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

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See configuration management system for approval history.

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Change Record

REVISION	DATE	ECO #	DESCRIPTION OF CHANGE
A	02/18/2014	ECO-01394	Initial release
B	01/22/2015	ECO-02632	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.

TABLE OF CONTENTS

LIST OF TABLES AND FIGURES

1 OVERVIEW

1.1 Background

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

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, capture efficiencies of standard methods to sample fish in wadeable streams are often less than 100% for fish abundance (i.e., biased) and are influenced by species and body size, stream habitat and hydrologic conditions, and the sampling method used (Bayley and Dowling 1990, Bayley and Peterson 2001, Price and Peterson 2010). Sampling bias for a particular method (e.g., gear) can also be influenced by sample timing. These biases can be particularly problematic for monitoring fish populations, because sampling efficiencies can potentially change temporally due to sample timing (e.g., season, diel period) and fish behavior (e.g., spawning movements). NEON's wadeable stream fish sampling protocol uses electrofishing, considered the most effective method of fish sampling at wadeable stream sites (Barbour et all 1999), and the most repeatable and standardized method at the Observatory level. Sampling each site twice a year (roughly spring and autumn) 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 mix of single pass and three pass depletion helps NEON data users better calculate sample 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 fish sampling 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.001155	NEON Training Plan
AD[05]	NEON.DOC.050005	Field Operations Job Instruction Training Plan
AD[06]	NEON.DOC.004104	NEON Science Data Quality Plan
AD[07]	NEON.DOC.002979	NEON Animal Care and Use Program: Training Plan for Personnel Working with Live Vertebrate Animals

2.2 Reference Documents

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

RD[01]	NEON.DOC.000008	NEON Acronym List
RD[02]	NEON.DOC.000243	NEON Glossary of Terms
RD[03]	NEON.DOC.002652	NEON Data Products Catalog
RD[04]	NEON.DOC.001271	AOS/TOS Protocol and Procedure: Data Management
RD[05]	NEON.DOC.003282	NEON Protocol and Procedure: Site Management and Disturbance Data Collection
RD[06]	NEON.DOC.005247	AOS/TOS Standard Operating Procedure: NEON Aquatic and Terrestrial Site Navigation
RD[07]	NEON.DOC.004257	NEON Standard Operating Procedure: Decontamination of Sensors, Field Equipment, and Field Vehicles
RD[08]	NEON.DOC.001152	NEON Aquatic Sample Strategy Document
RD[09]	NEON.DOC.003046	AOS Protocol and Procedure: Aquatic Macroinvertebrate Sampling
RD[10]	NEON.DOC.003162	AOS Protocol and Procedure: Wadeable Stream Morphology
RD[11]	NEON.DOC.005224	NEON Protocol and Procedure: 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)
Hz	Hertz
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**).

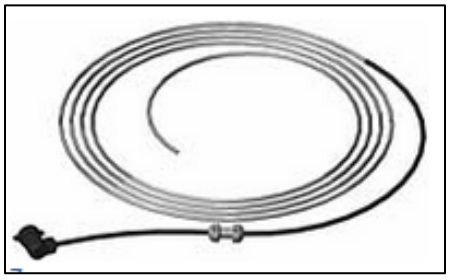

Figure 2. Cathode for backpack electrofishing unit (photo: store.smith-root.com)

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: Electronic data capture Software tool used to create NEON electronic data entry applications. Referred to as the remote data capturing device in this protocol.

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

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

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

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.

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. The protocol outlined here describes the use of electrofishing (i.e., DC or pulsed DC backpack electrofishing) to sample fish in wadeable streams at designated NEON sites (1 km stream).

Up to ten replicate non-overlapping ~100 m reaches 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 are sampled twice every year using a three-pass electrofishing depletion approach. 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. Three random reaches are sampled twice per year then a new set of three random reaches are sampled in subsequent years. Random reaches will be sampled via a single-pass electrofishing. Species, length and weight data will be collected as well as observations for deformities, eroded fins, lesions, tumors, and parasites.

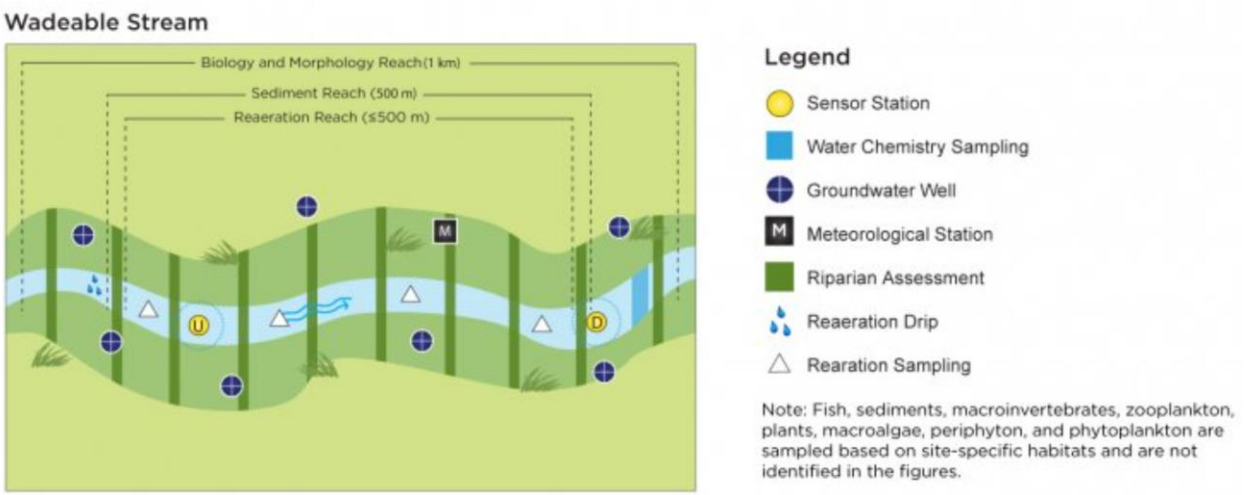

Figure 3. A generic wadeable stream site layout. Fish sampling will occur throughout entire biology and morphology reach.

The fish sampling protocol also includes non-destructive methods for collecting voucher specimen, and 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 fish species sampled at NEON sites 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 NEON ASU biorepository for future studies.

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

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

Quality assurance is performed on data collected via these procedures according to the *NEON Science Data Quality Plan* (AD[06]).

4 SAMPLING SCHEDULE

4.1 Sampling Frequency and Timing

Wadeable stream fish sampling will occur two times per year, once at the beginning of the growing season and once towards the end of the growing season at each site, roughly spring and autumn. The initial assessment of growing season and sample timing will be determined for each stream site using historical data including ice-out, water temperature (or accumulated degree-days), weather, and riparian peak greenness. Sample timing will be refined on a site-by-site basis by NEON Science based on data collected by the aquatic sensors and Field Operations. Sample timing shall be outlined in the *NEON Aquatic Sampling Strategy Document* (RD[08]). Fish sampling schedules shall be coordinated with Permitting at minimum seven days prior to the onset of sampling by field Science to ensure adequate sampling notifications and communications with state and federal wildlife agencies is completed prior to the field deployment at each site.

Fish sampling corresponds with the first and third (out of 3 total) aquatic biological sampling bout windows. Fish sampling must be scheduled within the specific biology bout window. Weather or hydrologic conditions at the site may push sampling outside of the bout window. Fish sampling should be scheduled as the last sampling activity of the biology bout to minimize impacts on other sampling objectives, the exception to this rule is at the D19 Caribou Creek wadeable stream site where fish sampling is allowed to be the first event during the 3rd (autumn) bio-bout, to ensure that the *Thymallus arcticus* (Arctic grayling) migration is sampled. Should fish sampling occur before other biological sampling events, you must consult the other biology bout protocols to ensure that they are scheduled in response to the potential disturbance created by fish sampling following the disturbance criteria outlined in them.

1. 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 on when the first on the ground sampling event is scheduled during the bout 2 window).
2. If conditions do not allow for fish sampling to occur during bout 3, then sampling shall occur when safe conditions allow up to 30 days beyond the end of bout 3, or until January 31, whichever occurs first.

A minimum of 2 weeks between fish sampling bouts shall be observed. Sampling bouts should not be longer than 5 days long assuming no weather or other unexpected schedule delays. All three passes in a fixed sampling reach must be sampled 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, there must be a minimum of 30 minutes between passes to allow fish to resettle in the reach. If there is not enough time complete a 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.

4.2 Criteria for Determining Onset and Cessation of Sampling

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

4.3 Timing for Laboratory Processing and Analysis

Individual domain facilities will temporarily store preserved voucher specimens of fish (target species) as well as any amphibians or reptiles (non-target specimens) fatally injured and euthanized, or killed during fish sampling activities. Vouchered specimens will be shipped to the NEON Biorepository at the Arizona State University for long-term storage. Fish and non-target voucher specimens may be stored for up to 12 months following the preservation guidelines outlined in **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 1. 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*

Table 2. Fish sample storage and holding times.

