AOS PROTOCOL AND PROCEDURE: FSL – FISH SAMPLING IN LAKES

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Change Record

REVISION	DATE	ECO #	DESCRIPTION OF CHANGE
A	02/18/2014	ECO-01394	Initial release
B	01/22/2015	ECO-02632	Migration to new protocol template
C	11/18/2015	ECO-03328	Major updates to include IACUC requirements and input from technicians, removed datasheets from appendices and created NEON.DOC.003106 Datasheets for AOS Protocol and Procedure: Fish Sampling in Lakes
D	05/02/2017	ECO-04507	CM updated with new template and changes based on feedback from FOPS. Fish tissue DNA sampling included. Fish euthanasia with AQUI-S20E. Minimized sample size per species and included bulk processing steps.
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.
G	05/12/2021	ECO-06536	Clarification of morphospecies naming. Formalizing the voucher specimen containers and label requirements. Temperature thresholds 18 C Salmonids, and 26 C no salmonids
H	03/16/2022	ECO-06781	• Update to reflect change in terminology from relocatable to gradient sites.
J	02/05/2025	ECO-07129	• Migrated to protocol template rev M • Updated bout duration from 5 days to 3 days • Updated number of sampling reaches to sample for bout minimum requirements based upon reductions in sampling • Updated maximum number of fish to process per species per transect from 50 to 30 • Updated requirement for MS-222 to be pharmaceutical grade • Updated to allow fish euthanasia using MS-222 if 10% eugenol is unavailable • Updated section 5 Safety to include safe lab techniques for using ethanol and flame sterilization together • Updated the barrier section to describe the changes for TECR, MCDI, and WALK in Appendix D.4 • Added table that describes the prioritization matrix for sampling fixed reaches. • Included an image of a mortality tracker that can be used to prevent post-hoc mortality exceedances • Updated some of the supplies list items to match what is currently in-use and added bucket cover options. • Substantial copy editing throughout document

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1 OVERVIEW

1.1 Background

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

Unlike rivers and streams where relatively few sampling methods (e.g., electrofishing, seines) are commonly used to characterize fish assemblages (Guy et al. 2009; Rabeni et al. 2009), numerous methods (e.g., electrofishing, seines, fyke nets, gill nets, trawling) are used to sample fish assemblages in lakes and impoundments (Miranda and Boxrucker 2009; Murphy and Willis 1996). Multiple methods are typically required because lakes and impoundments have two distinct zones (i.e., pelagic and littoral) that differ in physicochemical characteristics and fish assemblage structure. Substantial differences in physical characteristics (e.g., depth, water clarity, vegetation) and the selectivity of species and sizes of fish affect the efficiency of sampling methods in differing zones. For example, multiple gears are often necessary to sample both juvenile and adult fish of the same species because of differing habitat use and size biases associated with various equipment (Boxrucker et al. 1995). Although a few studies have evaluated multiple sampling equipment types, most studies have focused on a limited number of species (e.g., gizzard shad *Dorosoma cepedianum*, black crappie *Poxomis nigromaculatus*) at small spatial scales (e.g., Boxrucker et al. 1995; Guy et al. 1996; Allen et. al 1999).

The sampling method described in this protocol attempts to overcome traditional problems associated with characterizing fish assemblages in lentic habitats, by using multiple active and passive methods including 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.

1.2 Scope

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

1.2.1 NEON Science Requirements and Data Products

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

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

1.3 Acknowledgments

The design and implementation of lake fish sampling methods was based on the guidance from the NEON Fish Sampling Workshop. Specifically, D. C. Dauwalter, A. J. Davis, E. A. Frimpong, G. D. Grossman, K. G. Gerow, R. M. Hughes, C. P. Paukert, and D. M. Walters were instrumental in providing recommendations for the site-level fish sampling design at NEON lake sites. Additionally, the sampling protocols herein followed the guidelines recommended by the American Fisheries Society (Bonar et al. 2009) and were chosen to align with those of United States Environmental Protection Agency (USEPA 2007).

2 RELATED DOCUMENTS AND ACRONYMS

2.1 Applicable Documents

Applicable documents contain higher-level information that is implemented in the current document. Examples include designs, plans, or standards.

AD[01]	NEON.DOC.004300	EHS Safety Policy and Program Manual
AD[02]	NEON.DOC.004316	Operations Field Safety and Security Plan
AD[03]	NEON.DOC.000724	Domain Chemical Hygiene Plan and Biosafety Manual
AD[04]	NEON.DOC.050005	Field Operations Job Instruction Training Plan
AD[05]	NEON.DOC.004104	NEON Science Data Quality Plan

2.2 Reference Documents

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

RD[01]	NEON.DOC.000008	NEON Acronym List
RD[02]	NEON.DOC.000243	NEON Glossary of Terms
RD[03]	NEON.DOC.002652	NEON Data Products Catalog
RD[04]	NEON.DOC.001271	AOS/TOS Protocol and Procedure: DMP – Data Management
RD[05]	NEON.DOC.003106	Datasheets for AOS Protocol and Procedure: FSL – Fish Sampling in Lakes
RD[06]	NEON.DOC.001646	General AQU Field Metadata Sheet
RD[07]	NEON.DOC.001152	NEON Aquatic Sample Strategy Document
RD[08]	NEON.DOC.003282	NEON Protocol and Procedure: SIM – Site Management and Disturbance Data Collection
RD[09]	NEON.DOC.005247	AOS/TOS Standard Operating Procedure: NEON Aquatic and Terrestrial Site Navigation
RD[10]	NEON.DOC.004257	Standard Operating Procedure: Decontamination of Sensors, Field Equipment, and Field Vehicles
RD[11]	NEON.DOC.003046	AOS Protocol and Procedure: INV – Aquatic Macroinvertebrate Sampling
RD[12]	NEON.DOC.001197	AOS Protocol and Procedure: BAT – Bathymetry and Morphology of Lakes and Non-Wadeable Streams
RD[13]	NEON.DOC.001195	AOS Protocol and Procedure: RIP – Riparian Habitat Assessment in Lakes and Non-Wadeable Streams
RD[14]	NEON.DOC.005224	NEON Protocol and Procedure: SCS – Shipping Ecological Samples and Equipment

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
EHS	Environmental Health and Safety
Hz	Hertz
IACUC	Institutional Animal Care and Use Committee
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

Anode: The part of the fish sampling equipment that puts a positively charged electrode into the waterbody, electrofishing uses the interaction between a positive charge and a negative to temporarily immobilize fish. On backpack shockers used by the NEON program, the anode is a metal ring (or diamond) on a fiberglass shaft that is submerged in water and operated by a switch (**Figure 1**).

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: The part of the fish sampling equipment that puts a negatively charged electrode into the waterbody, electrofishing uses the interaction between a positive charge and a negative to temporarily immobilize fish. On backpack shockers used by the NEON program, the cathode is a steel cable that is dragged behind the operator (**Figure 2**).

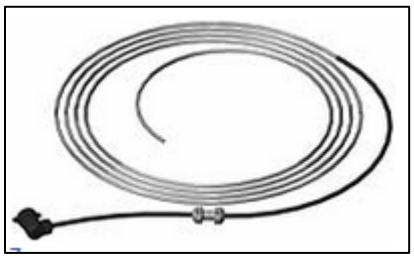

Figure 2. Cathode for backpack electrofishing unit (photo: store.smith-root.com)**.**

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.

Fulcrum: Software tool used to create NEON electronic data entry applications.

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

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

INAD: Clinical field trials to determine the efficacy of AQUI-S ® 20E as an anesthetic for use in a variety of fish species.

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.

ServiceNow: Software tool used for problem/incident tracking and resolution.

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°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 goal of sampling fish at NEON's lake sites is to determine the taxa diversity, the number of species present (richness), biomass, and to enable DNA analysis for fishes at each lake. These variables, particularly diversity and biomass, are tracked over time to detect changes in species loss, changes in the community structure and function, as well as the introduction and spread of invasive taxa (presence/absence). This protocol describes multiple active and passive methods (i.e., backpack electrofishing, mini-fyke nets, and gill nets) at different times throughout the year to capture these variables.

Fish sampling at NEON lake sites are delineated into ten fish sampling reaches and are designed so that each one includes a nearshore and offshore fish habitat. The ten fish sampling reaches are delineated so that permanent established plot markers (Riparian plot markers) evenly spaced along the circumference of the lake shoreline mark the boundary (start and end), generating pie shaped sampling reaches (**Figure 3**). Three sites are designated as permanent "fixed" reaches. Up to three fixed reaches are sampled twice per year with a backpack electrofisher using a three-pass electrofishing depletion approach in a netted off 10X10 littoral subsampling area, one mini-fyke net set, and one gill net set (Baker et al. 1997). The remaining seven reaches are "random" reaches and sampled following a random stratified design to ensure that the variety of habitat types are sampled equally over time. Up to seven random reaches are sampled using a rotating sampling design with initial random selection of shoreline reaches ensuring appropriate spatial coverage of habitat types within the lake (Baker et al. 1997). Random reaches are sampled in the spring and fall and use a single electrofishing pass (without block nets), one mini-fyke net set, and one gill net set. Sampling and net placement is located far enough apart to minimize interactions from each effort.

All fish captured during sampling are identified to the lowest possible taxonomic level, bulk counted, and inspected for deformities, eroded fins, lesions, tumors, and parasites. Up to thirty of each species caught per reach will be weighed and measured. Additionally, opportunistic voucher collection occurs when fish are mortally injured during sampling. These mortally injured fish are identified, weighed, measured and collected to be vouchered and stored at the NEON biorepository.

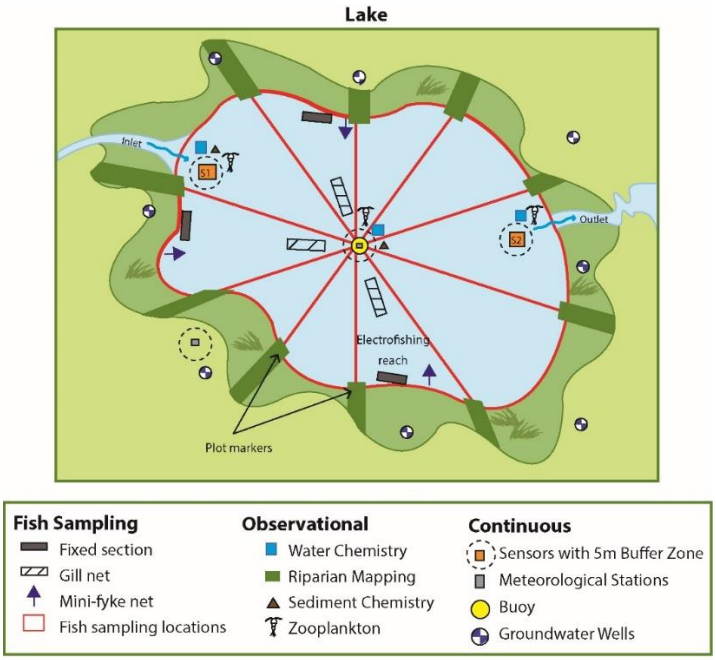

Figure 3. A generic lake site layout with fish sampling locations.

This protocol outlines non-destructive methods for collecting fish tissues from live specimens in the field. A subset of captured fish will undergo tissue removal (fin clipping) for DNA analysis. DNA analysis serves several purposes, including verifying the taxonomy of specimens, clarifying the taxonomy of rare or cryptic species, and characterizing diversity using molecular markers. The aim of sampling tissues from fish is to investigate the DNA of targeted species without causing mortal damage to the specimen. Field technicians prepare tissue samples collected in the field for shipping to an external lab, where genomic DNA is extracted and purified, a marker region is amplified using polymerase chain reaction (PCR), and the resulting PCR product is sequenced. Any remaining extracted DNA is archived at the external lab for future studies.

Standard Operating Procedures (SOPs), outlined in Section 7 of this document, provide detailed step-by-step directions, contingency plans, sampling tips, and best practices for implementing this sampling procedure. Field technicians must follow the protocol and associated SOPs to properly collect and process samples. NEON's problem reporting system should be used 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. If local conditions create uncertainty about carrying out these steps, it is critical that technicians document the problem and enter it in NEON's problem tracking system.

Quality assurance is performed on data collected via these procedures according to the NEON Science Data Quality Plan (AD[05]).

4 SAMPLING SCHEDULE

4.1 Sampling Frequency and Timing

Aquatic biological bouts are three pre-assigned date ranges in which aquatic biological data collection is scheduled, these dates roughly align with spring, summer, and fall. Bouts are determined per site, to ensure differences in latitude, longitude, seasons, altitude, and logistics are accounted for (Parker and Utz 2022). Bout timing is determined using stream flow, air temperature, and changes to riparian vegetation per site (Parker and Utz 2022).

Fish sampling in lakes takes place only during the first (spring) and third (fall) bio-bouts (**Table 1**). Sampling twice a year enables NEON fish data to not only provide information on the seasonal use of the site by species, but also recruitment and season biomass. Initial sample timing was determined for each lake site using historical data including ice-out, water temperature (or accumulated degree-days), weather, and riparian peak greenness. Sample timing will be refined on a site-by-site basis by NEON Science based on data collected by the aquatic sensors and Field Operations. Fish sampling must be scheduled within the specific biology bout window. Accommodations for local weather, hydrological conditions (e.g., late ice-off), or vegetation cover at the site may push sampling outside of the bout window in addition to events such as dangerous weather at the site, high flows on the stream, or logistical reasons. See the Aquatic Site Sampling Strategy (RD[07]) for additional details and scheduling preferences. Use NEON's problem reporting system to seek guidance and report sampling efforts that take place outside of the defined sampling window.

Table 1. Sampling frequency for fish sampling in lakes on a per SOP basis.

SOP	Site Type	Location	Bout Duration	Bouts Per Year	Bout Interval	Yearly Interval	Remarks
SOP B and SOP C	Lakes	Up to 6 reaches (three fixed and three random) per bout	3 days	2	Bouts conducted once in the spring and once in the fall	Annual	A minimum of one fixed and three random reaches must be complete to meet minimum bout completion requirements

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 2**) 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 three electrofishing passes will occur along with one mini-fyke and one gill net set. Random reaches 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 2**).

Table 2. Proposed lake sampling activities for a crew of 3 during a 3-day period.

Time of Day	Day 1	Day 2	Day 3
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
Night	Allow electrofishing reaches to recolonize	Electrofish in fixed reach(es)	Electrofish in fixed or random reaches

Scheduling Considerations

1. Sampling corresponds with the first and third sampling bout windows at lake sites (RD[07]).
    a. Fish sampling must be scheduled within the site-specific bout window.
2. Fish sampling should be scheduled as the last sampling activity of the biology bout to minimize impacts on other sampling objectives.
    a. Weather or other hydrological conditions at the site may push sampling outside of the bout window.

NOTE: *If fish sampling occurs before other bio-bout activities, you must consult the relevant protocols to ensure that they are scheduled in response to the potential disturbance created by fish sampling. If fish sampling occurs before periphyton or macroinvertebrate sample collection, a minimum of 14 days is needed between collection events.*

3. If conditions do not allow for fish sampling to occur during bout one, then sampling shall occur when safe conditions allow, up to two weeks before the scheduled start of the bout 2 window (i.e. two weeks before the calendar day the biology bout window opens, regardless of when the first on the ground sampling event is scheduled during the bout 2 window).
4. If conditions do not allow for fish sampling to occur during bout 3, then sampling shall occur when safe conditions allow, up to 30 days beyond the end of bout 3, or until January 31, whichever occurs first.
5. A minimum of 2 weeks between sampling bouts shall be observed. Sampling bouts should last no longer than 3 days assuming no weather or other unexpected schedule delays.

6. All three passes in a fixed sampling reach must be sampled within the same night.
    a. There must be a minimum of 30 minutes between passes to allow fish to resettle in the reach.
    b. No more than 2 hours can elapse between passes.
7. Do not start a new pass 30 minutes before sunrise.
8. The minimum requirement for a bout to be considered complete is completion of one fixed reach, this should be prioritized to be sampled first when possible.

4.2 Criteria for Determining Onset and Cessation of Sampling

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

4.3 Timing for Laboratory Processing and Analysis

Individual domain facilities will temporarily store preserved voucher specimens of fish (target species) as well as any amphibians or reptiles (non-target specimens) fatally injured and euthanized, or killed during fish sampling activities (**Table 3**). Vouchered specimens will be shipped to the NEON Biorepository at Arizona State University for long-term storage. Fish and non-target voucher specimens may be stored up to 12 months following the preservation guidelines outlined in **Table 4** and **SOP C**. For storage and shipping timelines see **SOP F**.

Fish fin clips may be taken from a maximum of 5 individuals from each species sampled/per site/per year for DNA analysis (e.g. if at site A, 4 species of fish are sampled during the year, up to 20 fin clips may be collected). Field Science may need to store fin clip samples at the Domain Support Facility until instructed to ship for processing. The tissue samples will be kept in the labeled sample vials with ethanol (70-95%) and refrigerated or frozen until directed to ship the samples. DNA tissue samples can be stored in these conditions for up to 12 months.

