AOS PROTOCOL AND PROCEDURE: ZOO – ZOOPLANKTON SAMPLING IN LAKES

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Change Record

REVISION	DATE	ECO #	DESCRIPTION OF CHANGE
A	05/28/2014	ECO-01127	Initial release
B	08/29/2014	ECO-02210	Minor updates based on feedback from the field
C	11/14/2014	ECO-02467	Migration to new protocol template
D	05/15/2015	ECO-02666	Minor updates including changes to the number of integrated samples taken, concentration of ethanol, updates to sample shipping and labeling, and the addition of sampling dates to appendix.
E	01/21/2016	ECO-03448	Removed Secchi/depth profile details, updates to Schindler sampling depth.
F	02/08/2017	ECO-04359	Update NEON template; Update sample ID template; Add DNA metabarcode SOP
G	02/13/2018	ECO-05297	Tape jar lids prior to shipping, add barcode label information, update decontamination protocol reference, move datasheets to appendix
H	12/19/2018	ECO-05968	Update barcode info, clarify DNA sterilization, lab processing, and storage
J	10/22/2019	ECO-06243	Change lab processing time to 24 hours, individually bag DNA samples, new template
K	03/16/2022	ECO-06781	• Update to reflect change in terminology from relocatable to gradient sites.
L	01/02/2025	ECO-07116	• Update reference documents • Update inlet, outlet sensors to littoral • No minimum ethanol swap time • Update reduced sampling guidelines • Update missed or incomplete sampling and biophysical criteria • New flow charts for all sampling SOPs • Allow bottle sizes smaller than 500 mL • Discard mesh square at ETOH swap • Use inventory app for samples in DSF > 2 days • Migrated to template rev M

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1 OVERVIEW

1.1 Background

Zooplankton inhabit all layers of a water body and constitute a major link between primary production and higher trophic levels in aquatic ecosystems. Unlike algae or phytoplankton, zooplankton are microscopic animals that do not produce their own food (**Figure 1**). These small invertebrates float freely in the water column of lakes and oceans. They are important as both prey and consumers in the aquatic food web. Zooplankton are often filter feeders (sometimes predators) that feed primarily on algae while also being the main food source for planktivorous fish. The zooplankton assemblage responds to environmental stressors such as nutrients, and such effects can be detected through changes in species composition, abundance, and body size distribution (USEPA, 2012).

Many zooplankton are capable of strong swimming movements and may migrate vertically from tens to hundreds of meters; others have limited mobility and depend more on water turbulence to stay afloat. Zooplankton can be classified according both to developmental stages: meroplankton and holoplankton; and to size (Sanders and Porter, 1990). Meroplankton spend only part of their life cycles as plankton, while holoplankton spend essentially their whole existence in the water column. Freshwater zooplankton are dominated by six major groups of animals: protozoa, rotifers, two subclasses of the Crustacea, the cladocerans and copepods, and *Mysis* and *Chaoborus* (**Figure 1 a-d**). The planktonic protozoa in particular have limited locomotion and are dominated by the meroplanktonic pelagic zooplankton (5 – 300 µm), characterized by a summer planktonic lifecycle, spending the rest of their life cycle in the sediments. Several non-planktonic rotifers (150 µm – 1 mm) are sessile (lower mobility) and mostly associated with the littoral zone (**Figure 2**). Most rotifers are non-predatory, and omnivorously feed on bacteria, small algae, and detrital particulate organic matter. The majority of Cladocera are small (0.2 to 3.0 mm) and have a distinct head and bivalve carapace covering the body. Locomotion is accomplished mainly by means of the large secondary antennae. Planktonic copepods (2-4 mm) consist of two major groups, the calanoids (*Diaptomus*) and the cyclopoids, distinguished by their body structure and length of antennae.

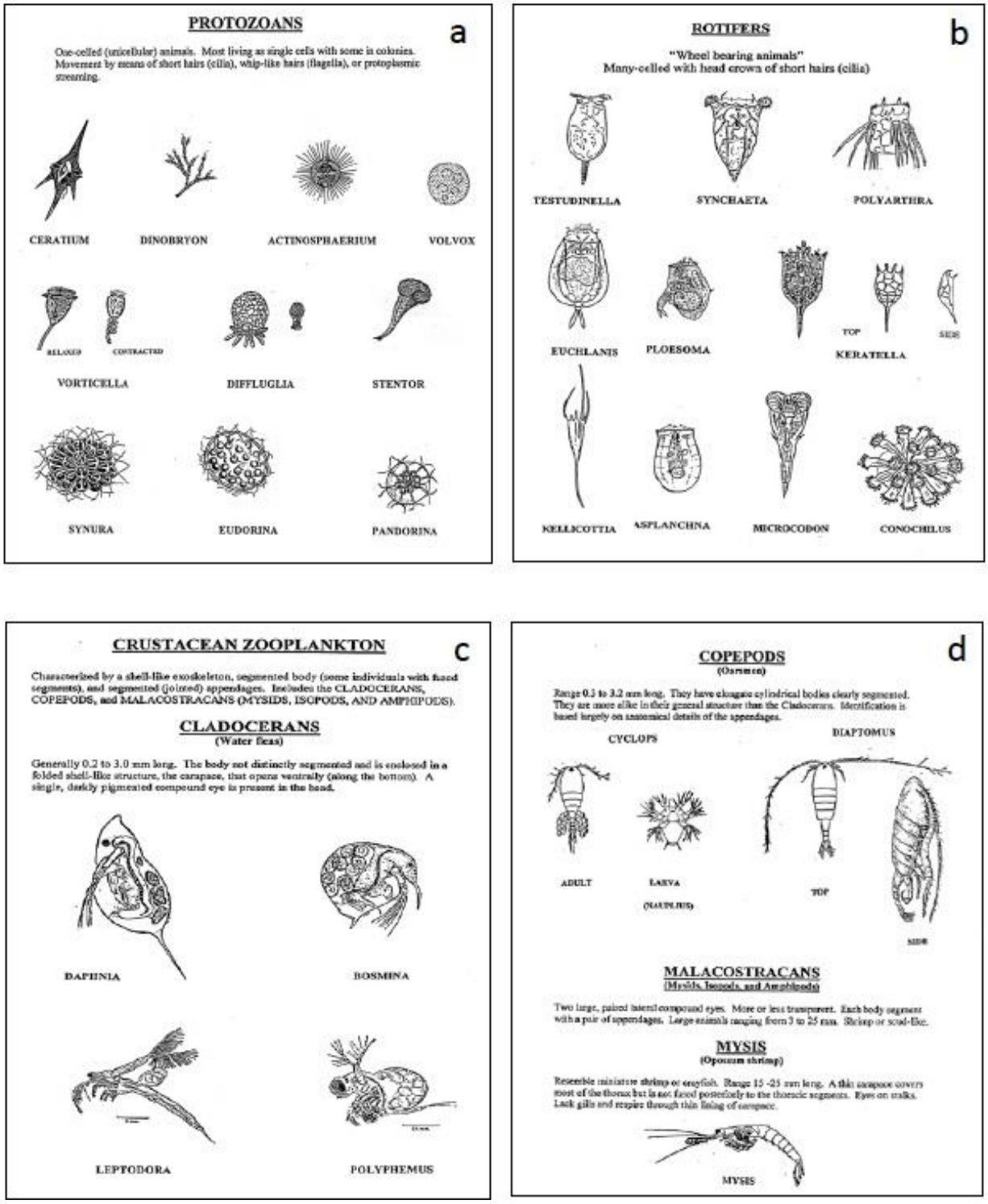

Figure 1. Classes of zooplankton (from Tharp).

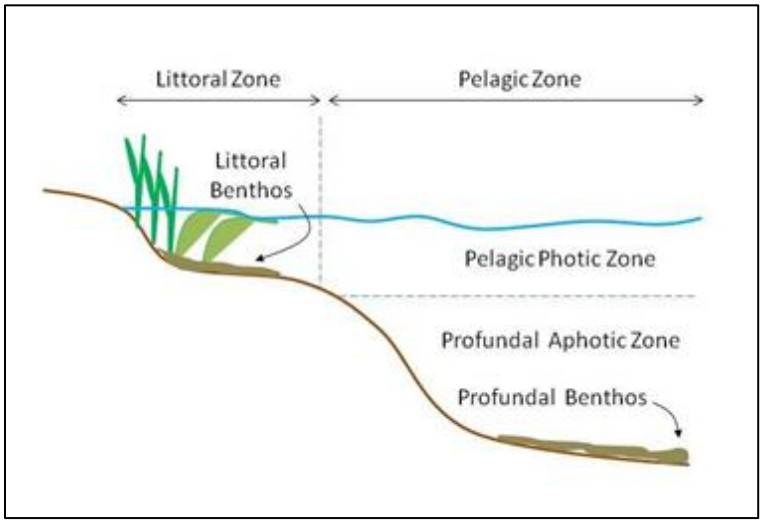

Figure 2. Zones of a lake.

Seasonality plays an important role in zooplankton species presence and abundance, with cyclopoid and calanoid copepods dominating the community in the winter months, with large cladocera peaking in mid-summer and small cladocera in later summer early autumn (Gerten and Adrian, 2002). A change in lake trophic conditions from oligotrophic to eutrophic has been shown to result in size-selective predation that potentially contributes to the decline of larger zooplankton, with ciliated protozoans and rotifers becoming more important (Wetzel, 2001).

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

This protocol is based on modified version of USEPA (2012a, b), Baker et al. (1997), and USEPA (2009).