Sample Type	Field Storage	Post-processing Lab Storage	Domain Hold Time
DNA Fin-clip	*1.5 mL cryo vial with ethanol (70-95%).* ***Never Formalin***	*Labeled Cryo vial with ethanol (70-95%).* ***Never Formalin***	*Up to 12 months if refrigerated or frozen and stored in 70-95% ethanol,*
Fish voucher < 200 mm	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 complete covered in 10% buffered formalin for 5 to 7 days	*Store at room temperature for 1 week, then remove from formalin and fix fish complete covered, in covered container, in 70% ethanol. 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 complete covered in 10% buffered formalin for 5 to 7 days.*	*Store at room temperature for 1 week, then remove from formalin and fix fish complete covered, in covered container, in 70% ethanol. Can be stored at room temperature.*	*Up to 12 months if stored in70-95% 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	*Store at room temperature for 1 week, then remove from formalin and fix bycatch complete covered, in covered container, in 70% ethanol. Can be stored at room temperature*	*Up to 12 months if stored in70-95% ethanol*

Sample Type	Field Storage	Post-processing Lab Storage	Domain Hold Time
	weight), for 5 to 7 days		
Invert by-catch	Fix in 95 ethanol in a labeled and sealed HDP container	*HDP bottle -voucher covered in 95% ethanol, labeled and lid taped and secured on*	*No Limit*

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 2 weeks between sample periods must be observed If during scheduled sampling environmental conditions interrupt sampling, follow the contingencies outline in **Table 3**. Always endeavor to fully sample all scheduled reaches, however if only a truncated sampling event can be carried out, at least 1 full (3 passes) fixed reach must be sampled for fish sampling to be considered a complete sampling bout and data to be published.

Table 3. 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 a flooding/high water event inhibit the ability to safely sample (for field staff or fish) occur 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	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

Delay/ Situation	Action	Outcome for Data Products
		days or beyond the extended contingency windows; submit a Service Now ticket to the protocol author.

4.5 Missed or Incomplete Sampling

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

- Logistics – e.g., insufficient staff or equipment
- Environment – e.g., deep snow, flooding, inclement weather
- 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. If sampling is impractical as a result of severe drought (dry), the stream is frozen, high flow (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 using sampling impractical this 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. 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* for more detail (RD[05]).

Missed or Incomplete Sampling Terms

Terms that inform Missed or Incomplete Sampling include:

- **Protocol Sampling Dates**: Bout-specific sampling window dates (Appendix D).
- **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 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 Service Now 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 and Permitting 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 4** below to determine required actions if scheduled activities are delayed or canceled. Guidance for this and other NEON protocols is summarized for ease of use in a table posted to a Field Science Sharepoint library. Defer to guidance in this protocol should any discrepancy exist.
2. Create a Fulcrum record for each Missed Sampling event in the field that cannot be rescheduled. As data are recorded in the field at the reach level, a record must be made for each reach missed.
    a. 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 5**).
4. For Rescheduled sampling events that occur outside of the defined Protocol Sampling Dates, a protocol-specific Flag must also be recorded see **Figure 4.**

Table 4. 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 protocol author.	Submit incident ticket informing Science of cancelation and create a Sampling impractical record for each scheduled reach that has been missed

Table 5. 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	*3X median discharge*
Location dry	*Location dry*
Location frozen	*Location frozen*
Water Temperature	➢ *> 18 C if salmonids are present, or > 26 C for non-salmonids, defer to temperature limits (both high and low) in site specific permits*
Permitting	*Any onsite conditions where fish sampling is prohibited in site permit*
Location snow covered	*Location inaccessible due to snow covered*
Other	*Sampling location inaccessible due to other ecological reason described in the remarks*
Extreme weather Management	*Events (e.g., thunderstorms, hurricanes) that compromise safety and access management activities such as controlled burn, pesticide applications, etc.*
Logistical	*Inability to field minimum sampling team (3 people) or equipment failure*

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 6**). 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 5 consecutive field days. There is no lab processing at the Domain Support Facility associated with this protocol. Fish tissue samples will be shipped to a central Domain Support Facility (D01) for processing annually. Additionally, tissue samples and/or voucher specimens (target and non-target species) may be preserved, archived, and shipped to external facilities by domain staff. Sample preservation and archival may require up to 10 minutes of processing per specimen for domain staff.

Table 6. 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 A1: Preparing for Data Capture	*1 h*	*1*	*1 h*

SOP	Estimated time	Suggested staff	Total person hours
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 C.4: Gill Net	*1 h*	*2*	*2 h*
SOP C.5: Mini-fyke Netting:	*1.5 h*	*2*	*3h*
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 5-day period per site. If field conditions appear unfavorable (e.g., prolonged thunderstorms, tropical storms, expected flooding) during the proposed sampling bout, postpone sampling until conditions are safe to sample and when the entire sampling bout can be completed in 5 days. If fish sampling is interrupted and the resumption of fish sampling is expected to occur three days past the biological bout, (or beyond the extended contingency windows; see Table 4 submit a trouble ticket (**Table 4**).
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.
        1) 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 NEON fish sampling protocol author, Domain Manager, Permitting Staff, and submit a Service Now ticket immediately after fish sampling ends that day. Science will work with the staff to identify if there is a problem or any changes needed with sampling.

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

      1) Electrofisher settings: voltage, duty cycle, and frequency

      2) Water quality: conductivity, dissolved oxygen, and temperature

      3) Field observations and suspected likely root cause of injury or mortality

      4) Description of species affected

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

5 SAFETY

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

Personnel working at a NEON site must be compliant with safe field work practices as outlined in the *Operations Field Safety and Security Plan* (AD[02]) and *EHS Safety Policy and Program Manual* (AD[01]). Additional safety issues associated with this field procedure are outlined below. The Field Operations Manager and the Lead Field Technician have primary authority to stop work activities based on unsafe field conditions; however, all employees have the responsibility and right to stop their work in unsafe conditions. In addition, the following general 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 (93 L/s; Lane and Fay 1997).
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.

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.
    a. The requirements for wader and boot selection have been adapted by the recommendations of the American Fisheries Society Professional Safety Committee (2008).
        1) Non-breathable materials provide the most insulation against electrical shock and include PVC, neoprene, or silicon. Non-breathable waders with built-in lug-sole boots are the preferred wader style.

2) Breathable materials including Gore-Tex® provide less insulation against electrical shock, but they may be more comfortable in warmer conditions and in environments with a lot of scrambling over boulders. The use of breathable waders with stocking feet is acceptable as long as the operator also wears dry clothing that covers any exposed skin while in the waders.

3) Studded-sole boots are allowed if they help secure footing in slippery conditions.

4) Stocking foot waders with the built-in gaiter ankle cuff are difficult to decontaminate. This style of wader may only be used if dedicated to a single site and not transferred across sites.

5) Felt-soles are prohibited because they are proven mechanisms of transportation of aquatic invasive species

b. 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.

4. Before sampling, inspect waders, boots, and gloves for leaks. Leave the water immediately if waders or gloves develop leaks.

5. A portable automated external defibrillator (AED) must be available in the field for all electrofishing work. The AED and First Aid Kit can be stored in a work vehicle, boat, or other known location as long as it is within a 6-minute walk from the active sampling location.

6. Avoid operating near bystanders, pets, or livestock that are in or near the water.

7. Electrofishing must be suspended if anyone feels a shock, however minor, for investigation and repair of equipment.

8. Avoid operating an electrofishing unit in heavy rain (light rain is acceptable) as this can increase the probability of electrical shock.

6 PERSONNEL

6.1 Training Requirements

All technicians must complete required safety training as defined in the *NEON Training Plan* (AD[04]). Additionally, technicians must complete protocol-specific training for safety and implementation of this protocol as required in *Field Operations Job Instruction Training Plan* (AD[05]). Refer to the *NEON Animal Care and Use Program: Training Plan for Personnel Working with Live Vertebrate Animals* (AD[07]).

All personnel participating in fish sampling for NEON are to be trained in fish sampling safety for water-based fieldwork. Specific training for fish sampling must also include electrofishing training for all field scientists. All lead aquatic field scientists and those designated by their manager shall be required to receive an electrofishing safety training prior to operating a backpack electrofisher. The backpack electrofisher manufacturer (Smith-Root) will provide this training. Additionally, all field scientists shall complete the U.S. Fish and Wildlife Service (USFWS) online Electrofishing Safety training course (National Conservation Training Center [NCTC] CSP 2202-OLT) once per season before the first sampling bout. Field scientists must pass the Electrofishing Safety Exam with a score >80%. Additional training resources include Wader Safety training created by the Victoria Department of Environment and Primary Industries. 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 health impacts of target fish species and non-target organisms (e.g. amphibians, reptiles) through the proper use of sampling equipment and technique. All field crews participating in fish sampling shall have one member that has received the manufacturer safety training; all crew members shall have completed the USFWS NCTC electrofishing safety training and possess a current CPR/AED/First Aid training.