Table 3. Sample type, activity and holding time for fish samples.

Sample type	Activity	Holding Time
DNA Fin clip	Ship to processing Domain	Up to 12 months
Fish voucher (<200 mm)	Ship to Biorepository	Up to 12 months
Fish voucher (>200 mm)	Ship to Biorepository	Up to 12 months
Non-target voucher	Ship to Biorepository	Up to 12 months
Invertebrate bycatch	Ship to Biorepository	Up to 12 months

Table 4. Fish sample storage and holding times.

Sample Type	Field Storage	Post-processing Lab Storage	Domain Hold Time
DNA Fin clip	Labeled 1.5 mL cryo vial with 70-95% ethanol. **Never Formalin**	Labeled cryo vial with 70-95% ethanol. **Never Formalin**	Up to 12 months if refrigerated or frozen, and stored in 70-95% ethanol
Fish voucher < 200 mm	Label and fill appropriate size HDPE wide mouth specimen jar (minimum 30mL bottle) with a 10% buffered formalin solution to fix specimens within one hour of euthanizing (10:1 formalin to fish tissue by weight); Store completely covered in 10% buffered formalin for 5 to 7 days	Store at room temperature for 1 week, then remove from formalin and completely cover voucher with 70-95% ethanol, store in covered container. Can be stored at room temperature.	Up 12 months if stored in 70-95% ethanol
Fish Voucher >200 mm	Fix with formalin through injection, within one hour of euthanizing (10:1 formalin to fish tissue by weight); Store completely covered in 10% buffered formalin for 5 to 7 days in a labeled, appropriate size HDPE wide mouth specimen jar.	Store at room temperature for 1 week, then remove from formalin and completely cover voucher with 70-95% ethanol, store in covered container. Can be stored at room temperature.	Up 12 months if stored in 70-95% ethanol
Non-target vouchers (reptiles and amphibians)	Fill appropriate size HDPE wide mouth specimen jar (minimum 30mL bottle) with a 10% buffered formalin solution to fix specimens within one hour of euthanizing (10:1 formalin to tissue by weight), for 5 to 7 days	Store at room temperature for 1 week, then remove from formalin and completely cover voucher with 70-95% ethanol, store in covered container. Can be stored at room temperature.	Up 12 months if stored in 70-95% ethanol
Invertebrate bycatch	Label and seal HDPE container with 70-95% ethanol	Store at room temperature in HDPE container with bycatch covered in 70-95% ethanol, with lid secured and taped	Up 12 months if stored in 70-95% ethanol

4.4 Sampling Timing Contingencies

A minimum of two weeks between sample periods must be observed. If environmental conditions interrupt scheduled sampling, follow the contingencies outline in **Table 5**. Always endeavor to fully sample all scheduled reaches, however if only a truncated sampling event can be carried out, at least one full (three passes) fixed reach must be sampled for fish sampling to be considered a complete sampling bout and data to be published.

Table 5. Contingency decisions for FSL – Fish Sampling in Lakes protocol.

Delay/ Situation	Action	Outcome for Data Products
Hours	If weather conditions deteriorate and conditions become unsafe (e.g. approaching thunderstorm, unsafe boating conditions), cease sampling and wait in a safe location for at least 30 minutes. If conditions improve, resume sampling. If conditions do not improve, captured fish should be released and sampling discontinued. If an entire pass cannot be completed, abandon data collection and start over on the next appropriate day. Wait a minimum of 12 hours before sampling the same reach.	None, as long as samples are collected within the pre-determined sampling window.
3-7 Days	If heavy rain or snowfall affects visibility, air temperatures drop to unsafe conditions, or flooding/high water occurs on or prior to the targeted sampling date, wait a minimum of 3 days to allow for safe conditions to return. This will also allow the fish community to recolonize habitats following a flooding/high water event.	None, as long as samples are collected within the pre-determined sampling window. If waiting for safe sampling conditions to return causes sampling to occur outside of the biological bout sampling window by more than three days or beyond the extended contingency windows, then submit a Service Now ticket to the Science team.

4.5 Missed or Incomplete Sampling

Sampling according to the schedule is not always possible, and multiple factors may impede work in the field at one or more plots or sampling locations in a given bout. For example:

- Logistics – e.g., insufficient staff or equipment
- Environment – e.g., deep snow, flooding, inclement weather, or
- Management activities – e.g., controlled burns, pesticide application
- Permitting – e.g., delay in issuance, conditional changes to permit conditions

Instances such as those listed above must be documented for scheduling, tracking long-term plot suitability, and informing end users of NEON data availability. Some types of missed sampling are due to events that should be recorded in the Site Management App; refer to the Site Management and Event Reporting Protocol for more detail (RD[08]).

If sampling is impractical as a result of severe drought (dry), the lake is frozen, access to portions of the reach are blocked, other site hazards, or logistical reasons such as the inability to field the minimum size 3-person crew, then indicate the status using sampling impractical for any affected reach on the mobile device. Should environmental conditions or resource availability impact the ability to fully execute the protocol for a complete sampling bout, a minimum of one fixed reach (all 3 passes, fyke and gill net set)

should be fully sampled. Note the cause of the minimized sampling effort on the "Reach Condition" section in the mobile field device.

Some types of missed sampling are due to events that should be recorded in the Site Management App; refer to the Site Management and Disturbance Data Collection Protocol for more detail (RD[08]).

Missed or Incomplete Sampling Terms

Terms that inform Missed or Incomplete Sampling include:

- **Protocol Sampling Dates**: Bout-specific sampling dates (**Appendix C**).
- **Scheduled Sampling Dates**: Bout-specific sampling dates scheduled by Field Science and approved by Science. These dates coincide with or are a subset of the Protocol Sampling Dates.
- **Missed Sampling**: Incidence of *scheduled sampling* that did not occur. Missed Sampling is recorded at the same resolution as data that are ordinarily recorded, in this case at the reach level as sampling impractical.
- **Sampling Impractical**: The field name associated with a controlled list of values that is included in the data product to explain a Missed Sampling event – i.e., why sampling did not occur, at the event and reach level.
- **Rescheduled**: Missed Sampling is rescheduled for another time according to one of the scenarios documented in **Figure 4**, resulting in no change to the total number of sampling events per year.

The documentation that must accompany missed sampling depends on the timing, subsequent action, and the audience appropriate for numerous scenarios (**Figure 4**).

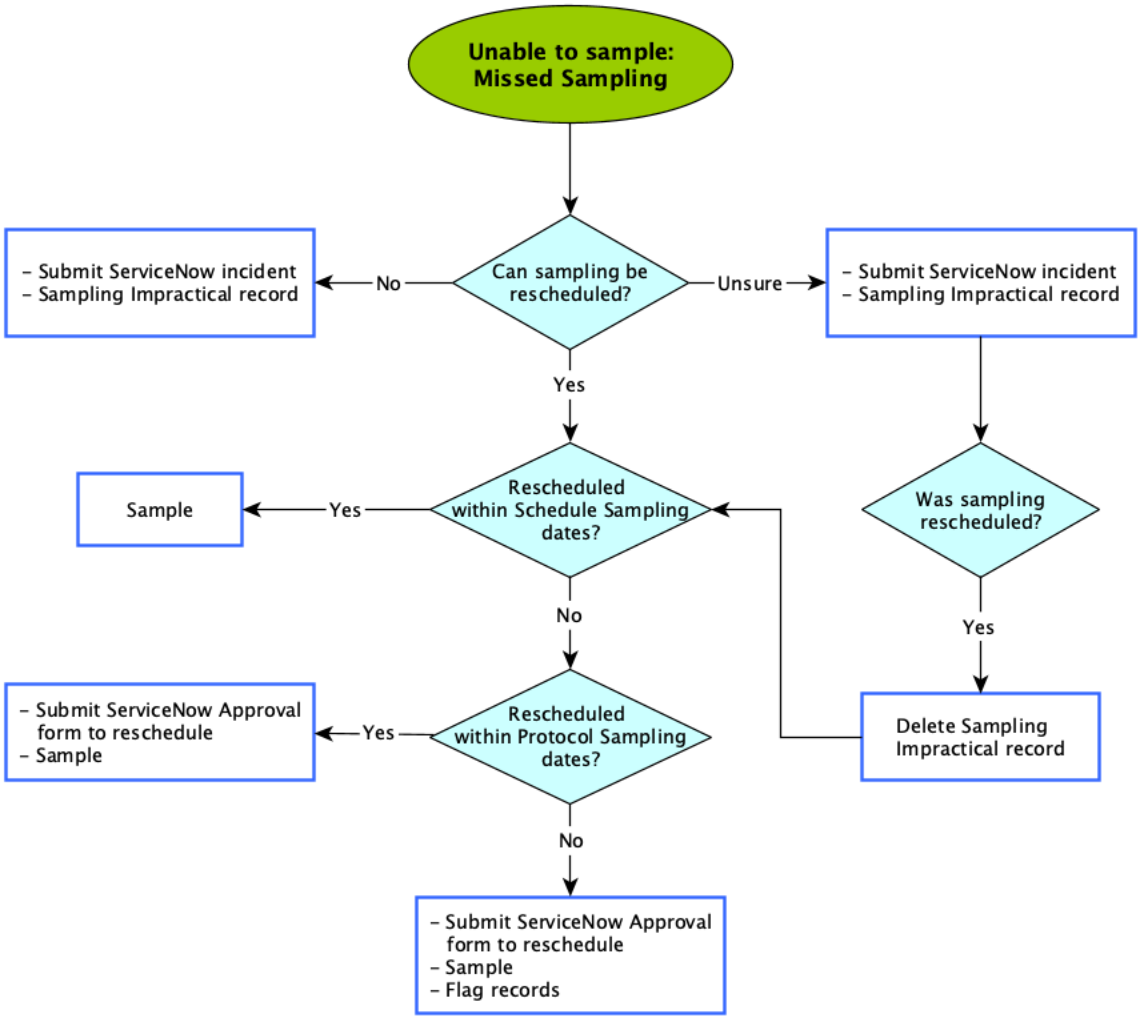

Figure 4. The documentation to account for a Missed Sampling event depends on the situation for each sampling unit not sampled per bout that is not sampled. Diamonds represent decision points and boxes describe the required action. Required actions may include: a) Submitting a ServiceNow incident, b) creating a Sampling Impractical record, c) creating a data Flag, d) creating a Site Management record, or e) some combination of (a) – (d).

To Report Missed or Incomplete Sampling:

1. Missed or Incomplete Sampling that cannot be rescheduled within the Schedule sampling dates must be communicated to Science by a ServiceNow Incident.
    a. For Missed Sampling that is Rescheduled, there are some cases that require approval by Science and Operations (**Figure 4**).
    b. Consult **Table 6** below to determine required actions if scheduled activities are delayed or canceled. This protocol is the ultimate source of information should any discrepancy exist

between this document and other summary materials – e.g., the 'Scheduled Field Activities – Delays and Cancellations' spreadsheet linked via the SSL.

2. Create a Fulcrum record for each Missed Sampling event in the field that cannot be rescheduled.
    a. As data are recorded in the field at the reach level, a record must be made for each reach missed.
    b. Missing data in downstream applications (e.g., Lab apps) are not recorded. For example,
3. For each Missed Sampling record, the **Sampling Impractical** field must be populated in the mobile collection device (**Table 7**).
4. For Rescheduled sampling events that occur outside of the defined peak green season, a protocol-specific biophysical criteria Flag must also be recorded (**Figure 4**).
    a. The biophysical criteria flag for fish sampling is: "conditions not met: outside bout window"

Table 6. Guidance for responding to delays and cancellations encountered during implementation of the Fish Sampling in Lakes protocol.

Activity Name	Days Delayed from Schedule	Delay Action	Cancellation Action
Fish Sampling	> 3-day past bio bout window	Submit incident ticket if rescheduling is delayed > 3 days past the end of the sampling bout. Flag data collected outside of protocol sampling dates. Sampling rescheduled outside of protocol contingency must be approved by Science.	Submit incident ticket informing Science of cancellation and create a Sampling impractical record for each scheduled reach that has been missed.

Table 7. Protocol-specific Sampling Impractical reasons entered in the Fulcrum application. In the event that more than one is applicable, choose the dominant reason sampling was missed.

Sampling Impractical reason	Description
High water velocity	High water velocity
Location dry	Location dry
Location frozen	Location frozen
Location snow covered	Location snow covered
Other	Sampling location inaccessible due to other ecological reason described in the remarks
Logistical	Site or plot access compromised, staffing issues, errors (e.g., equipment not available in the field)

4.6 Estimated Time

The time required to implement a protocol will vary depending on a number of factors, such as skill level, system diversity, environmental conditions, and distance between sample plots. The timeframe provided below is an estimate based on completion of a task by a skilled two-person team (i.e., not the

time it takes at the beginning of the field season). Use this estimate as framework for assessing progress (**Table 8**). If a task is taking significantly longer than the estimated time, a problem ticket should be submitted. Please note that if sampling at particular locations requires significantly more time than expected, Science may propose to move these sampling locations.

This protocol requires a minimum of three qualified field scientists for up to 3 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 (D01) for processing annually. 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.

Table 8. Estimated staff and labor hours required for implementation of the Fish Sampling in Lakes protocol.

SOP	Estimated time	Suggested staff	Total person hours
SOP A.1: Preparing for Data Capture	1 h	1	1 h per day
SOP B: Preparing for Sampling	3 h	2	6 h
SOP C.1: Electrofishing Reach Set-Up	*45 minutes fixed/random*	3 per fixed/random	2.15 *h per fixed/random*
SOP C.2: Electrofishing Field Setup	*30 minutes per day*	2	*1 h per day*
SOP C.3: Backpack Electrofishing	*45 minutes per pass*	3-6 per pass	2:15 h (3 people) to 4:30 h (6 people) per pass
SOP C.4: Gill Net	*1 h*	*2*	*2 h*
SOP B.5: Mini-fyke Netting	1.5 h	2	3 h
SOP D: Fish Handling	1-2 h per reach	3	3-6 h per reach

4.7 Sampling Specific Concerns

1. Under ideal conditions fish sampling shall be completed within a 3-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 three 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 **Table 5**) submit a trouble ticket.
2. Permit restrictions: review site specific permits related to fish sampling and ask questions when unsure about meaning or intention of permit restrictions to NEON permitting and or Science.
3. Reasonable efforts should be made to minimize mortality to fish during sampling. This includes the use of best fish handling practices:

a. Check for maximum water temperature electrofishing limits, unless otherwise (higher or lower) stated in site specific permit, then defer to permit's stated temperature limit.

   i. At lakes sites, night electrofishing may mean decreasing water temperatures, wait half an hour and check the temperature again.

b. Frequently change lake water in fish holding buckets, and frequently conduct bucket water temperature checks.

c. The use of aerators in fish holding buckets is required to ensure oxygenated holding water.

d. Keep a mesh netting or 5-gallon bucket cover on hand to cover the bucket during enumeration so no fish can jump out.

e. Keep holding buckets in well shaded areas, and/or put vegetation in holding water as micro-shade.

f. Use anesthetic when necessary to reduce handling stress.

g. Segregate predator species from prey-species.

4. Fish sampling-related injuries and mortalities resulting from electrofishing, netting, and processing should affect < 3% of fish captured cumulatively per sampling bout. In order to ensure fish health and safety, if 3% injury/mortality is exceeded during any day of fish sampling, contact the Science team, Domain Manager, Permitting Staff, and submit a Service Now ticket immediately. Science staff will work with domain staff to identify if there is a problem and/or if any changes are needed with sampling. Field staff will keep a running total of the fish caught, the number of mortalities, and the number of injuries during sampling. Additionally, the mobile data entry application keeps a tally of the number of mortalities against the number of fish caught and will notify when 3% injury/mortality is exceeded.

   a. The following information must be submitted in the Service Now ticket when reporting fish injuries and mortality that exceeds 3%:

      i. Electrofisher settings: voltage, duty cycle, and frequency

      ii. Water quality: conductivity, dissolved oxygen, and temperature

      iii. Field observations and suspected likely root cause of injury or mortality

      iv. Description of species affected

      v. Include any additional information that could help identify the root cause and for developing a solution (e.g. anode ring diameter and shape)

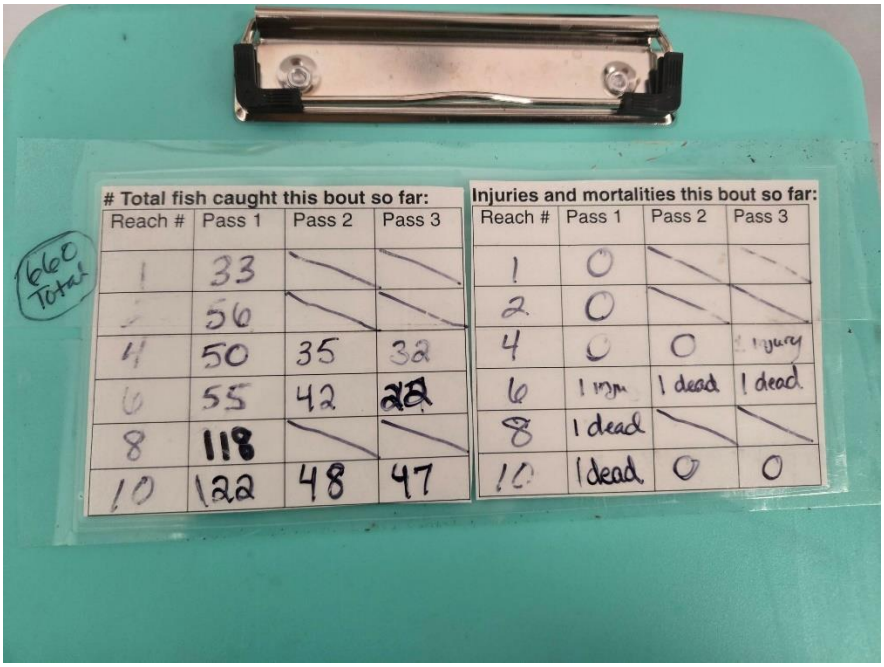

Figure 5. An example mortality tracker that can be used in the field during sampling. The mobile data entry application also has its own mortality tracker in case of any transcription errors during manual tracking.