2 RELATED DOCUMENTS AND ACRONYMS

2.1 Applicable Documents

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

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

2.2 Reference Documents

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

RD[01]	NEON.DOC.000008	NEON Acronym List
RD[02]	NEON.DOC.000243	NEON Glossary of Terms
RD[03]	NEON.DOC.002652	NEON Data Products Catalog
RD[04]	NEON.DOC.001271	AOS/TOS Protocol and Procedure: DMP – Data Management
RD[05]	NEON.DOC.002302	Datasheets for AOS Protocol and Procedure: Zooplankton Sampling in Lakes
RD[06]	NEON.DOC.001646	NEON General AQU & GAG Field Datasheet
RD[07]	NEON.DOC.002191	Datasheets for AOS Protocol and Procedure: Secchi Disk and Depth Profile Sampling
RD[08]	NEON.DOC.001152	NEON Aquatic Sample Strategy
RD[09]	NEON.DOC.004257	Standard Operating Procedure: Decontamination of Sensors, Field Equipment and Field Vehicles
RD[10]	NEON.DOC.002792	AOS Protocol and Procedure: DEP – Secchi Disk and Depth Profile Sampling in Lakes and Non-Wadeable Streams
RD[11]	NEON.DOC.014050	TOS Protocol and Procedure: BET – Ground Beetle Sampling
RD[12]	NEON.DOC.003282	NEON Protocol and Procedure: SIM – Site Management and Disturbance Data Collection
RD[13]	NEON.DOC.003600-NEON.DOC.003618	Aquatic Site Sampling Design – NEON Domain ##
RD[14]	NEON.DOC.005224	NEON Protocol and Procedure: SCS – Shipping Ecological Samples and Equipment

2.3 Acronyms

Acronym	Definition
^{o}C	degrees Celsius
DI	Deionized
DSF	Domain Support Facility

EMAP	Environmental Monitoring and Assessment Program (USEPA)
mL	milliliter
NLA	National Lakes Assessment (USEPA)
PFD	Personal Flotation Device
USEPA	United States Environmental Protection Agency
USGS	United States Geological Survey

2.4 Definitions

Epilimnion: Top layer of water of a stratified lake, denoted by highest temperatures and least dense water. Typically occurs in the summer.

Euphotic zone (or "Photic zone"): The upper layer of the water column water where sunlight penetrates and photosynthesis can occur. Specifically, the depth to which 1% of surface light penetrates and measured using a Secchi disk.

Eutrophic: An ecosystem with high nutrient concentration. In lakes, this often equates to algal proliferation or algal blooms.

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

Hypolimnion: The dense bottom layer of a stratified lake that sits below the thermocline. This layer is cooler than the surface water and has less circulation.

Littoral: Shallow, submerged, near-shore areas of lakes and rivers where sunlight is able to penetrate to the bottom.

Metalimnion: The layer of water in a stratified lake that sits between the hypolimnion and the epilimnion. Often equated with the thermocline.

Oligotrophic: An ecosystem with low nutrient content. In lakes, this often equates to very clear water and little algal production.

Pelagic zone: The part of the lake that is not near shore or close to the bottom, e.g., open water.

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

Stratified: Layers within the system (e.g., warm and cold water layers indicate thermal stratification in a lake).

Thermocline: A distinct layer in a body of water where the change in temperature is more rapid than increasing depth - usually a change of more than 1 ^{o}C per meter. The denser and cooler layer below the thermocline is the hypolimnion, the warmer upper layer is the epilimnion.

3 METHOD

The goals of the Zooplankton Sampling in Lakes Protocol are to quantify biodiversity, number of species present, and biomass (or biovolume) at each lake site. These variables will be used to build a database over time, in order to track changes in zooplankton community structure and function, and introduction of invasive species.

Samples shall be collected as an integrated water column sample at the central location of the lake. Two additional samples are taken at the littoral sensors of the lake. Samples are collected 3 times per year in order to capture differences in community composition, abundance, diversity, and biomass across seasons.

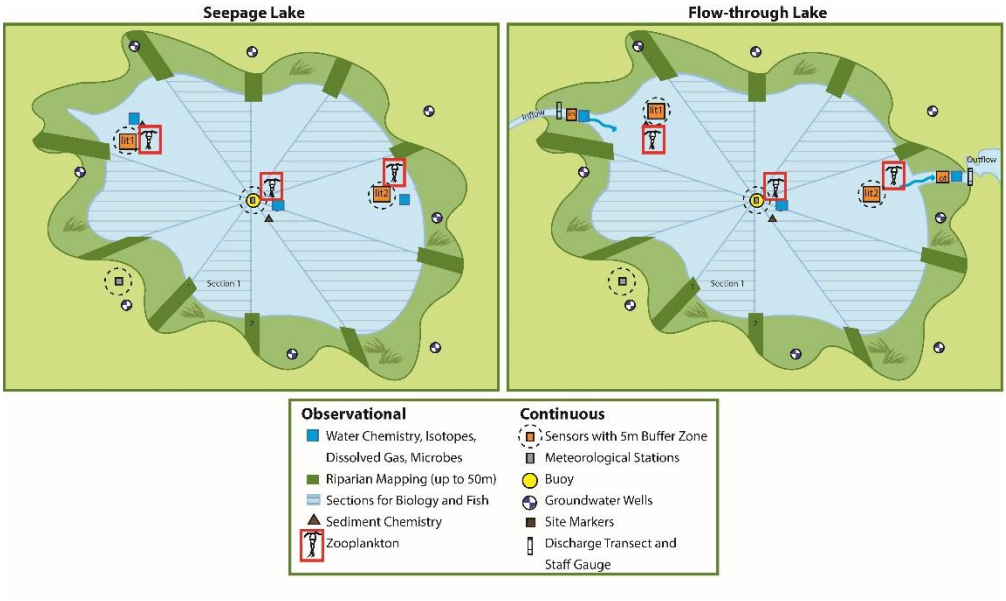

Figure 3. A generic lake site layout with zooplankton sampling locations. Samples are collected near the buoy and two littoral sensor sets.

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

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

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

4 SAMPLING SCHEDULE

4.1 Sampling Frequency and Timing

Lake zooplankton sampling occurs three times per year at each lake site, roughly spring, summer, and autumn (Parker and Utz 2022, RD[13]). Sampling must be initially scheduled within the first 21 days of the 1 month window specified in RD[13] with a minimum of two weeks between sampling dates. Accommodations for local weather conditions (e.g., late ice-off) may be made that cause the sample date to fall later in the bio bout or outside of the pre-determined window. See the appropriate Aquatic Site Sampling Design for your domain for additional details and scheduling preferences. Use NEON's problem reporting system to seek guidance and report sampling efforts that take place outside of the defined sampling window.

Zooplankton sampling occurs in the water column, therefore, takes place on the same day as Secchi Disk and Depth Profile Sampling in Lakes and Non-Wadeable Streams (RD[10]).

Table 1. Sampling frequency for zooplankton procedures on a per SOP per site type basis.

SOP	Site Type	Location	Bout Duration	Bouts Per Year	Bout Interval	Yearly Interval	Remarks
SOP B	lake	buoy, littoral1, littoral2	1 day	3	Minimum of 2 weeks between bouts	annual	SOP B and C must be done on the same day
SOP C	lake	buoy, litoral1, littoral2	1 day	3	Minimum of 2 weeks between bouts	annual	Bout 2 samples sent for analysis, Bout 1 and 3 samples sent to archive

Scheduling Considerations

1. All samples for a bout must be collected during the same day (includes SOP B and SOP C) and must meet the minimum number of samples. If weather or other conditions force sampling to stop during collection and sampling cannot be rescheduled, contact Science to discuss.
2. **Field Work and Laboratory Processing:** After zooplankton samples are collected, the following points are critical with respect to timing:
    a. Preserve samples in the field within 30 minutes of collection.
    b. Keep samples cool (4 °C) during transportation and storage from the field to the DSF.
    c. Replace ETOH in DNA samples after returning to the DSF and within 24 hours of collection and taxonomy samples within 72 hours of collection.
    d. Store all samples at 4 °C until shipping.

4.2 Criteria for Determining Onset and Cessation of Sampling

A range of dates for each site were determined *a priori*, based on historical data including ice on/ice off, the accumulation of degree days, weather, and riparian phenology (Parker and Utz 2022, RD[13]).

4.3 Timing for Laboratory Processing and Analysis

Zooplankton samples must have their preservative changed between arriving back at the DSF and 24 hours of field collection for DNA and 72 hours for taxonomy. Though samples should ideally be shipped to external processing facilities within 30 days of collection, preserved and refrigerated samples may be held longer at the domain support facility if necessary.

4.4 Sampling Timing Contingencies

All samples from this protocol must be collected within one day from one sampling site per bout (i.e., all samples per lake/non-wadeable stream as detailed in this protocol) because of the fluctuating nature of aquatic habitats and sensitivity to precipitation. Spreading sample collection over multiple days increases the likelihood of variability among samples. Sampling may be rescheduled due to weather or environmental conditions within the bout window (+ 3 days) provided in the Site Sampling Designs (RD[13]). A minimum of 2 weeks between sample collection and the start of the next bout window shall be observed, with the exception of specific domains that have a limited growing season (e.g., D18).

Table 2. Contingency decisions for zooplankton collection.

Delay/ Situation	Action	Outcome for Data Products
Hours	If weather conditions deteriorate and the lake becomes unsafe (e.g., approaching thunderstorm) or becomes too windy (>35 km hr^{-1}) and has unsafe wave heights (>1 m) so that the boat cannot be held stationary over a sampling point while at anchor, return to shore and wait in a safe location for 30 minutes (or appropriate wait time based on conditions). If conditions improve, resume sampling, if not, discard samples, return to the Domain Support Facility and reschedule sampling.	None as long as samples are collected within the pre-determined sampling window. If waiting for favorable conditions causes sampling to occur outside of the sampling window, data may be flagged.
	If field conditions cause reduced sampling, collect a minimum of 3 samples in a single habitat type. If you are not able to collect 3 samples in a single habitat type, re-attempt sampling on another day.	Reduced sample replication
6 Months	Preserved zooplankton samples may be held for up to 6 months (or longer if necessary) at 4 °C in the domain support facility if circumstances do not allow shipping to the external laboratory.	Holding samples >30 days affects external lab schedules, staffing, and budgets and delays data release on the NEON portal. However, sample integrity is not affected.

