AOS PROTOCOL AND PROCEDURE: FSS – FISH SAMPLING IN WADEABLE STREAMS

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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	Protocol migration
C	12/16/2015	ECO-03402	Major updates to include IACUC requirements and input from technicians
D	05/16/2017	ECO-04493	CM updated with new template and changes based on feedback from FOPS. Fish tissue DNA sampling included. Fish euthanasia with AQUI-S20E.
E	04/03/2017	ECO-05577	Major updates include revised sample contingency timing. An updated equipment list for voucher containers, working gloves, and mobile field device. Modified wader requirements. Added a step for recording water quality before every electrofishing pass, measuring each fish sampling reach, option for using two backpack electrofisher units, and net tending procedures for by-catch and handling birds or small mammals. Procedures for establishing fish sampling reaches where the permitted reach is less than 500 m. Included additional resources 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 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. Updated the reach condition function in the Fulcrum application to allow “heavy

			vegetation" as a constraint. Revised the AQUI-S20E dose with recommendations from the USFDA for salmonids and non-salmonids. Stressed the importance for using the morphospecies ID format. Emphasized the requirement to use anesthesia when collecting fish tissue for DNA where permitted.
G	05/06/2021	ECO-06524	• Updated to new template (NEON.DOC.050006 Rev K) • Permanent fish barrier method • Upper temperature criteria
H	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 at BLUE in Appendix D.4 • Updated maximum number of fish to process per species per transect from 50 to 30 at SYCA when stream temperature is between 22-26°C in Appendix D.4 • 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 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 helping researchers understand natural and anthropogenic changes to environmental conditions. Researchers commonly use fish as environmental indicators in bio monitoring assessments because most species have well-known diversity of tolerances and life histories (Simon 1998). Consequently, assessments of fish assemblages 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 represent a diverse taxonomic group with a broad range of habitat requirements and life history strategies. For example, fish assemblages commonly consist 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 biologists can easily sample.

Wadeable stream fish sampling presents challenges; the same characteristics that make fish excellent indicators (e.g., diversity of species and associated habitat requirements in lotic ecosystems) can be problematic when trying to adequately quantify fish presence and abundance in streams. Specifically, standard methods to sample fish in wadeable streams often have less than 100% capture efficiencies for fish abundance (i.e., relative abundance) and are influenced by species and body size, stream habitat and hydrologic conditions, fish behavior, the sampling method used, and the timing of the sampling (Bayley and Dowling 1990, Bayley and Peterson 2001, Price and Peterson 2010). This protocol specifically uses single-pass and three-pass electrofishing because it is considered the most effective method of fish sampling at wadeable stream sites (Barbour et al. 1999) and is the most repeatable and standardized method at the NEON Observatory level. Sampling each site biannually (roughly spring and fall) for the life of the project allows NEON to address some of the temporal biases inherent in fish sampling. Sampling a mix of fixed and random reaches and a mix of single pass and three pass depletion helps data users better calculate fish abundance, species composition, and capture efficiencies.

1.2 Scope

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

1.2.1 NEON Science Requirements and Data Products

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

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

1.3 Acknowledgments

The design and implementation of stream fish sampling methods was based on the guidance from the NEON Fish Sampling Workshop (6-8 March, 2013, Boulder, CO) and attendees D. C. Dauwalter, A. J. Davis, E. A. Frimpong, G. D. Grossman, K. G. Gerow, R. M. Hughes, C. P. Paukert, and D. M. Walters. The sampling protocols herein follows the guidelines recommended by the American Fisheries Society (AFS; Bonar et al. 2009) and have been chosen to align with those of USGS National Water-Quality Assessment (NAWQA; Meador et al. 1993) and USEPA National Aquatic Resources Survey (NARS; Peck et al. 2006, USEPA 2013). NEON would also like to acknowledge the contribution to the evolution of fish sampling methodology, by the Aquatic Biology technical work group.

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.003107	Datasheets for AOS Protocol and Procedure: FSS – Fish Sampling in Wadeable Streams
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.003162	AOS Protocol and Procedure: Wadeable Stream Morphology
RD[13]	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-S®20E	10% eugenol; fish anesthetic
Cm	Centimeter
DC	Direct Current
DNA	Deoxyribonucleic acid
EMAP	Environmental Monitoring and Assessment Program (USEPA)
EHS	Environmental Health and Safety
Hz	Hertz
IACUC	Institutional Animal Care and Use Committee
Km	Kilometer
M	Meter
mL	Milliliter
Mm	Millimeter
MS-222	Tricaine methanesulfonate
NARS	National Aquatic Resources Survey (USEPA)
NAWQA	National Water-Quality Assessment (USGS)
NRSA	National River and Streams Assessment (USEPA)
SL	Standard Length
TL	Total length
USEPA	U.S. Environmental Protection Agency
USGS	U.S. Geological Survey
V	Volt
W	Watt
YOY	Young of the Year

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)

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

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

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

Cathode: 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**).

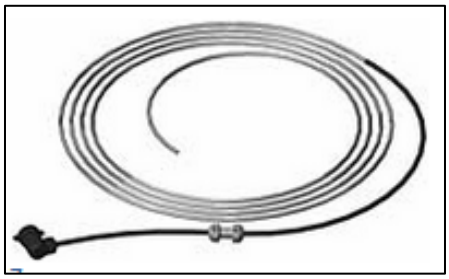

Figure 2. Cathode for backpack electrofishing unit (photo: store.smith-root.com)

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 per unit of time. In relation to pulsed-DC electrofishing, the frequency is measured in pulses per second (i.e., Hz) and can be adjusted. High frequency pulses are associated with increased injuries to, or mortality of, fish.

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

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

Hertz (Hz): Unit of frequency of electrical waves defined as one cycle 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.

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

Pulsed DC: Direct electrical current that is interrupted rapidly.

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

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

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

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

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

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

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

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

3 METHOD

The objective for this document is to outline the sampling protocol and procedures for sampling fish at NEON wadeable stream sites. This protocol describes the use of backpack electrofishing (i.e., DC or pulsed DC backpack electrofishing) to sample fish in wadeable streams at designated NEON sites (1 km stream (500 m McDiffett Creek, D06)).

At each NEON wadeable stream site, up to ten replicate non-overlapping reaches in lengths ranging from 40 – 130 m have been established and are sampled to estimate fish species composition, species diversity, relative abundance, and an indication of the distribution of species within the NEON wadeable stream reach (**Figure 3**). Three permanent "fixed" reaches have been established and up to all three are sampled twice every year using single-pass electrofishing. The remaining seven reaches established are "random" reaches and are sampled following a stratified random design to ensure that the variety of habitat types are sampled equally over time. Up to three random reaches are sampled twice per year (in the spring and fall) then a new set of up to three random reaches are sampled in subsequent years. Random reaches will be sampled via single-pass electrofishing.

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

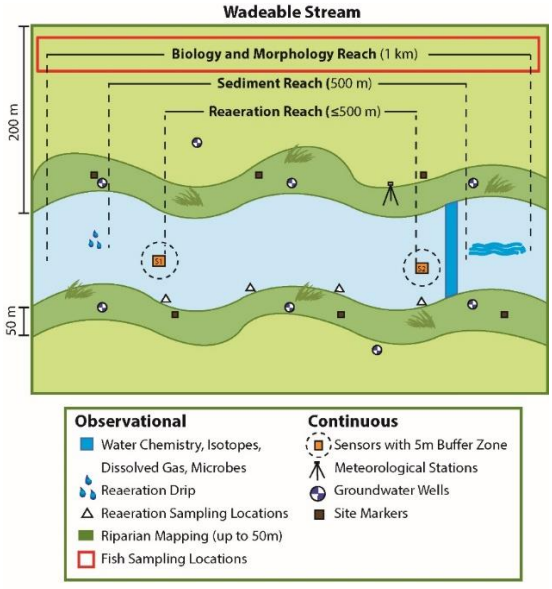

Figure 3. A generic wadeable stream site layout. Fish sampling will occur throughout entire biology and morphology reach (red box).

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

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

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

Quality assurance 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 wadeable streams takes place only during the first (spring) and third (fall) bio-bouts (**Table 1**). Initial sample timing was determined for each stream site using historical data including ice-out, water temperature (or accumulated degree-days), weather, and riparian 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 or hydrological conditions (e.g., late ice-off) 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 wadeable streams on a per SOP basis.

SOP	Site Type	Location	Bout Duration	Bouts Per Year	Bout Interval	Yearly Interval	Remarks
SOP B and SOP C	Wadeable Streams	Up to 6 reaches (three fixed and three random) per bout	3 days	2	Biannual: one bout 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

Scheduling Considerations

1. Sampling corresponds with the first and third sampling bout windows at wadeable stream sites (RD[07]).
    a. Fish sampling must be scheduled within the site-specific bout window but may be pushed passed the bout window due to events such as dangerous weather at the site, high flows on the stream, or logistical reasons. Sampling pushed passed the bout window require Science approval.
2. Fish sampling is 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.

   i. At some sites, flow conditions may be such that sampling may occur earlier than other protocols.

b. Other exceptions to scheduling requirements are:

   i. At D19 Caribou Creek fish sampling may occur first during the 3rd (autumn) bio-bout, to ensure that the *Thymallus arcticus* (Arctic grayling) migration is sampled.

   ii. At D11 Blue River fish sampling may occur first during the 1st (spring) bio- bout, to take advantage of low flow conditions.

   iii. At D14 Sycamore Creek fish sampling may occur outside of the normal bout window to account for low flow conditions and water temperatures that exceed the minimum fish sampling temperature constraints.

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 1, then sampling shall occur when safe conditions allow up to 2 weeks before the start of the biology 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 December 29th, whichever occurs first.

5. A minimum of 2 weeks between fish sampling bouts shall be observed. Sampling bouts should last no longer than 5 days and no less than 3 days, assuming no weather or other unexpected schedule delays.

6. *All three electrofishing passes in a fixed sampling reach must be completed before sundown* of the same day the reach was started, use the National Weather Service projected sundown time to assess if there is enough time to complete all three passes. Fish counting after the third electrofishing pass can continue after sundown.

   a. There must be a minimum of 30 minutes between passes to allow fish to resettle in the reach.

7. The minimum requirement for a bout to be considered complete is completion of one fixed reach and three random reaches, this should be prioritized to be sampled first when possible.

8. If there is not enough time complete the fixed reach, it is permitted to move upstream to the next random reach and sample that reach before the end of the day, and then sample the fixed reach on the proceeding day of sampling.
    a. Fish are to be discarded below the unfished fixed reach if any upstream random reaches are completed before the downstream fixed reaches.