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 EHS Safety Policy and Program Manual (AD[01]) and Operations Field Safety and Security Plan (AD[02]). Additional safety issues associated with this field procedure are outlined below. If an employee witnesses any unsafe conditions or uncontrolled hazards that present an imminent danger, they should immediately take action to stop work and report such conditions to their manager. Employees must also report all workplace injuries, illnesses, incidents, or releases to the environment as soon as possible, regardless of the severity.

In addition, the following general fishing safety guidelines are provided:

1. All employees shall have access to a form of communication for constant contact with other team members such as a two-way radio.
2. 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.).
3. Activities should only be performed when flow conditions are safe. Wading safety will be determined by the field crew at time of sampling. Do not attempt to wade in a lake past waist deep.
4. Safety datasheet information shall be readily available to field scientists working with chemicals included in this protocol. Field scientists must also be trained in safe handling of formalin (AD[03]).
5. 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.
6. 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. The lead ecologist on site 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 6.1 Training Requirements).
2. Audible signals must be used to alert field scientists when electrofishing equipment is in operation.
3. Chest wades and heavy-duty rubber lineman gloves (rated to 1,000V AC/1,500V DC) must be worn while working near an electrofishing unit.

4. The requirements for wader and boot selection have been adapted by the recommendations of the American Fisheries Society Professional Safety Committee (2008).
    a. 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.
    b. 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 only if 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.
    c. Studded-sole boots are allowed if they help secure footing in slippery conditions.
    d. 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.
    e. Felt soles are prohibited, they are proven mechanisms of transportation of aquatic invasive species.
5. Before sampling, inspect waders, boots, and gloves for leaks. Leave the water immediately if waders or gloves develop leaks.
6. 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 only if it is within a 6-minute walk from the active sampling location.
7. Avoid operating near bystanders, pets, or livestock that are in or near the water.
8. Electrofishing must be suspended if anyone feels a shock, however minor, for investigation and repair of the electrofishing equipment or the correct outerwear of all of the field scientists.
9. Avoid operating an electrofishing unit in heavy rain (light rain is acceptable) as this can increase the probability of electrical shock.
10. 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.

When processing DNA barcoding samples in the laboratory, additional safety precautions are required due to the use of highly flammable ethanol and flame sterilization in concurrence. Extreme caution must be used. Precautions include:

1. Separating the container of ethanol from the flame by at least 16 inches and following all PPE requirements in the Chemical Hygiene Plan (AD[03]), including the use of a lab coat, gloves and safety glasses.

2. Loose hair and dangling jewelry should be secured or removed.
3. Before using any flame, take care to ensure that all flammable objects and chemicals are removed from the immediate workspace and shelves above the area, and that domain staff are aware of the location of fire extinguishers.
4. Be aware that ethanol fumes can ignite and spread to the container of ethanol if it is too close to where the flame sterilization is occurring.

6 PERSONNEL

6.1 Training Requirements

All technicians must complete protocol-specific training as required in the Field Operations Job Instruction Training Plan (AD[04]). Additional protocol-specific required skills and safety training are described here.

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 an abridged video version of 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. 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:

Required for all crew members each year

USFWS NCTC CSP2202-OLT Electrofishing Safety Course: Resources include presentation (PowerPoint and video) and the final exam (free; registration is not required). Only the relevant sections, sections pertaining to boat electrofishing are not required.
https://fws.rev.vbrick.com/#/videos/088b3b02-302e-48bf-9e10-74636454c194

Wader Safety
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

Optional

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

6.2 Specialized Skills

Crew lead must have Smith Root training and the ability to use field guides to identify fish.

7 STANDARD OPERATING PROCEDURES

SOP Overview

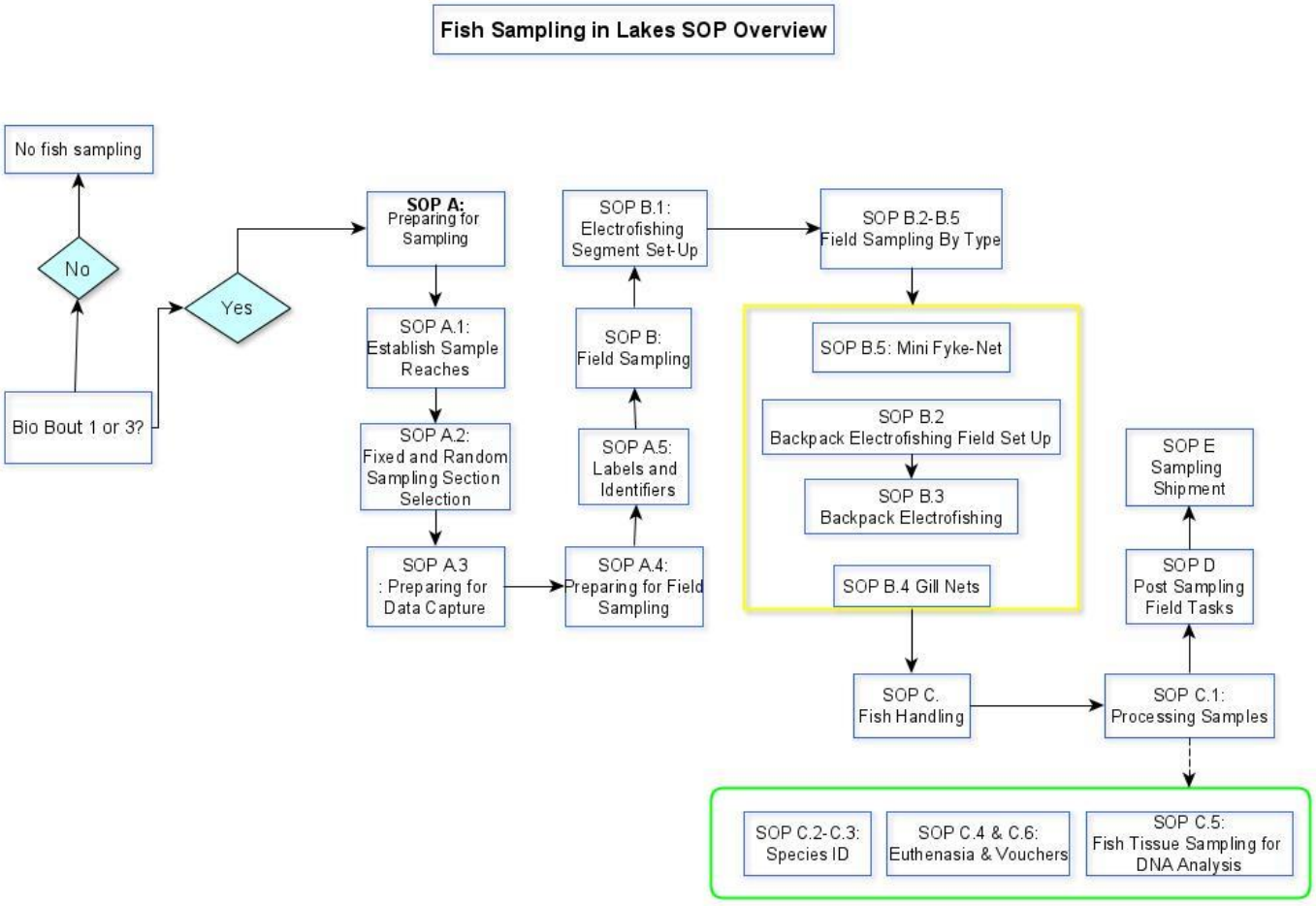

Figure 6. A high-level workflow diagram that visually shows how the separate SOPs are sequentially connected.

- **SOP A**: Preparing for Sampling
- **SOP B**: Field Sampling
- **SOP C**: Fish Handling
- **SOP D**: Post-Field Sampling Tasks
- **SOP E**: Data Entry and Verification
- **SOP F**: Sample Shipment

SOP A Preparing for Sampling

A.1 Preparing for Data Capture

Mobile applications are the preferred mechanism for data entry. Mobile devices should be synced and fully charged at the beginning of each field day.

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.

A.2 Preparing for Field Sampling

1. Permit Compliance
    a. Review the federal and/or state collection permit thoroughly.
    b. Notify the site host of the dates and times of the fish sampling activities prior to sampling.
    c. If required, notify responsible state agency of the dates and times of the fish sampling activities prior to sampling.
    d. Retain a copy of the permit with the sampling crew during the sampling activities.
    e. Take note of state bycatch reporting requirements.
2. Plan and save sampling routes for field teams using standard site navigation procedures (RD[09]). Route planning enhances sampling efficiency and helps avoid accidental foot traffic within NEON plots.
3. Field preparation
    a. It is good practice to test the equipment at least one month ahead of the sampling date. This will allow field staff to fix or borrow any broken equipment and still maintain their scheduled sampling dates.

VERY IMPORTANT: Charge (or replace) batteries for backpack electrofishing unit, GPS unit, mobile field data recording device with camera, portable scale, temperature/conductivity meter, and portable aerators overnight or longer. Start charging batteries at least two days before going to the field to allow batteries to fully charge.

    b. 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 to the anode using an abrasive pad or steel wool to gently scrub the surface.

***NOTE**: NEON keeps a spare electrofishing unit at HQ in cases where the Domain shocker in not working.*

c. Inspect waders for holes and tears. Repair waders if necessary. If your waders have holes in them, you will most likely get shocked through that hole!

d. Inspect lineman gloves, DO NOT repair torn lineman gloves. Discard them and order a replacement pair.

e. Inspect and repair fyke nets, gill nets, dip nets and block nets for rips, tears, and holes.

f. Inspect portable aquarium pumps, diffusion stones, and batteries.

g. Inspect buckets to ensure handles are present and functioning.

h. Ensure that all equipment has been decontaminated since last use (see RD[10]).

i. Verify that the mobile data entry device is charged and synced prior to use.

j. Print datasheets and specimen labels (RD[05]) on waterproof paper.

k. Ensure the MS-222 is pharmaceutical grade.

l. Check both AQUI-S®20E and MS-222 to ensure that the drugs have not expired.

m. Check with INAD group lead to make sure that a study request has been filed.

4. Prepare for field anesthesia/euthanasia.

- Fish will be anesthetized and euthanized with 10% eugenol (AQUI-S®20E).
- Non-target species will not be anesthetized; however, mortally injured non-target vertebrate species shall be euthanized with pharmaceutical grade Tricaine methanesulfonate (MS-222).
- All chemicals must not be past the expiration date.

a. Both **AQUI-S®20E** and **MS-222** must be stored in clearly labeled containers.

i. Use of the wrong chemical or any accident must be reported to Science immediately by creating a Service Now incident.

b. **10% eugenol (AQUI-S®20E) – fish anesthetic:**

- 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.
- Each aquatic field science lead is responsible for treating fish according to protocol, the proper handling of AQUI-S, and reporting field results to your Domain's INAD group leader.
    - NEON INAD Groups
        - Group 1: D01, D02, D04, D05
        - Group 2: D06, D07, D08, D09
        - Group 3: D10/13, D11, D12, D14

- Group 4: D15, D16, D17, D18/19

- Any questions regarding this program or how to complete the field datasheets should be directed to the INAD group leader monitor or NEON Science.

i. Field science must report field treatments to INAD group leaders within 20 days of field treatment.

ii. Report to INAD group lead must include daily:

1) dosage used
2) volume used
3) species treated
4) number of each species treated
5) average size and weight
6) mortalities
7) treatment effect
8) time to effect
9) recovery time
10) number of fish treated in each bucket
11) water temperature

iii. Be sure to bring along the INAD reporting mechanism in the field when conducting fish sampling activities.

iv. Make sure that the batch of AQUI-S®20E has not expired.

v. 10% eugenol should be added directly to the treatment water (native water in bucket). Do not make stock solutions or other dilute solutions of 10% eugenol.

vi. You must label bottles "**AQUI-S®20E**" in large, easy to read letters, in addition to any Chemical Hygiene Plan requirements.

c. **Tricaine methanesulfonate (MS-222) – non-target vertebrate euthanasia**:

i. Mix stock solution of pharmaceutical grade MS-222 in the Domain Support Facility.

ii. There are site-specific regulations where and how MS-222 can be mixed. Please follow the proper EHS permits for your site to confirm the MS-222 regulations and reach out to permitting for any questions.

iii. Wear nitrile gloves and eye protection, as MS-222 is a skin and eye irritant.

iv. Weigh 20 g of MS-222 powder and 50 g NaHCO3.

v. Mix 20 g MS-222 + 50 g NaHCO3 in a bucket with 1-liter tap water.

vi. Pour the stock solution into two 1 L amber HDPE bottles.

vii. You must label bottles "**MS-222**" in large, easy to read letters, in addition to any Chemical Hygiene Plan requirements.

viii. 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.

1) Discard unused solution in the lab following the NEON Domain Chemical Hygiene Plan and Biosafety Manual guidelines (AD[03]).

A.3 Labels and Identifiers

Barcodes are useful for minimizing transcription errors and tracking samples from the domain support facility to external locations. **All field samples must use a barcode and all samples being shipped from the DSF must have a barcode.**

Fish sampling produces four potential sample types:

1. Fish vouchers
2. DNA fin clips
3. Potential vertebrate bycatch vouchers
4. Potential invertebrate bycatch vouchers

All four sample types need to be labeled on the outside of the container with a human readable label containing the sample ID, and a barcode. Vouchers will have an additional identifier placed in the voucher container; with the voucher specimen id written on write in the rain paper in ethanol proof marker.

All barcodes need to be applied to dry containers for 30 mins before use. Labels produced during fish sampling are as follows: Fish vouchers use Type I (prefix A, plus 11 numbers); they have a tolerance from 4C to 105C and still scan (**Figure 7**). DNA fin clips use Type IV (prefix D, plus 11 numbers); they use the same material as Type II (cryo safe) but are a smaller size that accommodates 1.5 mL cryotubes (**Figure 8**).

Figure 7. Example Type I barcode label used for voucher specimens.

Figure 8. Example Type IV barcode label used for DNA fin clips.

1. Prepare voucher sample containers by affixing one Type I adhesive barcode label to each HDPE container or bag used per sample. Prepare DNA fin clip samples by affixing one Type IV adhesive barcode label to each 1.5 mL cryotube used per sample.

    a. Adhesive barcode labels should be applied to dry, room temperature containers in advance of their use in the field (at least 30 minutes prior but may be applied at the start of the season).

    b. For both HDPE containers and 1.5 mL cryotubes, barcode labels should be oriented such that it is possible to scan them; the scanner will not work on a curved surface. This means aligning the barcode lengthwise along the container, not horizontally wrapping around (**Figure 9**).

    c. Barcode labels must be associated with a unique sample and each barcode must be mapped to one sample in the database. Barcodes are unique, but are not initially associated with a particular sample, so you are encouraged to adhere barcode labels to needed containers in advance.

Figure 9. Example of proper orientation of barcode label on vial.

2. All fish and bycatch voucher containers must also include human readable labels, consisting of the fish voucher sampleID ("FSH.siteID.reachNo.YYYYMMDD.passNo.specimenNo" or bycatch sampleID ("FSH.siteID.reachNo.YYYYMMDD.passNo.specimenNo.nonFish") taped to the outside of the HDPE container (**Figure 10**).