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 sampling locations in a given bout. For example:

- Logistics – e.g., insufficient staff or equipment
- Environment – e.g., ice cover, inclement weather, or
- Management activities – e.g., controlled burns, herbicide application

Instances such as those listed above must be documented for scheduling, tracking long-term site suitability, and informing end users of NEON data availability. Some types of missed sampling are due to events that should be recorded in the Site Management App; refer to the Site Management and Event Reporting Protocol for more detail (RD[12]).

Missed or Incomplete Sampling Terms

Terms that inform Missed or Incomplete Sampling include:

- **AOS biology sampling bout window**: See RD[13] for site-specific dates
- **Canceled Sampling**: Incidence of *scheduled sampling* that did not, and will not, occur. Canceled Sampling is recorded at the same resolution as data that are ordinarily recorded (e.g., each missed zooplankton sample gets its own record).
- **Sampling Impractical**: The field name associated with a controlled list of values that is included in the data product to explain a Canceled Sampling event – i.e., why sampling did not occur. This field is also used to indicate any individually missed samples within an otherwise successful sampling bout, e.g., if a stream is drying and contingent decisions are used.
- **Biophysical Criteria**: This field name associated with a controlled list of values that is included in the data product to explain changes to the schedule.
- **Rescheduled**: Missed Sampling is rescheduled for another time within the *protocol sampling dates*, 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**).

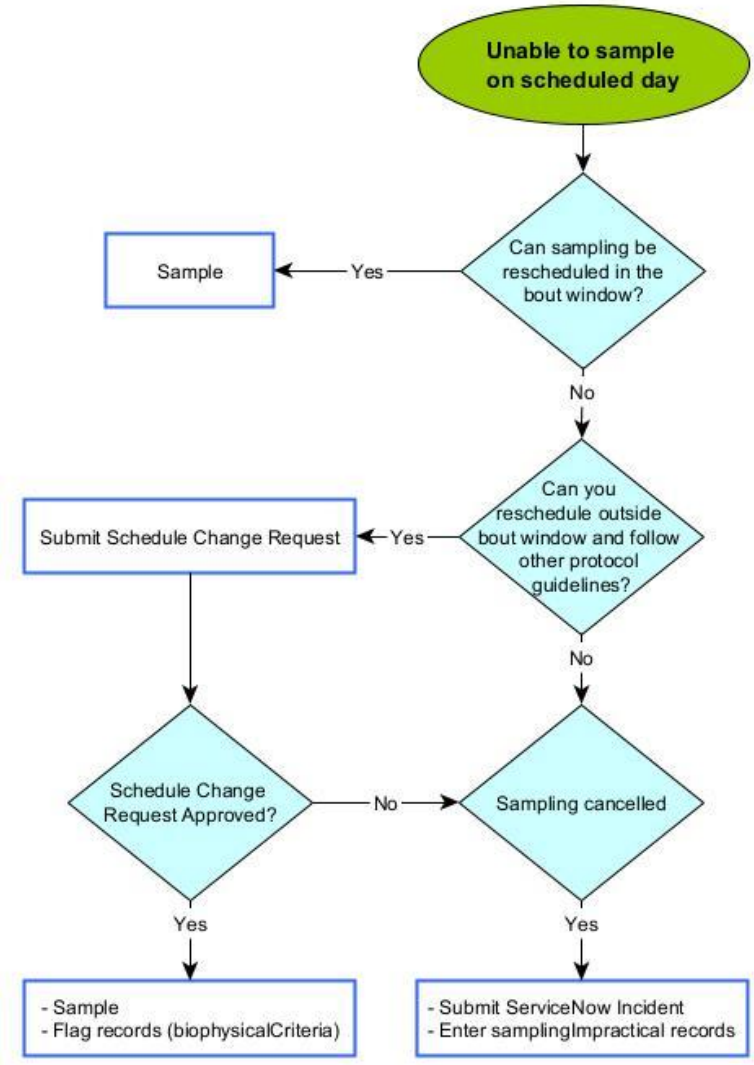

Figure 4. The documentation to account for a Missed Sampling event depends on the situation for each plot of each bout that is not sampled. Required delay and cancellation actions are outlined for each protocol in the 'Scheduled Field Activities – Delays and Cancellations' spreadsheet available on the SSL. Missed Sampling events may also require a Data Quality flag and/or creation of a Site Management record.

To Report Missed or Incomplete Sampling:

1. Canceled or rescheduled sampling must be communicated to Science by a Service Now Incident for cancellations or Schedule Change Request if sampling is rescheduled outside of the AOS Bio Bout window. Attempts to reschedule should be made prior to cancellation.
    a. The lead Field Ecologist should consult **Table 3** to best determine when reporting is required (**Figure 4**).

2. **Canceled** sampling: For each missed sample that is not rescheduled, the **Sampling Impractical** field must be populated in the mobile collection device (**Table 4**).
    a. Three zooplankton taxonomy samples should be collected during each sampling event, else sampling should be rescheduled.
    b. Total number of field sampling records expected per event (bout): 3 taxonomy + 3 DNA/ARC.
3. **Rescheduled** sampling events that occur outside of the defined AOS biology sampling bout window must be approved by Science in a Schedule Change Request.
    a. biophysicalCriteria – An indicator of whether sampling coincided with the intended biophysical conditions (i.e., within the AOS biology sampling bout window)
    b. windAffected – An indicator of whether winds were >35 km hr^{-1} at the time of sampling

Table 3. Guidance for responding to delays and cancellations encountered during implementation of Zooplankton Collection.

Activity Name	Days Delayed from Schedule	Delay Action	Cancellation Action
Zooplankton Sampling	> 3 days outside bio bout window or rescheduling after fish (streams only)	IS/OS Schedule Change Request	Submit incident ticket

Table 4. Protocol-specific Sampling Impractical and Biophysical Criteria entered in the Fulcrum application. In the event that more than one is applicable, choose the dominant reason sampling was missed.

Field name	Dropdown list option	Description
Sampling Impractical	Location dry	Location dry
Sampling Impractical	Location frozen	Location frozen
Sampling Impractical	Location snow covered	Location snow covered
Sampling Impractical	Logistical	Site or plot access compromised, staffing issues, errors (e.g., equipment not available in the field)
Sampling Impractical	Other	Sampling location inaccessible due to other ecological reason described in the remarks
Biophysical Criteria	OK – within bout window	Sampling occurred within the bout window, no known issues
Biophysical Criteria	conditions not met: sampled after fish	Sampling does not reflect the target biophysical conditions, benthic sampling occurred after benthos was disturbed during seasonal fish sampling
Biophysical Criteria	conditions not met: outside bout window	Sampling was conducted outside of the AOS sampling window

4.6 Estimated Time

The time required to implement a protocol will vary depending on a number of factors, such as skill level, system diversity, environmental conditions, and distance between sample plots. The timeframe

provided below is an estimate based on completion of a task by a skilled two-person team (i.e., not the time it takes at the beginning of the field season). Use this estimate as framework for assessing progress. If a task is taking significantly longer than the estimated time, a problem ticket should be submitted. Please note that if sampling at particular locations requires significantly more time than expected, Science may propose to move these sampling locations.

Table 5. Estimated staff and labor hours required for implementation of the Zooplankton Sampling in Lakes protocol.

SOP	Estimated time	Suggested staff	Total person hours
SOP A.1 and A.2: Preparing for data collection and sampling	1 h	1	1 h
SOP B: Zooplankton taxonomy collection	3 h	2	6 h
SOP C.1: Sterilize DNA equipment	1 h	1	1 h
SOP C.2: Zooplankton DNA collection	1 h	2	2 h (combined with SOP B)
SOP D: Post-Field Sampling Tasks	0.5 h	1	0.5 h
SOP E: Laboratory Sampling and Analysis	1 h	1	1 h
SOP F: Data Entry and Verification	1 h	1	1 h
SOP G: Shipping	1 h	1	1 h

5 SAFETY

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

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

Safety Data Sheets (SDS) shall be readily available and reviewed for all chemicals (ethanol) used during this task. Whenever chemicals are used, follow requirements of the site-specific Chemical Hygiene and Biosafety Plan (AD[03]) for Laboratory Safety and NEON EHSS Policy, Program and Management Plan (AD[01]).

See Section 8 in the NEON Operations Field Safety and Security Plan (AD [02]) for aquatic-specific field safety requirements. In addition, the following safety requirement must be followed:

1. In lakes, site-specific hazards may be encountered may necessitate sampling from the boat, without dismounting from the vessel. In addition, use extra caution in waters where alligators are present and maintain a safe distance from hazards.
2. All personnel must be wearing a personal flotation device (PFD) prior to entering the boat.
3. All personnel shall have access to a form of communication with other team members such as a two-way radio.
4. Be aware of any site-specific hazards and to the waters of that particular location (i.e., current status, tidal charts, etc.).

6 PERSONNEL

6.1 Training Requirements

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

All personnel required to operate a boat shall be trained through an approved program. All others shall be aware of boating safety procedures.

Personnel will be trained in field protocols associated with this document and trained in safe working practices for aquatic field work. Personnel must also be trained in safe handling of ethanol (AD[03]).

6.2 Specialized Skills

Where applicable, personnel will be licensed to operate a boat and able to safely handle a motor and drive a boat safely.

