

<i>Title:</i> AOS Protocol and Procedure: Fish Sampling in Lakes		<i>Date:</i> 02/18/2014
<i>NEON Doc. #:</i> NEON.DOC.001296	<i>Author:</i> J. Fischer	<i>Revision:</i> A

## AOS PROTOCOL AND PROCEDURE: FISH SAMPLING IN LAKES

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## 1 DESCRIPTION

### 1.1 Purpose

The primary purpose of this document is to provide a change-controlled version of Observatory protocols and procedures. This document provides the content for training and field-based materials for NEON staff and contractors. 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.

This document is a detailed description of the field data collection, relevant pre- and post-field tasks, and safety issues as they relate to this procedure and protocol.

### 1.2 Scope

This document relates the tasks for a specific field sampling or laboratory processing activity and directly associated activities and safety practices. This document does not describe:

- General safety practices
- Site-specific safety practices
- General equipment maintenance

It does identify procedure-specific safety hazards and associated safety requirements such as safe handling of small mammals or safe use of required chemicals and reagents.

### 1.3 Acknowledgments

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

## 2 RELATED DOCUMENTS AND ACRONYMS

### 2.1 Applicable Documents

Applicable documents contain information that shall be applied in the current document. Examples are higher level requirements documents, standards, rules and regulations.

AD [01]	NEON.DOC.004300	EHS Safety Policy and Program Manual
AD [02]	NEON.DOC.004316	Operations Field Safety and Security Plan

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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

## 2.2 Reference Documents

Reference documents contain information complementing, explaining, detailing, or otherwise supporting the information included in the current document.

RD [01]	NEON.DOC.000008	NEON Acronym List
RD [02]	NEON.DOC.000243	NEON Glossary of Terms
RD [03]		NEON DOORS Requirements
RD [04]	NEON.DOC.005003	NEON Scientific Data Products Catalog
RD [05]	NEON.DOC.014051	Field Audit Plan
RD [06]	NEON.DOC.000824	Data and Data Product Quality Assurance and Control Plan
RD [07]	NEON.DOC.001152	NEON Aquatic Sample Strategy Document
RD [08]	NEON.DOC.001204	AOS Protocol and Procedure: Macroinvertebrate Sampling in Lakes and Non-wadeable Streams
RD [09]	NEON.DOC.001164	NEON Bathymetric Mapping Protocol
RD [10]	NEON.DOC.001162	NEON Lake Riparian Mapping Protocol
RD [11]	NEON.DOC.001154	NEON Aquatic Decontamination Protocol

## 2.3 Acronyms

A	Ampere or amp
AFS	American Fisheries Society
cm	Centimeter
DC	Direct current
Hz	Hertz
m	Meter
mL	Milliliter
mm	Millimeter
MS-222	Tricaine methanesulfonate
PFD	Personal flotation device
SL	Standard length
TL	Total length
USEPA	United States Environmental Protection Agency
USGS	United States Geological Survey
V	Volt
W	Watt

## 2.4 Definitions

**Amperage** – A measure of electrical current strength expressed as amperes.

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**Ampere** (Amp or A) – A standard unit of electrical current used to measure strength. Current (A) = Power (W) / Voltage (V).

**Anode** – A positive electrode that is commonly a ring on a fiberglass pole for backpack electrofishing.

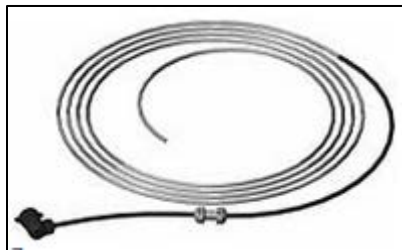


**Figure 1.** Electrode pole (anode) for backpack electrofishing unit (photo: store.smith-root.com)

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

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

**Cathode** – A negative electrode which is commonly a stainless steel cable that is dragged behind the operator for backpack electrofishing.



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

**Crepuscular** – Of or relating to twilight, both dawn and dusk.

**Direct current (DC)** – The unidirectional flow of electricity.

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**Duty cycle** – The fraction of time an entity is considered active. In relation to pulsed-DC electrofishing, the duty cycle refers to the proportion of electrical waveforms the current is on and is expressed as a percentage.

**Electrode** – A metallic conductor through which electrical current leaves (i.e., anode) or enters (i.e., cathode).

**Electrofishing** – The use of electricity to temporarily immobilize fish for collection of biological data (e.g., taxonomic identification, length weight).

**Frequency** – The number of times an occurrence repeats. In relation to pulsed-DC electrofishing, the frequency is measured in pulses per second (i.e., Hz) and can be adjusted. High frequency pulses commonly have been associated with increased injuries to fish.

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

**Lentic** – Of or relating to still waters, e.g., lakes.

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

**Pulsed DC** – Direct electrical current that is interrupted rapidly.

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

**Thermocline** -- A distinct layer in a body of water where the change in temperature is more rapid than increasing depth - usually a change of more than 1°C per meter. The denser and cooler layer below the thermocline is defined by the hypolimnion. The warmer upper layer is termed the epilimnion.

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

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

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

### 3 BACKGROUND AND OBJECTIVES

#### 3.1 Background

Aquatic organisms have long been used to understand natural and anthropogenic changes to environmental conditions. Fish are particularly useful indicators of ecological integrity because they are influenced by a variety of processes and regimes (i.e., resource availability, anthropogenic physiochemical disturbances), have the ability to alter aquatic ecosystems as top consumers, and are



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relatively long-lived species. Consequently, fish assemblage assessments can quantify assemblage structure and function in aquatic environments and provide a temporally integrated measurement of ecosystem health. Fish are used to assess ecosystem health because they are a diverse taxonomic group with a broad range of habitat requirements and life history strategies: fish assemblages represent a variety of feeding guilds, reproductive strategies, life spans, and tolerances to environmental degradation. Additionally, fish are a highly visible taxonomic group that can be easily sampled by biologists.

Assessing fish assemblages in lakes and impoundments is challenging due to numerous sampling biases (e.g., gear, season, location) that affect accurate characterization of populations and assemblages (Hayes et al. 1996). The quantitative assessment of fish assemblages is often limited by the cost associated with sampling because multiple sampling methods conducted across large temporal and spatial scales are required. Most research identifying appropriate gears for sampling fishes in lakes has focused on sport fish populations (Hubert 1996; Reynolds 1996). Although sport fishes are important from ecological and social perspectives, non-game fishes may be fundamental to ecosystem function and provide a better reflection of ecological integrity (e.g., Simon 1998). Consequently, little information is available on the appropriate methods to accurately and precisely estimate fish assemblage composition in lentic habitats.

**3.2 NEON Science Requirements**

This protocol fulfills Observatory science requirements that reside in NEON’s Dynamic Object-Oriented Requirements System (DOORS; RD [03]). Copies of approved science requirements have been exported from DOORS and are available in NEON’s document repository, or upon request.

**3.3 NEON Data Products**

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, which are documented in the NEON Scientific Data Products Catalog (RD [04]), available on the NEON website.

