AOS PROTOCOL AND PROCEDURE: ZOOPLANKTON SAMPLING IN LAKES

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See configuration management system for approval history.

The National Ecological Observatory Network is a project solely funded by the National Science Foundation and managed under cooperative agreement by Battelle. Any opinions, findings, and conclusions or recommendations expressed in this material are those of the author(s) and do not necessarily reflect the views of the National Science Foundation.

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

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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.

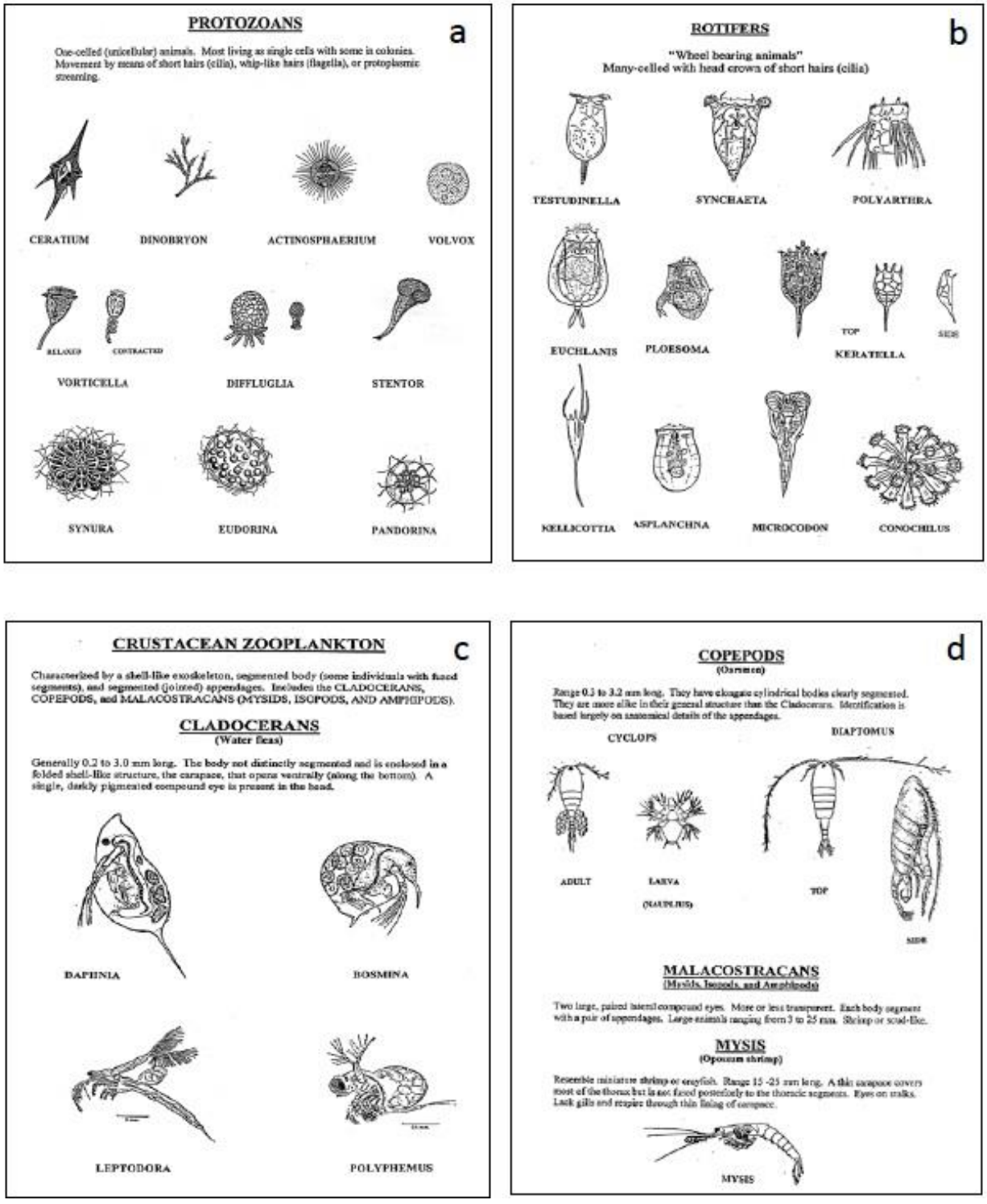

Figure 1. Classes of zooplankton (from Tharp).