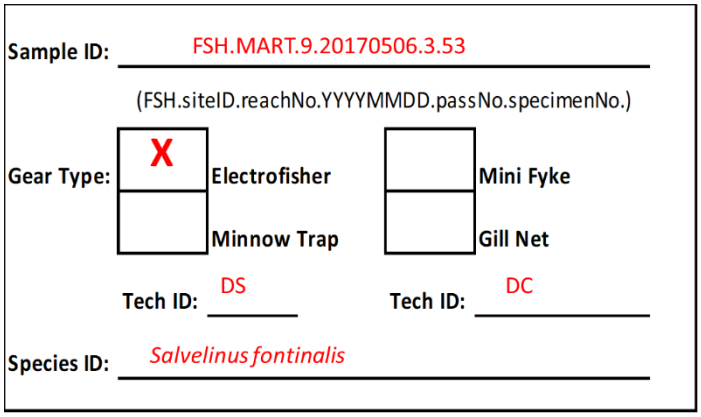

Figure 10. Example specimen label for a fish/bycatch voucher.

3. All DNA fin clip 1.5 mL cryotube samples must have a human readable label with the sampleID taped to the cryotube. Tape label so that a Type IV barcode label can also be taped to cryotube (**Figure 11**).

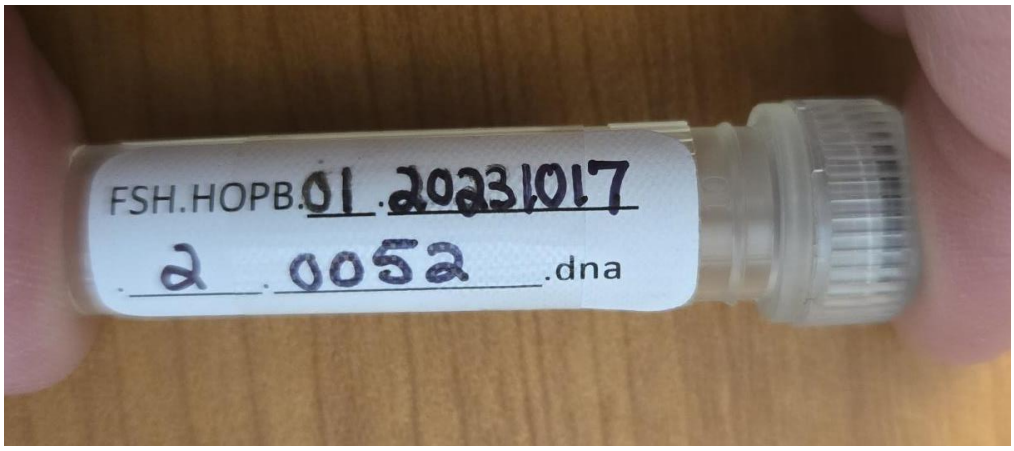

Figure 11. Example human readable sampleID label for DNA fin clip sample.

Table 9 provides a quick reference to the types of samples this protocol generates that require barcodes. The final disposition of all vialed samples must have a barcode affixed to assist in the shipping and receipt of samples destined for the Biorepository or an external laboratory.

Table 9. Barcode requirements for sample types generated by the Fish Sampling in Lakes protocol.

Sample Type	Description	Example Identifier	Fulcrum App	Container Type	Barcode Used	Barcode Required?	Barcode Qty
DNA fin clip	Fin clip for DNA analysis	FSH.CARI.01.20190520.1.0001.DNA (FSH.siteID.reachNo.YYYYMMDD.passNo.specimenNO. DNA)	(AOS) Fish [PROD]	1.5 mL cryotubes	Type IV	**Always Required**	1 per cryotube
Fish voucher samples	Voucher specimen	FSH.CARI.01.20190520.1.0001 (FSH.siteID.reachNo.YYYYMMDD.PassNo.specimenNO)	(AOS) Fish [PROD]	HDPE (minimum 30 mL) or plastic bag	Type I	**Always Required**	1 per voucher
Bycatch voucher samples	Vertebrate and invertebrate bycatch voucher samples	FSH.CARI.01.20190520.1.0001 (FSH.siteID.reachNo.YYYYMMDD.PassNo.specimenNO)	(AOS) fsh_non Target Species [PROD]	HDPE (minimum 30 mL) or plastic bag	Type I	**Always Required**	1 per voucher

SOP B Field Sampling

Data for field sampling are entered in the (AOS) Fish [PROD] and if appliable the (AOS) fsh_nonTargetSpecies [PROD] mobile applications. Instructions for the use of these applications can be found in the Sampling Support Library in the document "Manual for Fulcrum Application: (AOS) Fish [PROD]".

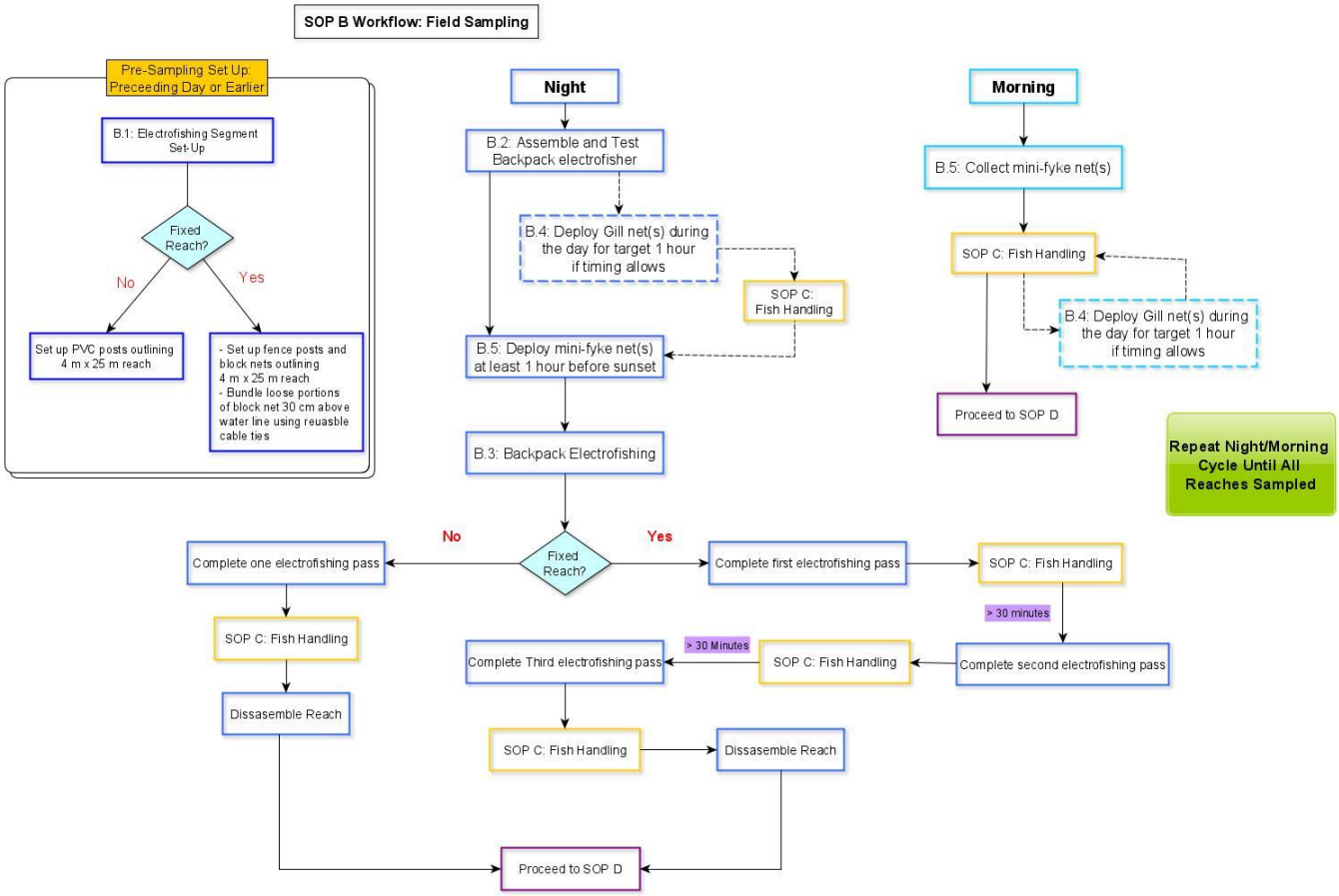

Figure 12. An expanded workflow diagram of SOP B: Field Sampling.

B.1 Fish Sampling Site Set Up

1. Navigate to the first prioritized fixed reach for sampling via the selected riparian sites using GPS points, the morphology map, or plot survey markers.
2. Setup fence posts and block nets for electrofishing in <u>fixed</u> reaches. For random reaches, setup fence posts or PVC pipe to mark <u>random</u> reaches 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.

VERY IMPORTANT: All field scientists MUST be trained in the use of fence post drivers/pullers prior to deploying with field team. See the Post Driver safety training on the NEON Safety SharePoint Page.

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. Start at the shoreline.
    b. Minimize the disturbance to the sampling area inside the 4 x 25 m electrofishing reach.
    c. Space fence posts a maximum of 4 m apart.
    d. Ensure that the deep edge is ≤ 1 m deep to allow for safe electrofishing.
    e. Ensure that the net is secured to the bottom, using decoy weights.
    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 reaches 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 reaches (up to 3) will be sampled via non-enclosure electrofishing (i.e., no block nets), using the fence post to delineate the electrofishing sampling area.

B.2 Backpack Electrofishing Set Up

Assemble backpack electrofisher and test settings on the backpack electrofisher before sampling begins. After settings are determined record them, they will be used for the remainder of the sampling bout. If settings must be changed during electrofishing, be sure to record new setting.

VERY IMPORTANT: All field scientists MUST wear necessary personal protective equipment before stepping into the water, including waterproof chest waders with lug-soled boots (felt waders are not allowed at NEON sites), rubber lineman gloves to insulate the wearer from electrical shock, and polarized sunglasses to increase the efficiency of fish capture. Headlamps must also be worn during nighttime sampling.

1. Assemble the Electrofisher
    a. Assemble anode pole (**Figure 1**).

b. Connect the cathode (**Figure 2**) and anode to the backpack electrofishing unit (**Figure 13**).

Figure 13. Cathode and anode connections on backpack electrofishing unit.

c. Connect the battery to backpack electrofishing unit and secure the batteries with the strap to the backpack frame. 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 14**).

Figure 14. Left: Battery location and secure placement in the backpack electrofishing frame. Right: lithium-ion battery adapter.

2. Test the Electrofisher

a. Test the backpack electrofishing unit >50 m away from the designated electrofishing reach. Select an area of the lake shoreline that has characteristics similar to that of the sampling reach (e.g., similar depth or vegetation).

b. Measure the water temperature and specific conductivity using the handheld YSI multimeter.

   i. Effective electrofishing depends on matching the backpack output voltage with the water conductivity.

   ii. High water temperatures can cause fish mortalities, do not fish if temperatures exceed the thresholds given here (> 18 C for salmonids, > 26 C for non-salmonids).

c. The crew member wearing the backpack electrofisher should wade into the lake ensuring that the cathode (i.e., rattail) and anode ring are both submerged.

d. While the electrofisher operator is standing in the lake, turn on unit and set the initial shock settings:

   i. Initial setting - Frequency to 30 Hz, duty cycle to 5%, and output voltage to 100 V.

   ii. Adjust setting as needed, using the site conductivity value for guidance on maximum allowable settings (**Table 10**).

Table 10. Guidelines for maximum settings for backpack electrofishing based on conductivity (NOAA NMFS 2000).

Settings	Initial Settings	Maximum Settings	
Voltage	100 V	Conductivity (µS/cm) < 100	Max Voltage 1,100 V
		100-300	800 V
		> 300	400 V
Duty Cycle%	5%	<100 (µS/cm)	10%
		100-300 (µS/cm)	20%
		>300 (µS/cm)	40%
Pulse Rate (Frequency)	30 Hz	60 Hz	

3. Begin electrofishing in shallow water (e.g., < 50 cm).

4. 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.

5. The anode ring must always be submerged before depressing the activation switch. When removed from the water, the unit will automatically turn off.

6. Press and hold the activation switch down and observe the behavior of fish.

a. Signs that fish are responding to the electrofisher settings include swimming toward the anode ring and flashing of the ventral (belly) portion of the fish. Be sure to check for immobilized fish near the cathode.

7. If no fish are sampled during test, experiment by slowly increasing settings. Be sure to review the collection permit requirements and conductivity maximum setting guidelines in **Table 10** and stay within the approved electrofisher settings.
8. The goal is to immobilize fish using the lowest settings possible at the site to avoid harming sampled fish.
9. Start by increasing **voltage** in 50-volt increments.
    a. Do not increase above maximum setting allowed for conductivity reading **Table 10**.
10. If fish still have not reacted, **lower voltage** to original test setting and in 5% increments Increase **duty cycle**.
    a. Do not increase duty cycle above maximum setting allowed for conductivity reading **Table 10**.
11. If fish still have not reacted, lower both voltage and duty cycle and increase hertz (pulse rate) by 5 Hertz increments, do not exceed 60 Hz.
12. Testing should take no more than 20 minutes and or 5 captured fish, whichever comes first.
    a. Domain ecologists are encouraged to make sure electrofisher backpacks are set at safe (for samplers and fish) and effective settings. If on-site they believe they need more than 20 minutes or 5 fish to find safe/effective setting, they should and can use their best professional judgment, and test past 20 minutes or 5 fish until they feel confident in the settings.
    b. It is possible and likely that no or few fish will be sampled in the short (space and time) test reach, in these cases use best professional judgment, conductivity, water temperature, site knowledge and set the electrofisher at the most conservative and safe settings for those parameters.
    c. As you start sampling you may need to adjust settings as you observe the reaction of the fish to your settings (lower if there are signs of injury or undue stress, increase if fish appear unaffected).
    d. You must record the settings at the start of sampling and also record any changes to settings as sampling proceeds.
13. If fish are immobilized during testing, use dip nets to capture individuals and place in a bucket ½ - ¾ full of lake water with battery-operated aerator. Once fish are swimming normally release in test reach in an area they will not be shocked again.

14. Examine captured fish for signs of injury (e.g., bent backs, dark bruising, hemorrhaging of the gills (**Figure 15**. Examples of electrofishing injuries to fish, a) bruising or "burn" marks, b) spinal injury, and c) internal hemorrhaging. Photos from Snyder 2003.). Record injury rate on the mobile device. Reduce settings until fish are no longer injured, if it is not possible to find a setting that does not injure fish stop sampling and report situation to domain manager and protocol author.

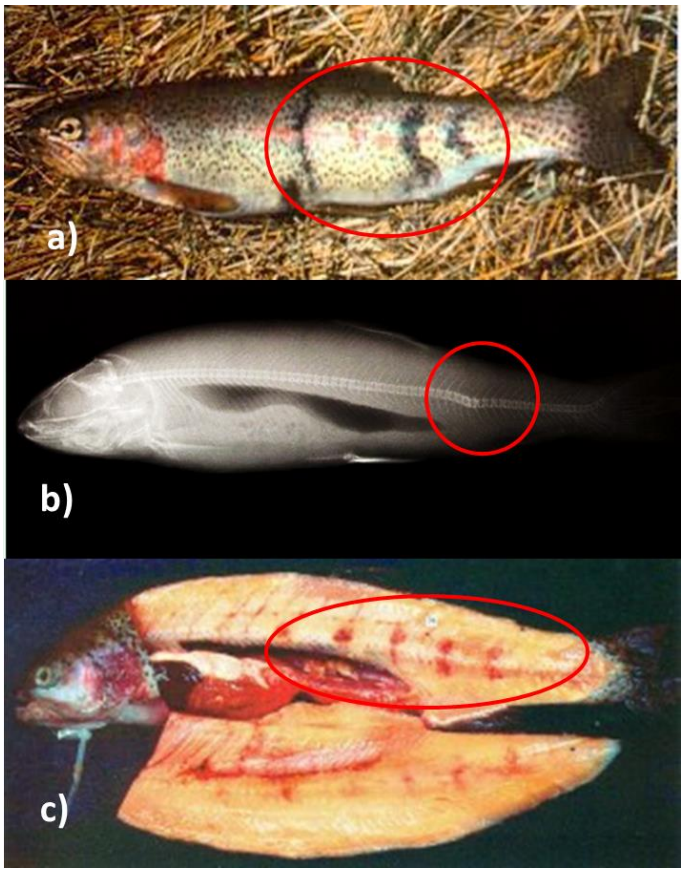

Figure 15. Examples of electrofishing injuries to fish, a) bruising or "burn" marks, b) spinal injury, and c) internal hemorrhaging. Photos from Snyder 2003.

a. Contact with the cathode or prolonged exposure to electricity due to failure to remove fish from the dip net quickly will increase injury rates.

b. If fish are injured, allow them to recuperate in a separate bucket with an aerator before releasing.

c. For any fish that do not recover, proceed to Euthanizing Fish and Non-Target Species (**SOP C.3**).

d. Record vouchers, injuries and mortalities on mobile app, otherwise fish should not be tallied.

e. Monitor injured fish for signs of normal respiration (gills open and close steadily) and swimming (upright, not listing) behavior for 10 minutes. If, after 10 minutes, fish are still on their side, upside-down, or injured, return to lower electrofishing settings. For moribund or injured fish, see **SOP C.3**.