**4 PROTOCOL**

The objective of this document is to outline the sampling protocol and procedures used for sampling fish in lakes at NEON sites. Unlike rivers and streams where relatively few sampling methods (e.g., electrofishing, seines) are commonly used to characterize fish assemblages (Guy et al. 2009; Rabeni et al. 2009), numerous methods (e.g., electrofishing, seines, fyke nets, gill nets, trawling) are used to sample fish assemblages in lakes and impoundments (Miranda and Boxrucker 2009; Murphy and Willis 1996). Multiple methods are typically required because lakes and impoundments have two distinct zones (i.e., pelagic and littoral) that differ in physicochemical characteristics and fish assemblage structure. Substantial differences in physical characteristics (e.g., depth, water clarity, vegetation) and the selectivity of species and sizes of fish affect the efficiency of sampling methods in differing zones.

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For example, multiple gears are often necessary to sample both juvenile and adult fish of the same species because of differing habitat use and size biases associated with various equipment (Boxrucker et al. 1995). Although a few studies have evaluated multiple sampling equipment, most studies have focused on a limited number of species (e.g., gizzard shad *Dorosoma cepedianum*, black crappie *Poxomis nigromaculatus*) at small spatial scales (e.g., Boxrucker et al. 1995; Guy et al. 1996; Allen et. al 1999). Herein we describe a sampling method that attempts to overcome traditional problems associated with characterizing fish assemblages in lentic habitats, by using multiple active and passive methods (i.e., backpack electrofishing, mini fyke nets, gill nets) at different times throughout the year to capture fish of a variety of size classes and habitat preferences.

In this protocol, permanent segments should be sampled biannually with a backpack electrofisher using multiple pass depletion, mini-fyke nets, and short-set gill nets at reaches within each segment (Figure 3; Baker et al. 1997). Sampling and net placement should be located far enough apart to minimize interactions. The number of random segments selected for sampling should be positively related to lake size, but should not cover the entire shoreline. In any given sampling year, up to 40% of the shoreline should remain unsampled. A rotating sampling design with initial random selection of shoreline segments ensures appropriate spatial coverage of habitat types within the lake (Baker et al. 1997). The same random segments that were systematically chosen for additional sampling should be sampled in spring and fall.

## 5 QUALITY ASSURANCE AND CONTROL

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

When unexpected field conditions require deviations from this protocol, the following field implementation guidance must be followed to ensure quality standards are met:

- 1) If wind is >9 km/hr such that the boat cannot be held stationary over a sampling point, stop sampling and wait for up to 30 minutes for wind to subside. If wind subsides, resume sampling, if not, return to the Domain Support Facility and sample at another time.
  - a) If electrofishing activities are interrupted due to unsafe field conditions, captured fish should be released and sampling discontinued. If an entire segment cannot be completed, recollect all data on the next appropriate day.
  - b) Do not begin sample collection unless there is enough time to complete an entire sampling segment (i.e., all passes of an electrofishing segment, or a 1-hour gill net set).
- 2) If heavy rainfall or that affects visibility or flooding/high water occurs on or prior to the targeted sampling date, wait a minimum of 3 days to allow the fish community to recolonize habitats.

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**5.1 Sampling-specific Concerns**

- 1) Fish sampling should not occur while other aquatic sampling activities that could disturb sediments or hydrology (e.g., macroinvertebrate sampling) are occurring in the lake.
- 2) Fish sampling must be completed within a 5-day period per site. If field conditions appear unfavorable (e.g., prolonged thunderstorms, tropical storms, expected flooding) during the proposed sampling bout, postpone sampling until the next appropriate time when the entire sampling bout can be completed in 5 days.
- 3) Reasonable efforts should be made to minimize mortality to fish during sampling. This includes the use of best fish handling practices (e.g., frequent changes of lake water in buckets, aerators) and limited use of collected specimens.
- 4) Electrofishing-related injuries should affect < 1% of fish captured. If this number is exceeded at the site, stop sampling and contact the NEON Aquatic Ecologist.

**6 SAFETY**

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.

See Section 10 in the NEON Operations Field Safety and Security Plan (AD 01]). In addition the following general fishing safety guidelines are provided:

- 1) Technicians are required not to put hands and feet in waters where alligators are present and to make sure a safe distance from hazards is maintained. Electrofishing will not be conducted at these sites.
- 2) All employees shall have access to a form of communication with other team members such as a two-way radio.
- 3) Technicians should be aware of any site-specific hazards and to the waters of that particular location (i.e. current status, tidal charts, etc.).
- 4) Activities should only be performed when flow conditions are safe. Do not attempt to wade in a lake past waist-deep.

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

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

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

**7 PERSONNEL REQUIREMENTS**

This protocol requires three qualified technicians for 5 consecutive field days. There is no lab processing at the Domain Support Facility associated with this protocol.

**8 TRAINING REQUIREMENTS**

All technicians must complete required safety training as defined in the NEON Training Plan (AD [04]). Additionally technicians complete protocol specific training for safety and implementation of this protocol as required in Field Operations Job Instruction Training Plan (AD [05]).

Personnel are to be trained in fish sampling in lakes and safe working practices for boat-based fieldwork. Specific training for lake fish sampling must also include electrofishing training for all technicians.

**9 FIELD STANDARD OPERATING PROCEDURE**

**9.1 Sampling Frequency and Timing**

Ranges of sample timing are provided on a site-by-site basis by Science Operations based on data collected by the aquatic sensors and Field Operations. Sample timing shall be outlined in the NEON Aquatic Sampling Strategy Document (RD [07]).

**9.1.1 Criteria for Determining Sampling Dates**

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

**9.1.2 Sampling Frequency**

Lake fish sampling will occur two times per year during the growing season at each lake site, roughly spring and autumn.

**9.1.3 Sampling Timing Parameters**

Sampling corresponds with the first and third sampling windows for Macroinvertebrate Sampling in Lakes and Non-wadeable Streams (RD [08]). Fish sampling must occur within a 1 month window of the specified sampling date (2 weeks before – 2 weeks after) depending on weather conditions at the site and should occur after macroinvertebrate sampling (RD [07]).

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A minimum of 2 weeks between sampling bouts shall be observed. Sampling bouts should not be longer than 5 days long. All three passes in a fixed sampling segment must be sampled within the same day, with at least 30 minutes between passes to allow fish to resettle in the reach.

Lake fish assemblage characterization requires multiple sampling methods that are optimal for sampling fish at different times of the day. Electrofishing will occur after sunset and before sunrise (or during lowest-light hours at Arctic sites). Gill nets will be set and sampled during daylight hours, with a preferred set time of 1 hour and maximum set time of 2 hours. Gill nets should be set in the morning or early afternoon to allow for processing time. Mini-fyke nets will be set before sunset and allowed to remain in the water until after sunrise the following morning (Table 1). Fyke nets should be set in late afternoon, ensuring that all nets will be set at least 1 hour before sunset.