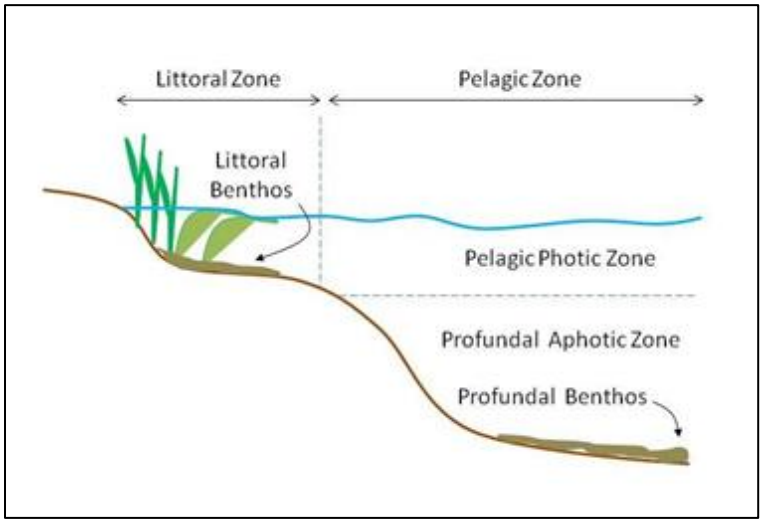

Figure 2. Zones of a lake.

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	EHSS Policy, Program and Management Plan
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 Performance QA/QC 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 Level 1, Level 2, Level 3 Data Products Catalog
RD[04]	NEON.DOC.001271	NEON Protocol and Procedure: Manual Data Transcription
RD[05]	NEON.DOC. 002302	Datasheets for AOS Protocol and Procedure: Zooplankton Sampling in Lakes
RD[06]	NEON.DOC.001646	General AQU Field Metadata Sheet
RD[07]	NEON.DOC.002191	Datasheets for Secchi Depth and Depth Profile Sampling
RD[08]	NEON.DOC.001152	NEON Aquatic Sample Strategy Document
RD[09]	NEON.DOC.004257	NEON Standard Operating Procedure (SOP): Decontamination of sensors, field equipment and field vehicles
RD[10]	NEON.DOC.001197	AOS Protocol and Procedure: Bathymetry and Morphology of Lakes and Non-Wadeable Streams
RD[11]	NEON.DOC.002494	Datasheets for AOS Sample Shipping Inventory
RD[12]	NEON.DOC.002792	AOS Protocol and Procedure: Secchi Depth and Depth Profile Sampling

2.3 Acronyms

Acronym	Definition
DI	Deionized
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 lake water where sunlight penetrates and photosynthesis can occur. Specifically, the depth to which 1% of surface light penetrates.

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

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.

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: The part of the lake that is not near shore or close to the bottom.

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 °C per meter. The denser and cooler layer below the thermocline is the hypolimnion, the warmer upper layer is termed 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 inlet and outlet of the lake or a location downwind of the predominant wind direction. Samples are collected 3 times per year in order to capture differences in community composition, abundance, diversity, and biomass.