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 attributes. 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 2**). 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 3** 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 2. 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 3. 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

Fish sampling in wadeable streams shall occur only during daylight hours for safety and consistency of capture efficiency. A minimum of two weeks between sample periods must be observed. If environmental conditions interrupt scheduled sampling, follow the contingencies outline in **Table 4**. Always endeavor to fully sample all scheduled reaches, however if only a truncated sampling event can be carried out, one fixed reach at minimum must be sampled for fish sampling to be considered a complete sampling bout and data to be published.

Table 4. Contingency decisions for FSS – Fish Sampling in Wadeable Streams protocol.

Delay/ Situation	Action	Outcome for Data Products
Hours	If weather conditions deteriorate and conditions become unsafe (e.g. approaching thunderstorm, rapid increase of water level in the wadeable stream), 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 each bout. For example:

- Logistics – e.g., insufficient staff or equipment
- Environment – e.g., deep snow, flooding, inclement weather
- 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 Disturbance Data Collection Protocol for more detail (RD[08]).

If sampling is impractical as a result of severe drought (dry), the stream is frozen, high flows (unsafe to wade), 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) should be fully sampled. Note the cause of the minimized sampling effort on the "Reach Condition" section in the mobile field device.

Missed or Incomplete Sampling Terms

Terms that inform Missed or Incomplete Sampling include:

- **Protocol Sampling Dates**: Bout-specific sampling window 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**).

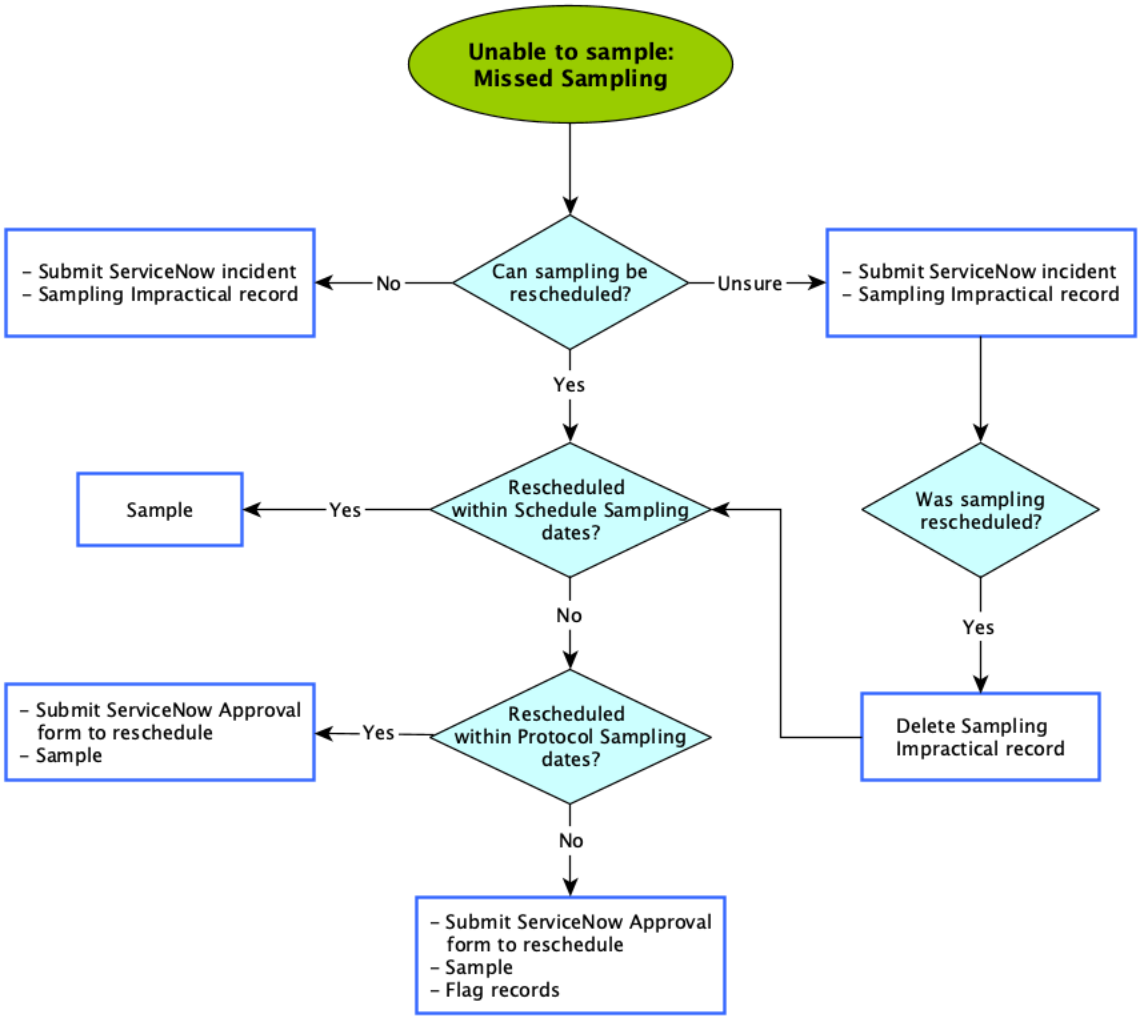

Figure 4. The documentation to account for a Missed Sampling event depends on the situation for each sampling unit not sampled per bout that is not sampled. Diamonds represent decision points and boxes describe the required action. Required actions may include: a) Submitting a ServiceNow incident, b) 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 through a ServiceNow Incident.
    a. For Missed Sampling that is Rescheduled, there are some cases that require approval by Science and Operations see **Figure 4**.
    b. Consult **Table 5** 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.
3. For each Missed Sampling record, the **Sampling Impractical** field must be populated in the mobile collection device (**Table 6**).
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 5. Guidance for responding to delays and cancellations encountered during implementation of the Fish Sampling in Wadeable Streams 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 6. 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 three-person team (i.e., not the

time it takes at the beginning of the field season). Use this estimate as framework for assessing progress (**Table 7**). If a task is taking significantly longer than the estimated time, submit a Service Now incident. 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 (D08) 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 7. Estimated staff and labor hours required for implementation of the Fish Sampling in Wadeable Streams 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 Segment Set-Up	45 minutes fixed/random	3 per fixed/random	2:15 h per fixed/random reach
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 D: Fish Handling	1-2 h per reach	3	3-6 h per reach

4.7 Sampling Specific Concerns

1. Fish sampling should not occur while other sampling activities are occurring upstream in the NEON reach that may disturb sediments, impair water clarity, move fish or otherwise affect hydrology of the system.
2. 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 4**) submit a schedule change request.
3. 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.

4. 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 >18 C in salmonid bearing streams, >26 C in non-salmonid streams, unless otherwise (higher or lower) stated in site specific permit, then defer to permit's stated temperature limit.
        i. If temperature is above criteria at the reach you planned to sample, and you believe that another scheduled reach might have cooler water temperatures due to shading or groundwater inputs, it is permitted to fish that reach if temperatures are below threshold.
    b. Frequently change stream 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 holding buckets in well shaded areas, and/or put vegetation in holding water as micro-shade.
    e. Use anesthetic to reduce handling stress.
    f. Segregate predator species from prey-species.
5. 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 after fish sampling ends that day. Science staff will work with domain staff to identify if there is a problem and/or if any changes are needed with sampling. It is strongly recommended that field staff 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 a trouble 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)

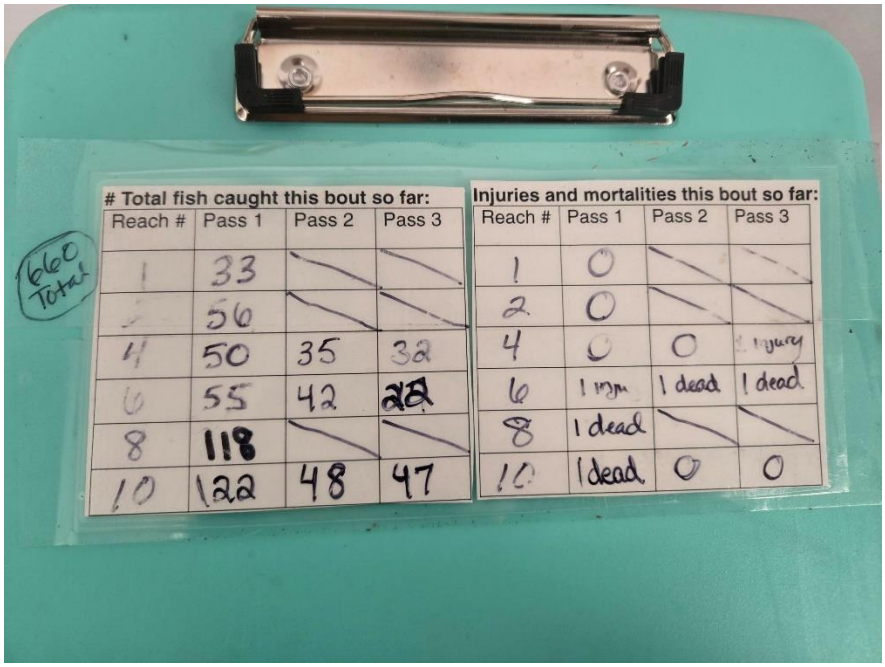

Figure 5. An example mortality tracker that can be used in the field during sampling. The mobile data entry application also has its own mortality tracker in case of any transcription errors during manual tracking.

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 on site communication with other team members if working outside of voice range, such as a two-way radio.
2. Field scientists should be aware of any site-specific hazards of that particular location (i.e. current status, potential for flash floods, poisonous snake presence etc.).
3. Activities in streams should only be performed when flow conditions are safe. Do not attempt to wade a stream where velocity x depth is ≥ 0.93 m2/s; (Lane and Fay 1997). Please refer to the Open Flow Application in Aviary to confirm the most recent discharge of your site and whether or not it is exceeding safe fishing
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. Field scientists are strongly encouraged to stretch and warm up before fishing. Wading through the stream is physically exerting on the body and improper preparation can result in injury.

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

1. One member of the fish sampling crew must be fully trained by the equipment manufacturer and all other associated personnel shall be trained on safe operations through established and approved training program (see Section 6.1 Training Requirements).
2. Audible signals must be used to alert field scientists when electrofishing equipment is in operation.
3. Chest waders and heavy-duty rubber lineman gloves (rated to 1,000V AC/1,500V DC) must be worn while working near an electrofishing unit.