**Table 1.** Proposed lake sampling activities for a crew of 3 during a 5 day period

	Day 1	Day 2	Day 3	Day 4	Day 5
Day	AM/PM: Set electrofishing block nets  PM: Set mini-fyke nets	AM: Pull mini-fyke nets  AM/PM: Run gill nets  PM: Set mini-fyke nets	AM: Pull mini-fyke nets  AM/PM: Run gill nets  PM: Set mini-fyke nets	AM: Pull mini-fyke nets  AM/PM: Run gill nets  PM: Set mini-fyke nets ( <i>if needed</i> )	AM: Pull mini-fyke nets ( <i>if needed</i> )  AM/PM: Run gill nets ( <i>if needed</i> )
Night	Allow electrofishing segments to recolonize	Electrofish in fixed segments	Electrofish in fixed or random segments	Electrofish random segments ( <i>if needed</i> )	

## 9.2 Equipment and Materials

**Table 2.** Field equipment list

Maximo Item No.	Item Description	Quantity	Habitat-Specific	Special Handling
<b>Field preparation</b>				
	Tricaine methanesulfonate (MS-222)	20 g		Y
	NaHCO <sub>3</sub> (buffering agent for MS-222)	50 g		
	Clove oil (is not using MS-222)	10 mL		
	HDPE bottle, amber, 1 L	2		
	Nitrile gloves (pair)	1		
	Lab safety glasses (pair)	1		
	Field data sheets (print on waterproof paper, write in pencil)	10		
	Specimen labels (waterproof paper)	2 sheets		

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Maximo Item No.	Item Description	Quantity	Habitat-Specific	Special Handling
	Battery charger (electrofishing batteries)	1		
<b>Segment establishment</b>				
	Site-specific bathymetry map	1		
	Site-specific riparian map	1		
	Plot survey markers (aluminum, site-specific)	10		
	Flagging tape	1 roll		
	Meter tape (50 or 100 m)	1		
	Handheld GPS (with batteries, ± 1 m accuracy)	1		
<b>Electrofishing</b>				
	Steel studded fence posts (i.e., T-post)	30		
	Fence post driver or small sledge	1		
	Fence post puller	1		
	3 mm block nets with lead lines and top lines with floats (35 m long × 2 m wide)	3		
	L-type block net stakes (e.g., ~45 cm long, 10 cm handle, 1 cm diameter stainless rod)	15		
	Net repair kit: <ul style="list-style-type: none"> <li>• needle</li> <li>• string</li> <li>• butane lighter</li> <li>• zip ties</li> </ul>	1		
	Battery-powered backpack electrofishing unit	1		
	Anode pole (1.5-2.0 m) with attached anode ring	1		
	Cathode (rattail type; 1-2 m long, 10-15 mm diameter)	1		
	Electrofischer batteries (rechargeable)	3		
	Abrasive pad to clean anode rings	1		
	6.4 mm mesh dip nets with fiberglass handles	4		
	Rubber lineman gloves (Class 0, rated for max use voltage 1,000V AC/1,500V DC)	1 pair per person		
	5 gallon buckets	15		
	Handheld water conductivity/temperature meter	1		
	Chest waders (approved for electrofishing)	1 pair per person		
	Chest wader repair kit (e.g., Aquaseal) or extra waders	1		
	Head lamps (with batteries)	1 per person		
<b>Gill nets</b>				
	Gill net tubs	6		
	Gill net hooks	6		
	Depth finder	1		
	Experimental monofilament sinking gill nets			

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Maximo Item No.	Item Description	Quantity	Habitat-Specific	Special Handling
	Panel dimensions – 3.1 m long × 1.8 m deep Mesh bar size – 19, 25, 32, 38, 44, 51, 57, 64 mm Mesh order – 38, 57, 25, 44, 19, 64, 32, 51 mm Hanging ratio – 0.5			
	Net floats	18		
	12.7 mm diameter rope (3-4 m in length) for floats	18		
	Net anchors	18		
<b>Mini-fyke nets</b>				
	Mini modified fyke nets Mesh – 6.35 mm bar knot-less with asphalt coating Lead – One, 7.6 m long × 0.6 m deep Trap – Two 0.6 m × 1.2 m rectangular frames, two 0.6 m diameter hoops with one funnel, cod end with purse string closure.	6		
	Reusable nylon cable ties (46 cm)	50		
	T-type block net stakes (e.g., ~45 cm long, 20 cm handle, 1 cm diameter stainless rod)	6		
	Waterproof blinking LED light	6		
<b>Fish processing</b>				
	Fish and top predator taxonomic ID key specific to location or region (denotes endangered species)	1		
	Portable aerators (batteries, diffusion stone)	15		
	Small dip net (3.2 mm mesh)	5		
	Fish measuring boards (50 cm)	2		
	Portable digital scale (batteries, charger)	1		
	Plastic tray (for weighing fish)	2		
	Nitrile gloves (pair)	10 pairs		
	Digital camera (batteries, memory card)	1		
	Pencils	5		
	Clipboard	1		
	HDPE wide mouth specimen jars (1 L)	50		
	25-50 mL graduated cylinder, plastic	1		
	Nitrile gloves (pair)	10		
	MS-222 or clove oil stock solution	1 L		Y
	10% buffered formalin (37-40% formaldehyde)	20 L		Y
<b>General boating equipment</b>				
	Boat	1		
	Anchor with rope	1		
	Oars	2		
	Trolling electric motor	1		
	Battery (12 volt)	1		

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Maximo Item No.	Item Description	Quantity	Habitat-Specific	Special Handling
	Personal flotation devices (PFDs)	1 per person		
	Safety kit for boat (e.g., flares, bailer, float with rope)	1		
	First aid kit	1		

### 9.3 Preparation

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

- 1) Charge or replace batteries for backpack electrofishing unit, boat motor, GPS unit, camera, portable scale, temperature/conductivity meter, portable aerators, and headlamps batteries overnight or longer.
- 2) Inspect electrofishing unit for normal operation (e.g., no frayed cathode or broken anode, no error message when turned ON, functioning activation switch).
- 3) Inspect boat, trailer, and motor for normal operation.
- 4) Inspect lineman gloves and waders for holes and tears, repair if necessary.
- 5) Inspect dip nets, block nets, gill nets, and fyke nets for rips, tears, and holes. Repair if necessary.
- 6) Inspect portable aquarium pumps, diffusion stones, and batteries.
- 7) Inspect buckets to ensure handles are present and functioning.
- 8) Ensure that all equipment has been decontaminated since last use (see RD [11]).
- 9) Print data sheets (Appendix A, Appendix B) and specimen labels (Appendix C) on waterproof paper.
- 10) Select random sampling segments if this is the first sampling date for the year (Section 9.5).
- 11) Mix MS-222 or clove oil stock solution (site-specific, depends on EHS permits) in the Domain Support Facility.
  - a) MS-222
    - i) Wear nitrile gloves and eye protection, as MS-222 is a hazardous substance.
    - ii) Weigh 20 g of MS-222 powder and 50 g Na<sub>2</sub>CO<sub>3</sub>.
    - iii) Mix 20 g MS-222 + 50 g Na<sub>2</sub>CO<sub>3</sub> in a bucket with 1 liter tap water.
    - iv) Pour the stock solution into two 1 L amber HDPE bottles.
    - v) Label bottles "MS-222 stock solution".
  - b) Clove oil
    - i) Clove oil is low in toxicity and not carcinogenic, however nitrile gloves and eye protection are recommended.
    - ii) Mix 10 mL clove oil with 1 L of tap water.
    - iii) Pour stock solution into 1 L amber HDPE bottles.
    - iv) Label bottles "clove oil stock solution".
  - c) Stock solution must be stored in dark bottles in a room-temperature (~70 °F) environment. Stock solution may be reused over sampling bouts.