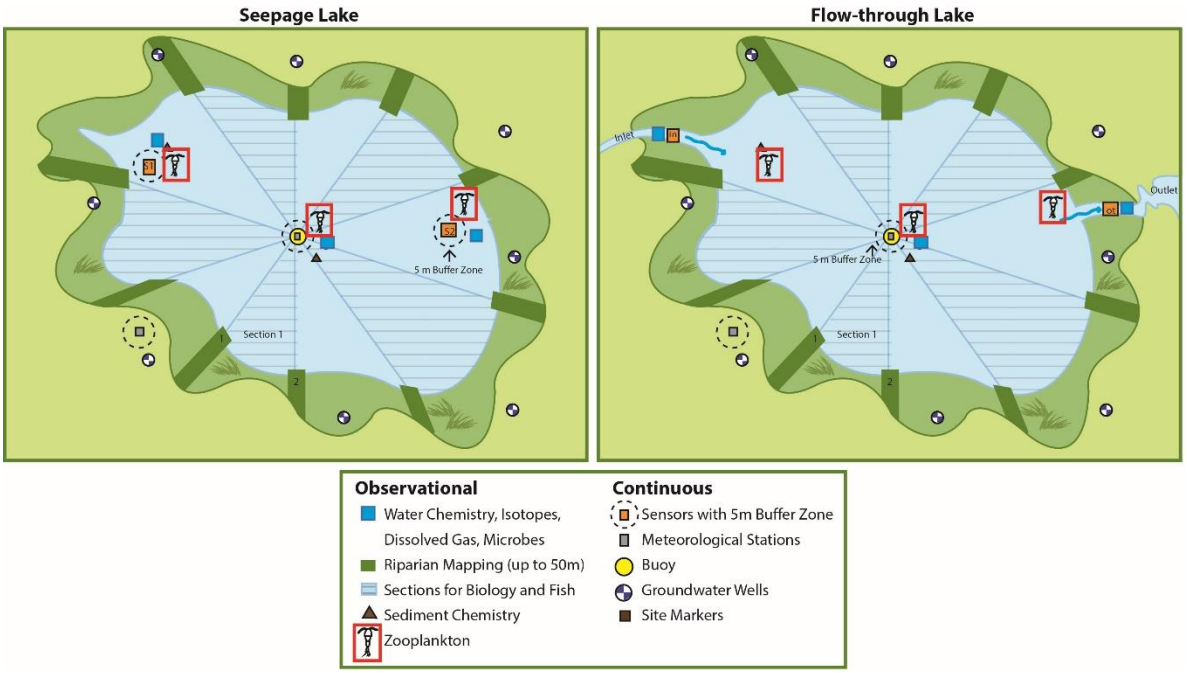

Figure 3. A generic lake site layout with zooplankton sampling locations. Seepage lakes have no true inlet or outlet stream. In flow-through streams, inlet and outlet infrastructure are located in the inlet or outlet stream channel.

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 will be performed on data collected via these procedures according to the NEON Science Performance QA/QC 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. Sampling must be scheduled within the first 21 days of the 1 month window specified in Appendix D 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 outside of the pre-determined window. Use NEON's problem reporting system to report sampling efforts that take place outside of the defined sampling window.

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 (Appendix D).

4.3 Timing for Laboratory Processing and Analysis

Though samples should ideally be shipped to external processing facilities within 7 days of collection, they may be held for up to 30 days at the domain support facility if necessary.

4.4 Sample Timing Contingencies

All samples from this protocol in one sampling bout must be collected within one day (i.e., all samples per lake/non-wadeable stream as detailed in this protocol) because of the fluctuating nature of aquatic habitats. Spreading sample collection over multiple days increases data variability among samples. A minimum of 2 weeks between sample periods shall be observed.

Table 1. Contingent decisions

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 (>32 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. If conditions improve, resume sampling, if not, return to the Domain Support Facility and sample at another time.	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 must be flagged.
	If you are able to return to the site to sample within 24 hours, you may keep samples from the previous day. If you are not able to return within 24 hours, discard any previously collected samples in the water or at the Domain Support Facility and start over.	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 must be flagged.
6 Months	Preserved zooplankton samples may be held for up to 6 months 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 and samples do not need to be flagged if held for ≤ 6 months.

4.5 Criteria for Permanent Reallocation of Sampling within a Site

Zooplankton sampling will occur on the schedule described above at 3 locations per site. Ideally, sampling will occur at these sampling locations for the lifetime of the Observatory (core sites) or the duration of the site's affiliation with the NEON project (relocatable sites). However, circumstances may arise requiring that sampling within a site be shifted from one particular location to another. In general, sampling is considered to be compromised when sampling at a location becomes so limited that data quality is significantly reduced. If sampling at a given location becomes compromised, a problem ticket should be submitted by Field Operations to Science.

There are two main pathways by which sampling can be compromised. Sampling locations can become inappropriately suited to answer meaningful biological questions (e.g., a terrestrial sampling plot becomes permanently flooded or a stream channel moves after a flood). Alternatively, sampling locations may be located in areas that are logistically impossible to sample on a schedule that that is biologically meaningful.

A common occurrence in zooplankton sampling may occur when the inlet and outlet sampling locations become too shallow for sampling. In that case, sampling may be moved to a location that has a more appropriate depth, and does not necessarily compromise sampling. Enter a problem ticket if moving more than 20 m from original location.

4.6 Sampling-specific Concerns

1. 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.
2. Samples must be preserved within 30 minutes in the field.
3. 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.

5 SAFETY

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

Personnel working at a NEON site must be compliant with safe field work practices as outlined in the Operations Field Safety and Security Plan (AD[02]) and EHSS Policy, Program and Management Plan (AD[01]). Additional safety issues associated with this field procedure are outlined below. The Field Operations Manager and the Lead Field Ecologist 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.

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 10 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 AND EQUIPMENT

6.1 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 2. Equipment list – General equipment

Item No.	Supplier	Supplier ID	R/S	Description	Purpose	Quantity	Special Handling
Durable items							
RD[10]			R	Site-specific Bathymetry Map	Determining sampling locations	1	N
			S	Work gloves	Used with the samplers for safe handling of the rope	1 pair	N
MX111388	CDW-G	4452963	R	Mobile data entry tablet	Field data entry	1	N
			R	Cooler with ice packs	Keeping samples cool	1	N
Consumable items							
RD[06] MX103942	Ben Meadows Co., Inc. Forestry Suppliers, Inc.	010510-1 49247	R	Aquatic Field Metadata Sheet (all-weather paper)	Recording metadata	1	N