External Training Resources:

Required all crew each year

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

Wader Safety
https://fws.rev.vbrick.com/#/videos/3acea0c2-b819-43e8-896b-5c75067d221c

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

7 STANDARD OPERATING PROCEDURES

SOP Overview

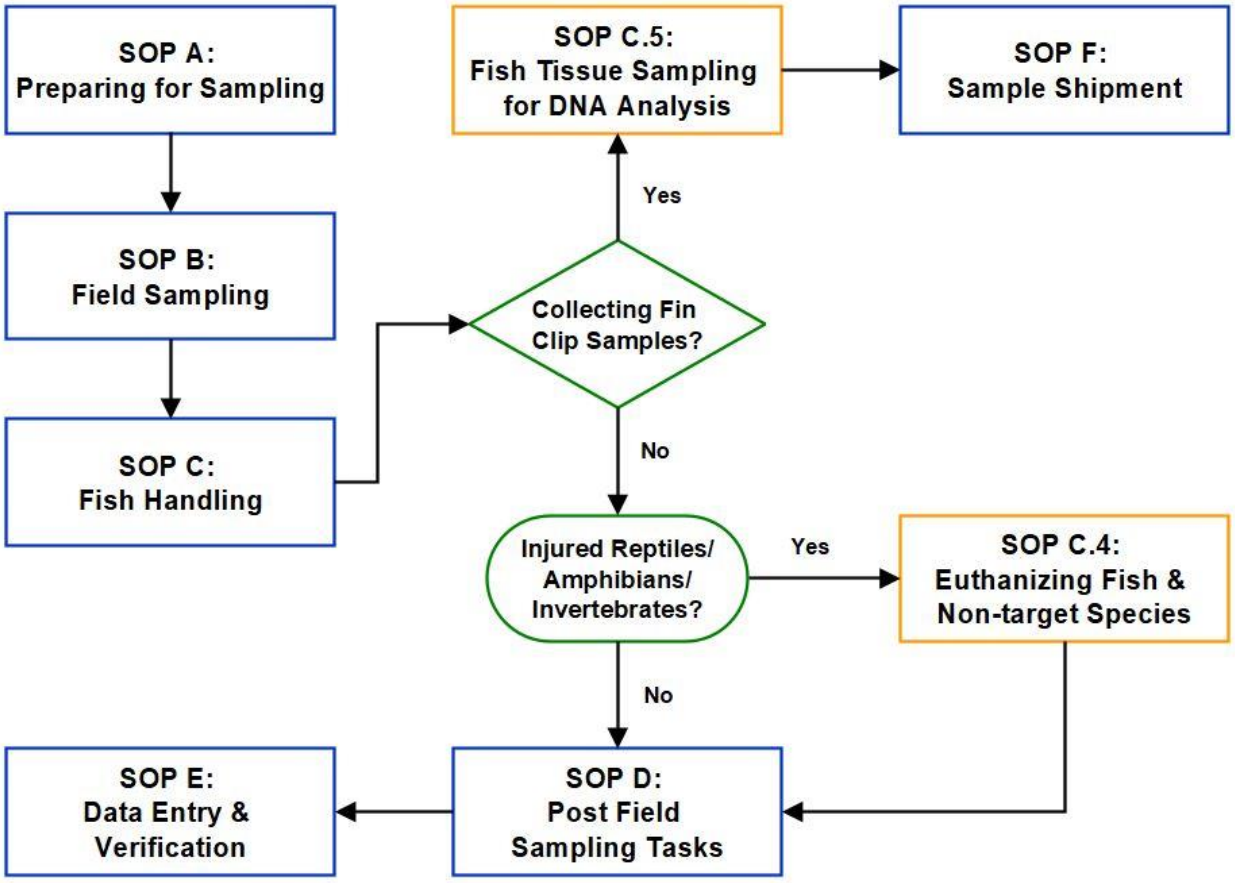

Figure 5. A high-level workflow diagram that visually shows how the separate SOPs are sequentially connected.

- 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 devices should be fully charged at the beginning of each field day.

Preparing for Field Sampling

1. Permit Compliance
    a. Review the federal and/or state collection permit thoroughly.
    c. Be sure to notify the site host of the dates and times of the fish sampling activities.
    d. If required, you must notify responsible state agency of the dates and times of the fish sampling activities prior to sampling
    e. Retain a copy of the collection permit during the sampling activities.
2. 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.
    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.
    c. NEON keeps a spare electrofishing unit at HQ in cases where the Domain shocker in not working.
    d. 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. Start charging batteries at least two days before going to the field to allow batteries to fully charge.
    e. 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
    f. Inspect lineman gloves, DO NOT repair torn lineman gloves. Discard them and order a replacement pair.
    g. Inspect dip nets and block nets for rips, tears, and holes, repair if necessary.
    h. Inspect portable aquarium pumps, diffusion stones, and batteries.
    i. Inspect buckets to ensure handles are present and functioning.
    j. Ensure that all equipment has been decontaminated since last use (see RD[07]).

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

3. 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 Tricaine methanesulfonate (MS-222).

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

      1) Use of the wrong chemical or any accident must be reported immediate using by creating a Service Now incident. -

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

      1) This drug is currently available under the USFWS Investigational New Animal Drug (INAD) program; Study Protocol 11-741.

      2) Each field aquatic 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.

         a) NEON INAD Groups

            i. Group 1: D01, D02, D04, D05

            ii. Group 2: D06, D07, D08, D09

            iii. Group 3: D10/13, D11, D12, D14

            iv. Group 4: D15, D16, D17, D18/19

      3) As such, NEON must comply with data reporting requirements including drug acquisition and handling as well as fish treatment and disposition.

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

         b) Report to INAD group lead must include daily:

            i. dosage used

            ii. volume used

            iii. species treated

            iv. number of each species treated

            v. average size and weight

            vi. mortalities

            vii. treatment effect

            viii. time to effect

            ix. recovery time

x. number of fish treated in each bucket

xi. water temp

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

5) Make sure that the batch of AQUI-S®20E has not expired. Any questions regarding this program or how to complete the field datasheets should be directed to the INAD group leader monitor or NEON Science.

6) 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.

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

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

1) Mix stock solution of MS-222 (in the handling of all MS-222; follow all site-specific requirements found in the site Environment Health and Safety Plan, landowner requirements, and state and local permits in the in the Domain Support Facility).

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

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

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

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

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

7) MS-222 stock solution must be stored in dark bottles away from light and refrigerated at 4°C. Stock solution may be stored and used in one month from the date of preparation.

a) Discard unused solution in the lab following the NEON EHS chemical hygiene guidelines (AD[03]).

A.2 Labels and Identifiers

Fish sampling produces four potential sample types: fish vouchers and DNA fin-clips. Occasionally there will also be vertebrate bycatch vouchers and invertebrate bycatch vouchers. All four sample types will need to be labeled on the outside of the container with a human readable label containing the sample ID, and a barcode. Vouchers will have an additional identifier placed in the voucher container; with the voucher specimen id written on write in the rain paper in ethanol proof marker.

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

1. All fish and bycatch voucher labels will have human readable labels, consisting of the fish voucher sample ID ("FSH.siteID.reachNo.YYYYMMDD.passNo.specimenNo.") taped to the outside of the HDPE container (**Figure 6**).

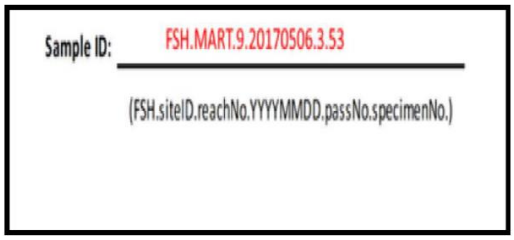

Figure 6. Example fish/bycatch voucher label.

2. All fish and bycatch vouchers must have Type I barcode label taped to the outside of HDPE container or bag (prefix A, plus 11 numbers) (**Figure 7**).

Figure 7. Type I barcode label taped to the outside of fish voucher container.

3. All DNA fin-clip 1.5 mL cryotube samples must have a human readable label with the sample ID taped to the cryotube. Tape label so that a Type IV barcode label can also be taped to cryotube (**Figure 8**).

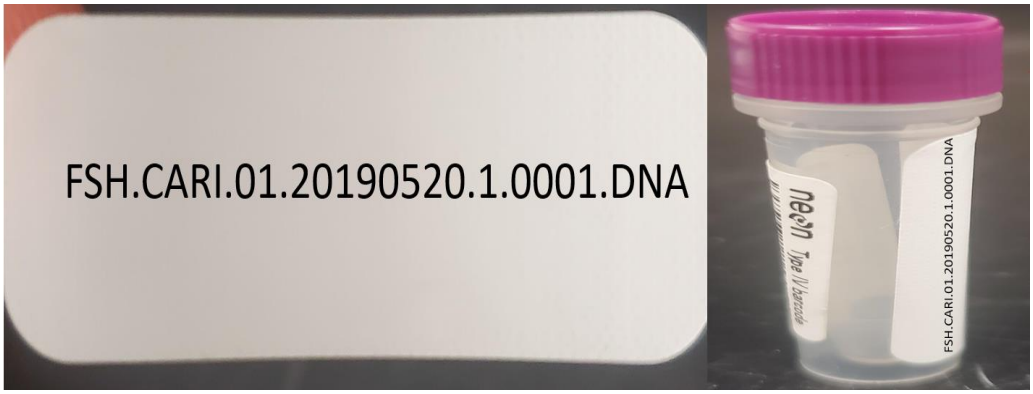

Figure 8. DNA human readable sample ID label.