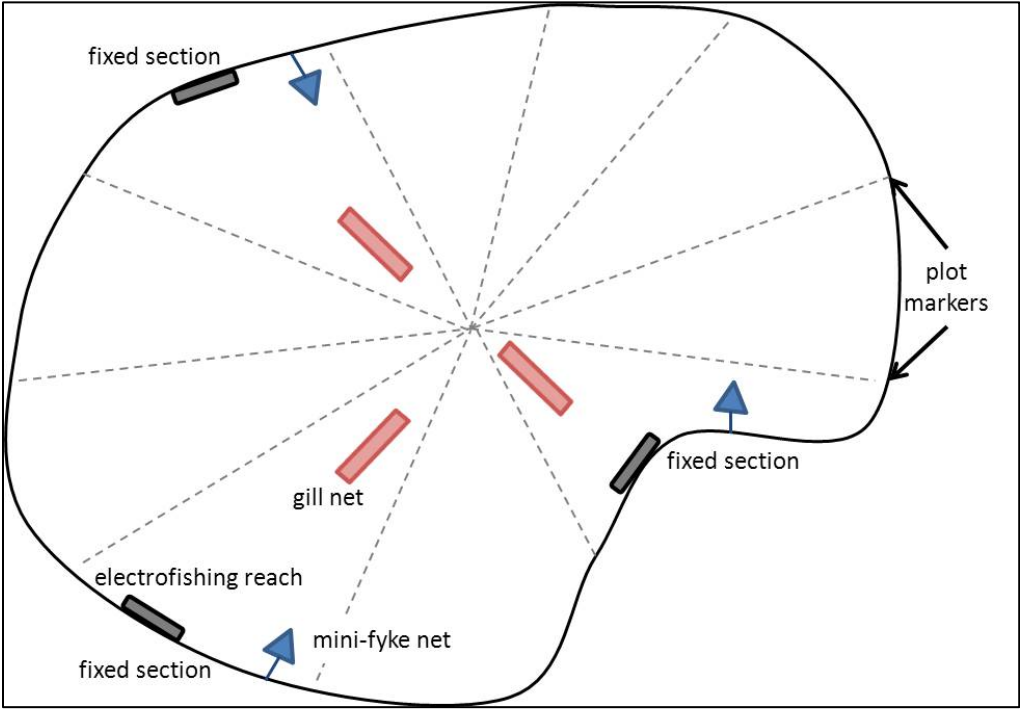


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### 9.4 Establishing Sampling Reaches

Establish sampling segments during the first year of sampling. Segments may need to be reestablished if significant morphological changes have occurred since the last sampling bout. Use the pre-determined 10 riparian habitat sections (see Lake Riparian Mapping Protocol, RD [10]).

- 1) Using the site-specific Riparian Map (RD [10]), determine the length of shoreline contained in each of the 10 riparian segments.
  - a) Fish sections ideally include at least 300 m of shoreline so that sampling sets (i.e., fyke nets, gill nets, electrofishing segments) are not too close together. If sections are <300 m, combine riparian sections.
- 2) Install an aluminum plot survey marker on the shoreline at each section boundary (if not already present; Figure 3). Record the location of each marker on the handheld GPS unit and on the Reach Delineation Data Sheet (Appendix A).
  - a) Add GPS points to the lake bathymetry map (RD 09] at the Domain Support Facility.
  - b) If you are unable to install plot survey markers due to permitting restrictions, record GPS data so you can return to the location.



**Figure 3.** Schematic of NEON lake site delineated into 10 riparian habitat sections (RD [10]), three of which are designated as “fixed”.

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### 9.5 Fixed and Random Sampling Section Selection

Section selection occurs during the first year of sampling. Sections will be revisited over the following years.

- 1) Up to six sections (three fixed and three random) will be sampled during each sampling bout (Table 3) depending on the size of the lake.
- 2) Select three of the 10 riparian sections to be the “fixed” sections. Fixed sections will be sampled two times per year throughout the duration of NEON measurements.
  - a) The three fixed 100 m reaches should be chosen to best represent the habitat variability throughout the lake (e.g., presence or absence of vegetation, substratum type; Figure 3). Fixed reaches will be selected by the NEON Aquatic Ecologist or Domain Aquatic Technician.
  - b) Avoid having sensor sets within electrofishing reaches. Electrofishing must occur  $\geq 5$  m away from all in-lake electronics.
- 3) Select three of the remaining seven random sections to be sampled annually. Refer to Appendix D for randomized order of sections for each lake site.
- 4) Use the same three random sections for all sampling dates within one year (Table 3).
- 5) For each year of sampling, continue down the list of randomized sections not sampled previously. In year three (if the lake contains 10 sections), there should only be one section that has not yet been sampled. Return to the first random section when all sections have been sampled.
- 6) Follow this pattern for the remainder of the study.

**Table 3.** Example of rotating section design for sampling one lake site over 10 years. Gray boxes denote when a section is sampled. Randomized order for each site is presented in Appendix D.

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

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## 9.6 Sample Collection in the Field

### 9.6.1 Electrofishing Segment Set-up

- 1) Navigate to the first riparian section selected for sampling using GPS points, the morphology map, or plot survey markers.
- 2) Setup block nets for electrofishing in fixed segments and fence posts (no nets used) to mark random segments.
  - a) Electrofishing shall only be conducted the night following block net set up or later to allow fish to acclimate after disturbing the area.
- 3) Drive fence posts using the fence post driver into the lake substrate outlining the 4 m × 25 m electrofishing reach, parallel to the shoreline.
  - a) Start at the shoreline.
  - b) Do not disturb the area inside the 4 x 25 m sampling area.
  - c) Space fence posts a maximum of 2 m apart.
  - d) Ensure that the deep edge is ≤ 1 m deep to allow for safe electrofishing. It is ok if the reach is less than 4 m x 25 m as long as the size has been measured and recorded.
- 4) Attach the top of the block net to each fence post in the fixed reaches with a reusable nylon cable tie approximately 30 cm above the water line.
  - a) Fence posts in random segments are used to delineate the electrofishing area, but will not have nets attached to them.
- 5) Bundle the remaining net and secure to the fence post with a reusable nylon cable tie, keeping the net is above the water line.
  - a) The random segments (up to 3) will be sampled via non-enclosure electrofishing (i.e., no block nets).

### 9.6.2 Backpack Electrofishing Field Set-up

Test settings on the backpack electrofisher before sampling begins. After settings are determined, they will be used for the remainder of the sampling bout.

- 1) **VERY IMPORTANT:** All technicians MUST wear necessary personal protective equipment before stepping in the water, including waterproof chest waders with appropriate fitting rubber lugged-soled boots, rubber lineman gloves to insulate the wearer from electrical shock. Head lamps must also be worn during nighttime sampling.
- 2) Assemble anode pole (Figure 1).
- 3) Measure and record water temperature and conductivity using the handheld conductivity meter. Record on Field Data Sheet (Appendix B).
- 4) Connect the cathode and anode to the backpack electrofishing unit (Figure 4).