Item No.	Supplier	Supplier ID	R/S	Description	Purpose	Quantity	Special Handling
RD[05], RD[07] MX103942	Ben Meadows Co., Inc. Forestry Suppliers, Inc.	010510-1 49247	R	Field data sheets (all-weather paper)	Recording data	2	N
MX103942	Ben Meadows Co., Inc. Forestry Suppliers, Inc.	010510-1 49247	R	Pre-printed adhesive labels	Labeling sample bottles, human-readable	1 sheet	N
			S	Adhesive barcode labels	Labeling sample bottles with barcode-readable	1 sheet	N
			S	Laboratory nitrile gloves	Preventing preservative contact with skin	3 pair	N
			R	Pencils	Recording data	4	N
MX102002	Grainger, W.W.	1JU51	R	Permanent markers	Labeling samples	4	N

R/S=Required/Suggested

Table 3. Equipment list – Sampling equipment

Item No.	Supplier	Supplier ID	R/S	Description	Purpose	Conditions Used	Quantity	Special Handling
Durable items								
MX100397	Ben Meadows Co., Inc.	426A10	R	Plankton tow net, 12" mouth, 63 µm mesh	Collecting samples	Water depth >4 m	1	N
			R	Nylon safety line (10-50 m)	Used with tow net and Schindler-Patalas	Water depth >4 m	1	N
MX105580	Ben Meadows Co., Inc.	217004	R	Schindler-Patalas sampler, 12 L, 63 µm mesh size	Collecting samples	Water depth ≤4 m	1	N
MX100308	Fisher Scientific Company	0340910E	R	500 mL Wash bottle	Rinsing the sampler net	All	1	N
MX100338	BioQuip Products Inc. Fisher Scientific Company	1426B NC0095946	S	Plastic sampling tray	Removing debris from the sample	All	1	N
MX102975	BioQuip Products Inc.	4748	S	Forceps	Removing debris from the sample	All	1	N
MX109276	Amazon Capital Services Inc.	B00X0WT8MQ	S	Depth finder	Determining depth at sampling location	All	1	N

Item No.	Supplier	Supplier ID	R/S	Description	Purpose	Conditions Used	Quantity	Special Handling
Consumable items								
MX100652	Fisher Scientific Company	033134E	R	500 mL Nalgene sample bottles	Sample container	All	3	N
MX100665	Thomas Scientific, Inc.	1709C04	S	1 L Nalgene sample bottles	Sample container for large samples	Large samples	3	N
MX100213 MX100202	Fisher Scientific Company Thomas Scientific, Inc.	4355601 C954K61	R	95% Ethanol	Preservative	All	1	Y
			R	DI water or tap water	Rinsing	All	2L	N
MX104844	Grainger, W.W.	5LH30	S	Resealable bags (gallon)	Organizing sample bottles	All	3	N

R/S=Required/Suggested

Table 4. Equipment list – Shipping supplies

Item No.	Supplier	Supplier ID	R/S	Description	Purpose	Quantity	Special Handling
Durable Items							
(None)							
Consumable Items							
			R	Cardboard box, UN-rated, Group II	Shipping samples to taxonomist	1	N
MX109205	Grainger, W.W.	30RD13	R	Vermiculite, Grade 2	Absorbing liquid leaks and cushioning shipment	TBD	N
MX102717	Uline	S-5106	R	Heavy duty plastic trash bag	Lining the shipping container	1	N
			R	Appropriate labels and forms for limited quantity ground shipments (see AD[03])	Shipping paperwork and external shipping labels	TBD	N
MX101876	Grainger, W.W.	2A227	S	Electrical tape	Taping jar lids prior to shipping	1 roll	N

Item No.	Supplier	Supplier ID	R/S	Description	Purpose	Quantity	Special Handling
			R	Shipping inventory (RD[11])	Provides sample information to external lab	1	N

6.2 Training Requirements

Field staff must complete protocol-specific training for safety and implementation of this protocol as required in Field Operations Job Instruction Training Plan (AD[04]).

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]).

6.3 Specialized Skills

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

6.4 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.

Field sampling requires two field ecologists for four hours per site plus travel to and from the site. There is no domain lab processing associated with this protocol.

7 STANDARD OPERATING PROCEDURES

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 and synced 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 Zooplankton Field Sampling

1. Collect and prepare all equipment, including sample bottles and labels.
    a. Load GPS sampling coordinates in GPS (accuracy ±4 m).
2. Pre-print human-readable adhesive labels (Figure 4) for sample bottles and data sheets on all-weather paper (label template are available in the Sampling Support Library or RD[05]).
    a. Labels must be pre-printed or filled out using pencil or ethanol-safe pen in the field.
    b. Use the following naming convention for sample IDs (see Table 5). Sample IDs written on the physical sample label must match the sample ID generated by the mobile app.
    SITE.YYYYMMDD.samplerType.sampleNumber[.DNA]
        i. SITE = 4 letter site code
        ii. YYYYMMDD = Collection date
        iii. samplerType = Type of sampling equipment used, see Table 5.
        iv. sampleNumber = Numbers 1-3, corresponds to one of three samples. Does not need to be directly linked to a specific location.
        v. [DNA] = Append to sample ID if this is a DNA sample.