Note: *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.*

Note: *The electrofisher settings established on the first day of sampling can be used on subsequent days so long as the conductivity stays with (+/-) 50 µS/cm.*

f. Continue monitoring fish health throughout fish sampling.

B.3 Backpack Electrofishing

1. Navigate to fish reach that will be sampled.
   a. If unable to sample the reach, indicate by recording sampling impractical.
2. At every fish sampling reach, there are several details to record on the mobile device.
   a. Record the dominant habitat type as littoral when electrofishing.
   b. Electrofishing activities must take place at least 5 m away from any in-lake electronic instrumentation (sensor sets).
3. 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. The water quality measurements may be copied for each additional pass within one fish reach (i.e. if sampling at a fixed reach the water quality data collected before the first pass can be applied to the second and third pass).
4. If reach is a fixed slowly enter the lake (so as not to disturb fish) and begin lowering the block net by releasing the reusable nylon cable ties.
   a. Secure the bottom of the block net with stainless block net stakes or added weights (decoy lead weight).
   b. In random reaches, 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.
5. Record the pass start date and time on the mobile device so that conductivity, turbidity, and other water quality measurements from the in-lake sensor sets can be coupled with the fish sampling bout. The pass start and pass end times are for recording the full length of time (including processing) for sampling each reach. This is different from the Electrofisher (EF) time which is the amount of time (in seconds) that the unit is actively shocking.
6. It is critical that the EF timer on the back electrofisher is reset before every pass.

7. The crew member operating the backpack electrofisher should walk into the lake at one end of the sampling reach, ensuring that the cathode (i.e., rattail) is submerged as much as possible, while holding the anode pole in one hand (anode submerged), the electrofisher should be off at this point.
    a. 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).
    b. The electrofisher operator may, but is not required to, hold a dip net in the other hand if they feel comfortable.
    c. A minimum of three netters are recommended when electrofishing.
8. The other crewmembers will enter the lake behind the electrofisher operator.
    a. The primary netter(s) will stay close to the electrofisher operator to net stunned fish.
    b. The remaining crewmember serves as the lead and as the back-up netter. As such, the lead crew member will carry the bucket and net any other stunned fish that are missed by the electrofisher operator or the primary netter(s). They will also monitor the electrofishing operation by: ensuring that fish are effectively immobilized, that captured fish in the buckets remain healthy, and that the crew is operating safely, in an efficient manner, and netting fish correctly. The lead will also ensure that any potential bystanders are not entering the water.
    c. At some sites where many fish are typically captured, it may be useful to add additional crewmembers to help distribute the work effort.
9. Turn the electrofishing unit on and notify the other field scientists. Confirm that all field scientists are ready to begin.
10. Ensure that the electrofishing unit settings (frequency, duty cycle, and output voltage) are at the lowest level that allows for the effective capture of fish and **that the timer ("EF time") has been reset to "0".**
    a. Record the initial electrofishing settings at the beginning of each pass.
11. Depress and hold the activation switch on anode pole to begin electrofishing.
    a. The anode ring must always be submerged before depressing the activation switch and should never be taken out of the water with the switch depressed. The unit will automatically turn off if the anode is removed from the water or if the operator bends over forward.
12. 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.

13. 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.
14. As the anode is moved across the stream, the netters will capture drifting, immobilized fish.
    a. This may require walking slowly from side to side.
    b. Dip nets should be held as close to the lake substrate as possible without picking up excessive substrate or debris.
    c. There should always be one net behind the anode.
    d. Fish are often attracted to the cathode (rattail). Netters should periodically check this area for stunned fish.
    e. Netters should be aware that immobilized fish may not always be visible, particularly benthic species (e.g., darters, sculpin), and netters should frequently inspect their nets to minimize injury to fish by continuous exposure to electricity.
    f. Crewmembers with dip nets should CALMLY net immobilized fish without excessive disturbance.
    g. Never put hands in the water to capture fish while activation switch is depressed (i.e., while electrical current is pulsing through the water).
        i. If the netter cannot capture a fish using the net, 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 their hand (or use the small dip net) in the water.
        ii. After capturing the fish, the netter removes their hands from the water and verbally confirms that they have 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.

    h. If any endangered species (review collection permit before sampling) are caught, identify, take a photo, and release immediately. Stop all fish sampling activities and inform domain manger and protocol author upon returning from the field. Follow the guidance of the collection permit for specimen vouchering and reporting procedures.
15. Always remove fish from dip nets and place in buckets or a live well to minimize injury to the fish.
16. At all NEON lake sites by-catch (any non-fish caught during fish sampling), must be processed and recorded following the sites applicable federal and state regulations require.

17. Sampling will continue along the shoreline in a zig-zag pattern (**Figure 16**) in a single pass with attention to sampling all complex cover (e.g., overhanging or aquatic vegetation, woody debris, undercut banks).

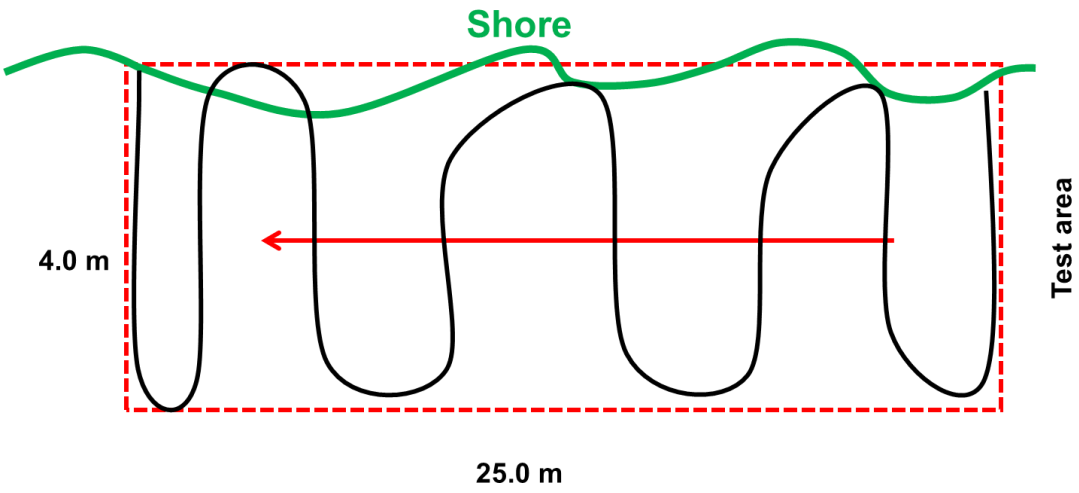

Figure 16. Lake electrofishing sampling pattern.

18. 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:
    a. Releasing the activation switch on the anode pole.
    b. Inserting the anode into cover and holding the anode temporarily still.
    c. The electrofisher operator then depresses the activation switch as netters hold dip nets immediately downstream of the anode and cover.
    d. The activation switch should continue to be depressed until the abundance of observable immobilized fish decreases.
    e. Continue electrofishing by moving the anode around the cover to immobilize additional fish, before sampling up the reach.
    f. Netters should make a few sweeps through any cover that was shocked to pull out immobilized individuals that are stuck in the cover or didn't follow the anode out.
        i. If unable to net immobilized fish (e.g. they are tangled in the complex cover), cease shocking to prevent prolonged exposure to electrical current.
19. 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 lakebed 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, record the Net Integrity as:

   i. Not compromised – No noticeable gap that would allow fish to enter or exit the study area.

   ii. < 10% compromised – 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.

   iii. > 10% compromised – 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.

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.*

b. Follow the guidelines below depending upon the Net Integrity recorded:

   i. If < 10% of the Net Integrity is compromised, fix the net, and continue to sample, make sure < 10% compromised is recorded in the mobile app when pass is complete.

   ii. If > 10% of the Net Integrity is compromised at fixed reaches during the first pass and it is partially or completely sampled – STOP fishing and:

      1) Release fish to the sampling area and discard any collected data.

      2) Re-establish and secure the block nets a half an hour after the initial sampling attempt or when safe to do so. Then, re-initiate sampling at that location.

   iii. If > 10% of the Net Integrity is compromised at fixed reaches during the second pass and it is partially or completely sampled – STOP fishing and:

      1) Release fish to the sampling area and discard any data collected for the second pass.

      2) Report compromised net to Science upon returning from the field. Science may decide to accept the first pass and request you to continue fishing the next reach. If you do not hear back from Science before the transect is scheduled to be repeated, then proceed to carry out the full 3 pass sampling procedure. The priority is to complete the fixed reach once it has been started.

      3) Re-establish and secure the block nets a minimum of 12 hours after the initial sampling attempt or when safe to do so. Then, re-initiate sampling at that location beginning with the first pass.

4) Do not discard any data collected from 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.

iv. If > 10% of the Net Integrity is compromised at a fixed reach during the third pass - STOP fishing and:

1) Release fish to the sampling reach, and discard any data collected for the third pass.

2) Report compromised net to Science upon returning from the field. Science may decide to accept the first two passes. If you do not hear back from Science before the transect is scheduled to be repeated, then proceed to carry out the full 3 pass sampling procedure. The priority is to complete the fixed reach once it has been started.

3) Re-establish and secure the block nets 12 hours after the initial sampling attempt or when safe to do so. Check with science before continuing at this site Then, re-initiate sampling at that location beginning with the first pass.

4) 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!*

20. 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 rising 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 lake temperature.

i. Segregate predatory fish from prey species in separate buckets

ii. 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 lake.

i. If enough staff are available, it is strongly encouraged that there is a designated individual in charge of the buckets (i.e., assisting with the net-to-bucket fish transfer, ensuring fish remain in bucket, and prevent overcrowding).

c. Secure a mesh cover across the top of the bucket if fish are able to jump out or when transporting them.

d. Place buckets of fish out of direct sunlight. Placing some floating vegetation in the bucket can also provide cover or shade for captured fish and reduce stress. Buckets can also be placed in the lake to allow the ambient water temperature to cool down the bucket water temperature.

e. The duration that fish are retained in the holding buckets should be kept to less than one hour when possible. This may require that fish captured in the beginning of the reach are processed before the reach is fully sampled. At sites where this is the case, field science needs to make sure that there are enough staff on hand such that the ID staffer can enumerate fish while the remaining staff continue sampling.

21. 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.

    a. Make sure the netters are in position. Then the backpack operator will sweep the anode along the base of the block net.

    b. Inspect net and remove any fish or bycatch stunned and trapped in net.

22. Once the entire sampling reach (pass) has been sampled, read and record the EF time in seconds from the back of the electrofishing unit on the mobile device.

    a. Electrofisher time is critical for calculating sampling effort.

    b. Record the final electrofisher settings as they may have been changed while sampling, in the mobile device at the end of each pass.

    c. Record the total number of times the battery was changed.

23. Turn the electrofisher off, remove and place on the bank with anode and cathode still attached.

24. Once the entire pass has been fished and all the fish have been processed (**SOP C.1**) record the pass end date and time on the mobile device.

25. If this is a random reach electrofish sampling is complete.

26. If this is a fixed reach, repeat Steps 4-25 until three passes have been completed.

    a. Observe a minimum of 30 minutes between the end of the previous pass and the beginning of the second or third pass within a fixed reach. This allows for fish that were not captured to recover.

    b. Depletion is determined when two consecutive passes result in sequential decreases in total number of individuals sampled after the first pass (e.g., 1000 fish on first pass, 200 fish on second pass, and 50 fish on third pass).

        i. If the number of sampled fish increase with each pass do the following:

            1) Re-check that the settings on the backpack electrofisher have not changed

2) Inspect the block net for holes and that the lead line is laying across the stream bed

3) Ensure that your anode sweeping technique is even and consistent

4) Make sure the netters are alert during each pass

ii. If this issue continues in a subsequent fixed sample reach, contact the domain manager and submit a Service Now ticket to Science.

27. If three passes have been completed with < 10% net integrity, electrofish sampling is completed at this reach.
    a. Take down nets.
28. After the last pass of the night break down the backpack electrofishing unit.
    a. Disconnect the cathode and anode from the backpack electrofishing unit.
    b. Disconnect the battery from the backpack electrofishing unit and remove battery from the backpack frame.
    c. Place backpack electrofishing unit in case.
    d. Disassemble anode pole and store with backpack electrofishing unit.
    e. Inspect the anode and cathode for corrosion and clean as described in SOP A Preparing for Sampling section above.
    f. Place recently used battery separate from charged batteries where it can be easily distinguished for charging.

B.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 reach using GPS.
3. Prepare the gill net to be deployed by attaching net anchors to each end of leaded bottom line and attaching the float rope (with float attached) to the net anchor (**Figure 17**).
    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.
4. Ensure that the float line is long enough to float on the water surface.

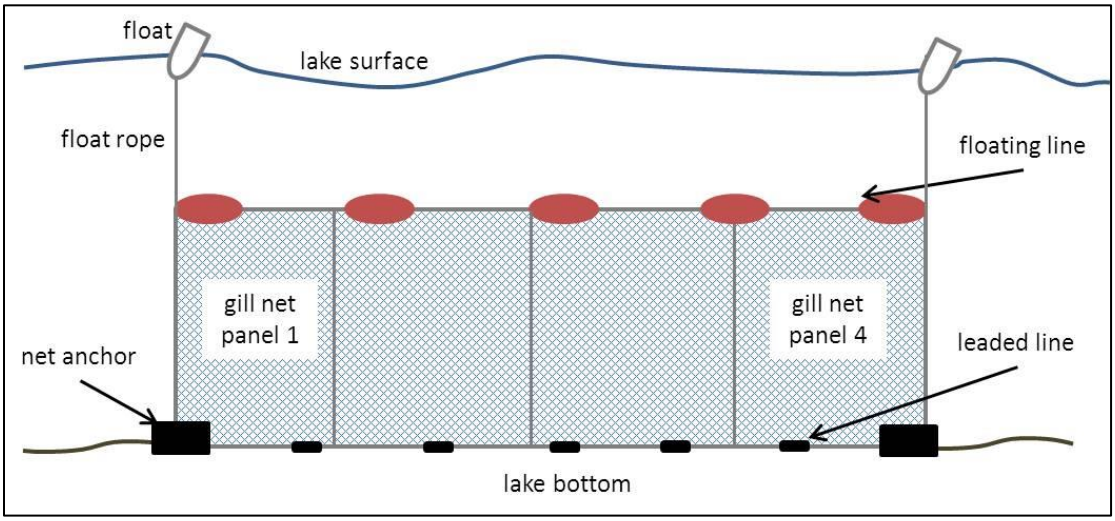

Figure 17. Example of gill net setup. The dimensions of this net are approximately 3.1 m long and 1.8 m deep.

5. 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.
6. Record the pass start date and time (24-hour time plus time zone, e.g., 13:30 MDT) on the mobile device. 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.
7. Begin slowly releasing the gill net into the water, ensuring that the net is not twisted. Begin maneuvering the boat slowly in reverse, perpendicular to, and away from, the shore while the gill net is being deployed.
    a. Setup gill nets at additional sampling locations if fish density is relatively low at the site and fish processing will take < 1 hour per net. 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.
    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.
    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.

8. 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.
        i. 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.
        ii. If the animal is fatally injured, 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.
        iii. Record the non-target species information on the mobile device.
9. After 1 hour (or other set time depending on permit requirements), proceed to the first net set and begin pulling in the net.
10. Untie the float line and net anchor and set aside.
11. Record the net deployment end date and time on the mobile device.
12. 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.
13. Place net anchors and float lines in appropriate buckets or tubs.
14. 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.
    a. Using the mobile device, record if the gill Net Integrity has:
        i. No compromise – no noticeable opening (hole) or restriction (tangled or collapsed) that would allow fish to pass through the net without being captured.
        ii. < 10% compromised – 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.

iii. > 10% compromised – 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 gill Net Integrity tracking described in the previous step, the following actions are recommended:

i. If < 10% of the gill Net Integrity is compromised, process and record captured fish. Repair the net before deploying it at other locations.

ii. If > 10% of the gill net is compromised, release captured fish, repair the net, and re-deploy the net.

iii. Do not discard any data until the re-deployment is complete and new data have been recorded.

15. Process all fish (**SOP C.1**) from one net before pulling the next net. Record pass end date and time on the mobile device or once all fish have been processed.

16. 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, contact the NEON Aquatic Ecologist, Domain Manager, and Permitting, 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%:

i. Gill net set time

ii. Water quality: conductivity, dissolved oxygen, and temperature

iii. Field observations and most likely root cause of injury or mortality

iv. Description of species affected

v. Include any additional information that could help identify the root cause and for developing a solution

B.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 reach using GPS. Record the pass start date and time on the mobile device. 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 reach and avoid setting the mini-fyke net directly within the electrofishing area (**Figure 18**).
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.
    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.