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**Figure 4.** Cathode and anode connections on backpack electrofishing unit

- 5) Connect the battery to backpack electrofishing unit and secure the batteries with the strap to the backpack frame (Figure 5).



**Figure 5.** Battery location and secure placement in the backpack electrofishing frame.

- 6) Test the backpack electrofishing unit >50 m away from the designated electrofishing reach.
  - a) Select an area of the lake shoreline that has characteristics similar to that of the sampling reach (e.g., similar depth or vegetation).
- 7) Wade into the lake ensuring that the cathode (i.e., rattail) is submerged and anode ring is submerged.
  - a) Begin electrofishing in shallow water (e.g., < 50 cm).
- 8) While the electrofisher operator is standing in the lake, set the frequency to 30 Hz, the duty cycle to 12% (i.e., 4 ms pulse width), and output voltage to 250 V and turn the electrofishing unit on.

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- 9) Pause to verbally confirm settings on electrofisher and that the unit is turned on. Also confirm that all technicians are ready to proceed before pressing the activation switch on the anode pole.
  - a) The anode ring must always be submerged before depressing the activation switch. When removed from the water, the unit will automatically turn off.
- 10) Press and hold the activation switch down, and observe the behavior of fish. If fish do not appear to be affected by electrofishing (e.g., are not momentarily stunned), release the activation switch on the anode pole and increase voltage by 100 V (e.g., from 250 V to 350 V) and repeat Steps 9-10.
  - a) The goal is to immobilize fish using the lowest settings possible at the site.
- 11) If 1,100 V is reached and fish are still not responding to electrofishing proceed decrease voltage to 250 V and increase the frequency by 10 Hz (e.g., from 30 Hz to 40 Hz) and repeat Steps 9-10
  - a) If 60 Hz and 1,100 V is reached and fish are present but not immobilized, stop electrofishing and contact the NEON Aquatic Ecologist.
  - b) If fish are immobilized during testing, use dip nets to capture individuals and place in a bucket  $\frac{1}{2}$  -  $\frac{3}{4}$  full of stream water carried by one of the netters and continue with Step 12.
- 12) Continue electrofishing until approximately 20 individuals spanning a variety of sizes are netted.
- 13) Place fish in a bucket with fresh lake water and a battery operated aerator.
  - a) If other top predators are captured, identify (if possible) and record species on field data sheet (Appendix B) and immediately release >50 m away from electrofishing activity.
- 14) Examine captured fish for signs of injury (e.g., bent backs, dark bruising, hemorrhaging of the gills). Record injury rate on Field Data Sheet (Appendix B). Less than 1% of the captured fish should be injured.
  - a) If > 1% of captured fish are injured, stop sampling and contact the NEON Aquatic Ecologist.
  - b) Contact with the cathode or prolonged exposure to electricity due to failure to remove fish from the dip net in timely fashion will increase injury rates.
  - c) If fish are injured, allow them to recuperate in a separate bucket or live well with an aerator before releasing.
  - d) For any fish that do not recover, proceed to euthanization (Section 9.7).
- 15) Monitor captured fish for signs of normal respiration and swimming behavior for 10 minutes. If, after 10 minutes, fish are still on their side, upside-down, or injured, return to lower electrofishing settings.
  - a) It is important to note that some fish species (e.g., blacknose dace) are sensitive to electrofishing and may exhibit higher injury or mortality rates.
- 16) Once fish are swimming normally release fish back into the lake at least 50 m away from where they were caught.
- 17) Maintain electrofisher settings at the lowest level that allows for the effective capture of fish. Record frequency, duty cycle, and voltage settings on the Field Data Sheet (Appendix B) and reset the timer on the electrofishing unit. These settings will be used for the entire sampling bout.

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### 9.6.3 Backpack Electrofishing

- 1) Slowly enter the lake (so as not to disturb fish) and begin lowering the block net by releasing the reusable nylon cable ties.
  - a) This activity is best accomplished by the two netters immediately after backpack electrofishing setting testing. Each netter can start on the shoreline and work towards each other while trying to minimize disturbance to the area.
  - b) If necessary (e.g., excessive vegetation) secure the bottom of the block net with stainless block net stakes.
  - c) In random reaches, no block-net is necessary. Fish will be electrofished along a ~4 x 25 m area qualitatively.
- 2) Record the start time on the Field Data Sheet (Appendix B) so that conductivity and turbidity from the in-lake sensors.
- 3) Beginning at one end of the sampling reach, walk into the lake ensuring that the cathode (i.e., rattail) is submerged as much as possible, while holding the anode pole in one hand (anode submerged).
  - a) The electrofisher operator may, but is not required to, hold a dip net in the other hand if he/she feels comfortable.
- 4) The other crewmembers with dip nets will enter the lake behind the electrofisher operator.
  - b) The primary netter will stay close to the electrofisher operator to net fish.
  - c) The secondary netter will carry the bucket and net any other stunned fish that are missed by the electrofisher operator or the primary netter.
- 5) Ensure that the electrofishing unit settings (frequency, duty cycle, and output voltage) are those determined in Section 9.6.2 and that the timer (“EF time”) has been reset to 0.
- 6) Turn the electrofishing unit on and notify the other technicians. Confirm that all technicians are ready to begin.
- 7) Depress and hold the activation switch on anode pole to begin electrofishing.
  - a) The anode ring must always be submerged before depressing the activation switch and should never be taken out of the water with the switch depressed. The unit will automatically turn off if the anode is removed from the water.
- 8) Slowly sweep the anode inside the end of the block net to target any fish that may be seeking cover in the net.
- 9) After sweeping the block net, the electrofisher operator should turn parallel to the shoreline and slowly sweep the anode from the shore to the block net to expose all available habitats to electricity.
  - b) This may require walking slowly from side to side.
- 10) As the anode is moved from side to side, the netters will capture drifting, immobilized fish.
  - a) Dip nets should be held as close to the lake substrate as possible without picking up excessive substrate or debris.
  - b) There should always be one net behind the anode.
  - c) Fish are often attracted to the cathode (rattail). Netters should periodically check this area for stunned fish.