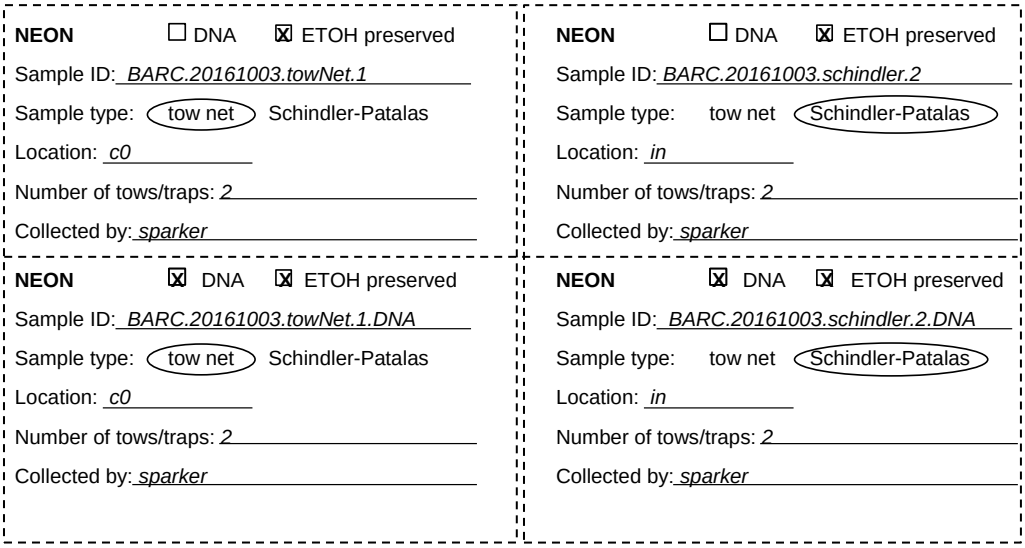

Figure 4. Example of human-readable adhesive field labels for zooplankton sampling.

3. When the system is available, adhesive barcode labels will be added to the sample containers and scanned by the mobile app (Figure 5). Add adhesive labels to zooplankton bottles prior to going in the field and getting the bottle wet.
    a. Keep a human-readable label on each bottle with a minimum of the sample ID printed to assist with organization and shipping.

Figure 5. Example of adhesive barcode labels.

Table 5. Examples of sample IDs generated by the mobile app.

Sampler type	Site type	Field app populates as:	Example Sample ID
Tow Net	Lake	townet	PRPO.20161027.townet.1
Schindler-Patalas	Lake	schindler	PRPO.20161027.schindler.1

4. 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.
5. Have ice or ice packs frozen and ready for cooler.
6. Check that all equipment is in good condition and all batteries are charged.
7. 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.

SOP B Field Sampling

B.1 Locating 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”
2. Near the major inlet in the littoral zone.
   a. Location ID = “in”
3. Near the outlet in the littoral zone.
   a. Location ID = “ot”

B.2 Collecting Samples

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 6): Integrate 2 to 3 Schindler traps to equal 1 sample. Measure to the bottom of the Schindler trap box (Figure 8). Record sample type, sample depths, and number of traps collected in mobile application (see example of paper datasheet in Appendix F).
   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

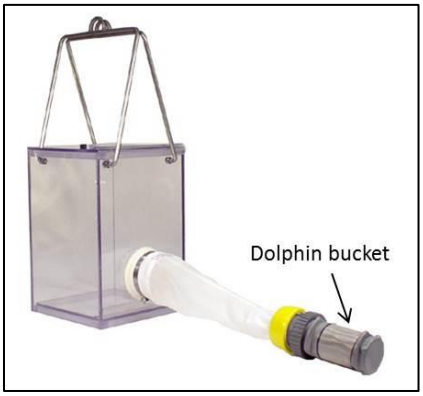

Figure 6. Example of a Schindler-Patalas sampler with Dolphin bucket.

6. When using the Schindler-Patalas trap:
   a. Ensure the dolphin bucket is attached (Figure 6)
   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.

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-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 500 mL sample bottle. Rinse dolphin bucket into collection bottle. Samples volume may be significantly less than 500 mL.
g. Repeat steps above to integrate Schindler-Patalas samplers.
h. Record data in the mobile app.
   i. If available, scan the barcode label with the tablet (Figure 7).
   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.

