of fin tissue will likely reduce their survivorship. Instead, euthanize the specimen, remove an adequate portion of fin tissue, and place in the tissue collection vial. The remaining specimen

can be collected as a voucher (see Section F.6 Whole Fish Voucher Specimen Preservation) or discarded following the guidance of the collection permit.

11. Place live fish that have had tissues samples collected into the recovery bucket.
12. Indicate on the mobile device or the Lake Fish Sampling Field Datasheet (RD[12]) that a DNA tissue sample was taken and provide a sample ID for each specimen.
13. Flame sterilize (or wipe) the cutting tool and forceps or wipe with an alcohol pad before collecting the next tissue sample to avoid cross-contamination. Be sure that no fish tissue, slime, or blood remains on the instruments.
14. Repeat Steps 1-9 until all targeted fish samples have had tissues collected. Up to 5 individuals per species per year are sampled for DNA analysis.
15. If a tissue sample was collected from a fish in a bulk sample then there needs to be an individual record included on the datasheet and a sample ID generated. Subtract the number of individuals used for tissue collection from the bulk sample count for each individual entry.
    a. For tissue storage and processing procedures, refer to Section 4.3 Timing for Laboratory Processing and Analysis and SOP H for shipping guidance.

F.6 Whole Fish Voucher Specimen Preservation

1. Fill jar with a 10% buffered formalin solution to fix specimens within one hour of euthanizing.
   a. If using concentrated formalin (37% formaldehyde), dilute 1 part concentrated formalin with nine parts water (can use native water).
   b. In general, use a ratio of 10:1 formalin to fish tissue by weight (example: use 1,000 mL formalin: 100 g fish).
      1) Use an appropriate size HDPE wide mouth specimen jar for the preservation of an individual specimen in a single jar. Groups of individuals of one species may be included in a single jar only if the individual has the Sample ID label physically attached to the specimen.
   c. For large fish greater than 200 mm total length, make a lateral 2-3 cm incision on the lower right side into the peritoneal. Open the body cavity, taking care not to damage bones or organs, will allow for better penetration of the fixative.
   d. Formalin should be injected into the dorsal muscles of specimens with muscle wider than 14 mm.
2. Secure lid tightly and store upright at room temperature (~70 °F). Be sure that the specimen label is included with the specimen and that the jar is properly labeled with the Sample ID in the following format “FSH.siteID.reachNo.YYYYMMDD.passNo.specimenNo.” (Figure 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%-95% ethanol or isopropanol, making sure that the specimen is fully submerged.
5. Secure lid tightly and store upright at room temperature (~70 °F) with appropriate specimen labels inside and outside of the container.
6. Should the fish sampling activities be suspended or cancelled due to a change in environmental conditions becoming unsafe, stop all specimen collection. If samples were collected, contact the domain manager and submit a trouble ticket through the NEON problem resolution system.

F.7 Ending the Sampling Day

1. Refreshing the sampling kit.
   a. Replace batteries for all battery-operated equipment (e.g., GPS unit, portable aerators).
   b. Refill/restock preservative and anesthetic stock solution containers.
2. Equipment maintenance, cleaning and storage.
   a. Wash all equipment that has been exposed to lake water according to the All Systems Standard Operating Procedure: Decontamination of Sensors, Field Equipment, and Field Vehicles (RD[07]).
   b. Dry all equipment thoroughly between sites and before storage.
   c. Check all nets for holes and patch if necessary using the net repair kit. Mending fish nets takes practice and patience. There are several resources available online. See the Oregon State University (OSU) 1989 reference for a resource to mend and patch fish nets.
   d. Inspect the anode ring on the electrofisher unit for corrosion. For light corrosion, use a steel wool pad to scrub the ring clean of rust. This will reduce unnecessary overloads on the backpack electrofisher. If corrosion is heavy, which is more common when operating in water with high conductivities, use fine grit sandpaper to remove rust.