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- d) Netters should be aware that immobilized fish may not always be visible, particularly benthic species (e.g., darters, sculpins), and netters should frequently inspect their nets to minimize injury to fish by continuous exposure to electricity.
  - e) Crewmembers with dip nets should CALMLY net immobilized fish without excessive disturbance.
  - f) Never put hands in the water to capture fish while activation switch is depressed (i.e., while electrical current is pulsing through the water). If the netter cannot capture a fish using the net (e.g., sculpin, young-of-year), notify the backpack electrofisher to stop shocking. The backpack electrofisher must release the activation switch and remove the anode from the water to ensure no pulse is being conducted, then verbally confirm that it is safe for the netter to put his/her hand (or use the small dip net) in the water. After capturing the fish, the netter removes his/her hands from the water and verbally confirms that he/she has done so. Only then may the backpack electrofisher place the anode in the water and depress the activation switch to continue fishing, notifying other technicians that the unit is on.
  - g) If any endangered species (technicians will be notified of likelihood before sampling) or other vertebrates (e.g., salamanders, turtles) are caught, identify, photograph if possible, and release immediately away from electrofishing activities.
- 11) Frequently remove fish from dip nets and place in buckets to minimize injury to the fish.
  - 12) Sampling will continue along the shoreline in a zig-zag pattern in a single pass with attention to sampling all complex cover (e.g., overhanging or aquatic vegetation, woody debris, undercut banks).
    - a) The electrofisher operator may take advantage of the response of fish to pulsed DC current (i.e., attraction of immobilized individuals towards the anode) in complex cover by:
      - i) Releasing the activation switch on the anode pole.
      - ii) Inserting the anode into cover from the previously sampled (i.e., undisturbed) direction and holding the anode temporarily still.
      - iii) The electrofisher operator then depresses the activation switch as netters hold dip nets immediately behind the anode and cover.
      - iv) The activation switch should continue to be depressed until the abundance of observable immobilized fish decreases.
      - v) Continue electrofishing by moving the anode around the cover to immobilize additional fish, before continuing electrofishing.
  - 13) While electrofishing, monitor the abundance of fish in the buckets to prevent escape or accidental spills.
    - a) Be aware of fish overcrowding in the buckets. If fish appear to be gasping at the water surface, they are likely short on oxygen due to water temperature or overcrowding. Place fewer fish in buckets and supplement with cooler water and aerators.
    - b) Bucket replacement and moving fish is easier for the netters to do as they will need to step out of the lake.
    - c) Place buckets of fish out of direct sunlight if possible.
  - 14) When the crew reaches the end of the block net, the electrofisher operator should slowly sweep the anode inside the block net as fish may have moved to avoid the electrical field.
  - 15) Once the entire sampling reach has been sampled, read and record the time (EF time) in seconds from the back of the electrofishing unit on the Field Data Sheet (Appendix B).
    - a) Electrofisher time is critical for calculating sampling effort.

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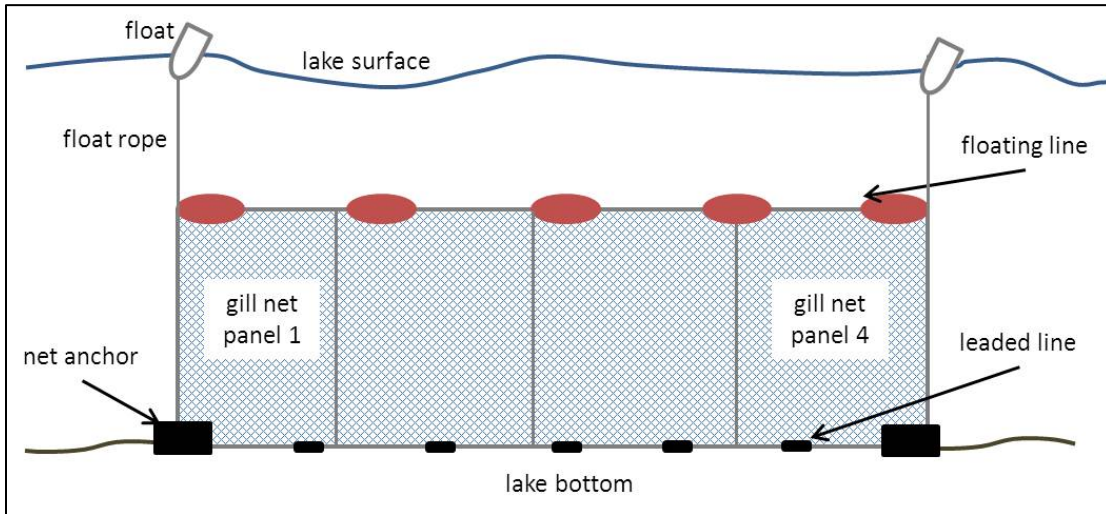
- 16) Turn the electrofisher off, remove and place on the bank with anode and cathode still attached.
- 17) Proceed to fish processing (Section 9.6.6).
  - a) If this is a fixed reach, repeat Steps 2-16 until three passes have been completed. If this is a random reach, complete only 1 pass.
    - a) Observe a minimum of 30 minutes between passes to allow fish that were not captured to recover.
    - b) Depletion is determined when two consecutive passes result in sequential decreases in total number of individuals sampled after the first pass (e.g., 1000 fish on first pass, 200 fish on second pass, 50 fish on third pass).
    - c) If this is a random reach, fish are sampled using only one pass.
- 18) Remove block nets and fence posts if all passes are complete.
- 19) Break down the backpack electrofishing unit if the crew cannot complete another reach during dark hours:
  - a) Disconnect the cathode and anode from the backpack electrofishing unit.
  - b) Disconnect the battery from the backpack electrofishing unit and remove battery from the backpack frame.
  - c) Place backpack electrofishing unit in case.
  - d) Disassemble anode pole and store with backpack electrofishing unit.
  - e) Place recently used battery separate from charged batteries where it can be easily distinguished for charging.

#### 9.6.4 Gill Nets

- 1) Load boat with necessary fish sampling equipment (e.g., gill nets in tubs, live wells, measuring board, digital scale, depthfinder).
- 2) Locate pre-selected riparian segment using GPS (Figure 3).
- 3) Prepare the gill net to be deployed by attaching net anchors to each end of leaded bottom line and attaching the float rope (with float attached) to the net anchor (Figure 6).
  - a) Start with the end of the net that will be deployed first (i.e., net end that is towards the top of the gill net tub).
  - b) The net can remain in the gill net tub with float lines and anchors attached until it is deployed.
  - c) Ensure that the float line is long enough to float on the water surface.



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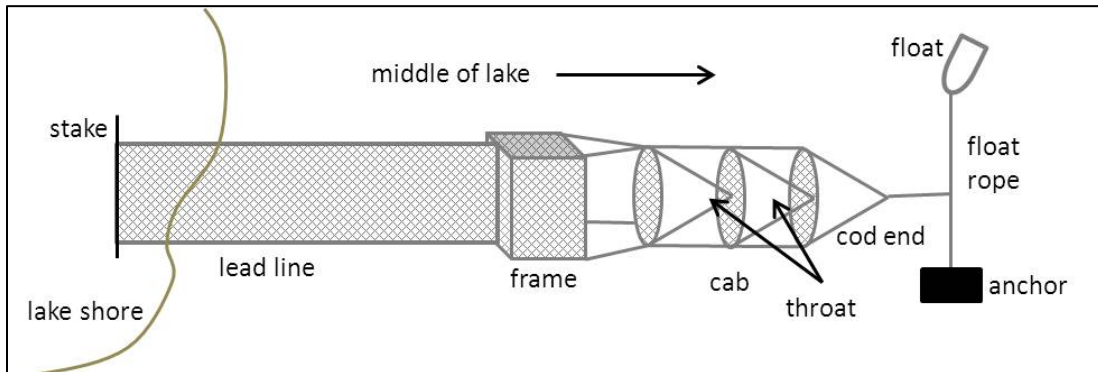
**Figure 6.** Example of gill net set up