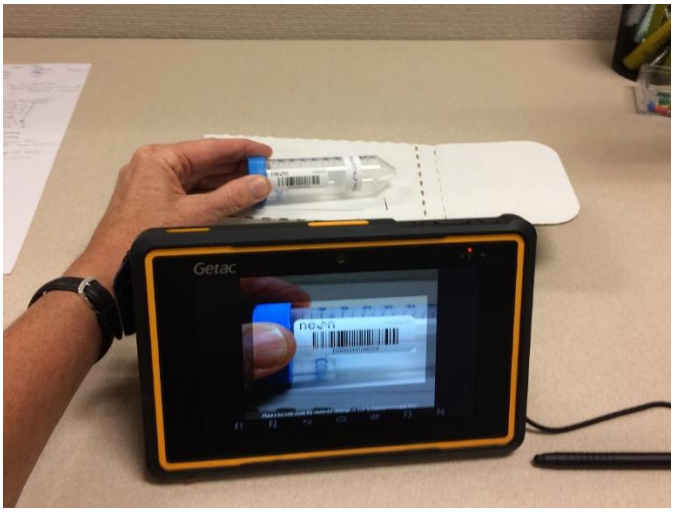

Figure 7. Barcode label scanning.

i. Preserve according to Sample Preservation (Section B.3) 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.

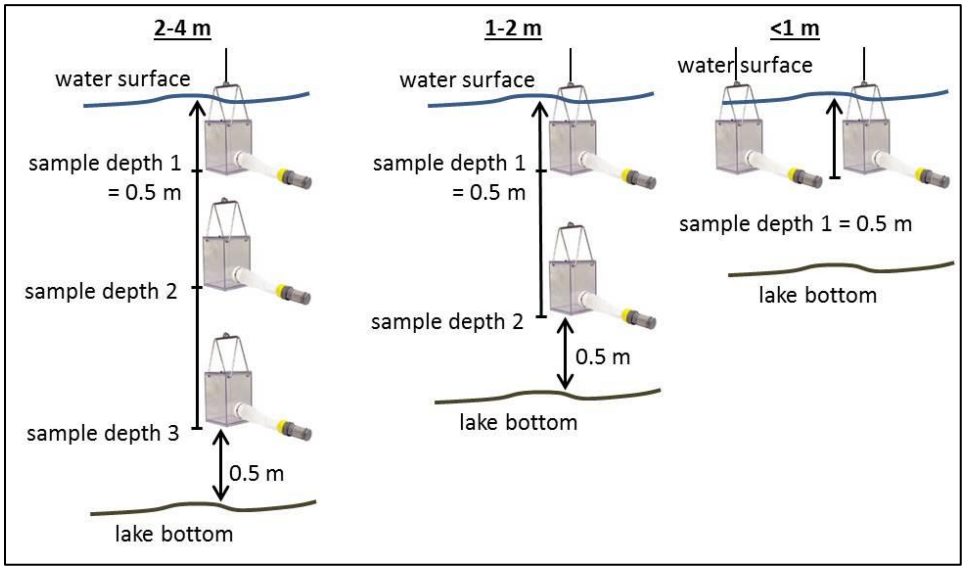

Figure 8. Schindler-Patalas sampling depths.

7. **Tow net sampler** (Figure 9): 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 9. 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 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 10) 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).
m. Record data in the mobile app.
    i. If available, 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 (Section B.3) 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.

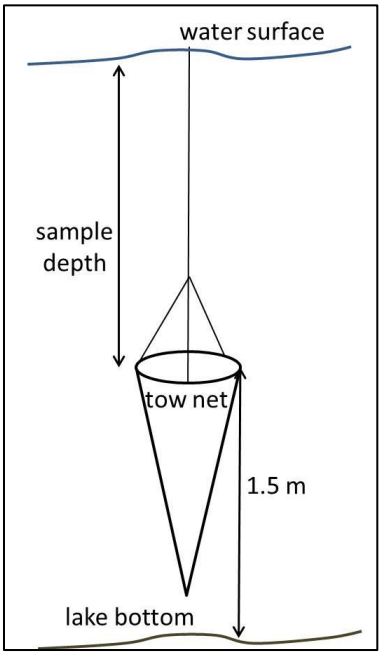

Figure 10. Tow net configuration.

B.3 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 500 mL sample bottle within 5 minutes of collection to reach a final concentration of ~30-40% ETOH.
    a. The sample can be <500 mL.
    b. Consider using a larger sample bottle (1 L) if there is not enough room to add ethanol to reach a final preservative concentration of 30-40%.
    c. 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
    d. 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 °C) to return to Domain Support Facility.

B.4 Ending the Sampling Day

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 that has come in contact with lake/non-wadeable stream water according to the NEON Aquatic Decontamination Protocol (RD 09)].
    b. Dry all equipment thoroughly before storage.
    c. Check all nets for holes and patch if necessary.

SOP C Zooplankton DNA Metabarcode Field Collection

Zooplankton 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.

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 DNA from the environment outside of the site (Laramie et al. 2015).