7 STANDARD OPERATING PROCEDURES

SOP Overview

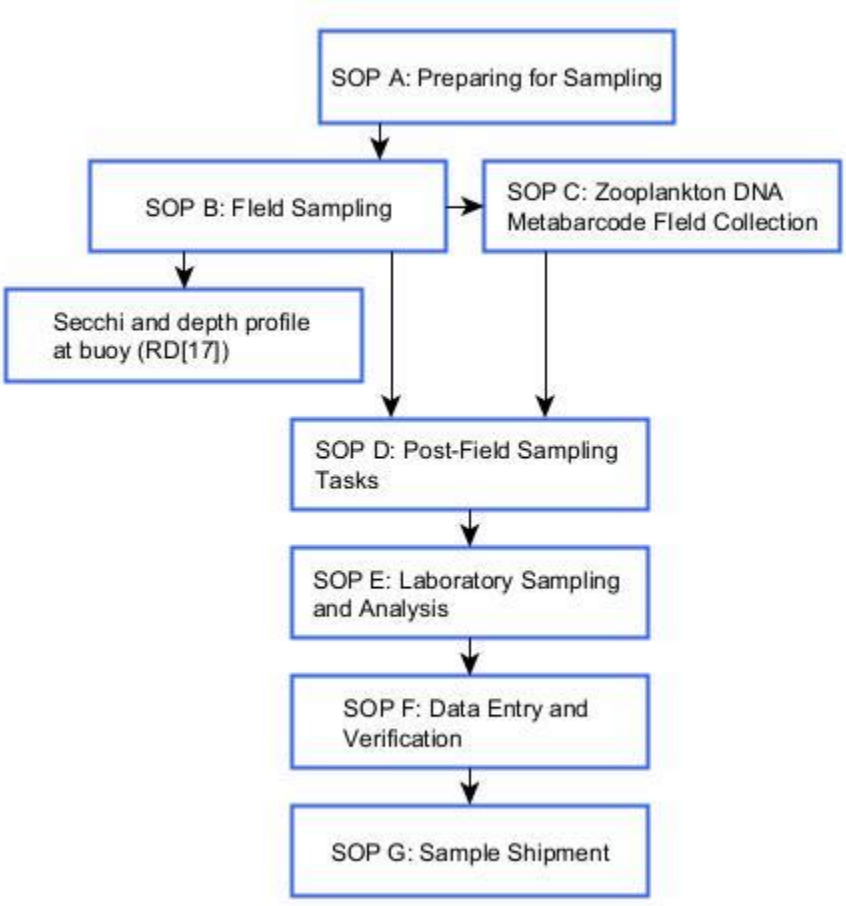

Figure 5. A high level workflow diagram that visually shows how the separate SOPs are sequentially connected.

- **SOP A**: Preparing for Sampling
- **SOP B**: Field Sampling
- **SOP C**: Zooplankton DNA Metabarcode Field Collection
- **SOP D**: Post-Field Sampling Tasks
- **SOP E**: Laboratory Sampling and Analysis
- **SOP F**: Data Entry and Verification
- **SOP G**: Sample Shipment

SOP A Preparing for Sampling

A.1 Preparing for Data Capture

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

However, given the potential for mobile devices to fail under field conditions, it is imperative that paper datasheets are always available to record data. Paper datasheets should be carried along with the mobile devices to sampling locations at all times.

A.2 Preparing for Field Sampling

1. Plan and save sampling routes for field teams using standard site navigation procedures. Route planning enhances sampling efficiency and helps avoid accidental foot traffic at NEON sites. Load GPS sampling coordinates on handheld GPS unit (±4 m accuracy) for lakes/rivers.
2. Collect and prepare all equipment, including sample bottles, sample bags, and pre-printed labels. Know which habitats are typically sampled at the site.
3. Have ice or ice packs frozen and ready for transportation cooler.
4. Once you know the approximate size of samples at your site, consider the size of jar you are using to collect sample. For ZOO-ARC, use the smallest jar you can where you can still have the appropriate ration of sample: ETOH (**Table 6**).
5. Check nets and sieves for holes, repair if necessary. Ensure that both are clean and free of debris and organic matter.
6. If collecting DNA/ARC samples, decontaminate all equipment used in DNA/ARC sample collection with 10% bleach following normal decontamination procedures (Section D.3 in RD[09]) prior to field collection. This bleach cleaning differs from normal decontamination as it is intended to remove any DNA existing on the equipment from storage that could contaminate the samples collected in the field.
    a. Equipment may also be bleach-cleaned after sampling and stored in decontaminated containers and bags equipment until the next bout.
7. Fill 1 L HDPE bottles (or 4 L HDPE jug) with 95% ethanol. Cap tightly. Clearly label bottle (suggest using Globally Harmonized System (GHS) labels) and transport to the field following EHS guidelines.
8. Enter general aquatic field metadata (RD[06]) and Secchi and depth profile (RD[07]) in their respective mobile applications once per day upon every field visit. If other protocols are done in the same day, one record for field metadata and one record for Secchi and depth profile are sufficient.

Table 6. Number of field samples labels and bottles required per sampling event.

	Sample type	Number of labels/containers	Sample container
Lakes	Zooplankton taxonomy	3	125-500 mL HDPE bottle
	Zooplankton DNA	3 (Bout 2)	125-250 mL HDPE bottle **AND** Whirl-pak®/zip-top bag
	Zooplankton ARC	3 (Bouts 1 and 3)	Smallest Nalgene container, 125-500 mL

A.3 Labels and Identifiers

Barcode labels are required on all zooplankton containers shipped to external labs. All barcodes need to be applied to dry containers for 30 mins before use. Zooplankton collection uses Type I barcodes (prefix A, plus 11 numbers).

Figure 6. Zooplankton sample suite, including 3 ZOO-tax and 3 ZOO-dna bottles with human readable and barcode labels.

1. All zooplankton samples will have a weather-resistant, adhesive, human readable label on the outside of the bottle (**Figure 7**).
    a. Add adhesive labels to sample bottles prior to going in the field and getting the bottle wet.
2. Adhesive barcode labels should be applied to dry, room temperature zooplankton bottles in advance of their use in the field, at least 30 minutes prior.
    a. Barcode labels should be aligned lengthwise along the bottle as the scanner will not work on a curved surface (i.e. horizontally wrapped around the bottle; **Figure 6**).
    b. Barcode labels must be associated with a unique sample and each barcode must be mapped to one sample in the database. Barcodes are unique, but are not initially associated with a

particular sample, so you are encouraged to adhere barcode labels to needed containers in advance.

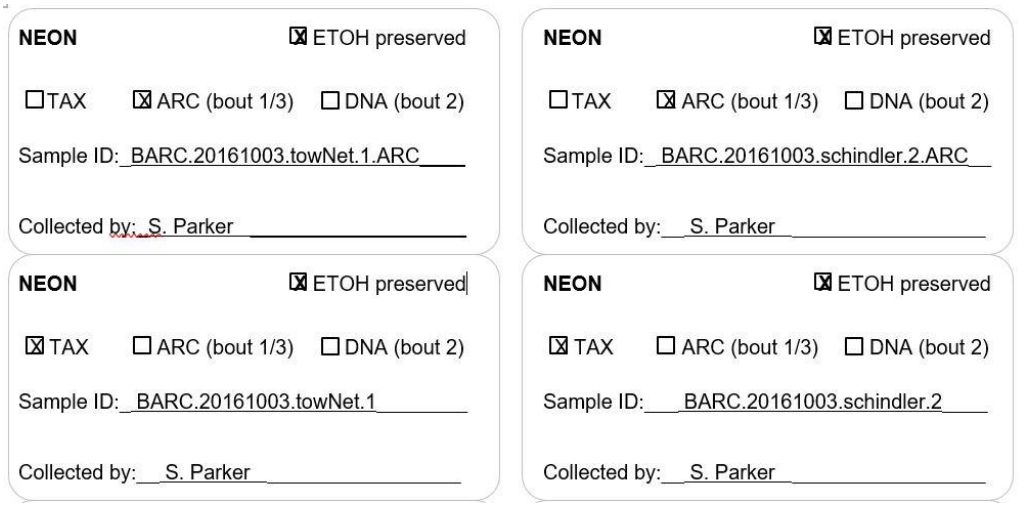

Figure 7. Example of human-readable adhesive field labels for zooplankton sampling.

About Barcode Uses and Placement

Barcodes are required for all samples shipped to an external lab. Barcodes should be placed vertically on sample bottles so that they are not curved for scanning.

Table 7. Sample ID and barcode requirements for field samples generated by the ZOO-Zooplankton Sampling protocol.

Sample Type	Description	Example Identifier	Fulcrum App	Container Type	Barcode Used	Barcode Required ?	Barcode Qty
ZOO-tax	Preserved zooplankton sample	PRPO.20161027.townet.1 OR PRPO.20161027.schindler.1	(AOS) Zooplankton Field Data [PROD]	125-500 mL HDPE bottle	Type I	Yes	1 per bottle, 3 per bout
ZOO-dna	Preserved zooplankton DNA sample (Bout 2)	PRPO.20161027.townet.1.dna OR PRPO.20161027.schindler.1.dna	(AOS) Zooplankton Field Data [PROD]	125-500 mL HDPE bottle	Type I	Yes	1 per bottle, 3 per bout 2
ZOO-arc	Preserved zooplankton ARC sample (Bouts 1 and 3)	PRPO.20161027.townet.1.arc OR PRPO.20161027.schindler.1.arc	(AOS) Zooplankton Field Data [PROD]	smallest Nalgene, 125-500 mL	Type I	Yes	1 per bottle, 3 per bouts 1 and 3

SOP B Field Sampling

Data for field sampling are entered in the (AOS) Zooplankton Field [PROD] mobile application. Instructions for the use of this application can be found in the Sampling Support Library in the document "Manual for Fulcrum Application: (AOS) Zooplankton [PROD]".