- 4) Maneuver boat to the appropriate depth (> 2 m or as deep as possible in shallower lakes) using the depth finder within the riparian section boundaries and hold the boat in a still position (using the motor or oars) with the aft facing the approximate center of the lake.
- 5) Record the start GPS location, depth from the depth finder, and start time (24-hour time plus time zone, e.g., 13:30 MDT) on the Field Data Sheet (Appendix B).
- 6) Begin slowly releasing the gill net into the water, ensuring that the net is not twisted.
  - a) Start maneuvering the boat slowly in reverse, perpendicular to, and away from, the shore (Figure 3) while the gill net is being deployed.
- 7) After the net is completely deployed, record the stop GPS location, stop time and lake depth on the Field Data Sheet (Appendix B).
  - a) Setup gill nets at additional sampling locations if fish density is relatively low at the site and fish processing will take < 1 hour per net. Subsequent gill nets may be set and sampled the following day.
  - b) If the site has high fish density, sample only 1-2 gill nets in one day.
  - c) Target gill net set time is 1 hour (maximum 2 hours) to minimize mortality.
  - d) Gill netting must occur during daylight hours, so nets should be set in the morning or early afternoon too allow for processing time.
- 8) After at least 1 hour, proceed to the first net set and begin pulling.
- 9) Untie the float line and net anchor and set aside.
- 10) Record stop time and gill net mesh panel number of the first panel pulled (Appendix B).
- 11) Remove fish from each mesh panel and place in live well filled with fresh lake water with a battery powered aerator. Fold the net back into the gill net tub until all panels have been processed.
- 12) Place net anchors and float lines in appropriate buckets or tubs.
- 13) Process all fish (Section 9.6.6) from one net before pulling the next net.

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### 9.6.5 Mini-fyke Netting

- 1) Begin setting mini-fyke nets in late afternoon, ensuring that all nets will be set at least 1 hour before sunset.
- 2) Load boat with necessary fish processing equipment (e.g., fyke nets, live well, measuring board, digital scale).
- 3) Locate pre-selected riparian segment using GPS (Figure 3).
- 4) Maneuver the boat near shore while keeping the boat perpendicular to the shoreline.
- 5) Wrap the end of the mini-fyke lead line around the t-bar stake and push the stake into the shore above the waterline (Figure 7).
  - a) Leave enough slack in the lead line so that the bottom fully contacts the substrate (e.g., so that fish cannot swim underneath).
  - b) Ensure that the float line is long enough to prevent the float from sinking.



**Figure 7.** Example of a mini modified fyke net

- 6) Attach the net anchor to the cod (narrow) end and float line (with float attached) to the net anchor.
- 7) Begin slowly maneuvering the boat in reverse while deploying the net from the front of the boat.
  - a) Fyke nets must be set tightly to decrease the chance of the net collapsing on itself. Use the float line to pull the net as tight as possible before letting the float line go.
  - b) The throat of the net must be underwater for fish to pass freely into the trap.
  - c) All a portion of the cab to remain above the water line for turtles or other vertebrates.
  - d) The trap of the net must be above the thermocline (see site-specific bathymetric map).
  - e) Affix blinking LED light to the float at sites where watercraft are present.
- 8) Record start GPS location, start time, and depth on the Field Data Sheet (Appendix B).
- 9) Repeat Steps 2-8 until all mini-fyke nets have been set (one net in each electrofishing section).
- 10) The following morning at least one hour after sunrise, begin pulling the first mini-fyke net set the previous day (maximum set time = 30 hours).
  - a) This may be done from shore with waders or with the boat depending on conditions and permitting considerations.
  - b) Fyke nets must not be pulled earlier than 30 minutes after sunrise and no later than 30 minutes before sunset.

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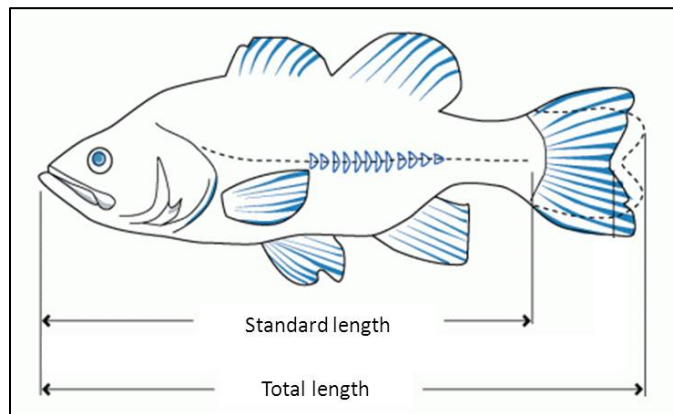
- c) Fyke nets must be set for 2 crepuscular periods (i.e., dusk and dawn).
- 11) Record stop time on Field Data Sheet (Appendix B).
- 12) Remove the net anchor and float line and set aside.
- 13) Untie the cod end and empty the fish into a live well filled with fresh lake water with a battery powered aerator by lifting the rectangular frames of the fyke net above the live well.
- 14) Remove T-stake and set aside.
- 15) Fold mini fyke net lead over the frame while wrapping corners and set aside in boat.
- 16) Place net anchors, float lines, and T-stakes in appropriate buckets or tubs.
- 17) Process all fish (Section 9.6.6) from each net before pulling the next net.

### 9.6.6 Fish Processing

- 1) Ensure that all technicians handling fish keep hands wet with lake water and free of chemicals (e.g., sunscreen, insect repellent) while processing fish.
- 2) Designate one technician to identify fish throughout the sampling bout for taxonomic consistency.
- 3) For any non-fish top predators (e.g., salamanders) collected, identify and record species to lowest practical taxon on the Field Data Sheet and release.
  - a) Photograph the specimen before releasing if possible.
- 4) Ensure that electrofishing time, electrofisher settings, and pass time, or stop time of nets, as appropriate, had been recorded on the Field Data Sheet (Appendix B).
  - a) For gill nets, record which panels were pulled first.
- 5) Setup the digital scale and a measuring board on a flat surface.
- 6) Place plastic measuring tray on scale pan and tare scale.
- 7) Mix anesthetic in one 5-gallon bucket.
  - a) Fill bucket approximately half full with stream water (~10 L).
  - b) For both MS-222 and clove oil, add 25 mL of anesthetic stock solution to 10 L stream water. Mix well (the small dip-net makes a good mixer).
  - c) Label bucket so all technicians knows it is anesthetic.
- 8) Remove fish from the first bucket or live well using the small handheld dip net.
  - a) Larger fish may need to be removed carefully by hand.
- 9) Place one fish at a time in the anesthetic bucket. Carefully monitor respiration 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.
  - a) If this dose of anesthetic is insufficient, add anesthetic stock solution in 5 mL increments until anesthetization is achieved.
  - 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.
- 10) Identify fish to species using the 4-letter species code (e.g., *Cottus cognatus* = COCO) and record on Field Data Sheet (Appendix B).
  - a) If the species cannot be identified or identification is uncertain, weigh and measure following Steps 11-13. Proceed to Section 9.7 to euthanize the specimen.
    - i) Do not collect more than 5 specimens of the same unknown species. Rather, morphotype and label with a unique identifier on the Field Data Sheets (Appendix B).