4. All DNA fin-clip 1.5 mL cryotube samples must have a Type IV barcode label (prefix D, plus 11 numbers), taped to the cryotube (**Figure 9**).

Figure 9. DNA Type IV barcode label, taped to the cryotube.

Table 7. 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)	*Fish: Single fish capture*	*1.5 mL cryotubes*	*Type I* V	***Always Required***	*1 per cryotube*
Fish and bycatch voucher samples	Voucher specimen	*FSH.CARI.01.20190520.1.0001 (FSH.siteID.reachNo.YYYYMMDD. PassNo.specimenNO)*	*Fish: Single fish capture*	*HDPE (minimum 30 mL) or plastic bag*	*Type I*	***Always Required***	*1 per voucher*

SOP B Field Sampling

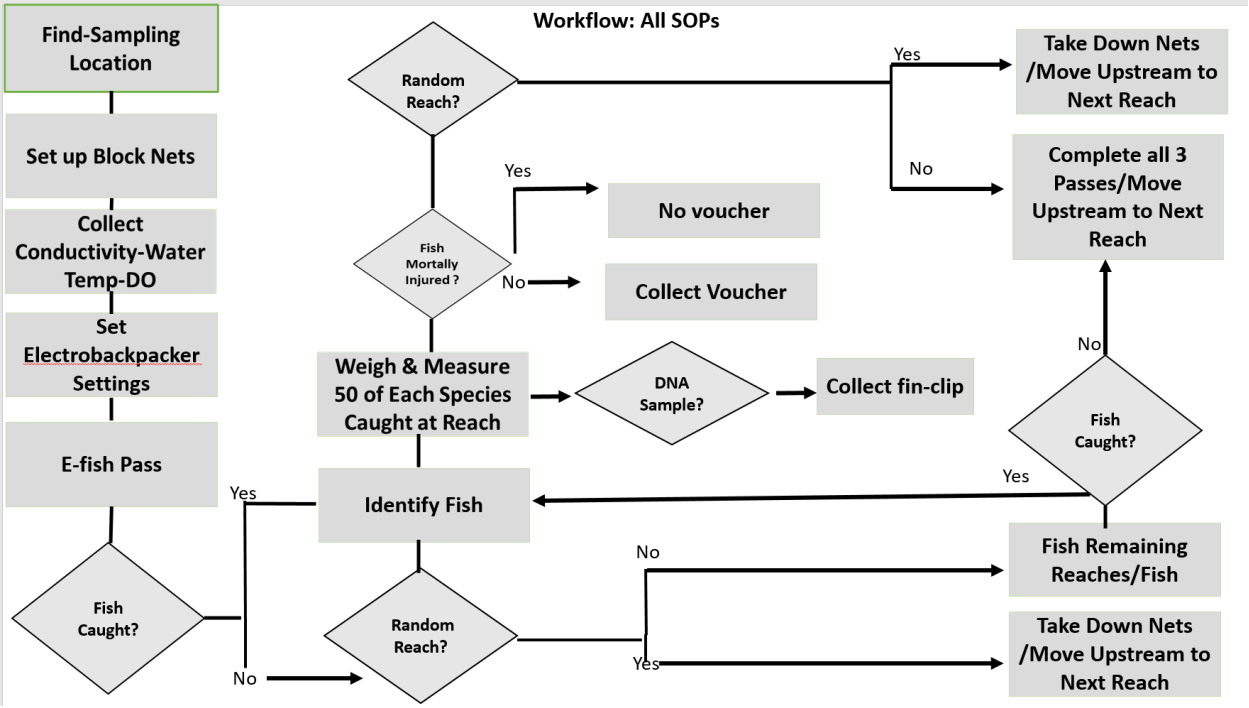

Figure 10. An expanded diagram of the workflow for a single field sampling SOP.

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 sampling reach and proceed 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.
    c. MCDI and TECR have permanent, reach bisecting fish barriers, set block nets at these barriers following the direction in bullet 1) and 2) below (How to sample these reaches is further discussed in **Appendix D**.

1) 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.

2) 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.

d. 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 12**).

e. 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.

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

g. Anchor lead line to substratum using large rocks (**Figure 12**) 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.

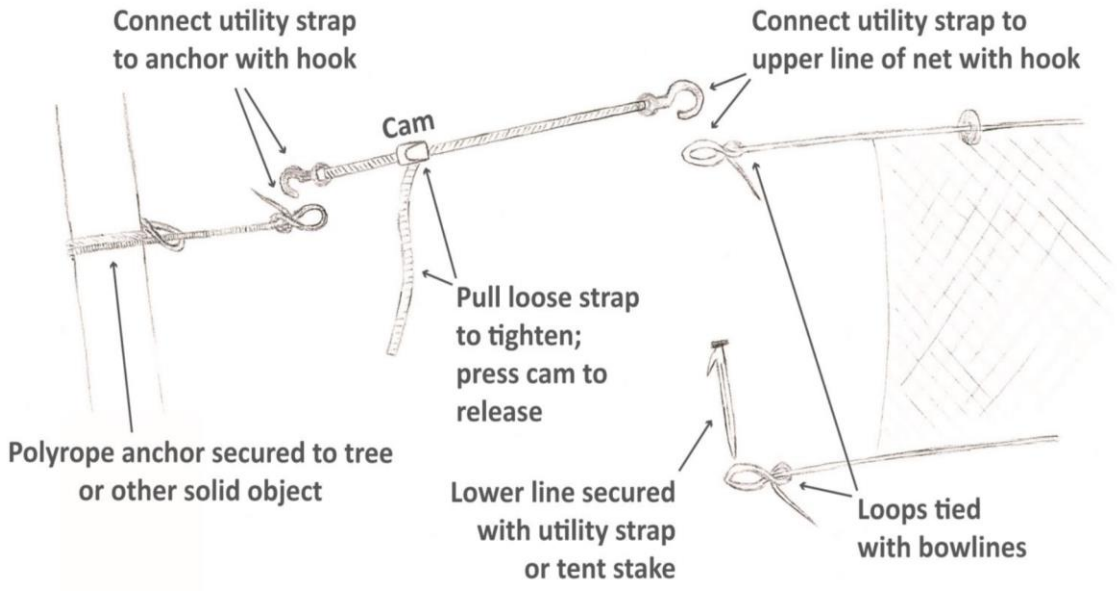

Figure 11. Example of wadeable stream block net set up.

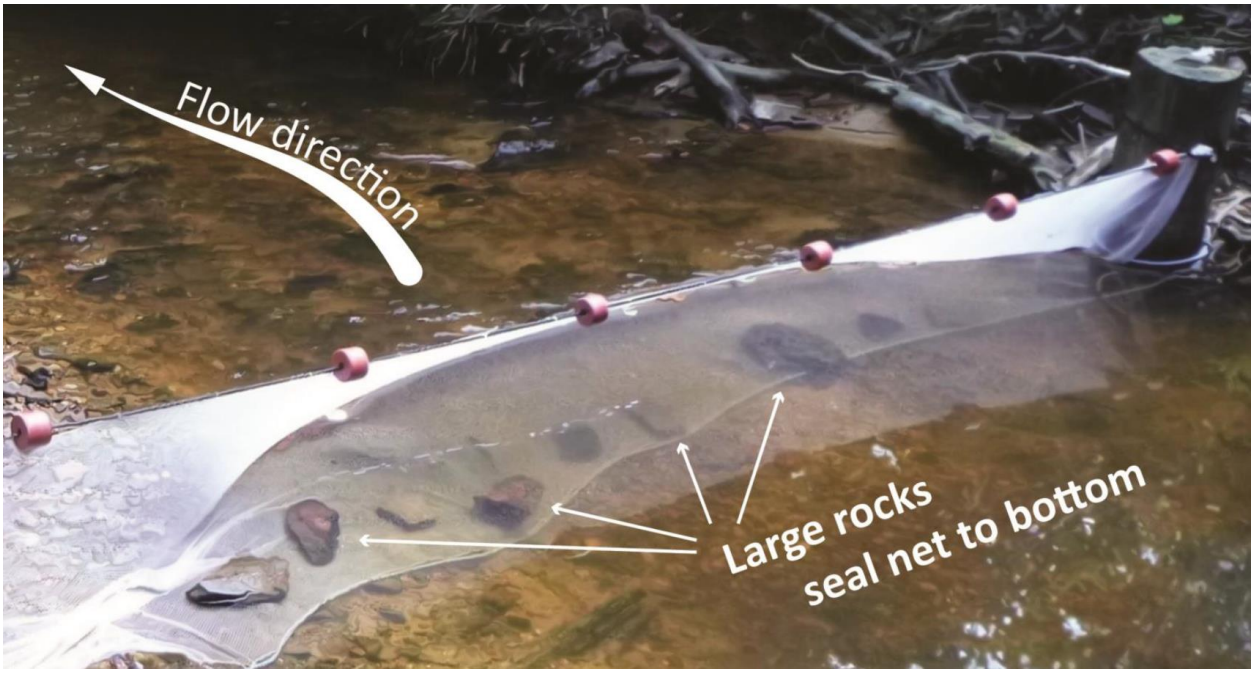

Figure 12. Potential net set up strategies for difficult block nets installations (Dan McGarvey).