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. At stream sites where the water level is less than knee deep at the maximum depth, rubber boots or hip waders are allowed. Follow the wader material guidance above.
6. Before sampling, inspect waders, boots, and gloves for leaks. Leave the water immediately if waders or gloves develop leaks.
7. A portable automated external defibrillator (AED) must be available in the field for all electrofishing work. The AED and First Aid Kit can be stored in a work vehicle, boat, or other known location as long as it is within a 6-minute walk from the active sampling location.
8. Avoid operating near bystanders, pets, or livestock that are in or near the water.
9. Electrofishing must be suspended if anyone feels a shock, however minor, for investigation and repair of equipment.
10. Avoid operating an electrofishing unit in heavy rain (light rain is acceptable) as this can increase the probability of electrical shock.
11. 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. Field scientists must pass the Electrofishing Safety Exam with a score >80%. Additional training resources include Wader Safety training which is available through the NCTC Electrofishing Safety training resource videos. Lastly, all field scientists shall complete the Collaborative Institute Training Initiative's (CITI) Institutional Animal Care and Use Committee (IACUC) fish and amphibian training. Field scientists must pass the final tests for each training (fish and amphibian) with an 80% or better. The CITI IACUC trainings are good for up to three years. Provide your manager with a copy of each applicable certificate documenting the successful completion of each required training. See the AFS Fisheries Safety Handbook for additional fish sampling safety information.

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

External Training Resources:

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.
http://nctc.fws.gov/courses/csp/csp2202/resources/index.html

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

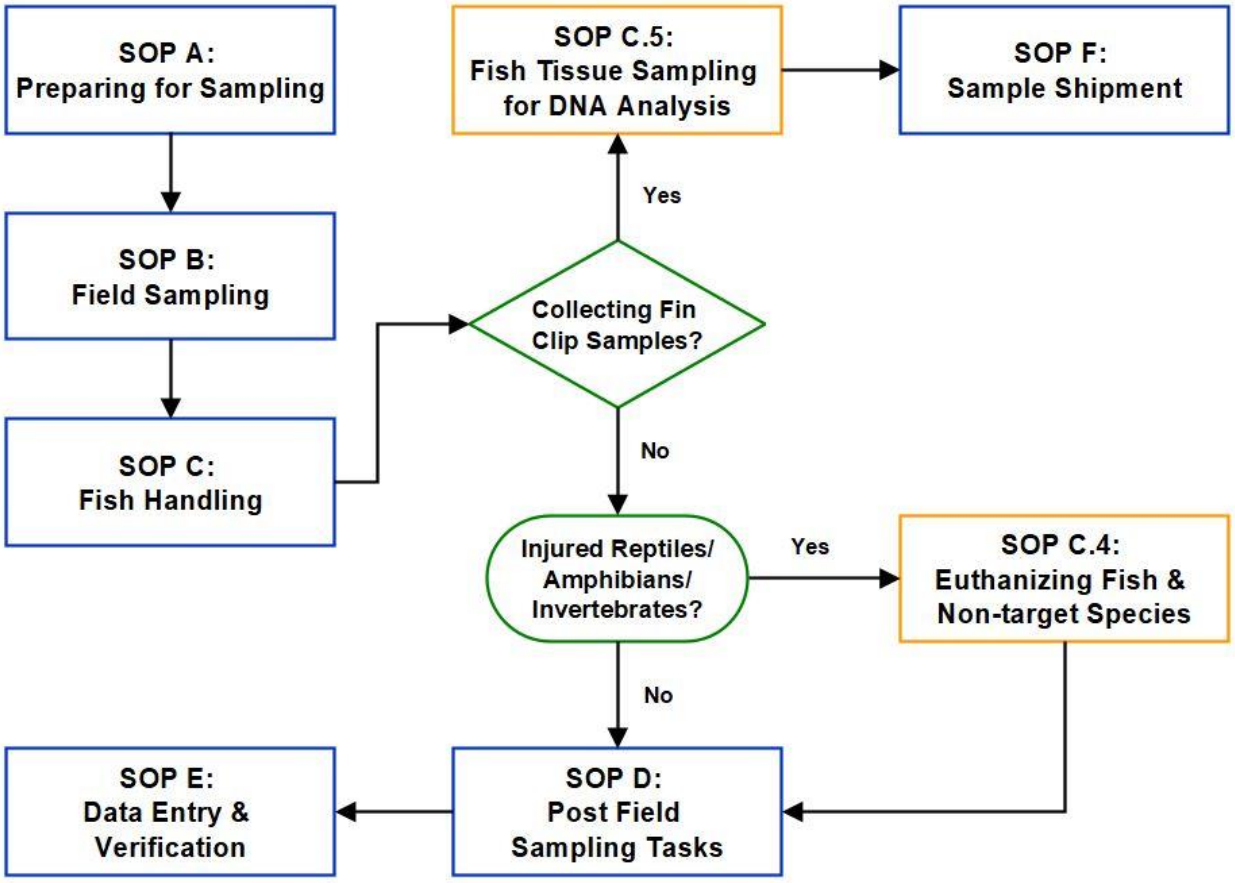

Figure 6. A high-level workflow diagram that visually shows how the separate SOPs are sequentially connected.

- **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.
    a. Notify the site host of the dates and times of the fish sampling activities.
    b. If required, notify responsible state agency of the dates and times of the fish sampling activities prior to sampling.
    c. Retain a copy of the collection permit with the sampling crew during the sampling activities.
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.

1) 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.

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

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

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

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

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

vii. 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 Rite 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 4°C to 105°C 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**).

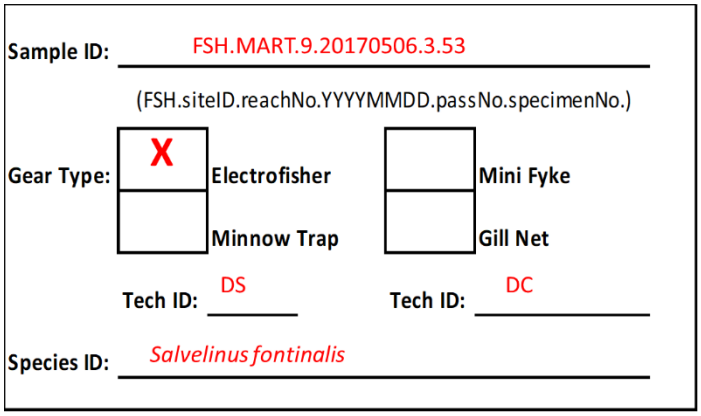

Figure 10. Example specimen label for a fish/bycatch voucher.

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**).

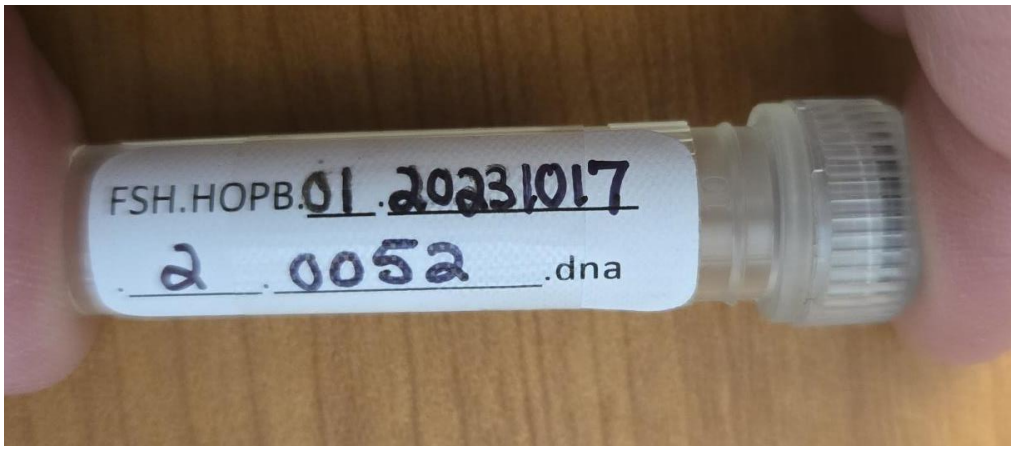

Figure 11. Example human readable sampleID label for DNA fin clip sample.

Table 8 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 8. Barcode requirements for sample types generated by the Fish Sampling in Wadeable Streams 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_nonTarget 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]".

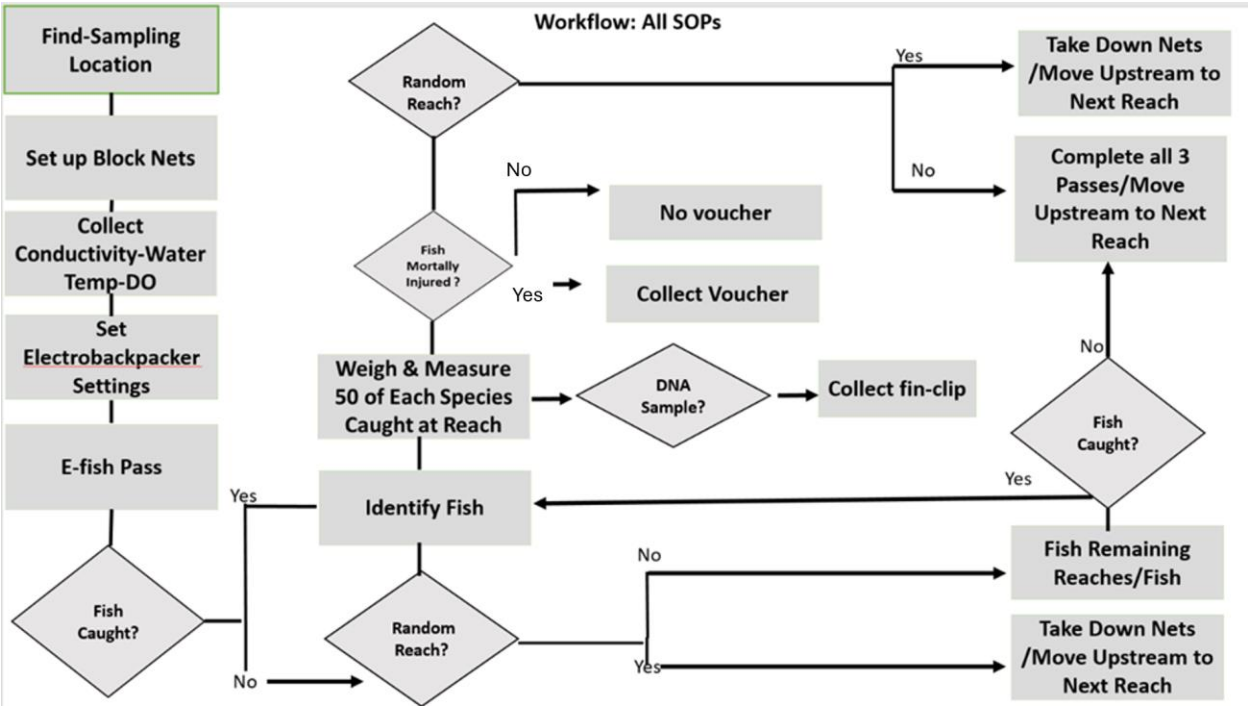

Figure 12. An expanded workflow diagram of SOP B: Field Sampling.