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- ii) Do not euthanize endangered species (site specific lists will be provided before sampling) or fish > 200 mm standard length.
- iii) For all specimens >200 mm standard length and any fish not able to be euthanized (i.e., due to permitting concerns), photograph specimen carefully and record the camera image number on the Field Data Sheet along with the relevant weight and length information about the fish (Appendix B).
- iv) Euthanize fish < 200 mm standard length using a lethal dose of anesthetic (10 mL stock solution of either MS-222 or clove oil/L stream water) in the field.
- v) Add 1 L of lake water and 10 mL of MS-222 or clove oil stock solution to a new 5 gallon bucket. Mix thoroughly.
- vi) Transfer fish from the holding live well or bucket to the bucket containing the anesthetic solution with the small handheld dip net.
- vii) Monitor fish until respiration ceases.
- viii) Place fish into appropriate sample container (e.g., wide mouth HPDE bottles) with completed specimen label (Appendix C). One taxon per specimen bottle.
  - b) Photo voucher 1 representative specimen from each taxon.
    - i. Include metric ruler for scale using the measuring board.
    - ii. Photograph 1: Lateral photo with fish's head facing to the left.
    - iii. Photograph 2: Ventral photo that includes the mouth (mouth position, lip structure, and barbels can be important distinguishing features).
- 11) Place the fish in the plastic tray on the tared digital scale. Determine weight to nearest 0.1 g and record on field data sheet (Appendix A).
- 12) With wet, clean hands, remove the fish from the plastic tray and place the fish on the measuring board with mouth at the "0" end of the board. Measure total length to the tip of the pinched-together tail (Figure 8) to the nearest mm and record on Field Data Sheet (Appendix A).



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

- 13) Inspect the fish for deformities, including eroded fins, external lesions, parasites, and tumors and electrofishing injuries (burn marks, bent spine, hemorrhage) and record on Field Data Sheet (Appendix A).
- 14) Place processed fish in a bucket containing fresh lake water and a battery powered aerator for later release.

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- a) Do not overcrowd fish in the reviving buckets, they need as much aerated water as possible.
- 15) Repeat Steps 8-14 until  $\leq 100$  fish per species are identified, weighed, measured, and inspected for deformities.
  - a) If more than 100 individuals in one species are captured, anesthetize, weigh, and measure the first 100 and simply count the remaining fish (no anesthetization) to speed processing time and alleviate stress to fish.
- 16) Release the processed, revived fish back into the lake outside of the blocknet or near the collection location.
  - a) If mortality occurs during processing, save individuals for collections (see Sample Preservation, Section 9.7).

### 9.7 Sample Preservation

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

### 9.8 Sample Shipping

- 1) Ship preserved samples within 1 month of the end of the sampling bout.
- 2) 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.
- 3) Follow shipping and Hazmat procedures for formalin.
- 4) Place package inside appropriately-sized cooler or other sturdy shipping container. Add packing material, as necessary, to take up excess space in container.
- 5) Tape and label container for shipping.
- 6) Ground ship to Fish Taxonomist (*to be determined pending lab contracts*) for identification and long-term preservation.

### 9.9 Data Handling

- 1) Download all images from the camera and save in folder named "SiteCode\_YYYYMMDD\_SpecimenID".
- 2) Enter all data from the Field Data Sheets (Appendix A, Appendix B) within 48 hours of returning to the Domain Support Facility.
- 3) Archive/file all paper field sheets at the Domain Support Facility.
- 4) Upload data to the NEON Cyber-Infrastructure database.

### 9.10 Refreshing the Sampling Kit

- 1) Replace batteries for all battery operated equipment (e.g., GPS unit, portable aerators).
- 2) Refill/restock preservative and anesthetic stock solution containers.

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### 9.11 Equipment Maintenance, Cleaning, and Storage

- 1) Wash all equipment that has been submerged in stream water according to the NEON Aquatic Decontamination Protocol (RD [11]).
- 2) Dry all equipment thoroughly between sites and before storage.
- 3) Check all nets for holes and patch if necessary.

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**APPENDIX A SECTION ESTABLISHMENT DATA SHEETS**

**NEON Lake Fish Sampling: Section Establishment**

Domain: \_\_\_\_\_  
 Site: \_\_\_\_\_  
 Date: \_\_\_\_\_  
 Time: \_\_\_\_\_

Technicians: \_\_\_\_\_  
 \_\_\_\_\_

Section ID	Plot marker 1 coordinate	Plot marker 1 ID	Plot marker 2 coordinate	Plot marker 2 ID	Shoreline length (m)	Habitat features
1						
2						
3						
4						
5						
6						
7						
8						
9						
10						

Notes:



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**APPENDIX B FIELD DATA SHEETS**

**NEON Lake Fish Sampling**

<b>Domain:</b> _____	<b>Weather:</b> _____
<b>Site:</b> _____	_____
<b>Date:</b> _____	<b>Technicians:</b> _____
<b>Section ID:</b> _____	_____
<b>Water temp (°C):</b> _____	<b>Frequency:</b> _____
<b>Conductivity:</b> _____	<b>Duty cycle:</b> _____
<b>Start time:</b> _____	<b>Voltage:</b> _____
<b>End time:</b> _____	<b>EF time:</b> _____
<b>(circle one) electrofish, gill net, fyke net</b>	<b>Depth (net):</b> _____

#	Pass	Species	Total length (mm)	Weight (g)	DELT, electrofish injury	Comment/Photo ID

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## NEON Lake Fish Sampling

Domain: \_\_\_\_\_ Page \_\_\_\_ of \_\_\_\_  
 Site: \_\_\_\_\_ (circle one) electrofish, gill net, fyke net  
 Date: \_\_\_\_\_  
 Section ID: \_\_\_\_\_

#	Pass	Species	Total length (mm)	Weight (g)	DELT, electrofish injury	Comment/Photo ID

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**APPENDIX C SPECIMEN LABELS**

Domain: \_\_\_\_\_  
 Site: \_\_\_\_\_  
 Date: \_\_\_\_\_  
 Section ID: \_\_\_\_\_  
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 Collector: \_\_\_\_\_

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**APPENDIX D    RANDOMIZED SECTION SELECTION PER SITE**

Randomized reach order is shown for each site below. Skip numbers that have either been chosen as a fixed reach, or do not exist at the site (i.e., sites that are < 1 km may have fewer than 10 reaches).

Domain	Site	Randomized reach order
D03	Lake Barco	10, 9, 2, 6, 8, 7, 4, 1, 5, 3
D03	Lake Suggs	9, 4, 5, 7, 2, 6, 3, 10, 8, 1
D05	Crampton Lake	3, 6, 1, 2, 4, 10, 5, 7, 9, 8
D05	Site to be determined	5, 9, 7, 10, 4, 2, 1, 6, 3, 8
D09	Prairie Lake	8, 5, 3, 10, 9, 7, 2, 4, 6, 1
D09	Prairie Pothole	9, 2, 10, 6, 3, 5, 8, 7, 4, 1
D11	South Pond at Klemme	7, 9, 1, 4, 5, 3, 3, 10, 2, 8
D18	Toolik Lake	2, 6, 5, 10, 7, 4, 1, 3, 8, 9