SOP G Data Entry and Verification

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

However, given the potential for mobile devices to fail under field conditions, it is imperative that paper datasheets are always available to record data. Paper datasheets should be carried along with the mobile devices to sampling locations at all times. As a best practice, field data collected on paper datasheets should be digitally transcribed within 7 days of collection or the end of a sampling bout (where applicable). However, given logistical constraints, the maximum timeline for entering data is within 14 days of collection or the end of a sampling bout (where applicable).

See RD[04] for complete instructions regarding manual data transcription.

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

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

SOP H Sample Shipment

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

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

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

H.1 Handling Hazardous Material

Follow shipping and Hazmat procedures for formalin.

H.2 Supplies/Containers

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

H.3 Timelines

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

H.4 Conditions

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

H.5 Grouping/Splitting Samples

N/A

H.6 Return of Materials or Containers

N/A

H.7 Shipping Inventory

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

H.8 Laboratory Contact Information and Shipping/Receipt Days

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

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APPENDIX A DATASHEETS

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

Table 17. Datasheets and mobile applications associated with this protocol

NEON Doc. #	Title	Mobile Application
NEON.DOC.001646	General AQU Field Metadata Sheet	(AOS) Field Metadata [PROD]
NEON.DOC.002494	Datasheets for AOS Sample Shipping Inventory	Shipping App [PROD]
NEON.DOC.003106	Datasheets for Fish Sampling in Lakes	(AOS) Fish [PROD]

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

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

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

Step 4 – If this is your first sampling year, establish and select random sampling 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-S®20E.

Step 7 – Identify fish to species using the 6-letter species code (e.g., *Cottus cognatus* = COTCOG) and record on the mobile device or the *Lake Fish Sampling Field Datasheet* (RD[12]). For uncertain species, follow the guidance provided in SOP F.2. For morphotype species and labeling with a unique identifier, refer to SOP F.3.

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

Step 11 – If any endangered species (review collection permit before sampling) or other vertebrates (e.g., salamanders, turtles) are caught, identify, take a photo, and release immediately and **STOP** all electrofishing activities within the permitted reach. Follow the guidance of the collection permit for reporting procedures.

Step 12 – Preserve euthanized specimen in a jar with a 10% buffered formalin (then ethanol for long-term storage. Ship to the external facility when directed to.

APPENDIX C REMINDERS

Before heading into the field:

- ☑ Collect and prepare all equipment including labels.
- ☑ Pre-print labels on waterproof paper.
- ☑ Ensure all batteries are fully charged.
- ☑ When mixing the stock solution, wear nitrile gloves and eye protection, as MS-222 is a skin and eye irritant.
- ☑ Assemble and review all required Safety Data Sheets for chemicals used in this protocol.

Sample collection:

- ☑ All field scientists MUST wear necessary personal protective equipment before stepping into the water, including waterproof chest waders with lug-soled boots (felt waders are not allowed at NEON sites), rubber lineman gloves to insulate the wearer from electrical shock, and polarized sunglasses to increase the efficiency of fish capture.
- ☑ While electrofishing, monitor the abundance of fish in the buckets to prevent escape or accidental spills.
- ☑ Sample all complex 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 release immediately. Stop all fish sampling activities. Follow the guidance of the collection permit for reporting procedures.
- ☑ Release the processed, revived fish back into the lake outside of the block net.

Sample processing:

- ☑ If an endangered species is morbidly injured, stop all fish sampling activities, follow the permit guidance before applying euthanasia. Also, follow the permit guidelines for the disposition of killed endangered species.
- ☑ If more than 50 individuals 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 bout windows at lake sites (RD[06]). Fish sampling must be scheduled within the site-specific bout window. Fish sampling should be scheduled as the last sampling activity of the bout to minimize impacts on other sampling objectives.

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 been chosen as a fixed reach. If sampling is impractical because of severe drought (dry) or that the lake is frozen then indicate this for any affected reach on the mobile device or the *Lake Fish Sampling Field Datasheet* (RD[12]). Should environmental conditions affect 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 mobile device or 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