B.1 Fish Sampling Field Set Up

1. Navigate to the most downstream sampling reach selected for this sampling bout using GPS points and fish plot markers.
    a. Sampling must begin at the most downstream fixed sampling reach first, and then proceed to the remaining random reaches working from downstream to upstream to minimize disturbance.
    b. For site specific random reach sampling order consult **Appendix D**.
2. Set up block nets.
    a. Setting up and maintaining net integrity is of vital importance to a successful fish sampling bout. This can be difficult at wadable streams sites because: flow is constantly pushing on the nets, stream debris floats into and gets caught in block nets, and substrate can be too hard or too soft for secure fence post installation.
    b. Always secure the downstream block net first, followed by the upstream net.

***NOTE**: MCDI and TECR have permanent, reach bisecting fish barriers. Set block nets at these barriers following the direction below. More details regarding how to sample these reaches is discussed in **Appendix D**.*

i. Section below the barrier: Sample the section downstream of the barrier first. Set up the downstream block net at the beginning of the reach and use the permanent barrier to block upstream fish movement while sampling.

ii. Section above the barrier: The reach section above the barrier must have two block nets, one to block the top of the reach, and the other directly before the barrier, preventing fish from being washed over the barrier.

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.

c. At all wadeable stream reaches, secure a 1/8 mesh size block net at the reach boundary using steel fence posts or existing vegetation (e.g., tree). The use of trees can be highly effective where and when fence post cannot be secured. However, if the choice to use vegetation is made, you must ensure that you have permission from the site host, your permit, and the Domain manager (**Figure 13**).

d. Relocate any debris (i.e., tumble weeds) in the stream that interferes with the block net deployment. Do not remove in-stream habitat features (i.e. large wood) to install block nets. Instead, move the net up or down stream of structure.

e. Pull the net across the stream ensuring enough slack in the lead line (bottom of net) to reach down to the stream substrate.

f. Anchor lead line to substratum using large rocks (**Figure 14**) or block net anchor stakes. Sandbags may be used where native sand, gravel, or rocky material is available and is used to fill the sandbags with.

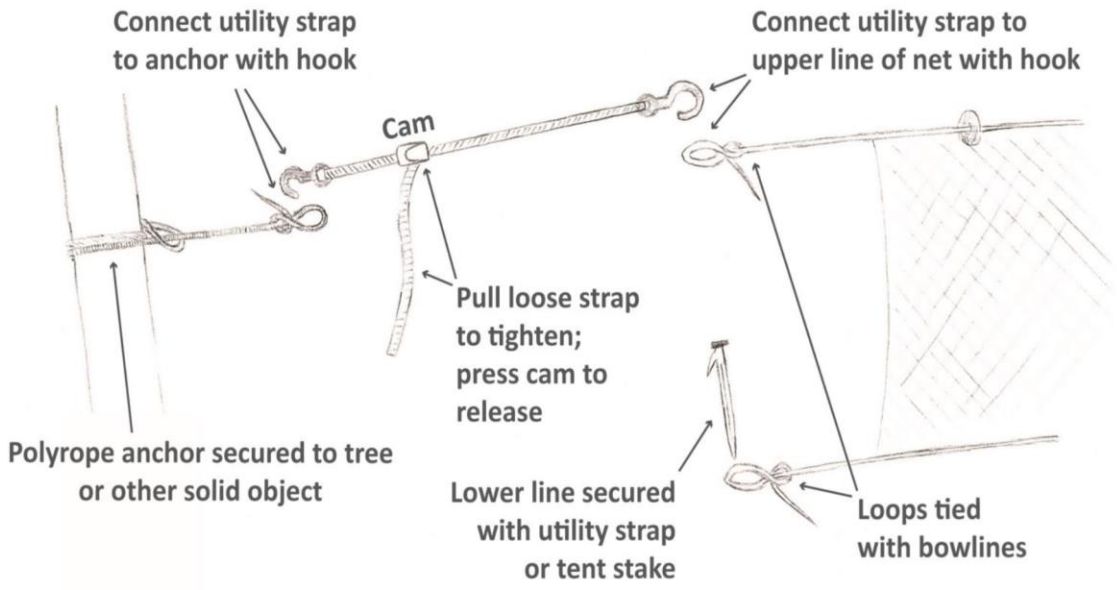

Figure 13. Example of wadeable stream block net set up.

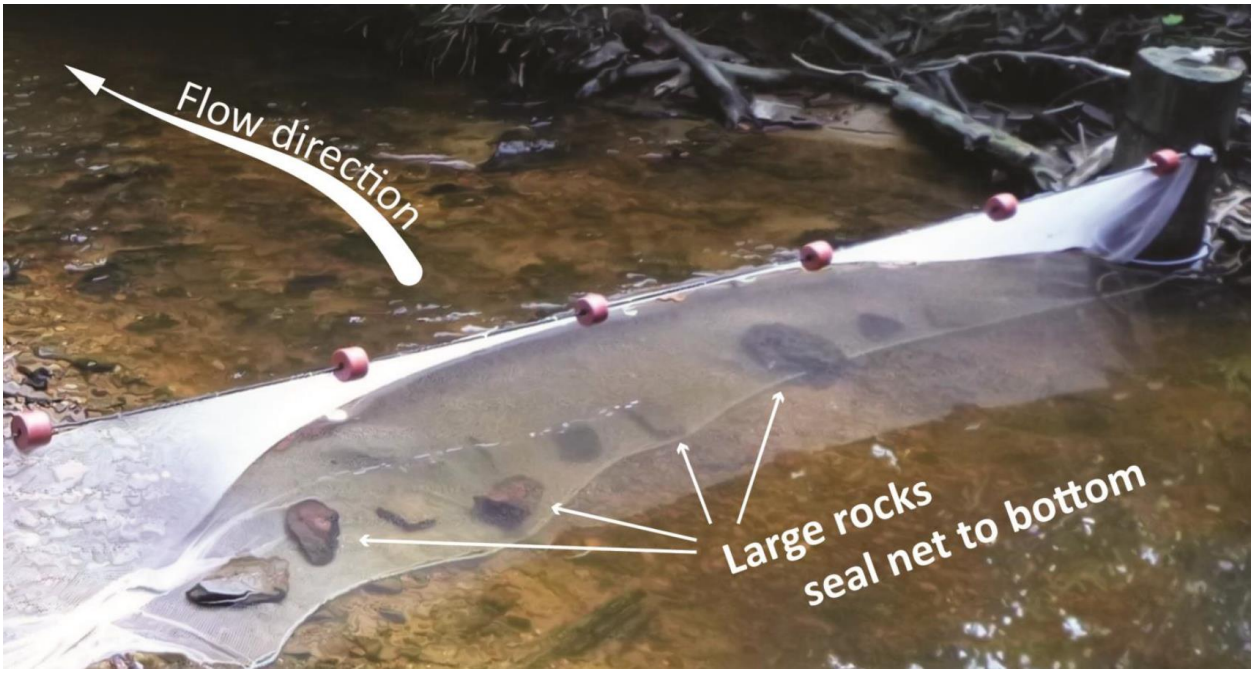

Figure 14. Potential net set up strategies for difficult block nets installations (Dan McGarvey).

3. Stage 5-gallon buckets filled with native water, with battery-operated aerators on the bank along the stream reach for holding fish during electrofishing. For stream sites where hundreds of fish are typically captured, use several buckets staged every 15-25 m though out the sampling

reach. Be sure to have one crew member rinse buckets with native water and monitor captured fish.

a. Be sure to have enough buckets to segregate predators from prey (e.g. char from sculpin)

b. Locate an appropriate shaded location (e.g., flat ground under a tree) as the fish processing site along the stream bank near the targeted sampling reach. Place processing equipment (e.g., fish measuring boards, digital scale, plastic weighing tray, and sample bottles, preservative) at this location.

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 appropriate waders for the site (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.

1. Assemble Electrofisher

   a. All sites with an average wetted width that during the sampling season is < 7 m, must only use one electrofishers.

      i. All sites with and average sampling season wetted width > 7 m, must use 2 electrofishers (currently only the BLUE site uses 2 electrofishers).

   b. Assemble anode pole (**Figure 1**).

   c. Connect the cathode (**Figure 2**) and anode to the backpack electrofishing unit (**Figure 15**).

   d. Connect the battery to backpack electrofishing unit and strap in the batteries 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 16**).

Figure 15. Cathode and anode connections on backpack electrofishing unit.

Figure 16. Left: Battery location and secure placement in the backpack electrofishing frame. Right: lithium-ion battery adapter.

2. Test the electrofisher
    a. Test the backpack electrofisher and shock settings in the section of stream between the downstream permit boundary and the most downstream sampling reach.
    b. Measure the water temperature and specific conductivity using the handheld YSI multimeter at the test reach.
        i. Effective electrofishing depends on matching the backpack output voltage with the water conductivity.

ii. High water temperatures can cause fish mortalities (> 18 C for salmonids, > 26 C for non-salmonids).

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

d. While the electrofisher operator is standing in the stream, 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 9**).

Table 9. 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 z	

3. 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.

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

5. 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.

6. 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 9** and stay within the approved electrofisher settings.

7. The goal is to immobilize fish using the lowest settings possible at the site to avoid harming sampled fish.

8. Start by increasing **voltage** in 50-volt increments.
    a. Do not increase above maximum setting allowed for conductivity reading **Table 9**.
9. 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 9**.
10. 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.
11. 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.
12. If fish are immobilized during testing, use dip nets to capture individuals and place in a bucket ½ - ¾ full of stream water with battery-operated aerator. Once fish are swimming normally release in test reach in an area they will not be shocked again.
13. Examine captured fish for signs of injury (e.g., bent backs, dark bruising, hemorrhaging of the gills (**Figure 17**). 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.