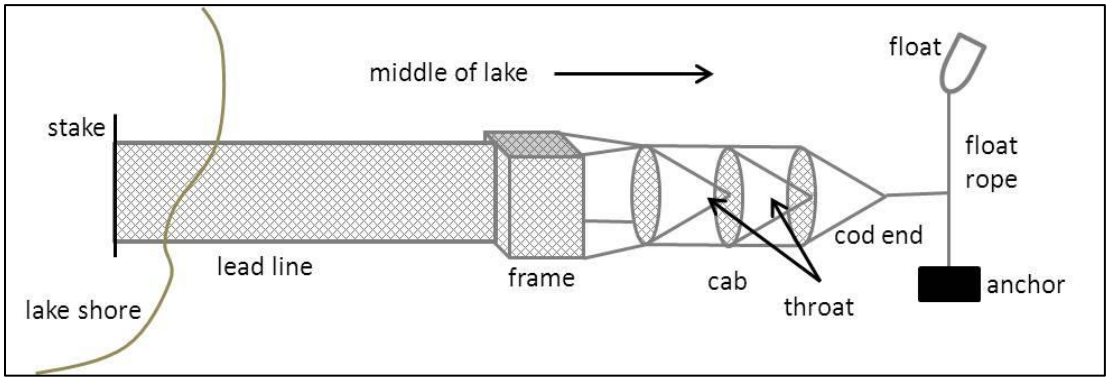

Figure 18. Example of a mini modified fyke net. The entire net from stake to cod end is approximately 10.5 m long with a net depth of 1.2 m.

6. Attach the net anchor to the cod (narrow) end and float line (with float attached) to the net anchor. Alternatively, a T-post can be used to stake the cod end as an anchoring method.
7. Begin slowly maneuvering the boat in reverse while deploying the net from the front of the boat.
    a. It may be more effective to deploy the entire net while wading so that the T-post does not become dislodged.
    b. 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.
    c. The throat of the net must be underwater for fish to pass freely into the trap.
    d. A portion of the cab should remain above the water line for turtles or other vertebrates to breathe.
    e. The trap of the net must be above the thermocline (see site-specific bathymetric map).
    f. 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.
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.
10. One mini-fyke net shall be set in each reach.
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.
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.
    a. Using the mobile device, record if the gill Net Integrity has:
        i. No compromise – no noticeable hole or restriction (tangled or collapsed) that would allow fish to pass through the min-fyke net without being captured.

ii. < 10% compromised – Approximately 10% (~0.5 m2) 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.

iii. > 10% compromised – More than 10% (~0.5 m2) 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:

i. If < 10% of the mini-fyke Net Integrity is compromised, process and record captured fish. Repair the net before deploying it at other locations.

ii. If > 10% of the mini-fyke net is compromised, release captured fish, repair the net, and re-deploy the net.

iii. Do not discard any data until the re-deployment is complete and new data have been recorded.

19. Process all fish (**SOP C.1**) from each net before pulling the next net. Record pass end date and time on the mobile device 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:

i. Before removing entangled animals protect yourself first. Protect your eyes with sunglasses or safety glasses and use gloves to handle the animal.

ii. 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.

iii. 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.

iv. Record the non-target species information on the mobile device.

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, contact the NEON Aquatic Ecologist, Domain Manager, and Permitting, 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%:
        i. Mini-fyke net set time
        ii. Water quality: conductivity, dissolved oxygen, and temperature
        iii. Field observations and most likely root cause of injury or mortality
        iv. Description of species affected
        v. Include any additional information that could help identify the root cause and for developing a solution

SOP C Fish Handling

After fish have been captured, they must be processed. Processing includes the identification and tally of all fish caught, and the measurement of weight and length for up to 30 individuals of each species captured at each reach. Processing must be carried out with minimal stress, harm, and suffering to fish and other species (e.g. reptiles, amphibians, invertebrates) caught during sampling

The recording of fish species requires using the NEON master taxon list of fish species codes. This list can be found on the NEON sampling support library within the Fish Sampling Protocol tab. The NEON master taxon lists also include codes for instances when identification below a given taxonomic rank (e.g., family, genus) cannot be made.

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).

C.1 Processing Samples

2. If no fish are caught within a sampling reach, indicate “No” in the “Target Taxa Present?” field on the mobile device. If fish are caught within the sampling reach, indicate “Yes” in the “Target Taxa Present?” field on the mobile device.
2. Ensure that all field scientists handling fish keep hands wet with native stream water and free of chemicals (e.g., insect repellent, sunscreen) while processing fish.
3. Field scientists shall confer with each other and the field guides when identifying fish species. Designate one field scientist to record fish throughout the sampling bout for taxonomic consistency.
4. At all sites except D04 sites, if bycatch (any animal except fish) are inadvertently caught during fish sampling, release unharmed and follow permit, landowner and site-host recording requirements. Permit and landowner requirements will dictate if and what type of bycatch your site is required to report. If the site is required to report bycatch, work with permitting to determine reporting process.
    a. D04’s aquatic sites are located in Puerto Rico, an oceanic island in which invertebrates often fill the niches that fish would otherwise fill, as such any collections need to be reported. Using the invertebrate bycatch table in the mobile device, record the taxon of each invertebrate caught during fish sampling. Once identified and recorded, release invertebrate bycatch.
5. Setup the digital scale and a measuring board on a flat surface and make sure that the scale is level.
6. Place plastic measuring tray on scale pan and tare.
7. Hastily measure the length of the fish.

8. Hastily measure the weight of the fish on the tared measuring tray.

***NOTE** – in some instances and if the fish are small enough, it is useful to take a Whirl-Pak, fill it with a very small volume of stream water, tare the Whirl-Pak with the plastic measuring tray, and then measure the fish's length and weight from inside the Whirl-Pak. It may introduce more error; however, this approach ensures that the fish remain in closer-to-ambient stream conditions and aren't exposed to the air. This is particularly important for smaller fish that are more susceptible to exposure.*

9. If a fin clip is collected for DNA sampling, Anesthetize the fish before taking a fin clip to reduce stress, see Section C.4.

10. Preparing Anesthesia for fin clippings: AQUI-S®20E (10% eugenol)

    a. AQUI-S reduces the stress experienced by the target species and minimizes the risk of injury to the field scientist (e.g. sharp spine pricks).

    b. 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 C.4**.

    c. Mix anesthetic in one 5-gallon bucket. Fill the bucket approximately half-full with native water (2.5 U.S. gallons or ~10 L) or more as needed.

    d. The dosage treatment of AQUI-S®20E is as follows:

        i. 25 - 40 mg/L for freshwater salmonids, expose to treatment no longer than 5 minutes.

        ii. 40 - 100 mg/L for freshwater non-salmonids, expose to treatment no longer than 5 minutes.

            1) Only use the minimum concentration which effectively renders fish species as handleable.

        iii. Refer to **Table 11** for calculated eugenol concentrations.

            1) Example: For a target concentration of 25 mg/L; 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).

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

Table 11. 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 holding bucket using the small handheld dip net.
    a. Larger fish may need to be removed carefully by hand. Be sure that your hands are clean and free of soap or sunscreen residue. You may also wear nitrile or latex-free gloves, but those should be wetted with native water before fish handling.
10. Place one fish at a time in the anesthetic bucket. Carefully monitor respiration, spontaneous movements, and swimming behavior to gauge the state of sedation and movements to determine when fish is anesthetized. If the fish can be easily handled without flipping its tail back and forth, it is sufficiently anesthetized. Sedation should be achieved in 1 to 5 minutes following emersion in the anesthetic solution. The required sedation time should be < 5 minutes.
    a. If this dose of anesthetic is insufficient, add 0.5 mL increments of AQUI-S®20E to increase the concentration until anesthetization is achieved within the limits described below.
        i. For salmonids, use 25 – 40 mg/L of AQUI-S®20E, **do not** exceed a concentration of 40 mg/L.

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

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.*

b. Do not exceed 5 fish in the anesthetization bucket at one time.

c. Leaving fish in the anesthetization bucket for too long can cause mortality. Monitor respiration and gill movement constantly.

d. Be sure to include required information within the *INAD Field Datasheets*.

11. Once fish is sedated, identify fish to species or lowest possible taxonomic level, using the mobile data device drop down species list for fish.

    a. If the species cannot be identified or identification is uncertain, weigh and measure and follow method in **SOP C.2** for handling uncertainty is fish species identification to record appropriate taxonomic information.

12. After fish has been identified weigh and measure fish length:

    a 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.

    b. The sensitivity of the scale in the field is not accurate below 0.5 g. Therefore, when recording fish weights that are less than 0.5 g, enter the value 0.25 g on the mobile data device.

    c. With gloved or bare hand wetted 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 19**) to the nearest millimeter and record on the mobile device.

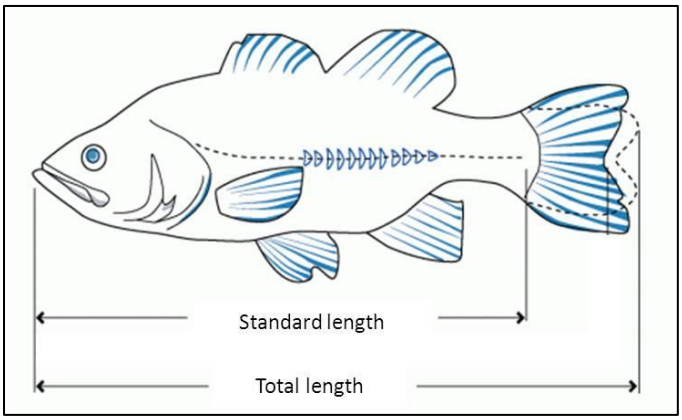

Figure 19. Measure the total length of each fish by pinching the fork together.

13. Inspect the fish for DELTS (deformities, including eroded fins, external lesions, parasites, and tumors) (**Figure 20**). DELTS should be considered 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.

    a. Document if the fish was killed or injured as a result of capture and processing. These could include electrofishing injuries (burn marks, bent spine, hemorrhage) as well as gill or mini-fyke net related injuries (damage to skin, gills, or fins) sustained while captured. Mortality or injury may also include damage as a result of other captured species. Record DELTS and capture/processing-related injuries on the mobile device.

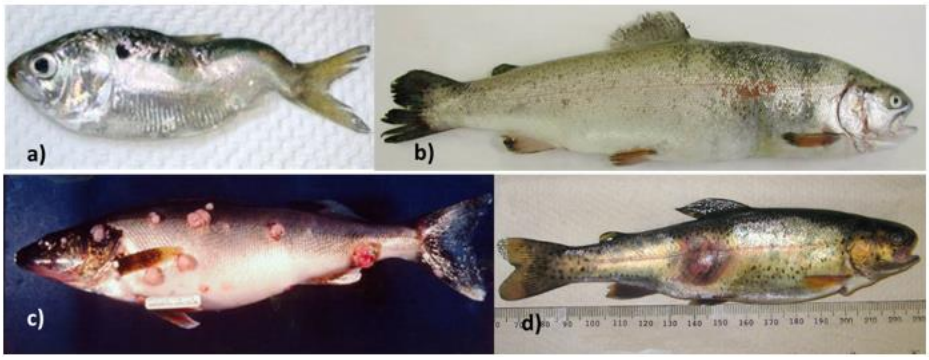

Figure 20. Example DELTS, a) deformity, b) eroded fins, c) tumors, and d) lesion. Photos by a) Kane 2005, b) Leander 2017, c) Cornell 2009, and d) Photo by Craig Banner 2004.

14. If collecting fin clips for DNA barcoding samples, refer to Fish Tissue Sampling for DNA Analysis (**SOP C4**).

15. Indicate and record the life stage of the specimen, only if field staff responsible for species identification has confidence in the ability to determine life stage (e.g. larval, young of the year, juvenile, adult, or gravid). The ease or even ability to determine age class will differ between site and species (juvenile salmonids are easy to identify, while often gravid females can be difficult). Only assign if you are positive of age class, otherwise leave blank.

16. Place processed fish in a bucket labeled “recovery bucket” containing fresh lake water and a battery-powered aerator for later release. Monitor fish for respiration and swimming behavior.

    a. Do not overcrowd fish in the recovery buckets; they need as much aerated water as possible. Use multiple buckets to reduce the concentration of captured fish.

    b. Segregate predator species from prey species, and large fish from small fish in buckets.

17. Repeat Steps 5-16 for up to 30 fish of each species captured at a reach. When less than 30 fish per species are identified, all of them shall be weighed, measured, and inspected for DELTS.

    a. If > 30 fish of a species are caught at the reach/sampling equipment level (start the count new for each sampling type), only the first 30 are weighed and measured, the remainder do

not need anesthesia or need to weighed/measured but should be bulk counted by species in the bulk count data table on the mobile device.

i. If holding buckets have greater than 30 individuals of one species, randomly choose the first 30 to weigh and measure.

ii. If bulk count is relatively low, actually count each individual per species, and record "Actual" in mobile app. If bulk count is too large to accurately count each individual per species, estimate number and record "Estimate" in mobile app.

iii. In some instances, for bulk species particularly salmonids, it will be easy to identify, bin, and bulk count YOY. In those cases, bin, count, and record YOY separately from the other bulk count of the same species. At sites where this is possible there will be two bulk counts for the same species: one count for YOY, and one count for other age classes. If identifying bulk YOY is not feasible, record all age classes per species together.

iv. Place the bulk counted individuals in the recovery bucket with the other processed individuals.

v. If it is desired to record a weight and length measurement of an individual or to collect a tissue sample or a whole specimen voucher for an individual fish identified in the bulk count group, create an individual specimen record. This will allow for an appropriate sample ID to be generated. Be sure to subtract the number of specimens that were recorded individually from the bulk count number.

18. Release the processed, revived fish back into the lake as close to the area and habitat they were sampled from (outside of the electrofish boundaries if electrofishing).

    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; see Voucher Specimen Preservation, **SOP C.5**.

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

C.2 Handling Uncertainty in Species Identification

All sampled fish must have a taxonID associated with them. During fish sampling, field ecologists must identify each fish species to the lowest possible taxonomic level. However, taxonomic identifications based on morphological features can involve uncertainty for a variety of reasons. There will be instances

where accurate identification to species is not feasible. 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:

- Assign an identification qualifier and a taxonID with finer taxonomic resolution.
- Assign a morphospecies and a taxonID with coarser taxonomic resolution.

Use an Identification qualifier if you are sure the fish is one of two or three choices.

1. Identification qualifiers contain information by using ID'q codes (**Table 12**) at the taxonomic level for which there is uncertainty.

    a. If there is confidence about the genus of a specimen and uncertainty in the species identification, then depending on your comfort level with the identification use the code **"CS"** which equals 'cf (not sure). species' or **"AS"** which equals 'aff. (similar to, but not) species'. This provides information to the user that the species identification is possibly incorrect.

    b. 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 use the code "**CG"** or the code "**AG"**, depending on field taxonomist level of certainty use one of these two codes, to indicate uncertainty in the genus-level assignment.

    c. If you do not know the genus species but can narrow the family down to between one and three families use the codes **"CF"** or **"AF"** depending on your level of certainty.

Table 12. Codes for identification qualifier entries

ID'q 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 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). 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.

1. 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.

2. When using morphospecies ID, you must use the following naming format: the domainID and the site name where the specimen was captured, the year of capture, and the word "Morph" followed by one or more unique letters. For example, "D15.REDB.2014.MorphA".

Note: *It is important to use the morphospecies ID to document, track, and resolve uncertain fish taxa.*

   a. At the site level, the letter at the end of the morphospecies ID (e.g., "A") should *never* be repeated during the same year for any other morphospecies than that for which it was originally designated. 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.REDB.2014.MorphAA").

3. **Never** use scientific or common species names in the "Morphos Species ID Remarks". Morphospecies data is public data, so be professional in descriptive comments used in the "Morphos Species ID Remarks".

4. 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).

5. The expectation is that field ecologists will eventually resolve morphospecies. It is the responsibility of field science to keep notes and pictures, and use field guides, consultations with local experts, and/or BOLD DNA results to resolve morphospecies identifications. Once a morphospecies has been resolved, document resolution of the fish morphospecies record in the fish sampling app.

C.2.1 Fish Not On Taxon List/Larval Fish ID

1. 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. Do not put the name, scientific or common, in the comment section of the data.

2. The taxon table will be updated for the subsequent year to make new species identified on site available to field scientists within that domain.

3. 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.

C.3 Euthanizing Fish, Reptiles, and Amphibians and Non-Target Species

Euthanizing Fish

1. Euthanize fatally injured fish using a lethal dose of 10% eugenol (AQUI-S®20E) at a concentration of 100 mg/L. Refer to **Table 13** 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.
2. 10% eugenol solution should be used whenever possible. In the event that 10% eugenol is not available, MS-222 can be used to euthanize fish (not for fin clips), following the same steps for Euthanizing Amphibians, outlined below **Euthanizing Amphibians p 63**.