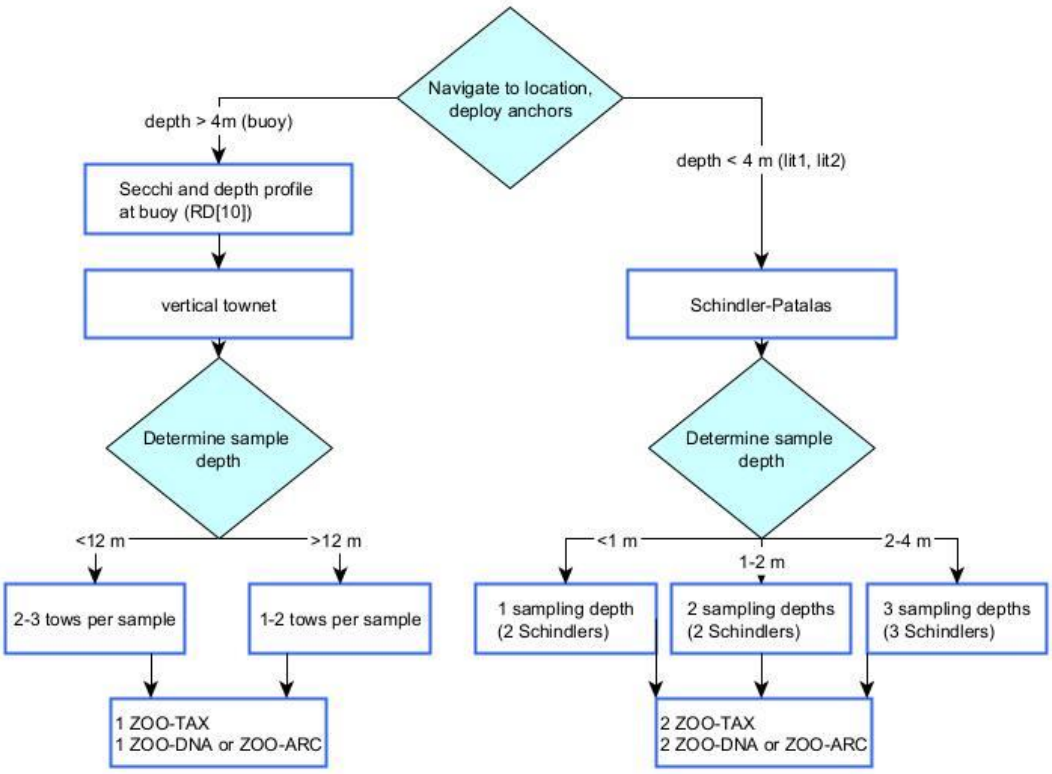

Figure 8. Workflow for field sampling by location for zooplankton collection.

B.1 Spatially and Temporally Linked Protocols

Field Metadata and Gauge Height

- Data are entered into the (AOS) Field Metadata and Gauge Height [PROD] app at the start and end of every aquatic field day.

Secchi Depth and Depth Profile Sampling

- Secchi and depth profile measurements must be recorded with every protocol that samples the water column, such as zooplankton (RD[10]).

B.2 Sampling Locations

1. In the deepest point in the lake (near buoy), determined by site map and pre-loaded GPS coordinates.

a. Location ID = “c0” (lake not stratified) or “c1” (lake stratified)

2. Near the littoral 1 sensor set.
   a. Location ID = “lit1”
3. Near the littoral 2 sensor set.
   a. Location ID = “lit2”

B.3 Collecting Samples

Zooplankton taxonomy (ZOO-tax) samples are collected during each sampling bout, three times per year. Collecting three samples per bout is the minimum required to maintain data quality.

1. Navigate to the sampling location. Do not sample within a 5 m radius of the aquatic instrumentation.
2. Gently lower anchors at the bow and allow boat to float back with wind or current to sampling location. Drop a second anchor at the stern to hold boat in place.
   a. Allow ~5 minutes for sediments to settle after lowering the anchor; you can use this time to prepare the sampling equipment.
   b. Using a bow anchor rope 2 times the water depth will minimize disturbance of the sediment at the sampling location.
   c. The boat must be anchored at the bow and stern so that the boat doesn’t rotate, in order to collect representative water column samples.

3. Always sample near the bow of the boat to minimize the effects of the motor on the water column. When anchored, the boat tends to orient itself with the bow into the wind or current.
4. Determine the total water depth from depth finder readings or a weighted rope.
   a. If depth is ≤4 m, or if phytoplankton clog the tow net, use a Schindler-Patalas sampler and proceed to Step 5.
   b. If depth is >4 m, use the tow net and proceed to Step 7.
5. **Schindler-Patalas sampler** (**Figure 9**): Integrate 2 to 3 Schindler traps to equal 1 sample. Measure to the bottom of the Schindler trap box (**Figure 11**). Record sample type, sample depths, and number of traps collected in mobile application.
   a. If depth is 2-4 m, integrate the following (3 traps):
      i. 0.5 m below surface (measure to bottom of Schindler trap)
      ii. Middle of water column
      iii. 0.5 m above lake bottom
   b. If depth is 1-2 m, integrate the following (2 traps):

i. 0.5 m below surface

ii. 0.5 m above lake bottom

c. If depth is <1 m, integrate the following (2 traps):

i. Sample in locations that are deeper than 0.8 m to prevent disturbing the sediment contaminating the sample with benthic material.

ii. 2 Schindler traps 0.5 m below surface

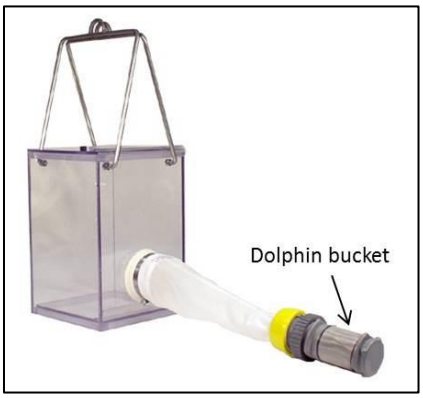

Figure 9. Example of a Schindler-Patalas sampler with Dolphin bucket.

6. When using the Schindler-Patalas trap:

a. Ensure the dolphin bucket is attached (**Figure 9**).

b. Lower sampler to appropriate depth at a consistent speed so trap doors do not close prematurely.

i. When the depth is reached and the sampler stops descending, the trap doors will close automatically.

c. Bring the sampler to the surface. The water will drain through the net.

i. When filled with water, this sampler is heavy. Work gloves may be worn to protect hands when pulling in the rope.

ii. Lift the sampler slowly to allow water to drain through the net and dolphin bucket.

1) Once above the water's surface, opening the lid of the Schindler breaks the suction and allows water to flow into the dolphin bucket faster. Do not allow additional source water into the sampler at this point.

d. Inspect the sample for organic and inorganic debris. If sediments were captured, discard sample and start again. If organic debris (e.g., leaves, plants) is noted accounting for >25% of sample, discard sample and start again. Smaller amounts of organic debris can be removed by hand.

i. Discard sample away from where you intend to collect the next sample.

e. Rinse the sampler and net until the entire sample is in the dolphin bucket.

i. Lake water can be used on the outside of the net

ii. DI, tap water, or 63 μm mesh

iii. -filtered source water (note: this is not the same mesh size used for macroinvertebrate sampling) must be used if rinsing the inside of the trap or net to ensure that no additional zooplankton are added to the sample.

f. Carefully remove the dolphin bucket and transfer sample to a 125-500 mL sample bottle. Rinse dolphin bucket into collection bottle.

g. Repeat steps above to integrate Schindler-Patalas samplers.

h. Record data in the mobile app.

i. Scan the barcode label with the tablet (**Figure 10**).

ii. Record the total number and sampling depths of the Schindler-Patalas samples added to the composite in the mobile app.

iii. Ensure that the human-readable sample ID matches the sample ID generated by the mobile app.

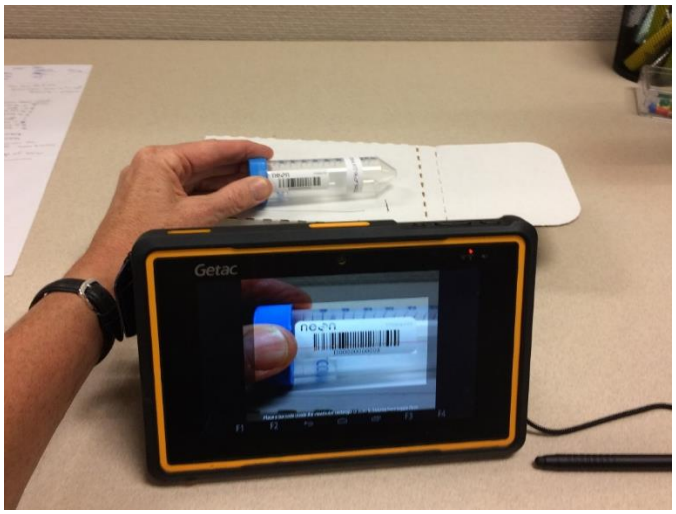

Figure 10. Barcode label scanning.

i. Preserve according to Sample Preservation (SOP B.4) and place sample bottle in cooler.

j. Proceed to next sampling location, rinse trap, net, and dolphin bucket well with lake water, and repeat steps above.

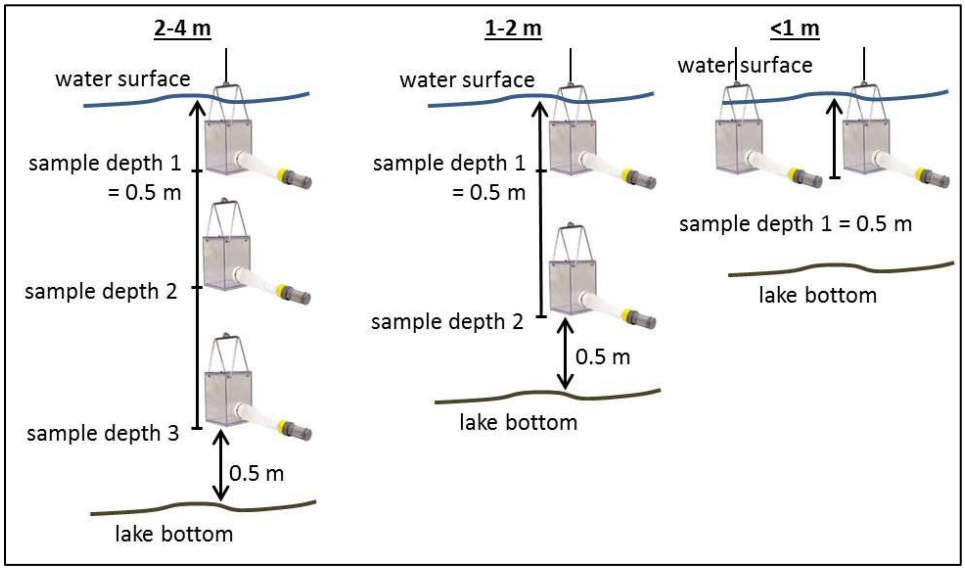

Figure 11. Schindler-Patalas sampling depths.