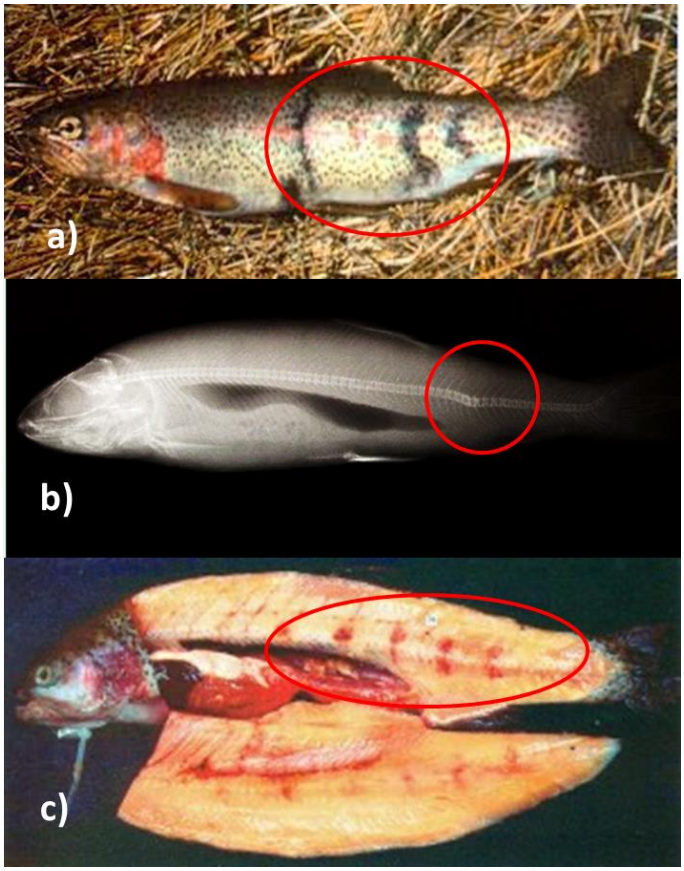

Figure 17. Examples of electrofishing injuries to fish, a) bruising or "burn" marks, b) spinal injury, and c) internal hemorrhaging. Photos from Snyder 2003.

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. Proceed to the downstream block net of the first sampling reach.

   a. Begin fish sampling at the furthest downstream scheduled reach and work upstream throughout the sampling bout. Sampling schedule and conditions may require that upstream reaches are sampled before a downstream reach. Minimize impacts to downstream reaches that have not been sampled when accessing upstream reaches.

   b. If unable to sample the reach, indicate by recording sampling impractical.

   c. Electrofishing activities must take place at least 5 m from any in-stream electronic instrumentation (sensor sets).

3. At every fish sampling reach, there are several details to record on the mobile device.

   a. Record the dominant habitat type (riffle, run, pool, step pool). Also, if there is a mix of habitats, record the subdominant habitat type.

   b. Document if the reach condition could affect the data collection (normal flow, segmented pools, disconnected side channel, other low flow <100 m sampled, high flow < 100% of reach sampled, heavy vegetation, skipped reach, beaver activity). If multiple reach conditions affect data collection, document the most impactful conditions.

3. Measure and record the water temperature, conductivity, and dissolved oxygen within the fish sampling reach 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. Record the pass start date and time on the mobile device so that conductivity, turbidity, and other water quality measurements from the in-stream 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.

5. It is critical that the EF timer on the back electrofisher is reset before every pass.

6. The crew member operating the backpack electrofisher should walk into the stream, ensuring that the cathode (i.e., rattail) is submerged as much as possible, while holding the anode pole in one hand (anode 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.

7. The other crewmembers will enter the stream 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 the stream is relatively large or where many fish are typically captured, it may be useful to add additional crewmembers to help distribute the work effort.

8. Turn the electrofishing unit on and notify the other field scientists. Confirm that all field scientists are ready to begin.

9. 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.

10. 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.

11. Slowly sweep the anode inside (i.e., upstream) along the downstream 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.

12. After sweeping the block net, the electrofisher operator should then turn upstream and slowly sweep the anode across the stream channel to expose all available habitats to electricity.

    a. This may require slowly walking from bank to bank in streams wider than 2 m.

13. As the anode is moved across the stream, the netters will capture drifting, immobilized fish.
    a. Dip nets should be held as close to the stream substrate as possible without picking up excessive substrate or debris.
    b. Be sure that the appropriate net size is used depending on the stream size or conditions. Generally, smaller nets are used in smaller streams or shallow conditions. Larger nets perform better in larger streams that are relatively deep with steady flow.
        i. Dip net mesh is a decision made at the site level by Field Science
            1) Mesh size should be small enough so that the smallest fish on site does not escape through the holes.
            2) But not so small that the push of water makes keeping the net submerged or sweeping the net inefficient.
            3) If help is needed to determine dip net mesh size, create a Service Now ticket asking for advice from Science.
    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.

14. Always remove fish from dip nets and place in buckets to minimize injury to the fish.
15. At all NEON stream sites by-catch (any non-fish caught during fish sampling), must be processed and recorded following the sites applicable federal and state regulations require.
16. Sampling will continue upstream in a zig-zag pattern across the channel with attention to sampling all complex instream cover (e.g., overhanging or aquatic vegetation, woody debris, undercut banks).
17. 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 from the downstream direction 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.
18. If field staff have reason to suspect that while fish sampling, block Net Integrity might be compromised, and there is enough staff, it is good practice for one field scientist to periodically check the downstream and upstream nets and ensure that nets stay securely installed. Remove debris that might compromise Net Integrity.
    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 and this is a random reach or the 1st pass of a fixed reach - STOP fishing and:

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

2) Re-establish and secure the block nets a minimum of 12 hours after you ended the last uncompleted sampling event and when safe to do so. Then, re-initiate sampling at that reach beginning with the first pass.

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

1) Release fish to the sampling reach, 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 reach 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 a minimum of 12 hours after the initial sampling attempt or when safe to do so. Then, re-initiate sampling at that reach 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!*

19. 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 stream 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 stream.

        i. If enough staff are available, it is strongly encouraged that there is a designated individual in charge of managing 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 stream 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 must ensure there are enough staff on hand such that the ID staffer can enumerate fish while the remaining staff continue sampling.

20. When the crew reaches the upstream block net, the electrofisher operator should slowly sweep the anode downstream of the block net as fish may have moved upstream to avoid the electrical field.
    a. Make sure the netters are in position. Then the backpack operator will sweep the anode along the base of the block net. For nets set in water deeper than a meter, be sure to sweep the block net along the mid water column.
    b. Inspect net and remove any fish or bycatch stunned and trapped in net.
21. 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.
22. Turn the electrofisher off, remove and place on the bank with anode and cathode still attached.
23. 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.
24. If the reach is braided and time constraints prohibit the electrofishing of each braid, start by fishing the braid with the "field estimated" greatest amount of flow.
    a. Continue fishing subsequent channels; working from the channel with the greatest amount of flow to the channel with the least amount of flow, fishing until time constraints requires you to stop.
    b. In instances where it is difficult to determine which channel has greater flow defer to the channel sampled during the last sampling bout.
    c. On the mobile device mark that the reach has multiple channels, after fishing indicate the number and percentage of channels fished.

Note: *This only pertains to dynamic systems with constantly changing braided channels, at sites where the reach has established multiple channels, the expectation is that all those channels are sampled.*

25. Once the reach has been completely sampled, measure the reach length from the downstream block net to the upstream block net following the thalweg. Record the length on the mobile device.

26. If this is a random reach proceed upstream to set up and sample the next reach if time allows.
27. If this is a fixed reach, repeat Steps 5-26 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.
28. Remove the downstream block net after Pass 1 (random reaches) or Pass 3 and processing (fixed reaches) have been completely fished.
29. Remove the upstream block net if it is not needed for the next reach (e.g., this net may serve as the downstream block net for the next reach if reaches are contiguous) or there is not enough daylight to continue with the next reach.
30. After the last pass of the day, 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.

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 50 individuals of each species captured at each reach, site dependent. 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

1. 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 in the measuring tray.

8. Hastily measure the weight of the fish on the 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 some error in the measurement; however, this approach ensures that the fish remain in closer-to-ambient stream conditions and aren't exposed to the air for as long. This is particularly important for smaller fish that are more susceptible to exposure.*

9. In the event that 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 10** 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 10. 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 18**) to the nearest millimeter and record on the mobile device.

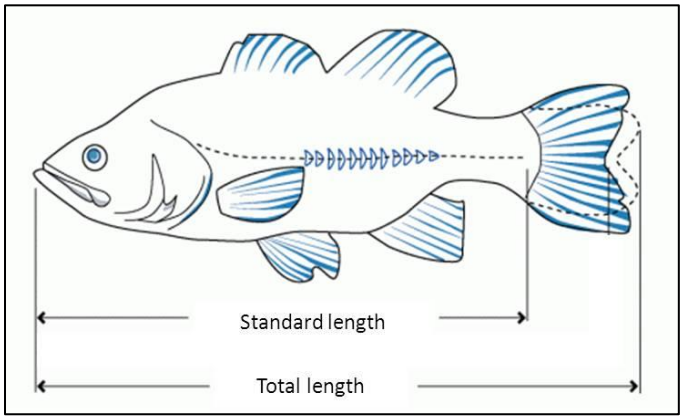

Figure 18. Measure the total length of each fish by pinching the fork together.

13. Inspect the fish for DELTS (deformities, including eroded fins, external lesions, parasites, and tumors) (**Figure 19**). 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.

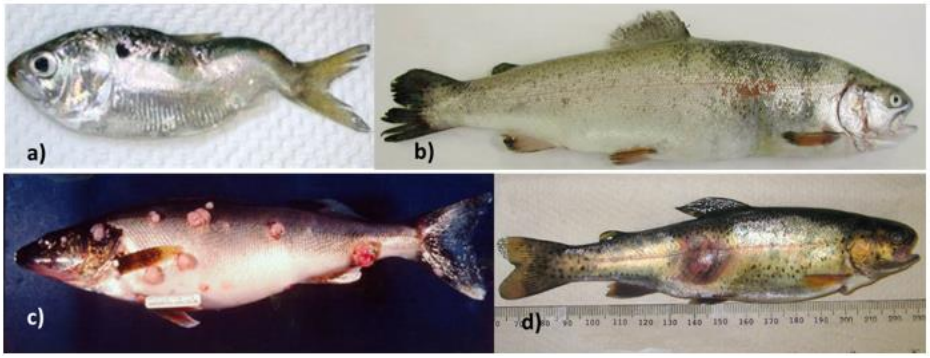

Figure 19. Example DELTS, a) deformity, b) eroded fins, c) tumors, and d) lesion. Photos by a) Kane 2005, b) Leander 2017, c) Cornell 2009, and d) Photo by Craig Banner 2004.