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 settings, be sure to record new setting if these are changed during electrofishing.

1. Assemble Electrofisher
    a. **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.

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

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

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

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

e. 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 14**).

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

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

2. Test 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 conductivity using the handheld YSI multimeter at the test reach.

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

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

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

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

      1) Frequency to 30 Hz, duty cycle to 10%, and output voltage to 100 V.

      2) Adjust setting as needed, using the site conductivity value for guidance on maximum allowable settings (**Table 8**).

Table 8. Guidelines for maximum settings for backpack electrofishing based on conductivity (NOAA NMFS 2000).

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

e. 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.

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

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

   1) 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.

h. 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 8** and stay within the approved electrofisher settings.

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

j. Start by increasing **voltage** in 50-volt increments

   1) Do not increase above maximum setting allowed for conductivity reading **Table 8**.

k. Still no fish reaction-**lower voltage** to original test setting and in 5% increments Increase **duty cycle**.

1) Do not increase duty cycle above maximum setting allowed for conductivity reading **Table 8**.

l. If still no fish reaction lower both voltage and duty cycle and increase hertz (pulse rate) by 5 Hertz increments, do not exceed 60 Hz

m. Testing should take no more than 20 minutes and or 5 captured fish, whichever comes first

1) 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.

2) 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.

3) 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).

4) You must record the settings at the start of sampling, and also record any changes to settings as sampling proceeds.

n. 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.

o. Examine captured fish for signs of injury (e.g., bent backs, dark bruising, hemorrhaging of the gills (**Figure 15**). Record injury rate on the mobile device. Reduce settings until fish are no longer injured, if it is not possible to find a setting that does not injure fish stop sampling and report situation to domain manager and protocol author.

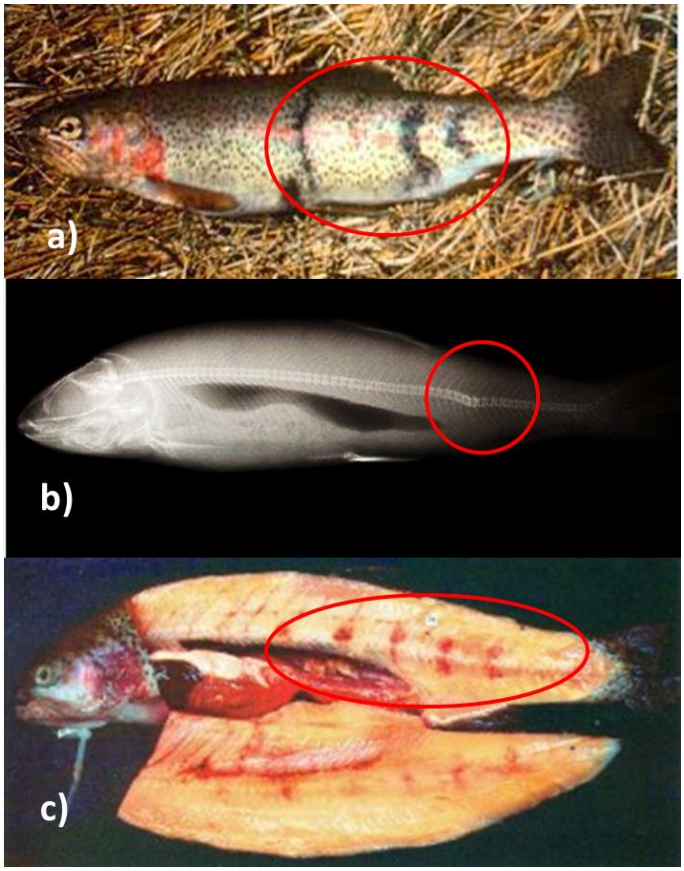

Figure 15. Examples of electrofishing injuries to fish, a) bruising or "burn" marks, b) spinal injury, and c) internal hemorrhaging. Photos from Snyder 2003.

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

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

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

s. Record vouchers, injuries and mortalities on mobile app.

t. 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, fish processing step 10.

***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.*

u. 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).
2. 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. Walk into the stream, operator should be wearing the backpack electrofisher, but electrofisher should be off at this point, make sure that the cathode (i.e., rattail) is submerged as much as possible, while holding the anode pole in one hand (anode ring submerged).

a. The electrofisher operator may, but is not required to, hold a dip net in the other hand if they feel comfortable.

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). This crew member will monitor the electrofishing operation; ensure that fish are effectively immobilized, that captured fish in the buckets are remaining healthy, and that the crew is operating safely. The lead will also ensure that any potential bystanders are not entering the water.
      1) Lead is responsible for ensuring that electrofishing is carried out in an efficient manner, that netters are netting correctly.
   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. 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.

1) Dip net mesh is a decision made at the site level by Field Science

   a) Mesh size should be small enough so that the smallest fish on site does not escape through the holes.

   b) But not so small that the push of water makes keeping the net submerged or sweeping the net inefficient.

   c) If help is needed to determine dip net mesh size, create a Service Now ticket asking for advice from Science.

b. There should always be one net behind the anode.

c. Fish are often attracted to the cathode (rattail). Netters should periodically check this area for stunned fish.

d. Netters should be aware that immobilized fish may not always be visible, particularly benthic species (e.g., darters, sculpin), and netters should frequently inspect their nets to minimize injury to fish by continuous exposure to electricity.

e. Crewmembers with dip nets should CALMLY net immobilized fish without excessive disturbance.

f. Never put hands in the water to capture fish while activation switch is depressed (i.e., while electrical current is pulsing through the water). If the netter cannot capture a fish using the net, 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. 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.

g. 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. 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).

a. The electrofisher operator may take advantage of the response of fish to pulsed DC current (i.e., attraction of immobilized individuals towards the anode) in complex cover by:

1) Releasing the activation switch on the anode pole.

2) Inserting the anode into cover from the downstream direction and holding the anode temporarily still.

3) The electrofisher operator then depresses the activation switch as netters hold dip nets immediately downstream of the anode and cover.

4) The activation switch should continue to be depressed until the abundance of observable immobilized fish decreases.

5) Continue electrofishing by moving the anode around the cover to immobilize additional fish, before sampling up the reach.

6) Netters should make a few sweeps through any cover that was shocked to pull out immobilized individuals that are stuck in the cover or didn't follow the anode out.

a) If unable to net immobilized fish (e.g. they are tangled in the complex cover), cease shocking to prevent prolonged exposure to electrical current.

16. 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.

a. Remove debris that might compromise net integrity.

b. If during sampling it is discovered that one of the block nets is ≤ 10% compromised, fix net, and continue. Record ≤ 10% compromised in mobile app when pass is complete.

c. If during sampling it is discovered that the block net is > 10% compromised and this is a random reach or the 1st pass of a fixed reach-STOP fishing and:

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

2) Re-establish and secure the block nets a minimum of 12 hours after the 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.

d. At a fixed reach if during sampling the second pass it is discovered that the block net is > 10% compromised 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 except the first pass and request you to continue fishing the next reach. If you do not hear back from Science accepting the first pass 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.

e. At a fixed reach if during sampling the third pass it is discovered that the block net is > 10% compromised 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 except the first two passes. If you do not hear back from Science accepting just the first two passes 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.

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!*

17. 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.

1) Segregate predatory fish from prey species in separate buckets

2) Separate different age classes to prevent larger fish from harming small individuals.

b. Bucket replacement and moving fish is easier for the netters to do, as they will need to step out of the stream.

c. Secure a mesh netting 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. This may require that fish captured in the beginning of the reach are processed before the reach is fully sampled.

18. 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.

19. 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.

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

21. 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.

22. Check and record net integrity.

    a. If net integrity is compromised follow steps outlined **steps 16 a, b, c, d, and e** of this section.

23. 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.

24. 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 5-22 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).

        1) If the number of sampled fish increase with each pass do the following:

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

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

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

            d) Make sure the netters are alert during each pass

        2) If this issue continues in a subsequent 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 daybreak 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 at least 50 individuals of each species captured at each reach. Processing must be carried out with minimal stress, harm, and suffering to fish and other species (e.g. reptiles, amphibians, invertebrates) caught during sampling

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

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

C.1 Processing Samples

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 fill otherwise, will collect and report invertebrate data caught during fish sampling using the invertebrate bycatch table in the mobile device. 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. Anesthetize fish before weighing and measuring to reduce stress.
8. Preparing Anesthesia: 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:

   1) 25 - 40 mg/L for freshwater salmonids, expose to treatment no longer than 5 minutes.

   2) 40 - 100 mg/L for freshwater non-salmonids, expose to treatment no longer than 5 minutes.

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

   3) Refer to **Table 9** for calculated eugenol concentrations.

      a) 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).

      b) Label the bucket so the crew knows it contains anesthetic.

Table 9. 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.
        1) For salmonids, use 25 – 40 mg/L of AQUI-S® 20E, **do not** exceed a concentration of 40 mg/L.

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

***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 following Steps 11 Only voucher mortalities (inadvertently killed fish) or specimens that required euthanasia due to injuries. Use method SOP C2 for handling uncertainty is fish species identification.

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 or the Wadeable Stream Fish Sampling Field Datasheet.