7. **Tow net sampler** (**Figure 12**): integrate at least 2 tow nets to equal 1 sample. Measure depth to the top of the tow net mouth. Note sample type, sample depths, and number of tows collected in the mobile app.
    a. If the sampling location is >12 m deep, assess the amount of zooplankton collected after 1 tow. If zooplankton fill at least half of the sample bottle, you do not need to collect a second tow.
    b. Ensure that the tubing end is firmly attached and clamp is closed over tubing prior to sampling. The tubing end comes loose easily.
    c. Lower the net to where the bottom tubing is 0.5 m above the bottom of the lake. If conditions are windy, add a small weight at the bottom of the net to help lower the tow net.
    d. Pull the nylon rope vertically at a rate of 0.5 m/s until top of net is out of the water.
    e. Inspect the sample for organic and inorganic debris. If sediments were captured, discard sample and start again. If organic debris (e.g., leaves, plants) is noted accounting for >25% of sample, discard sample and start again. Smaller amounts of organic debris can be removed by hand.
        i. Discard sample away from where you intend to collect the next sample.
    f. If algae clog the net, rinse the sides of the net down with the 500 mL wash bottle. If water cannot drain through the net, consider using the Schindler-Patalas sampler at this location.
        i. Lake water can be used on the outside of the net
        ii. DI, tap water, or 63 µm filtered lake water must be used if rinsing the inside of the trap or net to ensure that no additional zooplankton are added to the sample.

g. After the inspection, slowly lower the net into the lake to within inches of the net opening above the water surface and abruptly pull upwards out of the water to collect the contents of the net at the bottom. *Take care not to submerse the top of the net opening below the water surface and introduce more sample water*. Repeat several times until the net is fully rinsed.

Figure 12. Zooplankton tow net with bottom tubing and clamp.

h. Complete the rinsing of the net contents by spraying water against the **outside** of the net with a wash bottle filled with lake water. If rinsing the inside of the net, use DI, tap water, or 63 μm filtered lake water. Be sure to inspect and rinse the net seams carefully for trapped zooplankton.

i. Holding the net in a vertical position, carefully open the spigot on the hose and pour into the 125-500 mL sample bottle.

   i. If large organic debris is present, remove the large debris with forceps.

j. Rinse the concentrated zooplankton into the collection bottle using a squirt bottle filled with DI.

k. Enter the tow depth (distance in meters of water column through which the collecting unit was retrieved) in the mobile app (**Figure 13**) so sample volume can be calculated.

l. Collect two or more tows following the steps above to increase the number of organisms in the sample.

i. Leave space in bottle for preservative (bottle should be no more than ~1/2 full before preservative is added). Minimize bottle size based on the typical amount of organic material collected at the site.

m. Record data in the mobile app.

i. Scan the barcode label with the tablet.

ii. Record the total number and sampling depths of the individual tow nets added to the composite in the mobile app.

iii. Ensure that the human-readable sample ID matches the sample ID generated by the mobile app.

n. Preserve according to Sample Preservation (SOP B.4) and place sample bottle in cooler.

o. Proceed to the next sample location, and rinse the net, collection cup and retrieval rope thoroughly (2 to 3 times) with water from the site on the downwind side of the sampling location and repeat above steps.

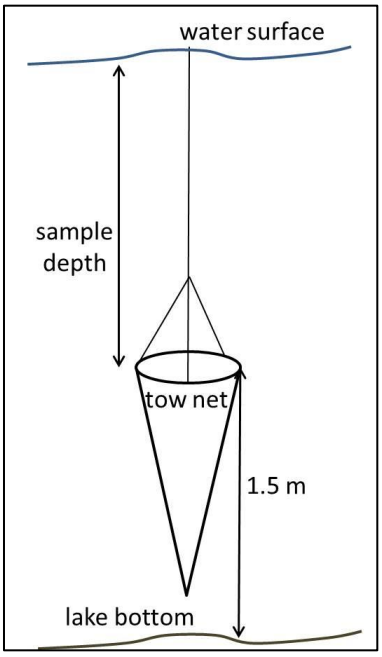

Figure 13. Tow net configuration.

B.4 Sample Preservation

All processing must be completed in the field. Should conditions not allow for this step to be undertaken in the field within 5 minutes of sampling, all preservatives shall be added within 30 minutes of collecting the sample in the field. If samples are not preserved within 30 minutes, discard and resample.

1. Add enough 95% ETOH solution to the sample bottle within 5 minutes of collection to reach a final concentration of ~30-40% ETOH.

a. Use 125 mL to 500 mL Nalgene. Minimize the container size depending on the amount of organic material typically collected at the site.

b. If the mouth of the bottle is small, you may use a funnel to assist in transferring the sample from the net or Dolphin bucket to the container.

c. Organic material should fill less ~50% of the container, then fill the bottle to the top with ethanol. The goal is to reach a final preservative concentration of 30-40%.

d. Use C1V1=C2V2 to calculate preservative volume, where:

   i. C1=concentration of ETOH before adding to sample (95%)

   ii. V1=**volume** of ETOH before adding to sample

   iii. C2=**concentration** of ETOH in final sample (30-40%)

   iv. V2= **volume** of final sample

e. As a rule of thumb, add enough 95% ETOH to slightly less than double the sample volume (i.e., <50% ETOH in final sample).

2. Carefully re-cap bottle, ensuring that no sample escapes, and gently invert bottle to mix.

3. Store in a cooler (~4 ^{o}C) to return to Domain Support Facility.

SOP C Zooplankton DNA Metabarcode Field Collection

Zooplankton DNA (ZOO-dna) samples are collected during each sampling bout, three times per year. Samples collected specifically for DNA metabarcoding will be analogs to three of the samples already being collected. This sampling approach uses the community metabarcoding approach, where replicate samples from the site are preserved in high-concentration ethanol for sequencing analysis. These samples are collected using field methods that are as "sterile" as possible, with the understanding that it is difficult to maintain sterility in the field. Equipment will be sterilized in the domain lab prior to sampling and rinsed with source water between samples at a site on a given day.

C.1 Sterilize Equipment

All equipment must be cleaned and sterilized prior to sample collection at the site to prevent contamination of DNA from the person collecting the sample, DNA from another site or sampling date, or DNA from the environment outside of the site (Laramie et al. 2015).

1. Cleaning equipment:
    a. Wear nitrile gloves to clean all nets and other equipment that come in contact with samples during zooplankton sampling in a 10% bleach solution. Because these are community samples and less sensitive to field contamination than eDNA, this cleaning is a dunk or dip in the bleach solution and is not a soak for a specified amount of time (Liu et al. 2020). Follow with a DI or tap water rinse or soak to remove the residual bleach (Jane et al. 2014, Laramie et al. 2015). Note that this is a higher concentration than is used in the Aquatic Decontamination Protocol (RD[09]).
    b. For hard surfaces like the Schindler-Patalas sampler, wipe Plexiglas walls with Kimwipes prior to bleach cleaning to remove additional residue.
    c. Equipment to decontaminate with stronger solution:
        i. Tow net
        ii. Schindler-Patalas and dolphin bucket
        iii. Collection bottles, if they do not come clean and capped from the lab
        iv. Forceps, sieves, or any additional equipment that comes in contact with the sample.
        v. Waders and boots if you plan to wade near the sampling location. If you remain in the boat, this is not necessary.
2. Wearing clean nitrile gloves, place equipment and consumables in a clean bag so as not to contaminate it on the way to the field site.

C.2 Field Collection

1. Fill out and place an adhesive label on the collection bottle. Check "DNA" on label.

2. Collect DNA samples after collecting taxonomy for consistency. Prioritize taxonomy sampling if contingent decisions are necessary.
    a. Collect an additional sample at each sample location (littoral 1, littoral 2, and buoy) using the same sampler already chosen to use for taxonomy samples at each of those locations (SOP B). Wear clean nitrile gloves while collecting DNA samples.
3. Prior to sampling, prime the sampler by rinsing well with local source water. Rinse from the outside of the net so zooplankton and other organisms are not trapped inside the net during rinsing. You may use surface water (this adds minimal DNA to the sample when compared to the actual organisms in the final sample). Wear clean nitrile gloves while priming equipment.
    a. Good practice is to collect both DNA and taxonomy samples while wearing clean nitrile gloves.
    b. You may either use new gloves for each sample or re-use and clean gloves with ETOH between samples.
        i. Use a clean ETOH wipe or spritz with ETOH and wipe to remove residual material.
4. Collect samples according to SOP B for the appropriate sample depth. Faster zooplankton may appear to be missing from the sample, however De Bernardi (1984) suggests that trade-offs are made with every sampler, and replicate samples collected from clear samplers improve estimates of zooplankton abundance.
5. Choose “DNA” in the mobile app, and the appropriate habitat and sampler metadata.
6. Sample ID = *SITE.YYYYMMDD.sampleType.sampleNumber.DNA* during Bout 2 and *SITE.YYYYMMDD.sampleType.sampleNumber.ARC* during Bouts 1 and 3. Check that the sample ID on the label matches the sample ID in the mobile app.
7. After sample collection, use forceps or gloved hands to pick out large organic matter, leaving the zooplankton in the sample.

a. Samples will be homogenized in a blender at the external facility. Make sure there are no large sticks or other material in the sample that could interfere with homogenization.