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 stream 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. Make sure the recovery buckets also have a mesh cover to prevent fish escape.
    c. Segregate predator species from prey species, and large fish from small fish in buckets.

Note: *At BLUE only sample up to 30 fish of each species per reach. If more than 30 are caught, follow bulk counting guidance below.*

Note: *AT SYCA only sample up to 30 fish of each species per reach IF stream temperature is between 22-26°C. If more than 30 are caught, follow bulk counting guidance below.*

17. Repeat Steps 5-16 for up to 50 fish of each species captured at a reach, site dependent. When less than 50 fish per species are identified, all of them shall be weighed, measured, and inspected for DELTS.
    a. If > 50 fish of a species are caught at the reach level, only the first 50 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 50 individuals of one species, randomly choose the first 50 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 stream immediately downstream of the downstream block net, unless the field ecologist determines it easier or better for the fish to be

released immediately above the upstream block net. Releasing fish above the upstream block net is not permitted if the upstream reach is scheduled to be sampled next.

a. If sampling at the furthest downstream reach (fish reach 1), place processed fish within the initial 20 m test reach within the permitted boundary.

b. If sampling is complete in a reach prior to completion of fish processing, upon completion of processing release fish back into reach.

c. If sampling disconnected pools, release fish in other suitable pools downstream or disconnected from the next upstream reach.

d. With the use of 10% eugenol (AQUI-S®20E), treated freshwater fish species may be immediately released following recovery; no withdrawal time is required.

e. 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 stream) will only occur where permitted. Field disposal of anesthetic will take place at least 10 m from the bank away from sensor sets or groundwater wells so as not to 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 11**) 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 11. 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 12** 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 58**.

Table 12. 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 stream water. Mix thoroughly.

b. Transfer fish from the holding bucket to the bucket containing the euthanizing solution with the small handheld dip net.

c. Monitor fish until respiration ceases. Fish should be exposed to the lethal dose of 10% eugenol for a minimum of 10 minutes.

d. 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 stream water in the field.

   a. Add 1 L of stream water and 10 mL of MS-222 stock solution to a new 5-gallon bucket. Mix thoroughly. Label the bucket so the crew knows that it contains the MS-222 euthanizing solution.

   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 20**). 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 20. 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. 1st stage injection solution - 1% MS-222:

      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 13. 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 21**).

Figure 21. 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 22**).

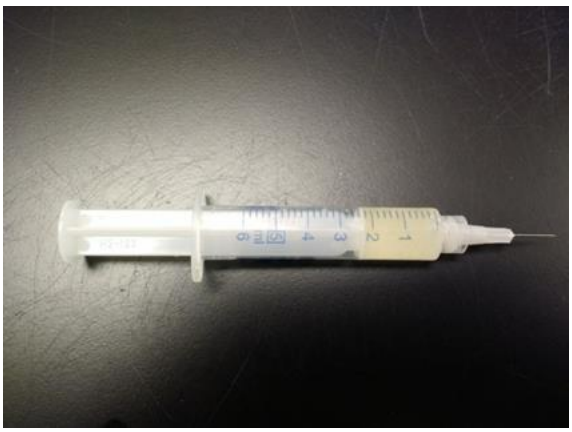

Figure 22. 2nd stage 50% (v/v) unbuffered pharmaceutical MS-222 solution. Note yellow cloudy appearance of solution.

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 21**).

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 23**).
    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 23** 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 Wadeable Stream Field Datasheet but do not collect a photo with a separate camera as the images will not be joined with the record.*

Figure 23. 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 24**). 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.

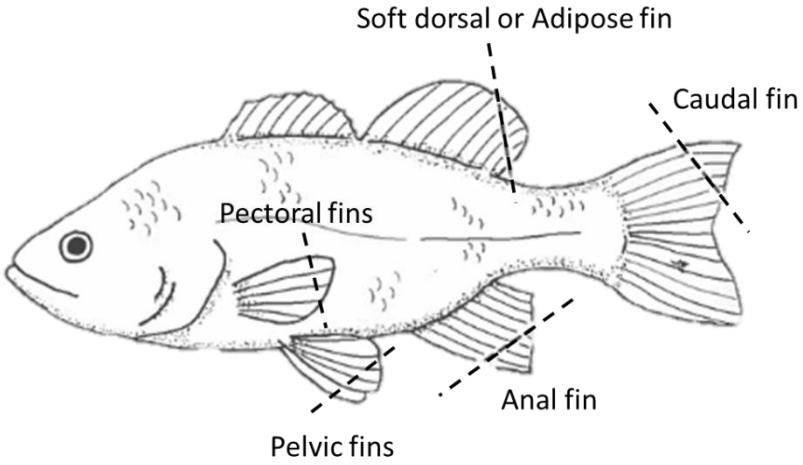

Figure 24. Optional fins to clip for tissue sampling.

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, Amphibian, and Reptile Voucher Specimen Preservation

1. Fish or 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 or other 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.

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 25**).

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

Figure 25. 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 Wadeable Streams 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 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.
2. To document whether a location is compromised according to the criteria above:
    a. Review **Sampling Impractical** records from the (AOS) Fish [PROD] application and Portal data to identify locations where sampling was scheduled but was not completed due to environmental or site management factors.
3. Create an incident with the following naming convention to document the missed sampling and compromised location: 'AOS Sampling Incomplete: FSH – [Root Cause Description]'
    a. Example: 'AOS Sampling Incomplete: FSH – Could not sample reach due to changes in stream morphology'

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

D.2 Ending the Sampling Day

1. Refreshing 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 come in contact with stream 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, plotIDs) 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 14. 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– Start sampling at the furthest downstream reach scheduled for sampling and work upstream. Set up block nets at downstream and upstream boundaries of the sampling reach and place 5 to 10 equally spaced 5-gallon buckets with battery-operated aerators on the bank along the stream reach. Take care to minimize habitat disturbances. Set up shaded level fish processing area prior to the start of electrofishing, to minimize stress on fish.

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 across the stream in a zigzag pattern and capture drifting, immobilized fish. Place immobilized fish in mesh covered 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 50 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 stream water and a battery-powered aerator for later release. Once revived, release the fish downstream 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.

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 instream cover types (e.g., overhanging or aquatic vegetation, woody debris, undercut banks).
- ☑ Observe a minimum of 30 minutes between passes to allow fish that were not captured to recover.
- ☑ Netters should frequently inspect their nets to minimize injury to fish by continuous exposure to electricity.
- ☑ Never put hands in the water to capture fish while activation switch is depressed.
- ☑ If endangered species are caught, identify and 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 stream downstream of the block net.

Sample processing:

- ☑ If an endangered species is mortally injured, stop all fish sampling activities, follow the permit guidance before applying euthanasia. Also, follow the permit guidelines for the disposition of killed endangered species.
- ☑ If more than 50 individuals of one species are captured, anesthetize, weigh and measure the first 50 (site dependent) and simply bulk 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 wadeable stream 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 random reaches in the order established in Appendix D.2, Table 16.

Table 15. Fixed reach prioritization for each site.

Domain	Site	Top priority fixed reach	Backup fixed reach	Last resort fixed reach
D01	Hop Brook	10	4	6
D02	Lewis Run	1	9	4
D02	Posey Creek	9	2	7
D04	Rio Guilarte	5	2	9
D04	Rio Cupeyes	8	6	3
D06	Kings Creek	1	4	3
D06	McDiffett Creek	4	2	NA
D07	LeConte Creek	1	10	4
D07	Walker Branch	5	1	3
D08	Mayfield Creek	9	2	7
D10	Arikaree River	9	1	6
D11	Pringle Creek	10	3	7
D11	Blue River	4	7	2
D12	Blacktail Deer Creek	2	3	6
D13	West St. Louis Creek	7	4	1
D14	Sycamore Creek	4	6	10
D15	Red Butte Creek	7	5	2
D16	McRae Creek	4	8	6
D16	Martha Creek	3	7	1
D17	Teakettle Creek	5	6	NA
D17	Upper Big Creek	1	7	4
D18	Oksrukuyik Creek	9	3	6
D19	Caribou Creek	10	1	6

D.2 Randomized Reach Selection Per Site

Randomized reach order is shown for each site below for sites with 10 fish reaches. See Appendix E.2 for sites with shortened reaches. Skip numbers that either have been chosen as a fixed reach, or do not exist at the site (i.e., sites that are < 1 km may have fewer than 10 reaches). If sampling is impractical as a result of severe drought (dry) or that the stream is frozen, then indicate this for any affected reach on the mobile device. Should environmental conditions affect the ability to fully sample a stream reach, commence with sampling but note the cause on the "Reach Condition" section in the mobile device.

Table 16. Randomized reach selection per site.

Domain	Site	Randomized reach order
D01	Hop Brook	9, 5, 3, 8, 1, 2, 7
D02	Lewis Run	6, 5, 7, 3, 2, 8, 10
D02	Posey Creek	6, 10, 4, 1, 5, 8, 3
D04	Rio Guilarte	8, 7, 4, 6, 1, 3, 10
D04	Rio Cupeyes	5, 10, 4, 7, 1, 2, 9
D06	Kings Creek	9, 8, 10, 2, 6, 5, 7
D06	McDiffett Creek	3, 1, 5
D07	LeConte Creek	3, 5, 9, 2, 6, 8, 7
D07	Walker Branch	9, 4, 2, 6, 10, 7, 8
D08	Mayfield Creek	4, 6, 8, 1, 3, 10, 5
D10	Arikaree River	7, 5, 2, 3, 4, 8, 10
D11	Pringle Creek	8, 6, 2, 5, 9, 4, 1
D11	Blue River	10, 5, 9, 3, 1, 8, 6
D12	Blacktail Deer Creek	7, 8, 1, 10, 4, 9, 5
D13	West St. Louis Creek	2, 9, 8, 10, 5, 3, 6
D14	Sycamore Creek	7, 9, 1, 8, 2, 5, 3
D15	Red Butte Creek	3, 4, 1, 9, 10, 6, 8
D16	McRae Creek	7, 9, 1, 2, 10, 5, 3
D16	Martha Creek	5, 6, 9, 2, 4, 10, 8
D17	Teakettle Creek	9, 7, 3, 4, 8, 10, 1, 2
D17	Upper Big Creek	2, 6, 3, 5, 9, 8, 10
D18	Oksrukuyik Creek	4, 5, 8, 10, 1, 2, 7
D19	Caribou Creek	5, 9, 8, 2, 3, 4, 7

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
HOPB.2025.spring	10	9, 5, and 3	4 and then 6
HOPB.2025.fall	10	9, 5, and 3	4 and then 6
HOPB.2026.spring	10	8, 1, and 2	4 and then 6
HOPB.2026.fall	10	8, 1, and 2	4 and then 6

Figure 26. Example scheduling of four fishing bouts for Hop Brook. (Note – this is an example and does not represent the existing random reach ordering that currently exists at HOPB).