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 16**) to the nearest millimeter and record on the mobile device.

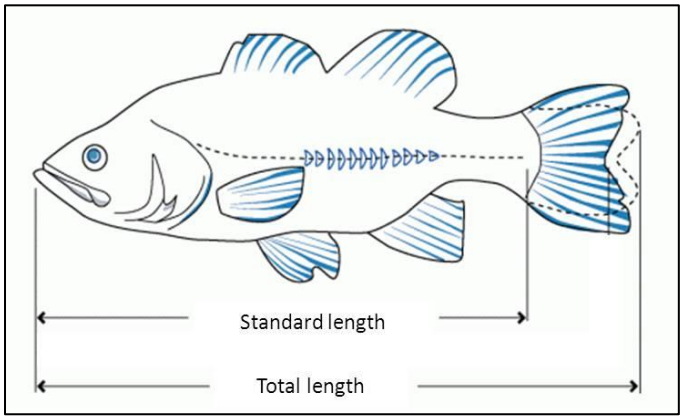

Figure 16. Measure the total length of each fish by pinching the fork together.

13. Inspect the fish for DELTS (deformities, including eroded fins, external lesions, parasites, and tumors) (**Figure 17**). DELTS should be considered as a pre-existing health condition that an individual fish may have been experiencing before being sampled. If there are multiple DELTS, indicate the single most impactful to the specimen.

    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.

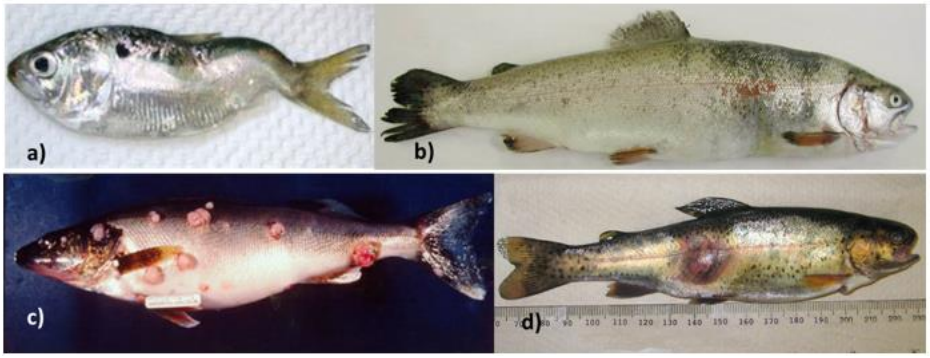

Figure 17. Example DELTS, a) deformity, b) eroded fins, c) tumors, and d) lesion. Photos by a) Kane 2005, b) Leander 2017, c) Cornell 2009, and d) Photo by Craig Banner 2004.

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 female can be difficult) only assign if you are positive of age class, otherwise leave blank.

16. Place processed fish in a bucket labelled "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. Segregate predator species from prey species, and large fish from small fish in buckets.
17. Repeat Steps 5-15 for the first 50 fish of each species captured at a reach. 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 is caught at the reach level. After the first 50 are weighed and measured, the remainder do not need anesthesia or to be weighed or measured but can be bulk counted by species in the bulk count data table on the mobile device.
        1) If holding buckets have greater than 50 individuals of one species, randomly choose the first 50 to weigh and measure.
        2) If bulk count is relatively low actually count each bulk species individual, record "Actual" in mobile app. If bulk count is too large to realistically count every individual estimate number and record "Estimate" in mobile app.
        3) 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.
        4) Place the bulk counted individuals in the recovery bucket with the other processed individuals.
        5) 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**.

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 ecologist 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:

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

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

1. Identification qualifiers contains information by using ID'q codes (**Table 10**) 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. Lethal dose of AQUI-S®20E for euthanizing fish.

Table 10. 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 names 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 ecologist 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 morpho species identification. Once 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

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 11** for calculated lethal dose concentrations of 10% eugenol. The American Veterinary Medical Association (AVMA 2013) recommends the use of eugenol as an acceptable agent for euthanizing fish. Fish exposed to a lethal dose of 10% eugenol are considered unsafe for human consumption.

Table 11. Lethal dose of AQUI-S®20E for euthanizing fish.

Target Concentration of AQUI-S20E (10% eugenol) mg/L	Volume of Treatment Water (gal)					
	2.5	5	10	15	20	25
100	8.4	16.8	33.6	50.4	67.3	84.1

a. Add 16.8 mL of 10% eugenol to 5.0 U.S. gallons of 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 follow the step in **SOP C.5**, depending on the size preserve specimen using the steps outlined in **SOP.C.5.** Place fish into appropriate sample container (e.g., wide mouth HPDE bottles-minimum 30mL) with completed specimen label and add 10% formalin preservative. One taxon per specimen bottle.

1) Adhesive Type I barcode labels will be added to the sample containers and scanned by the mobile app.

2) 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, then there needs to be an individual record included on the datasheet and a sample ID generated. Subtract the number of individuals pulled from the bulk sample count and create a record for each individual entry with a measured wet weight and length. The specimen voucher sample ID format is FSH.siteID.reachNo.YYYYMMDD.passNo.specimenNo.

2. Amphibians mortally injured as a result of fish sampling will be euthanized using a lethal dose of MS-222 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). Vouchers must be shipped to biorepository or stored on site. Seek guidance from NEON Science and Permitting about voucher storage.

e. Place the amphibian into an appropriate sample container (e.g., wide mouth HPDE bottles-minimum 30mL) with completed specimen label (**Figure 18**.) and add 10% formalin preservative. One taxon per specimen bottle. See steps on specimen preservation in **C.5**.

3. 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. 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 250 to 500 mg/kg of a pH-neutralized solution (0.7% to 1.0%) of MS-222. This will produce a loss of consciousness in less than five minutes (AVMA 2013).

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

Figure 18. 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.

c. 1st stage injection solution - 1% MS-222:

1) Prepare MS-222 and baking soda to be mixed with water in the field when needed)

2) 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.

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

d. Preparing 2nd stage injection solution - 50% (v/v) unbuffered MS-222:

1) Make a fresh solution in the field as needed.

2) Add one part MS-222 (g) to 2 parts water (mL); no baking soda (unbuffered).

3) Pre-weigh out 1 g of MS-222 at the Domain Support Facility into an appropriately sized container (ex. 20 mL scintillation vial covered in duct tape or foil to protect from light).

4) Add 1 g MS-222 in 2 mL tap water for 2 mL of 50% (v/v) MS-222 in the field when needed. A syringe can be used to measure out 2 mL of water. Shake the scintillation vial to mix the solution.

5) The solution will be thick with precipitates, cloudy, and pale yellow (**Figure 19**).

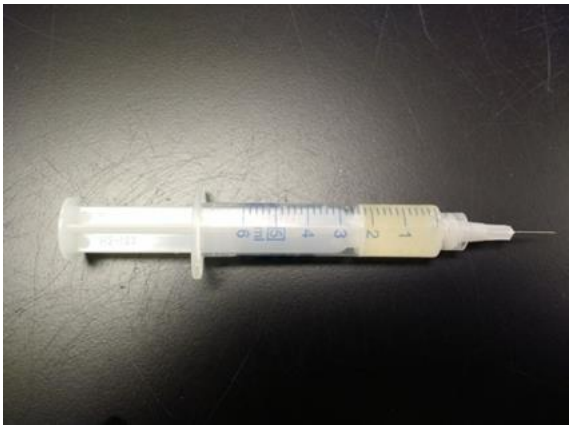

Figure 19. 2nd stage 50% (v/v) unbuffered MS-222 solution. Note yellow cloudy appearance of solution.

e. Administrating 1st stage injection instructions:

1) To inject 250 mg /kg into reptile use following formula:

a) Reptile weight (kg) x 250 mg/kg x 1 g/1000mg x 100 mL /1 g MS-222 = # mL of 1% MS-222 to inject into reptile. See **Table 12** for the 1st stage dosage calculator based on reptile weight in grams. The dose can be adjusted by adding or subtracting the 1% MS-222 solution volume using the table. For example, euthanizing a specimen that weighs 150 g with the 250 mg/kg concentration of 1% MS-222, inject 2.5 mL plus 1.25 mL (total 3.75 mL) of 1% MS-222.

b) **Table 12**. 1st Stage dosage calculator using 1% MS-222 for reptiles by weight (g).

2) To inject 250 mg /kg into reptile use following formula:

a) Reptile weight (kg) x 250 mg/kg x 1 g/1000mg x 100 mL /1 g MS-222 = # mL of 1% MS-222 to inject into reptile. See **Table 12** for the 1st stage dosage calculator based on reptile weight in grams. The dose can be adjusted by adding or subtracting the 1% MS-222 solution volume using the table. For example, euthanizing a specimen that weighs 150 g with the 250 mg/kg concentration of 1% MS-222, inject 2.5 mL plus 1.25 mL (total 3.75 mL) of 1% MS-222.

Table 12. 1st Stage dosage calculator using 1% MS-222 for reptiles by weight (g), number in white cells are volume of solution to inject, for reptiles that weigh amount indicated in dark cells.

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

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

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

5) 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 20**).

Figure 20. Injection into the inguinal fossa of a turtle. Photo by Kaufman 2017.