1. Cleaning equipment:
    a. Wearing nitrile gloves, clean all nets and other equipment that comes in contact with samples during zooplankton sampling using a 10% bleach solution. Follow with a DI rinse to remove the residual bleach (Jane et al. 2014). Note that this is a higher concentration than is usually used in the Aquatic Decontamination Protocol (RD[09]).
    b. 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.
3. Collect an additional sample at each sample location (inlet, outlet, 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.
4. 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. Wear clean nitrile gloves while priming equipment.
    a. Good practice is to collect both DNA and taxonomy samples while wearing clean nitrile gloves.
5. Collect samples according to SOP B for the appropriate sample depth.
6. Choose “DNA” in the mobile app, and the appropriate habitat and sampler metadata.

7. Sample ID = *SITE.YYYYMMDD.sampleType.sampleNumber.DNA*
8. After sample collection, use forceps or gloved hands to pick out large organic matter, leaving the zooplankton in the sample.
9. Minimize the amount of water in the sample by using a little water as possible for rinsing.
10. 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%.
11. Return sample to domain lab and store at 4 °C.

SOP D Laboratory Sampling and Analysis

1. For DNA samples (collected in SOP C), change out ethanol between 24 and 48 hours in the domain lab prior to shipping.
2. Wearing safety glasses and nitrile gloves, open field-preserved sample under fume hood.
3. Allow zooplankton to settle in the sample container, and decant or pipet ethanol off the top of the sample.
4. Replace ethanol, preserving as close to 95% as possible.
    a. Container does not need to be filled to the top with liquid.
5. Close sample container tightly.

SOP E Data Entry and Verification

Mobile applications are the preferred mechanism for data entry. Data should be entered into the protocol-specific application as they are being collected, whenever possible, to minimize data transcription error and improve data quality. If available, adhesive barcode labels should be used and scanned into the mobile application. 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. For detailed instructions on protocol specific data entry into mobile devices, see the NEON Internal Sampling Support Library (SSL).

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. As a best practice, field data collected on paper datasheets should be digitally transcribed within 7 days of collection or the end of a sampling bout (where applicable). However, given logistical constraints, the maximum timeline for entering data is within 14 days of collection or the end of a sampling bout (where applicable). See RD[04] for complete instructions regarding manual data transcription.

Data and sample IDs must be entered digitally and quality checked prior to shipping samples to an external lab.

SOP F Sample Shipment

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

1. Keep morphological taxonomy samples and DNA samples separate.
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.
3. 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.
4. Line a Group II cardboard box with a heavy-duty trash bag.
5. Place all sample jars right-side up inside the trash bag, inside the Group II cardboard shipping box. Add Grade 2 Vermiculite in the trash bag liner as needed to take up excess space in container and cushion samples.
6. Navigate to the "Shipping Information for External Facilities" document on CLA's NEON intranet site. Check whether items such as permits or cover letters are required in the shipment. Check this document often as instructions are subject to change.
7. Prepare a shipping inventory detailing the contents of the shipment, using the protocol-specific templates found on CLA's NEON intranet site (RD[11]. Include a printed copy of the shipping inventory in the shipment box in a zip-top bag.
8. Save the inventory with the following naming convention: "DXX_MOD_ShippingInventory_YYYYMMDD_XofX"
    a. Example: "D05_ZOO_ShippingInventory_20161202_1of1"
9. Complete packing slip, address shipment, and ship ground to the destination(s) specified in the CLA "Shipping Information for External Facilities" document on CLA's intranet site.
    a. Follow instructions for shipping ETOH in limited quantity ground shipments in AD[03].
    b. DNA samples do not need to be shipped on ice.
10. Email an electronic copy of the shipping manifest and tracking number to the email addresses listed in the CLA "Shipping Information for External Facilities" document, including the NEON CLA email address.

F.1 Handling Hazardous Material

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

F.2 Supplies/Containers

See section D.1 and Table 4 for specific shipping materials.

F.3 Timelines and Conditions

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 ^{o}C until shipping. Ship samples at ambient temperature.

F.4 Grouping/Splitting Samples

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.

F.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.

F.6 Shipping Inventory

Shipments are to have a hardcopy of the per sample tab of the shipping inventory (RD[11]) sent in each box as well as an electronic shipping inventory that is emailed to the receiving laboratory and to the contact in NEON Collections and Laboratory Analysis at the time of shipment. Also include the shipment tracking number in the email.

F.7 Laboratory Contact Information and Shipping/Receipt Days

See the Shipping Information for External Facilities on NEON's CLA intranet site.