Table 13. Lethal dose of AQUI-S®20E for euthanizing fish.

Target Concentration of AQUI-S®20E (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 lake 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. Determine if fish is under < 200 mm or > 200 mm and follow the steps outlined in **SOP C.5** depending on the size of the preserved specimen**.** Place fish into appropriate sample container (e.g., wide mouth HPDE bottles-minimum 30mL) with completed specimen label (**Figure 10**) and add 10% formalin preservative. One taxon per specimen bottle.

   i. Adhesive Type I barcode labels (**Figure 7**) will be added to the sample containers and scanned by the mobile app.

   ii. Keep a human-readable label on each bottle with the sample ID printed to assist with organization and shipping.

e. If the voucher specimen was pulled out of a bulk sample, there needs to be an individual record created in the mobile app with a sample ID generated. Subtract the number of individuals pulled from the bulk sample count and create a record for each individual entry with a measured weight and length. The specimen voucher sample ID format is: FSH.siteID.reachNo.YYYYMMDD.passNo.specimenNo.

Euthanizing Amphibians

1. Amphibians that are mortally injured as a result of fish sampling will be euthanized using a lethal dose of pharmaceutical grade MS-222, 200 mg/L of lake water in the field.
    a. Add 1 L of lake 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.
    b. Transfer amphibians from the holding bucket to the bucket containing the euthanizing solution with the small handheld dip net, be sure to completely submerge the voucher individual.
    c. Monitor the amphibians until respiration ceases.
    d. Euthanized amphibians must be vouchered (unless permit dictates otherwise) and shipped to biorepository.
    e. Place the amphibian into an appropriate sample container (e.g., wide mouth HPDE bottles-minimum 30mL) with completed specimen label (**Figure 10**) and add 10% formalin or 10% formaldehyde as preservative. One taxon per specimen bottle. See steps on specimen preservation in **SOP C.5**.

Euthanizing Reptiles

1. For mortally injured reptiles, a two-stage method of euthanasia is recommended. A fresh bottle of stage 1 and stage 2 reptile euthanasia solutions described below, should be prepared for each day of sampling (**Figure 21**). 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]).
    a. This procedure includes an intracoelomic injection of a minimum of 250 to a maximum of 500 mg/kg of a pH-neutralized solution (1.0%) of MS-222. This range of dosages will produce a loss of consciousness in less than five minutes (AVMA 2020).

Figure 21. 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.

b. <u>1st stage injection solution - 1% MS-222</u>:

i. Prepare 1% MS-222 and baking soda in the Domain Support Facility to be mixed with water in the field when needed: 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.

ii. Bring along 1 L of tap water 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.

iii. Dosing guidelines: Reptile weight (kg) x (250 - 500) mg/kg x 1 g/1000mg x 100 mL /1 g MS-222 = # mL of 1% MS-222 to inject into reptile. See Table below for the 1st stage dosage calculator based on reptile weight in grams.

Table 14. 1st stage dosage calculations using 1% MS-222 for reptiles based on reptile weight in grams.

Weight of Reptile (g)	mL of 1% MS-222 solution	
	250 mg/kg (minimum dose)	500 mg/kg (maximum dose)
10	0.25	0.5
50	1.25	2.5
100	2.5	5
200	5	10
300	7.5	15
400	10	20
500	12.5	25

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

v. Draw up the appropriate volume of the stage 1 solution into the syringe based on the reptile weight.

vi. 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 22**).

Figure 22. Injection into the inguinal fossa of a turtle. Photo by Kaufman 2017.

c. Following loss of consciousness, a second intracoelomic injection of unbuffered 50% MS-222 is administered.

i. Prepare MS-222 in the Domain Support Facility to be mixed with water in the field when needed: Pre-weigh 1 g of MS-222 into an appropriately sized container (e.g., 20 mL scintillation vial). Cover vial in duct tape or foil to protect from light.

ii. Add 2 mL tap water to the 1 g 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 well.

iii. Dosing guidelines: Inject 0.1 ml for specimens weighing less than 30 g. Add an additional 0.1 mL for every additional 30 grams of reptile.

iv. Using a 5 mL syringe, inject the appropriate dose of the 50% (v/v) MS-222 unbuffered solution into reptile, depending on the weight of the specimen. The solution will be thick with precipitates, cloudy, and pale yellow (**Figure 23**).

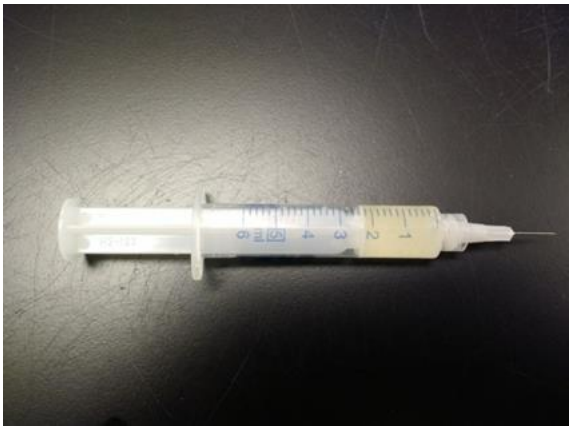

Figure 23. 2nd stage 50% (v/v) unbuffered pharmaceutical MS-222 solution. Note yellow cloudy appearance of solution.

v. 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 22**).

vi. Euthanized reptiles must be vouchered (unless permit dictates otherwise) and shipped to biorepository.

Note: *Do not dispose of specimens euthanized with MS-222 in the field or stream. Do not dispose of the MS-222 solutions in the field or stream.*

vii. Place the reptile into an appropriate sample container (e.g., wide mouth HPDE bottles-minimum 30mL) with completed specimen label (**Figure 10**) and add 10% formalin or 10% formaldehyde as preservative. One taxon per specimen bottle. See steps on specimen preservation in **SOP C.5**.

1. Aquatic invertebrate species, including arthropods and mollusks, 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).

2. In the event that a federal or state listed threatened or endangered species is mortally 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 and:

    a. Stop all fish sampling activities.

    b. Contact Permitting, protocol author, and the Domain manager to report the incident. Permitting will contact the regional U.S. Fish and Wildlife Service office and the local state fish and wildlife department to report the incident.

    c. Specimens will also be preserved following the methods described above.

    d. 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 Permitting who will then coordinate with the federal and state fish and wildlife authorities within the region where the specimen was collected.

C.4 Fish Tissue Sampling for DNA Analysis

Collect fish tissue from a maximum of 5 individuals per species per year, at each site (e.g. if the site has 5 species, a maximum of 25 fin clips a year are encouraged). NEON site DNA fin clip collections should capture the diversity of fish found at each site over time and space.

1. Put on gloves (nitrile or latex free).
2. Anesthetize target fish with AQUI-S®20E following **SOP C.1** (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 24**).

    a. Take the photo using the tablet and mobile application. Record the “photo view” which is auto-populated as lateral. If the photo is oriented as a dorsal or ventral view, select that from the photo view options.

    b. Be sure that the resulting image is in the “landscape” orientation. Include as much of the specimen in the field of view as possible. Minimize shadows and keep hands or other objects out of the image. See **Figure 24** for an example.

c. A caption can be entered but is not required. Click on the photo that was collected and enter a remark in the caption field.

d. Delete any photos that are not of suitable quality or otherwise should not be associated with the fish data.

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

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

Figure 24. 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% - 95%) 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.

Note*: A fresh (non-rusty), sterilized exacto knife is the perfect tool for getting very small fin clips.*

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.

VERY IMPORTANT: Fish tissues collected for DNA extractions cannot be exposed to or preserved in formalin.

6. Using the cutting tool, remove a piece of the target fin ray (**Figure 25**). 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.

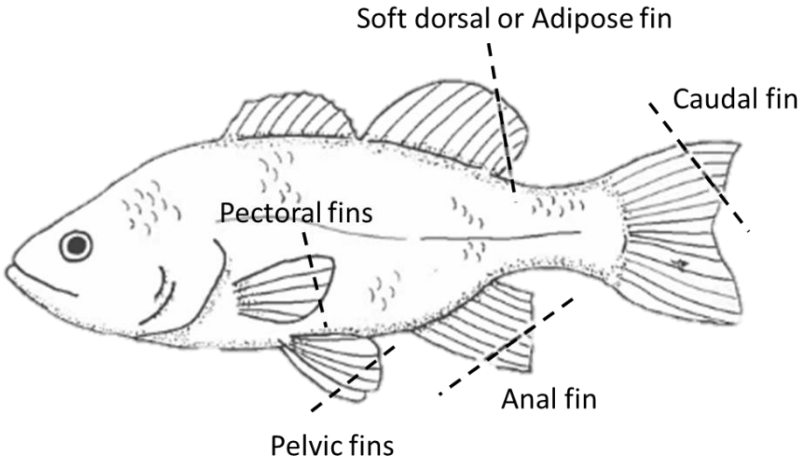

Figure 25. Optional fins to clip for tissue sampling.

7. 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" (**Figure 11**).
8. The fin clip vial must also have type IV barcode label attached the outside (**Figure 8**).
9. Collecting DNA tissue samples for fish that weigh less than 0.5 g and removing sufficient amounts of fin tissue will likely reduce their survivorship. Instead, euthanize the specimen, remove an adequate portion of fin tissue, and place in the tissue collection vial. The remaining specimen can be collected as a voucher (see **SOP C.5**) or discarded following the guidance of the collection permit.
10. Place live fish that have had tissues samples collected into the recovery bucket.
11. Indicate on the mobile device that a DNA tissue sample was taken and provide a sample ID format "FSH.siteID.reachNo.YYYYMMDD.passNo.specimenNo.DNA" for each specimen.

12. Flame sterilize (or wipe) the cutting tool and forceps or wipe with an alcohol pad before collecting the next tissue sample to avoid cross-contamination. Be sure that no fish tissue, slime, or blood remains on the instruments.
13. Repeat Steps 1-12 until all targeted fish samples have had tissues collected.
14. In order to capture the full potential diversity of the site spatial and temporal DNA, attempt to spread fin clip samples out across the reach and between spring and fall
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 procedures, refer to **Section 4.3** Timing for Laboratory Processing and Analysis and **SOP F** for shipping guidance.

C.5 Whole Fish and non-Target Vertebrates Voucher Specimen Preservation

1. Fish and other non-Target Vertebrates (i.e., Amphibians and Reptiles) < 200 mm:
    a. Fill jar with a 10% buffered formalin solution to fix specimens within one hour of euthanizing.
    b. Make sure to use an appropriately sized jar so that fish is not forced inside and damaged (bent or damage to the fins).
    c. If using concentrated formalin (37% formaldehyde), dilute 1-part concentrated formalin with nine parts water (can use native water).
    d. In general, use a ratio of 10:1 formalin to fish tissue by weight (example: use 1,000 mL formalin: 100 g fish).
        i. Use an appropriate size (minimum 30mL) HDPE wide mouth specimen jar for the preservation of an individual specimen in a single jar.
    e. Secure lid tightly and store upright at room temperature (~70°F). Be sure that the specimen label is included with the specimen and that the jar is properly labeled with the Sample ID in the following format “FSH.siteID.reachNo.YYYYMMDD.passNo.specimenNo.” (**Figure 10**).
    f. The voucher jar must also have a Type I barcode attached to the outside (**Figure 7**).
    g. The specimens must soak in 10% formalin for 7 days. After 7 days, transfer the specimen into an alcohol fixative; either 70% - 95% ethanol or isopropanol, making sure that the specimen is fully submerged. Discard the used formalin accordingly (AD[03]).
        i. Only ship voucher specimens in ethanol.
    h. Secure lid tightly and store upright at room temperature (~70°F) with appropriate specimen labels inside and outside of the container.
2. Fish and other non-Target Vertebrates (i.e., Amphibians and Reptiles) > 200 mm:

a. For large fish greater than 200 mm total length, make a lateral 2-3 cm incision on the lower right side into the peritoneal (the tissue that lines the fishes abdominal wall). Open the body cavity, taking care not to damage bones or organs, this will allow for better penetration of the fixative.

b. Place fish in a jar with enough 10% formalin solution to cover fish completely, place a watertight lid on jar, and soak in jar for 7-10 days.

   i. Make sure to use an appropriately sized jar so that fish is not forced inside and damaged (bent or damage to the fins).

   ii. After 7-10 days, decant formalin and soak fish in water just long enough that fish is rinsed of excess formalin.

   iii. Store in 70% ethanol or 50% isopropyl.

   iv. When shipping to the biorepository; wrap the specimen in water dampened cheese cloth with voucher number and site, then wrap in cheese cloth and package in double plastic bags (**Figure 26**).

   v. There must be a voucher and barcode label attached to the bag so that label can be read.

Figure 26. How to ship > 200 mm fish vouchers (American Museum of Natural History).

3. Discard used anesthetic solution according to NEON EHS chemical hygiene guidelines (AD[03]).

SOP D Post-Field Sampling Tasks

D.1 Document Incomplete Sampling Within a Site

Fish Sampling in Lakes is scheduled to occur at all prescribed sampling locations according to the frequency and timing described in Section 4 and Appendix C. Ideally, sampling will occur at these sampling locations for the lifetime of the Observatory (core sites) or the duration of the site's affiliation with the NEON project (relocatable sites). However, sampling may be shifted from one location to another when sampling is compromised. In general, a sampling location is compromised when sampling becomes so limited that data quality is significantly reduced.

There are two main pathways by which sampling can be compromised. First, sampling locations can become inappropriately suited to answer meaningful biological questions – e.g., a terrestrial sampling plot is compromised after road-building activities, or a stream moves after a flood and the location is no longer within the stream channel. Second, sampling locations may be located in areas that are logistically impossible to sample on a schedule that is biologically meaningful.

If sampling at a given reach is not possible during a given bout, a problem ticket should be submitted by Field Science staff.

To document locations not sampled during the current bout:

1. Review the completed sampling effort and create **Sampling Impractical** records as described in Section 4.5 for plots at which sampling was scheduled but was not completed.
4. To document whether a location is compromised according to the criteria above:
5. Review **Sampling Impractical** records from the (AOS) Fish [PROD] and Portal data to identify locations where sampling was scheduled but was not completed due to environmental or site management factors.
6. Create an incident with the following naming convention to document the missed sampling and compromised location: 'AOS Sampling Incomplete: FSH – [Root Cause Description]'
7. Example: 'AOS Sampling Incomplete: FSH – Could not sample reach due to excess vegetation at reach'.

Staff scientists review incident tickets periodically to determine whether a sampling location is compromised

D.2 End of the Sampling Day

1. Refresh the sampling kit.
    a. Charge and 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 Standard Operating Procedure: Decontamination of Sensors, Field Equipment, and Field Vehicles (RD[10]).
    b. Dry all equipment thoroughly between sites and before storage.
    c. Check all nets for holes and, if necessary, patch the net using the net repair kit. Mending fish nets takes practice and patience. The Oregon State University (OSU) 1989 reference provides a resource for how 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 E Data Entry and Verification

Mobile applications are the preferred mechanism for data entry. Data should be entered into the protocol-specific application as they are being collected, whenever possible, to minimize data transcription and improve data quality. Mobile devices should be synced at the end of each field day, where possible. Alternatively, devices should be synced immediately upon return to the Domain Support Facility.

However, given the potential for mobile devices to fail under field conditions, it is imperative that paper datasheets are always available to record data. Paper datasheets should be carried along with the mobile devices to sampling locations at all times. Data collected on paper data sheets must be transcribed within 14 days of collection or the end of a sampling bout (where applicable). See RD[04] for complete instructions regarding manual data transcription.

Note: No fish photos will be collected if the mobile device and application are not available.

Quality Assurance

Data Quality Assurance (QA) is an important part of data collection and ensures that all data are accurate and complete. Certain QA checks can be conducted in the field (i.e., before a field team leaves a plot or site), while others can be conducted at a later date in the office (typically within a week of collection). Field QA procedures are designed to prevent the occurrence of invalid data values that cannot be corrected at a later time, and to ensure that data and/or sample sets are complete before the sampling window closes. Invalid metadata (e.g. collection dates, eventIDs) are difficult to correct when field crews are no longer at a sampling location.

Office QA procedures are meant to ensure that sampling activities are ***consistent*** across bouts, that sampling has been carried out to ***completion***, and that activities are occurring in a ***timely*** manner. The Office QA will also assess inadvertently duplicated data and transcription errors to maintain data ***validity*** and ***integrity***. See the Data Management Protocol (RD[04]) for more discussion of QA measures.

Before samples ship to external facilities and/or their digital records load to the NEON database, the data must undergo thorough quality checks. The steps needed to accomplish this are outlined in the FSH QC Checklist, which is available on the NEON SSL.