8. Minimize the amount of water in the sample by using as little water as possible for rinsing.
9. Add 95% ETOH to sample jar to completely cover sample (Stein et al. 2012). Final ETOH concentration should be as close to 95% as you can get, and at least greater than 50%.
10. Return sample to domain lab and store at 4 ^{o}C.

SOP D Post-Field Sampling Tasks

1. Refresh the sampling kit
    a. Replace sample bottles.
    b. Print new field labels and field data sheets.
    c. Refill/restock preservative containers.
2. Equipment maintenance, cleaning and storage
    a. Decontaminate all equipment used at other sites that has come in contact with lake/non-wadeable stream water according to the NEON Aquatic Decontamination Protocol (RD 09)].
    b. Stainless steel sieves tend to rust. Clean and dry thoroughly prior to storage. To minimize rust, you may also do a methanol wipe to prevent corrosion.
        i. Put on nitrile gloves.
        ii. Wipe sieve with a methanol-wetted Kimwipe® to reduce methanol waste.
        iii. DO NOT rinse with DI water after methanol rise and wipe.
        iv. Allow methanol-rinsed equipment to air dry prior to storage.
    c. Dry all equipment thoroughly before storage.
    d. Check all nets for holes and patch if necessary.
3. Data QA/QC
    a. Required checks
        i. Check that the sample IDs generated by the mobile application(s) match the sample IDs written on the sample bottles.
        ii. Check that the barcode labels in in the mobile application(s) match the barcode labels adhered to the samples. At a minimum, check the last few numbers of the barcode.
    b. Nice to check
        i. Site ID, collect date, sampling protocol version
        ii. Sampling depths

D.1 Document Incomplete Sampling Within a Site

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

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

Zooplankton sampling locations may become compromised if the water level of the lake drops too low to samples near the inlet and outlet infrastructure. If sampling at a given location is not possible during a given bout a problem ticket should be submitted by Field Science staff.

Three zooplankton samples are the minimum that can be collected during a bout to ensure scientifically useful data. Therefore, it is preferable to collect a sample in a different location rather than not collect a sample at all.

To document locations not sampled during the current bout:

1. Review the completed sampling effort and create **Sampling Impractical** records as described in Section 4.5 for locations at which sampling was scheduled but was not completed.

2. To document whether a location is compromised according to the criteria above:

    a. Review **Sampling Impractical** records from the ZOO: Zooplankton Field Data [PROD] application and Portal data to identify locations where sampling was scheduled but was not completed due to environmental or site management factors.

3. Create an incident with the following naming convention to document the missed sampling: ‘AOS Sampling Incomplete: ZOO – [Root Cause Description]’

    a. Example: ‘AOS Sampling Incomplete: ZOO – Could not access inlet due to cows for 2 consecutive bouts’

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

SOP E Laboratory Sampling and Analysis

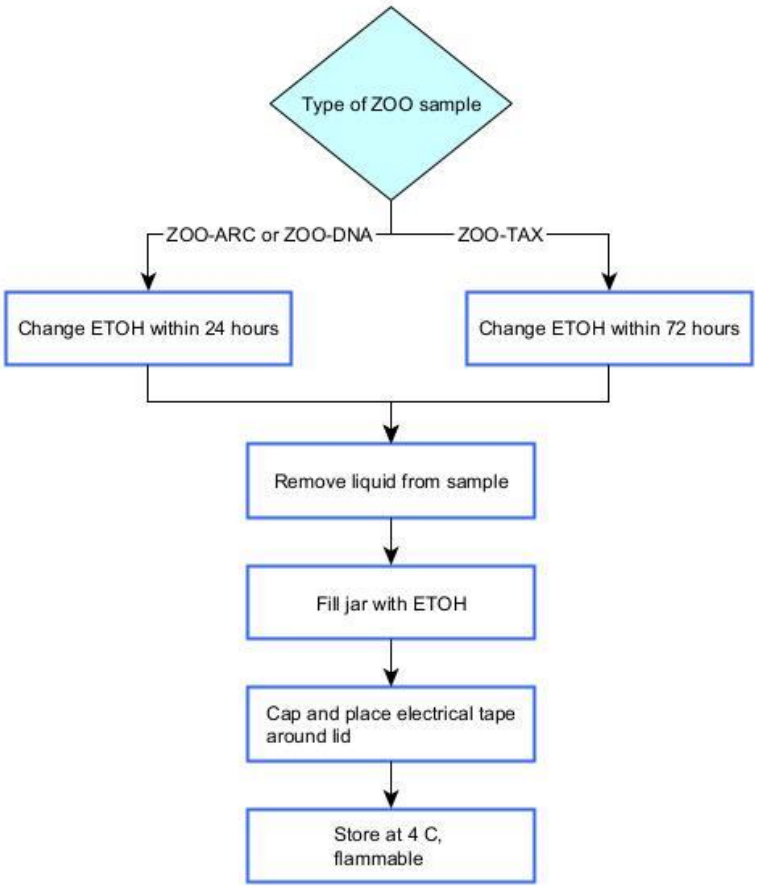

Figure 14. An expanded diagram of the lab workflow for the zooplankton lab SOP.

E.1 Preparation

1. Fill 500 mL wash bottle with 475 mL 95% undenatured ethanol for taxonomy preservation. Clearly label bottle for ETOH.

Table 8. Storage conditions, containers, and lab processing timing for ZOO samples.

Sample type	Time to preservative swap	Storage conditions	Container
ZOO-TAX	Arrival at DSF-72 hours	refrigerated (4 °C)	125-500 mL HDPE
ZOO-DNA	Arrival at DSF -24 hours	refrigerated (4 °C)	125-500 mL HDPE
ZOO-ARC	Arrival at DSF -24 hours	refrigerated (4 °C) or frozen (-20 °C)	125-500 mL HDPE - Smallest Nalgene that fits the sample, please downsize during ETOH swap if possible, to save storage space at the biorepository. A Nalgene is required because it has a better seal for long-term storage.

E.2 Processing Samples

1. For DNA samples (collected in SOP C), change out ethanol between returning to the DSF and 24 hours of collection in the domain lab prior to shipping. This helps to ensure that DNA is preserved quickly and will be in good condition for analysis.
2. Decontaminate the filter apparatus, nylon mesh, and any equipment that comes in contact with the DNA sample during processing with 10% bleach, or keep a separate, decontaminated filter apparatus for DNA.
3. Wearing safety glasses and nitrile gloves, open field-preserved sample. Perform ETOH swap under a fume hood. ETOH wipe gloves between samples for ZOO-DNA and ZOO-ARC.
4. Allow zooplankton to settle in the sample container and decant or pipet ethanol off the top of the sample. For all of the following methods, double check to make sure the mesh is in place and secure before you start to filter the sample to prevent sample loss.
    a. There are several ways change ETOH in zooplankton samples. Be sure that the following requirements are met regardless of which method is used:
        i. Sample material (zooplankton) is not lost from the sample bottle. Only liquid is removed and replaced.
        ii. Any equipment or consumables that come in contact with the sample material have been decontaminated with 10% bleach, rinsed with DI, and dried prior to the sampling day, and are rinsed with 10% bleach followed by DI between samples.
    b. Method 1: Allow samples to settle >4 hours. Decant or pipette liquid off the top of the sample over a 63 μm sieve in case the sample spills. Return any spilled sample to the bottle and replace liquid with ETOH.
    c. Method 2: Use the TOS beetle filter cup with 63 μm (zooplankton) mesh (RD[11]). Rinse material on mesh into sample bottle with ETOH, but do not include in sample. The TOS beetle filter cup should screw directly onto zooplankton collection bottles.

d. Method 3: Pour sample into dolphin bucket from the field sampling equipment. Use an ETOH-filled squirt bottle to rinse the contents of the cup back into the sample bottle. Be sure to decontaminate the dolphin bucket between samples.

e. Method 4: Make a filter lid using cut out jar or Nalgene lid. Place the 63 µm Nitex square over the top of the sample container and screw the modified lid firmly on top. Invert the jar over the waste container, and rinse sample material from the mesh lid back into the sample jar. This may work better at sites that have light or no sediment in samples.

5. If a Nitex mesh square was used, rinse animals and debris from the mesh into the sample container using ETOH and discard the mesh square. Do not leave the mesh square in the sample container to send to the external lab.

6. Replace ethanol, preserving as close to 95% as possible.

   a. Container does not need to be filled to the top with liquid as organisms will be homogenized with a blender at the external lab and do not need to remain intact.

7. Close sample container tightly.

8. ETOH wipe and sterilize filter apparatus or any equipment that comes in contact with the DNA sample material between samples.

9. Carefully clean and dry all equipment prior to storage.

E.3 Sample Storage

Store all samples at the domain support facility at 4 °C in a flammable-rated space until shipping. For shipping instructions see SOP G. If 4 °C space is an issue, DNA samples may also be stored at -20 °C until shipping.

Any samples stored for longer than 2 days should be entered into the Domain Support Inventory app.

SOP F Data Entry and Verification

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

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

Quality Assurance

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

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

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

Sample Identifiers & Barcodes

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

Adhesive barcode labels should be applied to dry, room temperature containers in advance of their use in the field (at least 30 minutes prior but may be applied at the start of the season). Barcodes are unique, but are not initially associated with a particular sample, thus it is encouraged to apply these in advance. Use the appropriate barcode label type with each container (i.e., cryogenic Type II barcode

labels only used for samples that are stored at -80°C, etc.). Note that a barcode label is applied *in addition to* a sample identifier (hand-written or printed).

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

SOP G Sample Shipment

Information included in this SOP conveys science-based handling and packaging instructions for the sample types included in this protocol. For shipping instructions, see RD[14].