6) A 250 mg/kg dose will be sufficient to anesthetize reptiles. In the event that the 500 mg/kg dose is needed, double the dose (**Table 12**).

f. Administrating 2nd stage injection instructions:

1) Using a 5 mL syringe, inject 0.1 mL to 1.0 mL of the 50% (v/v) MS-222 unbuffered solution into reptile, depending on the weight of the specimen.

2) 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 20**).

3) Inject 0.1ml for specimens weighing less than 30 g. Add an additional 0.1 mL for every additional 30 grams of reptile.

g. Euthanized reptiles (or other vertebrate bycatch) must be vouchered (unless permit dictates otherwise). Vouchers must be shipped to biorepository or stored on site. Seek guidance from NEON Science and Permitting about voucher storage.

h. Place the reptiles (or other vertebrate bycatch) into an appropriate sample container (e.g., wide mouth HPDE bottles-minimum 30mL) with completed specimen label (**Figure 18**.) and add 10% formalin preservative. One taxon per specimen bottle. See steps on specimen preservation in **C.5**.

4. Aquatic invertebrate species, including arthropods and mollusks, that are mortally injured while performing fish sampling tasks will be euthanized. Mortally 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).

   a. Consult NEON Science and Permitting for guidance on storage of invertebrate bycatch.

5. 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.

   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.3** (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 21**).

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

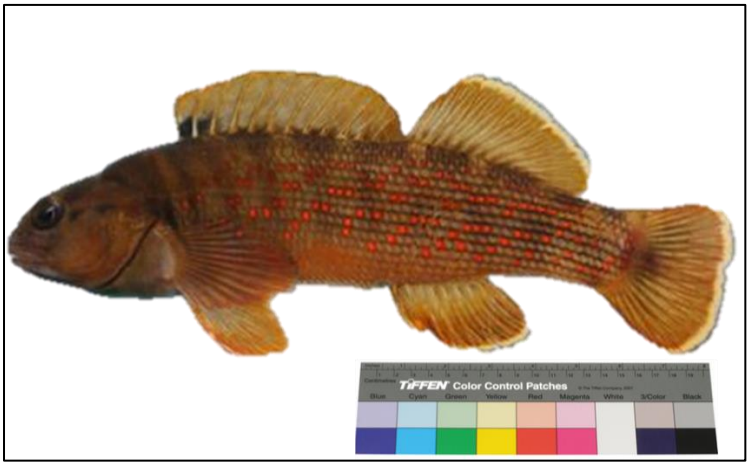

Figure 21. Example voucher photo. Lateral, head left, with color separation guide and scale bar. Photo from OSU 2017.

4. Dip the fin clipping scissors and forceps into high concentration ethanol (70% or greater) and ignite with lighter to flame sterilize the tissue sampling tools. If flame sterilization is unavailable, wet the instruments with native water after dipping in ethanol and wipe with a clean cloth.

***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.
6. **VERY IMPORTANT**: Fish tissues collected for DNA extractions cannot be exposed to or preserved in formalin.
7. Using the cutting tool, remove a piece of the target fin ray (**Figure 22**). 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.

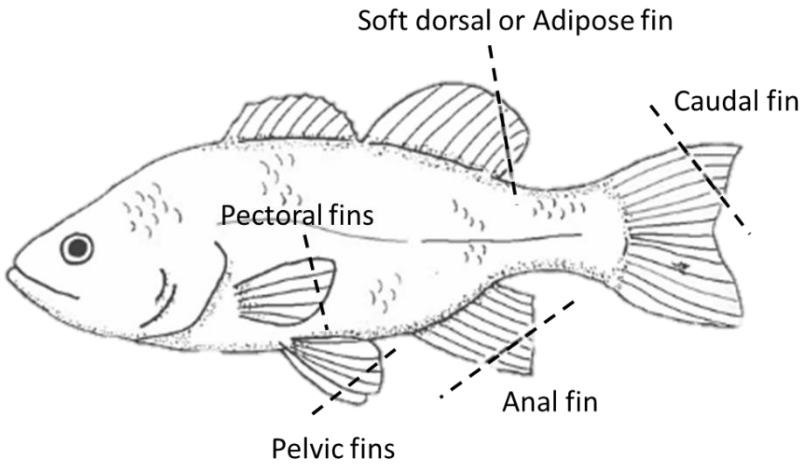

Figure 22. Optional fins to clip for tissue sampling.

8. 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".
9. The fin clip vial must also have type IV barcode label attached the outside.
10. 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** Whole Fish Voucher Specimen Preservation) or discarded following the guidance of the collection permit.

11. Place live fish that have had tissues samples collected into the recovery bucket.
12. Indicate on the mobile device or the Wadeable Stream Fish Sampling Field Datasheet that a DNA tissue sample was taken and provide a sample ID format "FSH.siteID.reachNo.YYYYMMDD.passNo.specimenNo.DNA" for each specimen.
13. Flame sterilize (or wipe) the cutting tool and forceps or wipe with an alcohol pad before collecting the next tissue sample to avoid cross-contamination. Be sure that no fish tissue, slime, or blood remains on the instruments.
14. Repeat Steps 1-13 until all targeted fish samples have had tissues collected.
15. 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
16. 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 Voucher Specimen Preservation

1. Fish < 200 mm:
    a. Fill jar with a 10% buffered formalin solution to fix specimens within one hour of euthanizing.
    b. If using concentrated formalin (37% formaldehyde), dilute 1-part concentrated formalin with nine parts water (can use native water).
    c. In general, use a ratio of 10:1 formalin to fish tissue by weight (example: use 1,000 mL formalin: 100 g fish).
        1) Use an appropriate size (minimum 30mL) HDPE wide mouth specimen jar for the preservation of an individual specimen in a single jar.
    d. 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 6**).
    e. The voucher jar must also have a Type I DNA barcode attached to the outside.
    f. Store in formalin for 5-7 days and then transfer to ethanol. Discard the used formalin accordingly (AD[03]). Transfer the specimens into an alcohol fixative; either 70%-95% ethanol or isopropanol, making sure that the specimen is fully submerged.

1) Ship voucher in ethanol only, unless otherwise directed.

g. Secure lid tightly and store upright at room temperature (~70°F) with appropriate specimen labels inside and outside of the container.

2. Fish > 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. 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 container with enough 10% formalin solution to cover fish, place a watertight lid on that jar, soak in jar for 7-10 days.

1) Decant formalin and soak fish in water.

2) Store in 70% ethanol or 50% isopropyl.

3) When shipping to biorepository; wrap in water dampened cheese cloth with voucher number and site, wrap the fish in cheese cloth, and package in double plastic bags (**Figure 23**).

Figure 23. 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 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 *NEON Standard Operating Procedure: Decontamination of Sensors, Field Equipment, and Field Vehicles* (RD[07]).
    b. Dry all equipment thoroughly between sites and before storage.
    c. Check all nets for holes and patch if necessary, using the net repair kit. Mending fish nets takes practice and patience. There are several resources available online. See the Oregon State University (OSU) 1989 reference for a resource to mend and patch fish nets (file://C:\\Users\dmonahan\Downloads\SGNO831989.pdf).
    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 in water with high conductivities, use fine grit sandpaper to remove rust.

D.2 Document Incomplete Sampling Within a Site

Fish Sampling in Wadeable Streams sampling is scheduled to occur at all prescribed sampling locations according to the frequency and timing described in Section 4 and in the site-specific sampling plan references in Appendix C. Ideally, sampling will occur at these sampling locations for the lifetime of the Observatory. However, sampling may be shifted from one location to another when sampling conditions are 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 Fulcrum records to determine which locations were scheduled for sampling but were not sampled.
2. Create an incident with the following naming convention to document the missed sampling: 'AOS/TOS Sampling Incomplete: MOD – [Root Cause Description]'.
    a. Example: 'TOS Sampling Incomplete: CDW – Could not access plot due to permanently closed road'.
3. Staff scientists review incident tickets periodically to determine whether a sampling location is compromised.

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.

SOP F Sample Shipment

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

Ground ship specimen vouchers to NEON ASU Biorepository for long-term preservation.

Ground ship fin clip tissues to the centralized Domain Support Facility (D01) for tissue processing and shipment to the external lab for sequencing (Canadian Centre for DNA Barcoding).

F.1 Handling Hazardous Material

Follow shipping and Hazmat procedures for ethanol, in the NEON Shipping document.

F.2 Supplies/Containers

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

F.3 Timelines

Ship samples fin clips when instructed to by Domain 1. Ship vouchers before the end of the calendar year.

F.4 Shipping Inventory

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

F.5 Laboratory Contact Information and Shipping/Receipt Days

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

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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 13. 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– 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 4 – Assemble backpack electrofisher and test the settings. Check anode and cathode for corrosion; remove with steel wool or other abrasive pad.

Step 5 – Move the anode across the stream in a zigzag pattern and capture drifting, immobilized fish. Place immobilized fish in 5-gallon buckets.

Step 6 – If this is a random reach, sample fish using only one pass. If this is a fixed reach, sample fish using three passes.

Step 7 – Anesthetize caught fish in a 5-gallon bucket with solutions of AQUI-S®20E.