Sample Identifiers & Barcodes

By default, each (sub)sample produced by this protocol receives a sample identifier, which contains information about the location, date, and sample type. Each (sub)sample will also be associated with a scannable barcode, which will not contain information about sample provenance, but will improve sample tracking and reduce transcription errors introduced by writing sample identifiers by hand.

Adhesive barcode labels should be applied to dry, room temperature containers in advance of their use in the field (at least 30 minutes prior but may be applied at the start of the season). Barcodes are

unique, but are not initially associated with a particular sample, thus it is encouraged to apply these in advance. Use the appropriate barcode label type with each container (i.e., cryogenic Type II barcode labels only used for samples that are stored at -80°C, etc). Note that a barcode label is applied *in addition to* a sample identifier (hand-written or printed).

Barcodes are scanned into the data entry application when a sample is placed into a container; only one barcode may be associated with a particular sample. Do not reuse barcodes. If a barcode is associated with multiple samples, the data ingest system will throw an error and refuse to pull in entered data.

SOP F Sample Shipment

1. Follow sample shipping timelines in Section 4.3 to maintain appropriate sample hold times and storage conditions.
    a. Discrepancies between this protocol document and the Shipping Protocol should be communicated to Science.
2. Follow instructions in the NEON Protocol and Procedure: Shipping Ecological Samples, Sensors, and Equipment in order to ship samples to external laboratories or the biorepository (RD[14]).
3. Fish DNA fin clips are sent from each domain to Domain 08 for processing.

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APPENDIX A QUICK REFERENCES

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

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

Table 15. Mix stock solutions of AQUI-S 20 in the Domain Support Facility.

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

Step 3 – Mix stock solutions of MS-222 in the Domain Support Facility.

Step 4 – Set electrofishing block nets, mini-fyke nets and gill nets according to the following timeline:

Table 16. Electrofishing block nets, mini-fyke nets and gill nets using the following timeline.

Time of Day	Day 1	Day 2	Day 3
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
Night	Allow electrofishing reaches to recolonize	Electrofish in fixed reaches	Electrofish in fixed or random reaches

Step 5 – Assemble backpack electrofisher and test the settings. Check anode and cathode for corrosion; remove with steel wool or other abrasive pad.

Step 6 – Move the anode along the shoreline in a zig-zag pattern and capture immobilized fish. Place immobilized fish in 5-gallon buckets.

Step 7 – If this is a random reach, sample fish using only one pass. If this is a fixed reach, sample fish using three passes.

Step 8 – Identify fish to species using the 6-letter species code (e.g., *Cottus cognatus* = COTCOG) and record on the mobile device. For uncertain species, follow the guidance provided in **SOP C.2**. For morphotype species and labeling with a unique identifier, refer to **SOP C.2**.

Step 9 – Measure the weight and length of each specimen (up to 30 of each species (per reach)) and inspect for deformities.

Step 10 – If collecting fish tissues for DNA analysis, target specimens **MUST BE** anesthetized with AQUI-S®20E.

Step 11 – Place processed fish in a bucket containing fresh lake water and a battery-powered aerator for later release. Once revived, release the fish outside of the block net.

Step 12 – If any endangered species (review collection permit before sampling) or other vertebrates (e.g., salamanders, turtles) are caught, identify, take a photo, and release immediately. **STOP** all fish sampling activities. Follow the guidance of the collection permit for reporting procedures.

Step 13 – Preserve euthanized specimens in a jar with a 10% buffered formalin, then in ethanol for long-term storage. Ship to the biorepository according to established shipping schedules.

APPENDIX B 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 30 individuals in one species are captured, anesthetize, weigh and measure the first 30 and simply count the remaining fish (no anesthetization).
- ☑ Do not exceed 5 fish in the anesthetization bucket at one time.

APPENDIX C ESTIMATED DATES FOR ONSET AND CESSATION OF SAMPLING

Sampling corresponds with the first and third sampling bout windows at lake sites. 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.

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

APPENDIX D SITE-SPECIFIC INFORMATION

D.1 Fixed Reach Prioritization Selection Per Site

Fixed reach sampling prioritization is shown for each site below for all stream sites. The "Top priority fixed reach" is sampled first in the fishing bout before any random reaches are sampled. If sampling is impractical at the top priority fixed reach because of some spurious event, then indicate this for any affected reach on the mobile device and then sample the "Backup fixed reach" or "Last resort fixed reach" should the backup also be unfishable. Should environmental conditions affect the ability to fully sample any fixed stream reaches, commence with sampling but note the cause on the "Reach Condition" section in the mobile device. Once the top priority fixed reach has been sampled, continue fishing the reaches in the order established in Appendix D.2, Table 18. After the three scheduled random reaches have been fished, and there is still time available, return to fishing the remaining fixed reaches (see Appendix D.3, Figure 26).

Table 17. Fixed reach prioritization for each site.

Domain	Site	Top priority fixed reach	Backup fixed reach	Last resort fixed reach
D03	Lake Barco – Not currently sampled	NA	NA	NA
D03	Lake Suggs – Not currently sampled	NA	NA	NA
D05	Crampton Lake	3	6	10
D05	Little Rock Lake	9	7	1
D09	Prairie Lake	5	1	7
D09	Prairie Pothole	1	9	5
D18	Toolik Lake	10	7	3

D.2 Random Reach Prioritization Selection Per Site

Randomized reach order is shown for each site below. If sampling is impractical as a result of severe drought (dry) or that the lake is frozen, then indicate this for any affected reach on the mobile device. 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 device.

Table 18. Randomized reach order per site.

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

D.3 Example Sampling Strategy for Two Years (Four Bouts)

Section **D.1** and **D.2** include tables that specify the prioritized fixed reach and the order of random reaches to sample. Below is a figure that serves as an example how the combined Tables 15 and 16, respectively, are combined to plan future bouts.

eventID	Prioritized Fixed Reach	Random Reaches	Fixed reaches to sample if time allows
CRAM.2025.spring	3	1, 2, and 4	6 and then 10
CRAM.2025.fall	3	1, 2, and 4	6 and then 10
CRAM.2026.spring	3	5, 7, and 9	6 and then 10
CRAM.2026.fall	3	5, 7, and 9	6 and then 10

Figure 27. Example scheduling of four fishing bouts for Crampton Lake. (Note – this is an example and does not represent the existing random reach ordering that currently exists at CRAM).

APPENDIX E INITIAL SAMPLING REACH ESTABLISHMENT AND SELECTION

Sampling reaches are established during the first year of sampling. Reaches may need to be re-established 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 ten riparian habitat sections (see RD[13] Riparian Habitat Assessment Protocol, as the boundaries between fish sampling reaches. 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 riparian reaches (**Figure 3**).

1. Using the site-specific Riparian Habitat Assessment locations, fish sampling will occur within each of the ten riparian reaches.
2. Navigate to the permanent aluminum plot survey markers on the shoreline at each section boundary if present. This marks the start of the sampling reach; the reach is a triangle bounded by the next riparian permanent marker and coming to point in the middle of the lake.

Fixed and random sampling sections occurred during the first year of sampling.

1. Three of the ten riparian sections “fixed” sections were selected. Fixed sections are sampled two times per year throughout the duration of NEON project.
2. The three fixed reaches were chosen to best represent the habitat variability throughout the lake (e.g., presence or absence of vegetation, substratum type).
3. The rest of the reaches are sampled using a rotating panel (**Table 18**) of randomized sections.

APPENDIX F 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 19. Equipment list – Field Preparation.

Supplier/ Item No.	Exact Brand	Description	Purpose	Quan-tity
Durable items				
	N	HDPE bottle, amber, 1 L	Stock solution (MS-222) container	2
	N	Lab safety glasses	Safe handling of chemicals	1 pair
	Y	Mobile field data recording device (Tablet)	Recording data	1
	N	Multisonde	Measuring % DO, temperature and salinity	1
Consumable items				
Fisher Scientific Company; Syndel	Y	Pharmaceutical grade Tricaine methanesulfonate (MS-222)	Euthanizing specimens	20 g
	N	NaHCO3 (baking soda)	Buffering agent for MS-222	50 g
AquaTactics Fish Health	Y	10% eugenol (AQUI-S®20E)	Anesthetizing specimens	50 mL
	N	Nitrile gloves (latex-free and powder-free; pair)	Safe handling of chemicals and fish	1
	N	Field data sheets (print on waterproof paper, write in pencil)	Recording data	10
	N	Specimen labels (waterproof paper)	Labeling specimens	2 sheets
	N	Adhesive barcode labels	Labeling sample bottles with barcode-readable labels	1 sheet
	N	Collection permit documents		1

Table 20. Equipment list – Reach Establishment.

Supplier/ Item No.	Exact Brand	Description	Purpose	Quan-tity
Durable items				
RD[12]	N	Site-specific bathymetry map	Navigating to sampling reaches	1
RD[09]	N	Site-specific riparian map	Navigating to sampling reaches	1
	N	Plot survey markers (aluminum, site-specific)	Establishing sampling reaches	10
	N	Meter tape (50 or 100 m)	Establishing sampling reaches	2
	N	Handheld GPS (with batteries, ± 1 m accuracy)	Navigating to sampling reaches	1
Consumable items				
	N	Flagging tape	Establishing sampling reaches	1 roll

Table 21. Equipment list – Fish Sampling.

Supplier/ Item No.	Exact Brand	Description	Purpose	Quan-tity
Durable items				
	N	Rubber mallet	Hammering PVC posts for random shore reaches	1
	N	PVC pipe 5 feet long	For setting random reach boundaries	24
	N	T-post insulator or caps with clips	Used to hang the block net on the T-posts	30
	N	Carabiners	Attaching weights to the block net	30
	N	Duck decoy weights (non-lead) 8 oz.	Anchoring the block net	36
	N	Wrench (9/16th)	Used to tighten the anode ring to the fiberglass pole	1
	N	Steel studded fence posts (i.e., T-post)	Securing block net at reach boundary	30
	N	Fence post driver or small sledge	Securing block net at reach boundary	1

Supplier/ Item No.	Exact Brand	Description	Purpose	Quan-tity
	N	Fence post puller	Securing block net at reach boundary	1
The Fish Net Company, LLC Duluth Nets	Y	3 mm mesh block nets with lead lines and top lines with floats (35 m x 1.5 m)	Catching drifting specimens	3
	N	Block net stakes or ropes to hold up nets (e.g., 1 cm diameter stainless rod, studded steel fence posts, ¼" Dacron Polyester rope)	Securing block net at reach boundary	15
	N	Reusable nylon cable ties, natural or white	Securing block nets	50
	N	Net repair kit: • needle • net string • butane lighter • zip ties	Repairing nets	1
	N	Gloves, Nitrile coated, Textured, Knitted cuff, Abrasion resistant, PIP or Equivalent	Handling T-posts, nets, and removing entangled birds or mammals	1
Smith-Root	Y	Battery-powered backpack electrofishing unit	Electrofishing	1
Smith-Root	Y	Anode pole (1.5-2.0 m) with attached anode ring	Electrofishing	1
Smith-Root	Y	Cathode (rattail type; 1-2 m and 10-15 mm in diameter)	Electrofishing	1
Smith-Root	Y	Electrofisher batteries (rechargeable)	Electrofishing	3
Smith-Root	Y	Battery charger (electrofishing batteries)	Charging the electrofisher	1
	N	Abrasive pad/steel wool to clean anode rings	Electrofishing	1
N&K Dip Nets	Y	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

Supplier/ Item No.	Exact Brand	Description	Purpose	Quan-tity
Smith-Root	Y	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 + 1 extra for group
	N	5 gallon buckets	Storing specimens	10
Challenge Plastics	Y	5-gallon bucket lids	Ensuring fish don't escape storage buckets	3
	N	Wrench (9/16th)	Used to tighten the anode ring to the fiberglass pole	1
	N	Chest waders (approved for electrofishing)	Safe wading and electro fishing	1 pair per person + 1 extra for group
	N	Chest wader repair kit (e.g., Aquaseal) or extra waders	Safe wading	1
	N	Polarized sunglasses (amber or brown lenses recommended)	Increasing efficiency of fish capture	1 pair per person + 1 extra for group
	N	Head lamps (with batteries)	Increasing efficiency of fish capture	1 per person and BRING EXTRA BATTERIES
Consumable items				
	N	Zip ties (multiple sizes)	Taking up slack on the block net	100+

Supplier/ Item No.	Exact Brand	Description	Purpose	Quan-tity
	N	Fish viewer	Viewing individual fish and taking photos	1
	N	Plastic weighing boat	For weighing fish under 75 mm	1

Table 22. Equipment list – Gill nets.

Supplier/ Item No.	Exact Brand	Description	Purpose	Quan-tity
Durable items				
	N	Gill net tubs (i.e., 5-gallon bucket)	Storing gill nets	6
	N	Depth finder	Navigating to sampling locations	1
Duluth Nets	Y	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
Duluth Nets	Y	• Net floats	Securing gill nets	18
	N	12.7 mm diameter rope (3-10 m in length) for floats	Securing gill nets	18
	N	Net anchors	Securing gill nets	18
	N	Live well (site-specific, i.e., 5-gallon bucket or tote)	For storing live fish on the boat for processing	1

Table 23. Equipment list – Mini-fyke nets.

Supplier/ Item No.	Exact Brand	Description	Purpose	Quan-tity
Durable items				
Duluth Nets	Y	Mini modified fyke nets: • Mesh – 6.35 mm bar knot-less with asphalt coating • Lead – One, 7.6 m long × 0.6 m deep • Trap – Two 0.6 m × 1.2 m rectangular frames, two 0.6 m diameter hoops with one funnel, cod end with purse string closure	For catching specimens	3
	N	Block net stakes or ropes to hold up nets (e.g., 1 cm diameter stainless rod, studded steel fence posts, ¼" Dacron Polyester rope))	Securing mini-fyke nets	4-6
	N	Waterproof blinking LED light	Marking mini-fyke net locations	6

Table 24. Equipment list – Fish Processing.

Supplier/ Item No.	Exact Brand	Description	Purpose	Quan-tity
Durable items				
	N	Fish and top predator taxonomic ID key specific to location or region (denotes endangered species)	Identifying specimens	1
	N	Portable aerators (batteries, diffusion stone)	Aerating buckets	15
	N	Small dip net (3.2 mm mesh)	Handling specimens	5
	N	Fish measuring boards (60 cm)	Measuring specimen length	2
	N	Portable digital scale (batteries, charger)	Weighing specimens	1
	Y	Plastic tray	Weighing specimens	2

Supplier/ Item No.	Exact Brand	Description	Purpose	Quan-tity
B&H Photo Corp	Y	Color separation guide	Photographing specimens	1
	N	25-50 mL graduated cylinder, plastic	Mixing anesthetic and euthanizing solution	1
Fisher Scientific Company	N	Fin clipping scissors	Cutting fish fins for DNA barcode samples	1
Fisher Scientific Company	Y	Refillable butane lighter	Flame sterilization of tissue sampling equipment	1
	N	Dish pan, plastic	Disinfecting tools with iodophor or betadine	1-12 qt.
Consumable items				
	N	Nitrile gloves (pair) Have small, medium, large, and extra-large gloves on hand	Safe handling of chemicals and fish	10
	N	HDPE wide mouth specimen jars (25 mL, 60 mL, 250 mL, 500 mL, and 1 L)	Specimen preservation containers	50
Fisher Scientific Company; Syndel	N	Pharmaceutical grade Tricaine methanesulfonate (MS-222) stock solution	Euthanizing specimens	1 L
AquaTactics Fish Health	Y	AQUI-S®20E stock solution	Anesthetizing specimens	1 L
Thomas Scientific, Inc.	N	10% buffered formalin (3.7-4.0% formaldehyde)	Preserving specimens	20 L
	N	Needle, disposable 1 inch, 25 gauge, sterile	Injecting reptiles with MS-222	1 pack
	N	Needle, disposable 1 inch, 27 gauge, sterile	Injecting reptiles with MS-222	1 pack
	N	Syringe, 5mL luer lock syringe	Measuring and injecting euthanasia fluids	1 pack

Supplier/ Item No.	Exact Brand	Description	Purpose	Quan-tity
	N	Ethanol (70 -95%)	For preserving fin clips for DNA and sterilizing DNA equipment	250 mL
	N	Ethanol (95%)	Euthanizing invertebrates	250 mL
	N	Iodophor or betadine disinfectant	Required by some States for decontaminating equipment	1 L
	N	50mL Centrifuge tube	Store syringe and needle, measure injection fluids	1
	N	60mL Clear bottle HDPE	Store dry MS-222 and baking soda for reptile euthanasia stage 1 solution	1
	N	20mL HDPE Scintillation vial	Store dry MS-222 for reptile euthanasia stage 2 solution	1
	N	Sharps Container, Portable, Slip Top	Receptacle for disposal of needles	1
Thomas Scientific, Inc.	N	Tissue containers (e.g. 1.5 mL microcentrifuge tubes)	For storing fin clips for DNA barcoding	100