1. Keep morphological taxonomy samples, DNA, and ARC samples separate.
    a. See CLA shipping information for details on shipping locations.
        i. Bout 2 DNA samples will be shipped to an external lab for analysis.
        ii. Bout 1 and Bout 3 ARC samples will be shipped directly to the archive facility.
2. Tape the lids of all sample jars and bottles with electrical tape. This helps prevent leaks and ethanol fumes from fading the sample labels.
    a. Tip: Assigning a different color of electrical tape to each site in the domain helps keep samples organized.
3. Bag samples
    a. ZOO-tax: Place sealed sample bottles into one or several gallon-sized resealable zip-top bags, grouped by site. Sample bottles are acceptable “inner containers” required for shipping.
    b. ZOO-dna: Individually bag each DNA sample in a zip-top bag to prevent contamination of DNA from sample to sample during shipment.
    c. ZOO-arc: Individually bag each ARC sample in a zip-top bag to prevent contamination of DNA from sample to sample during shipment.
4. See RD[14] for further shipping instructions.

G.1 Handling Hazardous Materials

Follow procedures for shipping ethanol in limited quantity ground shipments in AD[03] and RD[14].

G.2 Supplies/Containers

See RD[14] for specific shipping materials.

G.3 Timelines and Conditions

Taxonomy samples: Shipping should occur within one week of sampling if possible, however samples may be held for up to 30 days at the domain support facility if necessary. Samples may be stored at the domain support facility at 4 °C until shipping. Samples are shipped ground at ambient temperature.

DNA samples: Samples from Bout 2 must be held at 4 °C and will be shipped to the external facility for analysis on a CLA-defined schedule.

ARC samples: Samples from Bouts 1 and 3 will be shipped directly to the archive facility on a CLA-defined schedule.

All samples: Samples may be held for a maximum of 6 months if batch shipping is requested by the external lab. Samples are shipping ground at ambient temperature as this is a short enough time-period that sample degradation is not expected to occur.

G.4 Grouping/Splitting Samples

Taxonomy, DNA, and ARC samples are all shipped to different facilities and must stay separate.

Group samples by site per bout. Samples from multiple sites may be sent in the same shipment. Zooplankton samples may be shipped with macroinvertebrate samples if going to the same external lab or archive facility.

G.5 Return of Materials or Containers

Include return shipping label with WBS code if any shipping materials need to be returned to the domain support facility (e.g., cooler). Sample bottles will not be returned.

G.6 Shipping and Chain of Custody

The mobile shipping applications are used to create chain of custody documents. Shipments are to include a hardcopy of the shipping manifest (RD[14]) in each box as well as an electronic shipping manifest that is emailed to the receiving laboratory and NEON Collections and Laboratory Analysis at the time of shipment. The shipping manifest must accurately document the physical samples inside the shipping container.

G.7 Laboratory Contact Information and Shipping/Receipt Days

See the Shipping Information for External Facilities on the Stork app.

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APPENDIX A QUICK REFERENCES

Step 1 – Check the zooplankton field sampling kit to make sure all supplies are packed.

Step 2 – Prepare labels (2" x 4") and barcode labels. Sample ID = *SITE.DATE.samplerType.sampleNumber[.DNA or .ARC]*

Step 3 – Ensure the General (AOS) Field Metadata and Gauge Height (RD[06]) and (AOS) Secchi (RD[10]) apps are completed per field site visit.

Step 5 – Determine which sampler to use based on water depth.

1. If water depth is ≤4 m, use a Schindler-Patalas sampler.
    a. If 2-4 m collect samples at 0.5 m, mid-water column, and 0.5 m above the lake bottom and integrate.
    b. If 1-2 m collect sample from 0.5 m and 0.5 m above the lake bottom and integrate.
    c. If water is <1 m, collect 2 Schindler traps 0.5 m below water surface and integrate.
    d. If water depth is > 4 m, use the tow net and integrate at least 2 tows.

Step 6 – Collect 1 integrated sample for taxonomy and 1 integrated sample for DNA at:

1. In the deepest point in the lake (buoy), determined by site map and pre-loaded GPS coordinates.
    a. Location ID = "c0" or "c1" (depends on stratification)
4. Near the littoral 1 sensor
    a. Location ID = "lit1"
5. Near the littoral 2 sensor
    a. Location ID = "lit2"

Step 7 – Preserve samples in the field with ethanol (taxonomy final concentration 30-40%, DNA final concentration ~95%). Replace ETOH in DNA samples within 24 hours of collection.

Step 8 – Ship samples to zooplankton taxonomy lab, zooplankton DNA lab, or archive facility.

APPENDIX B REMINDERS

Before heading into the field:

- ☑ Collect and prepare all equipment including labels and barcodes.
- ☑ Check for holes in nets, assure that nets are clean and free of debris and organic matter and have been decontaminated (RD[09]) since last use.
- ☑ Bleach clean all equipment for DNA collection.

Sample collection:

- ☑ When making contingent decisions, a suite of 3 samples is the minimum number of samples in order to do statistics on the data. Do not collect fewer than 3 samples.
- ☑ Choose the appropriate sampler based on depth.
- ☑ Always sample near the bow of the boat to minimize the effects of the motor on the water column.
- ☑ Use deionized water, tap water, or 0.63 µm filtered lake water to rinse inside zooplankton sampling nets to prevent introduction of additional zooplankton to the samples. Do not introduce additional zooplankton to sample from rinsewater.
- ☑ Take care not to submerse the top of the net opening below the water surface after collecting the sample, during rinsing.
- ☑ Zooplankton often become trapped in the folds of the nets (near the stitching). Check net seams between samples to ensure that specimens are added to the correct samples, and do not remain in the net between sites.
- ☑ Wear gloves and use methods that are as sterile as possible for DNA collection.

Sample processing:

- ☑ Preserve samples in the field within 30 minutes of collection.
- ☑ Store at 4 °C until shipping to the external lab.
- ☑ Change ETOH in DNA samples after collection.

Data QA/QC:

Required checks

- Check that the sample IDs generated by the mobile application(s) match the sample IDs written on the sample bottles.
- Check that the barcode labels in in the mobile application(s) match the barcode labels adhered to the samples. At a minimum, check the last few numbers of the barcode.

Nice to check

- Site ID, collect date, sampling protocol version
- Sample depths

APPENDIX C ESTIMATED DATES FOR ONSET AND CESSATION OF SAMPLING

Preliminary date ranges for biological sampling bouts in lakes are based on the NEON temporal sampling strategy (Parker and Utz 2022). Refer to the Aquatic Site Sampling Design for your domain (RD[13]) for bout window start and end dates.

APPENDIX D SITE-SPECIFIC INFORMATION

Sampler types should be consistent at a location from year to year.

Domain	Site		Buoy (c0)	Littoral (lit1, lit2)
D03	Lake Barco	BARC	tow net	Schindler
D03	Lake Suggs	SUGG	Schindler	Schindler
D05	Crampton Lake	CRAM	tow net	Schindler
D05	Little Rock Lake	LIRO	tow net	Schindler
D09	Prairie Lake	PRLA	Schindler*	Schindler
D09	Prairie Pothole	PRPO	Schindler*	Schindler
D18	Toolik Lake	TOOK	tow net	Schindler

*May use tow net when water is high.

APPENDIX E 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 9. Equipment list – General equipment.

Supplier/ Item No	Exact Brand	Description	Purpose	Quantity
Durable items				
	N	Work gloves	Used with the samplers for safe handling of the rope	1 pair
	N	Mobile data entry tablet	Field data entry	1
	N	Cooler with ice packs	Keeping samples cool	1
Consumable items				
	N	Aquatic Field Metadata Sheet and Field data sheets (all-weather paper)	Recording metadata if tablet fails	1
	N	Pre-printed adhesive labels	Labeling sample bottles, human-readable	1 sheet
	N	Adhesive barcode labels (Type I)	Labeling sample bottles with barcode-readable	1 sheet
	N	Laboratory nitrile gloves	Preventing preservative contact with skin	3 pair
	N	Pencils	Recording data	4
	N	Permanent markers	Labeling samples	4

Table 10. Equipment list – Sampling equipment.

Supplier/ Item No	Exact Brand	Description	Purpose	Quantity
Durable items				
Forestry Suppliers, Inc.; 426A10	N	Plankton tow net, 12” mouth, 63 µm mesh	Collecting samples >4m water depth	1
	N	Nylon safety line (10-50 m)	Used with tow net and Schindler-Patalas	1
Forestry Suppliers, Inc.; 217004	R	Schindler-Patalas sampler, 12 L, 63 µm mesh size	Collecting samples ≤4 m water depth	1
	N	500 mL Wash bottle	Rinsing the sampler net	1
	N	Plastic sampling tray	Removing debris from the sample	1
	N	Forceps	Removing debris from the sample	1
	N	Depth finder	Determining depth at sampling location	1
Consumable items				
	Y	125 - 500 mL Nalgene sample bottles	Sample container, size is site-specific depending on how much organic material is in samples	3 – TAX, 3 – DNA/ARC
	N	Ethanol, 95%, undenatured	Preservative	1
	N	DI water or tap water	Rinsing	2L
Grainger, W.W.; 5LH30	N	Resealable bags (gallon)	Organizing sample bottles	3
Fisher Scientific Company; BPA4084	Y	Methanol (Spectranalyzed™), Fisher Chemical™	Drying metal sieves to prevent rust	As needed

Table 11. Equipment list – Laboratory processing (DNA samples).

Supplier/ Item No	Exact Brand	Description	Purpose	Quantity
Durable items				
	N	Sieve, 63 um	Changing preservative	N
	N	500 mL Wash bottle, for ETOH	Rinsing samples with ETOH	1
Consumable items				
	N	63 um Nitex mesh cloth, cut into squares for filter cup	Catching zooplankton over the filter cup	N
	N	Ethanol, 95%, undenatured	Preservative	1
	N	Filter cup (500 mL HDPE bottle and 63 um Nitex mesh square, see TOS beetle protocol)	Changing preservative	1
	N	Chlorine bleach (6%)	Decontaminating DNA equipment	1 gal
	N	Nitrile gloves	Maintaining sterile conditions	N