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 the specimen 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 specimen in a jar with a 10% buffered formalin, then ethanol for long-term storage. Ship to the external facility when directed to.

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 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

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

APPENDIX D SITE-SPECIFIC INFORMATION

D.1 Randomized Reach Selection Per Site

Randomized reach order is shown for each site below for sites with 10 fish reaches. See Appendix G 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 field device.

Table 14. Randomized Section Selection Per Site.

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

D.2 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 more 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.3 Sampling Reaches with Permanent Barriers

At TECR and MCDI reaches are bisected by a permanent fish barrier, have been identified and the following steps must be taken at these reaches.

1. **Record the presence of a permanent fish barrier that bisects the fish** reach:
    a. **record total reach length**
    b. **and** segment length from bottom of the reach to barrier length
    c. **and from barrier to top of** the reach length in mobile device
2. Start fishing the reach section from the downstream boundary to the permanent barrier.
    a. fix block net at the downstream reach boundary
    b. the permanent fish barrier can serve as a block to fish moving upstream.
3. Fish from the downstream transect boundary to the fish barrier, process fish and record them as **caught below the fish barrier** in mobile device.
4. Record eftime (seconds) from the backpack shocker for the lower area.
5. Release fish below downstream block net.
6. At fixed reaches, repeat steps 2 and 3.
7. At random reaches continue up and fish between the top of barrier and the top of the reach.
8. At fixed reaches fish between the permanent barrier and the upstream reach boundary for 3 passes.
9. Record the fish as caught above the fish barrier in mobile device.
10. In mobile device record EF time when complete with each pass for each segment.
11. Release fish above reach boundary, or if you have completed sampling release back into reach.

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. Complete the data entry on the mobile device or Wadeable Stream Fish Sampling.
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 24**), 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 24**). Each fish sampling reach is numbered sequentially beginning with "1" at the bottom (downstream) but just above the test reach (**Figure 24**). Record the coordinate at the plot marker location as well as the coordinate uncertainty using the Trimble data dictionary for AOS locations
    a. Refer to the Morphology Mapping (RD[10]) protocol for further descriptions and examples of stream habitats.
    b. 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. The right bank is preferred for consistency across sites.

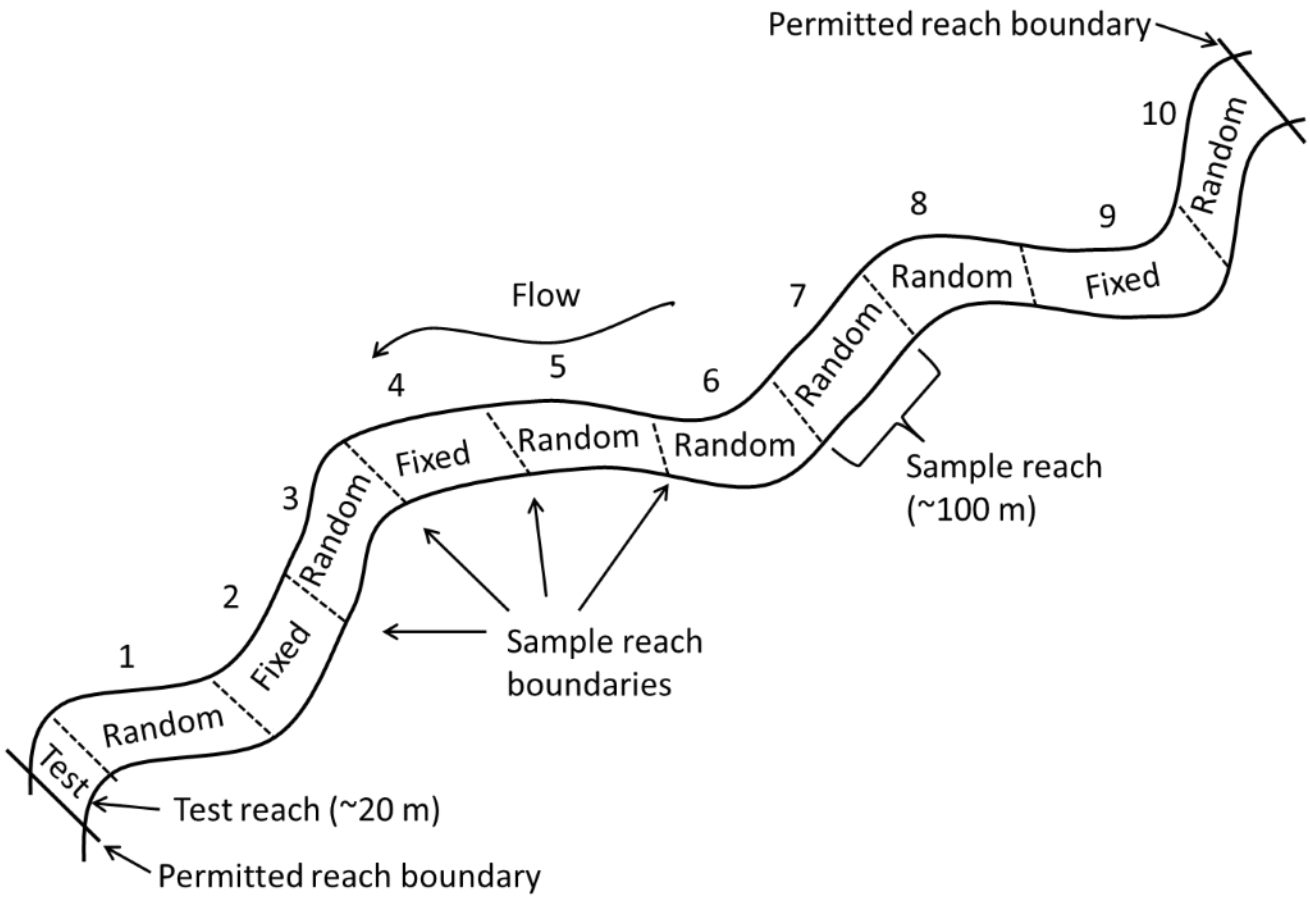

Figure 24. Schematic of a 1 km permitted NEON stream site delineated into ten 100 m reaches: 3 fixed and 7 random sampling reaches. Three random reaches will be chosen each year for sampling.

E1. 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, then it may be necessary to establish new reaches or the entire fish sampling reach. Submit a trouble ticket through the NEON problem resolution system.

1. Up to six 100 m (± 20 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 10 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** for 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 15**).
6. For each year of sampling, continue down the list of randomized reaches not sampled previously. In year three (if the stream contains 10 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 study.

Table 15. Example of fixed and rotating reach design for one site over 10 years. Gray boxes denote when a 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										

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 16. Equipment list – Field Preparation.

Supplier/ Item No.	Exact Brand	Description	Purpose	Quantity
Durable items				
	N	HDPE bottle, amber, 1 L	Stock solution ofMS-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:	Y	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
		Specimen labels (waterproof paper)	Labeling specimens	2 sheets

Supplier/ Item No.	Exact Brand	Description	Purpose	Quantity
	N	Adhesive barcode labels	Labeling sample bottles with barcode-readable labels	1 sheet
	N	Collection permit documents		1

Table 17. Equipment list – Reach establishment.

Supplier/ Item No.	Exact Brand	Description	Purpose	Quantity
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 18. Field fish sampling gear.

Supplier/ Item No.	Exact Brand	Description	Purpose	Quantity
Durable items				
	N	Steel studded fence posts (i.e., T-post)	Securing block net at reach boundary	8

Supplier/ Item No.	Exact Brand	Description	Purpose	Quantity
	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)	Securing block net at reach boundary	15
	N	Net repair kit: • needle • net string • butane lighter • zip ties	Repairing nets	1
	N	Gloves, Nitrile coated, Textured, Knitted cuff, Abrasion resistant, PIP or Equivalent	Handling 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

Supplier/ Item No.	Exact Brand	Description	Purpose	Quantity
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
	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
	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

Supplier/ Item No.	Exact Brand	Description	Purpose	Quantity
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
	N	Fin clipping scissors	Cutting fish fins for DNA barcode samples	1
Fisher Scientific Company:	Y	Refillable butane lighter	Flame sterilization of tissue sampling equipment	1
	N	Dish pan, plastic	Disinfecting tools with iodophor or betadine	1-12 qt.

Supplier/ Item No.	Exact Brand	Description	Purpose	Quantity
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
	N	Tricaine methanesulfonate (MS-222) stock solution	Euthanizing specimens	1 L
AquaTactics Fish Health:	Y	AQUI-S®20E stock solution	Anesthetizing specimens	1 L
	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

Supplier/ Item No.	Exact Brand	Description	Purpose	Quantity
	N	50mL Centrifuge tube	Store syringe and needle, measure injection fluids	1
	N	60mL Clear bottle HDPE	Store dry MS-222 and baking soda for reptile euthanasia stage 1 solution	1
	N	20mL HDPE Scintillation vial	Store dry MS-222 for reptile euthanasia stage 2 solution	1
	N	Sharps Container, Portable, Slip Top	Receptacle for disposal of needles	1
	N	Tissue containers (e.g. 1.5 mL microcentrifuge tubes)	For storing fin clips for DNA barcoding	100