D.4 Two Backpack Electrofishers Approach

At wadeable stream sites where the average wetted width of the entire biological reach is greater than 7 m, two backpack electrofishers can be used simultaneously to sample fish (Johnson et al 2007). The use of two backpack units improves the effectiveness and capture of fish in wider stream segments.

Follow the electrofisher field set-up procedures described in **SOP B**. Test each unit individually then together in the 20 m test reach. Be sure to avoid any anode-to-anode contact, this may result in damage to the backpack units. Also, if the electrical fields overlap, the backpack unit may produce an audible warning (or shut off). Should this happen, space out the anodes to minimize interference. Any changes should be made so that the initial settings are identical on both units. Maintain electrofisher settings at the lowest level that allows for the effective capture of fish. Record the initial frequency, duty cycle, and voltage settings on the mobile device for each electrofishing unit and reset the timer on both units before each pass.

1. Proceed to the first sampling reach, starting from the furthest downstream reach and working upstream. Follow the electrofishing guidance in **SOP B**.
2. Measure and record water temperature and conductivity using the handheld conductivity meter at each reach before every pass. Record on the mobile device.
3. The electrofisher operators should work side by side, each working one-half of the wadeable stream. The operators need to work together to push fish upstream while covering a variety of habitat types. Do not let one get ahead of the other. If you are too close, the electrofisher backpack will beep or shut down; if you are too far from each other, fish will escape.
4. There should be a minimum of three netters (four is ideal) to cover the two electrofisher operators. Each operator should have a designated netter with the third netter working between the groups to collect immobilized fish in a bucket. The third netter will serve as the crew lead and monitor the electrofishing operations. The lead will ensure that fish are effectively immobilized, that captured fish in the buckets are remaining healthy, and that the

crew is operating safely. The lead will also ensure that any potential bystanders are not entering the water.

5. If one operator is sampling along the shoreline, the second operator should be sampling at the mid-channel. Operators should not each be sampling the opposite shorelines simultaneously as fish will likely move downstream at the mid-channel. Maintain good communication between to two operators so that no habitat features are missed and to maximize spatial coverage while minimizing anode interference.
6. When encountering pool habitat or log jams, the two teams can work together. One operator should stay at the downstream edge of the pool or jam (acting like a fence) and the second operator can sample the perimeter of the pool or jam. At least one netter needs to be following the operator circumnavigating the pool or jam while the other netter(s) and the crew lead stay with the downstream operator.
7. Electrofisher settings may be changed for each unit independently while sampling. While the initial settings should be identical, the final settings may not be the same for both units. Record the final frequency, duty cycle, and voltage settings from each unit on the mobile device.
8. Once the entire reach has been sampled, read and record the time (EF time) in seconds from both of the electrofisher units on the mobile device.
9. Reset the timer, turn off the units, and proceed to fish processing (**SOP C**).

D.5 Sampling Reaches with Permanent Barriers

At TECR , WALK, and MCDI reaches are bisected by permanent fish barriers, therefore the following steps must be taken at these reaches within these sites:

TECR reach 1:

TECR reach 1 has been updated to a Random Reach starting in 2025, and fishing will ONLY occur below the barrier in that reach. This section is shorter than above the barrier; however, it is safer to fish which minimizes the risk of injury to staff and fish. The net for fishing will be placed immediately upstream of the barrier (to ensure no fish enter the reach being fished from upstream) and then all fishing will proceed from downstream to upstream until reaching the bottom of the barrier.

TECR reach 7:

TECR 7 will only be fished above the barrier starting in 2025. The net will be placed immediately upstream of the barrier, and fishing will proceed as normal from downstream to upstream and end at the bottom of reach 8.

WALK reach 6:

WALK reach 6 will ONLY be fished from the bottom of the reach up to 5m downstream from the sensor array. No fishing will occur within 5m of the sensors or anywhere near the culvert, the weir, and the reservoir upstream of the weir. In other words, no fishing will occur between the sensor set and the bottom of reach 7.

MCDI reach 5:

MCDI reach 5 will ONLY be fished below the barrier starting in 2025. The net will be placed at the top of the barrier and the top of reach 4, and fishing will proceed as normal from downstream to upstream and end at the bottom of the barrier.

D.6 Guidance for Fish Processing at SYCA and BLUE

SYCA

At SYCA, excessive fish mortality can occur from fish sampling during periods of high water temperature (22-26°C), this is especially true during bio bout three. To reduce the chance of unwanted fish mortality, the number of fish to measure has been reduced to no more than 30 per species and per transect when water temperature is between 22-26°C.

- After measuring 30 fish per species/per transect, start bulk counting that species.
- Restart species measurements at each new transect being sampled.
- Be sure to randomly select fish for measuring, so as not to bias larger fish. Random selection should involve no more effort than “blind” bucket selection during measurement.
- Additional effort to process fish rapidly will help reduce mortality.

BLUE

At BLUE, fish sampling can take a substantial amount of time to complete due to high rates of capture. To reduce the amount of time spent processing fish, the number of fish to measure has been reduced to no more than 30 per species and per transect.

- After measuring 30 fish per species/per transect, start bulk counting that species.
- Restart species measurements at each new transect being sampled.
- Be sure to randomly select fish for measuring, so as not to bias larger fish. Random selection should involve no more effort than “blind” bucket selection during measurement.
- Additional effort to process fish rapidly will help reduce mortality.

APPENDIX E ESTABLISHING SAMPLING REACHES

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 sample bout (e.g., riffles are now pools).

1. At the site, enter general aquatic field metadata on the mobile app (RD[06]). General field metadata only need to be filled out once per site per day, even if multiple protocols are implemented.
2. Using a field measuring tape, measure out ten, non-overlapping, 100 m (± 20 m) reaches, starting ~20 m above the downstream permit boundary (**Figure 27**), by following the thalweg of the main channel.
    a. Leave 20 m at the downstream boundary to allow for space to test the electrofisher and to release processed fish.
    b. Due to heterogeneous stream morphology and habitat characteristics, sampling reaches may deviate from 100 m in stream length to allow reaches to end at the natural boundaries of channel units (e.g., riffle, run, pool). A minimum length of 80 m and maximum of 120 m sampling reach lengths are acceptable. If natural channel units are longer than 120 m, then end the reach at 100 m.
3. Install a permanent aluminum plot survey marker on the bank at river right at each reach boundary (including the permit boundaries; **Figure 27**).
4. Each fish sampling reach is numbered sequentially beginning with “1” at the bottom (downstream) but just above the test reach (**Figure 27**).
    a. Record the coordinate at the plot marker location as well as the coordinate uncertainty using the Trimble data dictionary for AOS locations.
    b. Refer to the Morphology Mapping (RD[12]) protocol for further descriptions and examples of stream habitats.
5. If you are unable to install plot survey markers on the right bank (river right), use the left bank and note on the AOS Trimble data dictionary.
    a. The right bank is preferred for consistency across sites.

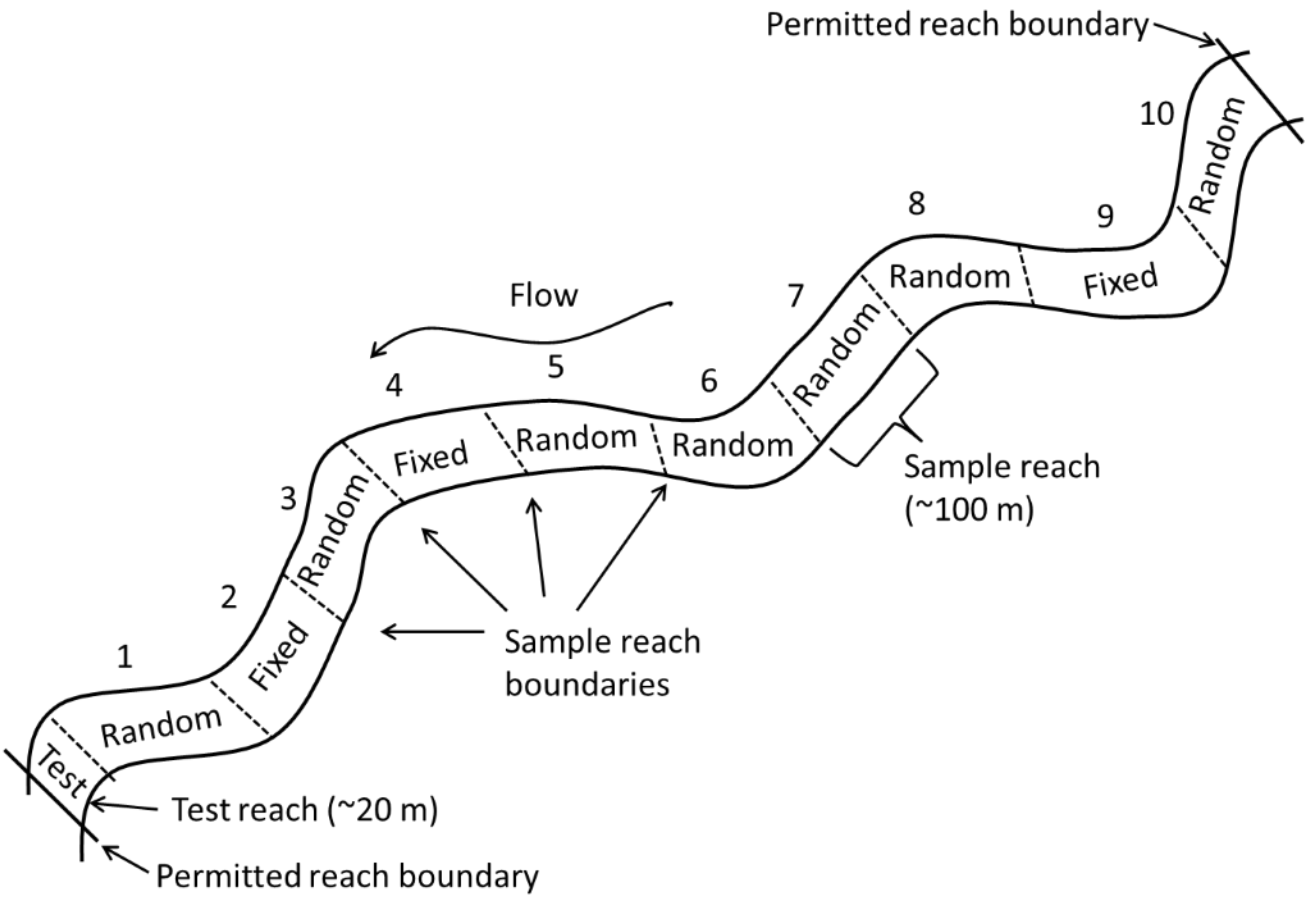

Figure 27. Schematic of a 1 km permitted NEON stream site delineated into ten 100 m reaches: 3 fixed and 7 random sampling reaches. Three random reaches will be chosen each year for sampling.

E.1 Fixed and Random Sampling Reach Selection

Reach selection occurs during the first year of sampling. Reaches will be revisited over the following years, unless the morphology of the stream significantly changes (e.g., riffles become pools). If the stream morphology changes significantly, as detected by the results of the Wadeable Stream Morphology Protocol (RD[12]), then it may be necessary to establish new reaches or the entire fish sampling reach. If this occurs, submit an incident via ServiceNow to inform Science staff.

1. Up to six 100 m (± 30 m) reaches (three fixed and three random) will be sampled during each sampling bout.
2. Electrofishing in reaches with sensors (S1 or S2) must occur ≥ 5 m away from all in-stream electronics.
3. Select three of the ten reaches to be the “fixed” reaches. Fixed reaches will be sampled two times per year throughout the duration of NEON measurements.

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

4. Select three of the remaining seven random reaches to be sampled annually. Refer to **Appendix D.2** a randomized order of reaches for each wadeable stream site.

5. Use the same three random reaches for the two sampling dates (bouts) within one year (**Table 17**).

6. For each year of sampling, continue down the list of randomized reaches not sampled previously. In year three (if the stream contains ten reaches), there should only be one reach that has not yet been sampled. Return to the first random reach on the list when all reaches have been sampled.

7. Follow this pattern for the remainder of the NEON data collection.

Table 17. Example of fixed and rotating reach design for one site over 10 years. Green boxes signify the top priority fixed reach to sample, yellow the second, and orange the third. Gray boxes denote when a random reach is sampled. Randomized reach order for each site is presented in Appendix D.1.

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

E.2 Shortened Sampling Reach

Establishing Shortened Sampling Reaches

The wadeable streams fish sampling design requires a minimum reach length of 1 km. The following information provides guidance for establishing fish sampling reaches at NEON sites that are 500 m in length, or half of a typical NEON aquatic stream reach. The spatial sampling design was modified in order to collect comparable fish data with other NEON wadeable stream sites that are 1 km. Procedures provided below should be implemented at other NEON wadeable stream sites where only a 500 m reach can be established. It should be noted that a 500 m reach is the absolute minimum length for a NEON wadeable stream site.

1. At the site, enter general aquatic field metadata on the mobile app (RD[06]). General field metadata only need to be filled out once per site per day, even if multiple protocols are implemented.
2. Using a field measuring tape, measure out five, non-overlapping, 80 m (± 20 m) reaches, starting 10-20 m above the downstream permit boundary (**Figure 28**), by following the thalweg of the main channel.
    a. Leave 10-20 m at the downstream boundary to allow for space to test the electrofisher and to release processed fish.
    b. Due to heterogeneous stream morphology and habitat characteristics, sampling reaches may deviate from 80 m in stream length to allow reaches to end at the natural boundaries of channel units (e.g., riffle, run, pool). A minimum length of 60 m and maximum of 100 m sampling reach lengths are acceptable. If natural channel units are longer than 100 m, then end the reach at 80 m.
    c. A minimum of five reaches must be present at wadeable stream sites that are less than 500 m.
3. Install a permanent aluminum plot survey marker on the bank at river right at each reach boundary (including the permit boundaries; **Figure 28**).
4. Each fish sampling reach is numbered sequentially beginning with “1” at the bottom (downstream) but just above the test reach (**Figure 28**).
    a. Record the coordinate at the plot marker location as well as the coordinate uncertainty using the Trimble data dictionary for AOS locations.
    b. Refer to the Morphology Mapping (RD[12]) protocol for further descriptions and examples of stream habitats.
5. If you are unable to install plot survey markers on the right bank (river right), use the left bank and note on the AOS Trimble data dictionary.
    a. The right bank is preferred for consistency across sites.

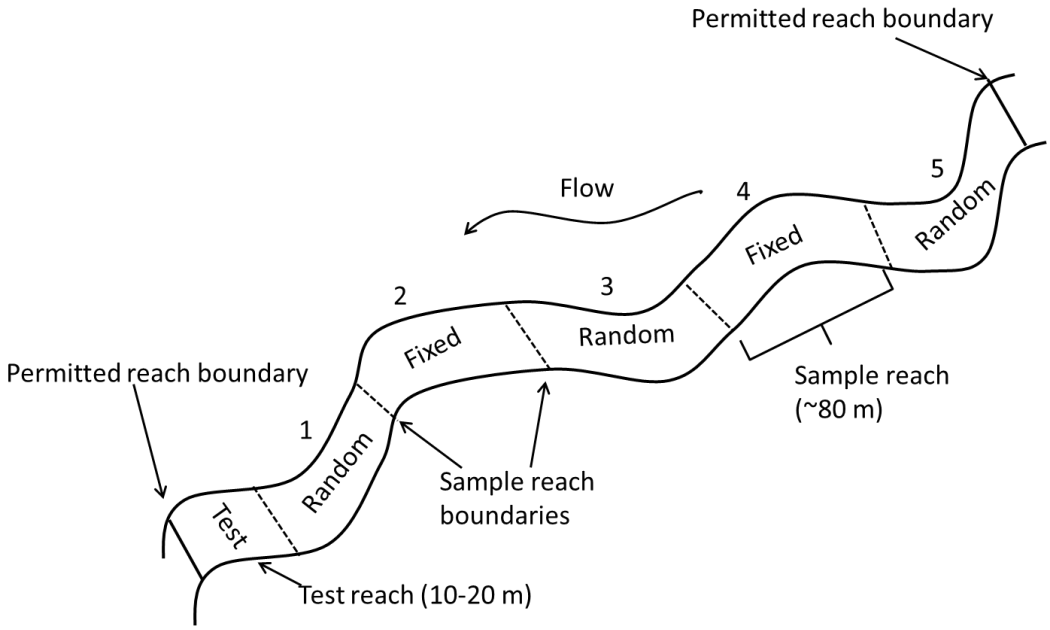

Figure 28. Schematic of a 500 m permitted NEON stream site delineated into five 80 m reaches: 2 fixed and 3 random sampling reaches. One random reach will be chosen each year for sampling.

Fixed and Random Shortened Sampling Reach Selection

Reach selection occurs during the first year of sampling. Reaches will be revisited over the following years, unless the morphology of the stream significantly changes (e.g., riffles become pools). If the stream morphology changes significantly, as detected by the results of the Wadeable Stream Morphology Protocol (RD[12]), then it may be necessary to establish new reaches or the entire fish sampling reach. If this occurs, submit an incident via ServiceNow to inform Science staff.

1. Up to three 80 m (± 20 m) reaches (one fixed and three random) will be sampled during each sampling bout.
2. Electrofishing in reaches with sensors (S1 or S2) must occur ≥ 5 m away from all in-stream electronics.
3. Select two of the five reaches to be the "fixed" reaches. Fixed reaches will be sampled two times per year throughout the duration of NEON measurements.

   a. The two fixed 80 m (+/- 20 m) reaches should be chosen to best represent the habitat variability throughout the 500 m permitted reach. For example, the inclusion of primary habitat (e.g., pools) and distinctive (e.g., waterfall and plunge pool) habitat features should be a priority when selecting permanent reaches. Fixed reaches will be selected by the NEON Aquatic Ecologist or Domain Aquatic Field scientist.
4. Select one of the remaining three random reaches to be sampled annually. Refer to **Appendix D.2** for a randomized order of reaches for each wadeable stream site.

5. Use the same random reach for the two sampling dates (bouts) within one year (**Table 18**).
6. For each year of sampling, continue down the list of randomized reaches not sampled previously. In year four, return to the first random reach on the list when all random reaches have been sampled.
7. Follow this pattern for the remainder of the NEON data collection.

Table 18. Example of fixed and rotating design for one site less the 500 m over 10 years. Gray boxes denote when a reach is sampled. Randomized reach order for each site is presented in Appendix D.

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

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 or 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				
	N	Site-specific morphology map	Navigating to sampling reaches	1
	N	Plot survey markers (aluminum, site-specific)	Establishing sampling reaches	12
	N	Meter tape (50 or 100 m)	Establishing sampling reaches	1
	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	Steel studded fence posts (i.e., T-post)	Securing block net at reach boundary	8
	N	Fence post driver or small sledge	Securing block net at reach boundary	1
	N	Fence post puller	Removing block net fence posts at reach boundary	1
The Fish Net Company, LLC Duluth Nets	Y	Mesh block nets with lead lines and top lines with floats (custom-built for each site)	Catching drifting specimens	3
	N	L-type block net stakes (e.g., ~45 cm long, 10 cm handle, 1 cm diameter stainless rod). ¼" Dacron Polyester rope works well for sites that can deploy block nets without stakes.	Securing block net at reach boundary	15
	N	Net repair kit: • needle	Repairing nets	1

Supplier/ Item No.	Exact Brand	Description	Purpose	Quan-tity
		• net string • butane lighter • zip ties		
	N	Gloves, Nitrile coated, Textured, Knitted cuff, Abrasion resistant, PIP or Equivalent	Handling gill 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
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
	N	5-gallon buckets	Storing specimens	10
Challenge Plastics	Y	5-gallon bait-bucket lids (has aerator hole and smaller hole in the lid so the entire lid doesn't need to be removed to net fish for enumeration)	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

Supplier/ Item No.	Exact Brand	Description	Purpose	Quan-tity
	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
Consumable items				
	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 – 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
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

Supplier/ Item No.	Exact Brand	Description	Purpose	Quan-tity
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)	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
	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

Supplier/ Item No.	Exact Brand	Description	Purpose	Quan-tity
	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