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APPENDIX A DATASHEETS AND MOBILE APPLICATIONS

The following datasheets and mobile applications are associated with this protocol:

Table 6. Datasheets and mobile applications associated with this protocol

NEON Doc. #	Title	Mobile Application
NEON.DOC.002302	Datasheets for AOS Protocol and Procedure: Zooplankton Sampling in Lakes	(AOS) Zooplankton Field Data [PROD]
NEON.DOC.001646	General AQU Field Metadata Sheet	(AOS) Field Metadata and Gauge Height [PROD]
NEON.DOC.002191	Datasheets for Secchi Depth and Depth Profile Sampling	(AOS) Secchi [PROD]
NEON.DOC.002494	Datasheets for AOS Shipping Inventory	Shipping App [PROD]

Datasheets can be found in Agile or the NEON Document Warehouse, user guides for mobile applications may be found in NEON's internal sampling support library.

APPENDIX B 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 if available. Sample ID = *SITE.DATE.samplerType.sampleNumber[.DNA]*

Step 3 – Ensure the General AQU Field Metadata Sheet (RD[06]) is 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.
2. 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"
2. Near the major inlet in the littoral zone
   a. Location ID = "in"
3. Near the outlet in the littoral zone
   a. Location ID = "ot"

Step 7 – Preserve samples in the field with ethanol (taxonomy final concentration 30-40%, DNA final concentration ~95%).

Step 8 – Ship samples to zooplankton taxonomy lab and/or zooplankton DNA lab.

APPENDIX C REMINDERS

Before heading into the field: Make sure you…

- ☑ Collect and prepare all equipment including labels.
- ☑ Pre-print adhesive labels.
- ☑ Check for holes in nets, assure that nets are clean and free of debris and organic matter and have been decontaminated (RD[08]) since last use.
- ☑ Check that all equipment is in good condition and all batteries are charged.
- ☑ Sterilize all equipment for DNA collection.

Sample collection: Be sure to…

- ☑ Choose the appropriate sampler.
- ☑ Always sample near the bow of the boat to minimize the effects of the motor on the water column. When anchored, the bow of the boat tends to orient itself with the bow into the wind or current.
- ☑ Triple rinse sampling equipment with source water.
- ☑ Take care not to submerse the top of the net opening below the water surface after collecting the sample, during rinsing.

Sample processing: Be sure to…

- ☑ Preserve samples in the field.
- ☑ Store at 4 ^{o}C until shipping to the external lab.
- ☑ Ship DNA samples within 24 hours or change ETOH.

APPENDIX D ESTIMATED DATES FOR ONSET AND CESSATION OF SAMPLING

Preliminary date ranges for biological sampling bouts in lakes. General guidelines for developing these dates are presented in the NEON Aquatic Sample Strategy Document (RD[08]). Also see the Site Specific Sampling Strategy Document on NEON's FOPS intranet site.

Domain	Site	Bout 1	Bout 2	Bout 3
D03	Lake Barco	9Feb-9Mar	27Jun-25Jul	29Oct-26Nov
D03	Lake Suggs	9Feb-9Mar	27Jun-25Jul	29Oct-26Nov
D05	Crampton Lake	20Apr-18May	5Jul-2Aug	13Sep-11Oct
D05	Little Rock Lake	20Apr-18May	5Jul-2Aug	13Sep-11Oct
D09	Prairie Lake	18Apr-16May	5Jul-2Aug	11Sep-9Oct
D09	Prairie Pothole	20Apr-18May	5Jul-2Aug	11Sep-9Oct
D18	Toolik Lake	21May-18Jun	29Jun-27Jul	6Aug-3Sep

APPENDIX E SITE-SPECIFIC INFORMATION: SAMPLER RECOMMENDATIONS

Domain	Site	c0	in	ot
D03	Lake Barco	tow net	Schindler	Schindler
D03	Lake Suggs	Schindler	Schindler	Schindler
D05	Crampton Lake	tow net	Schindler	Schindler
D05	Little Rock Lake	tow net	Schindler	Schindler
D09	Prairie Lake	Schindler*	Schindler	Schindler
D09	Prairie Pothole	Schindler*	Schindler	Schindler
D18	Toolik Lake	tow net	Schindler	Schindler

*May use tow net in wetter years.

APPENDIX F PAPER DATASHEET EXAMPLE

See also RD[05] for blank paper datasheets.

NEON Zooplankton Collection

Lakes

Site ID: *SUGG* **Sampling protocol:** *NEON.DOC.001193* **Rev:** *G*

Date: *2017-09-26* **Recorded by:** *sparker@battelleecology.org*

Local time: *10:30* **Collected by:** *jstewart@Field-ops.org*

Location ID	Sampling impractical?	Sampler type	DNA sample collected?	Sample number	Number of tows/traps	Sample depth 1 (m)	Sample depth 2 (m)	Sample depth 3 (m)	Sample ID
c0		*tow net*	*N*	*1*	*2*	*3.6*	*3.6*	*NA*	*SUGG.20170926.townet.1*
c0		*tow net*	*Y*	*1*	*2*	*3.6*	*3.6*	*NA*	*SUGG.20170926.townet.1.DNA*
in		*Schindler*	*N*	*2*	*2*	*0.5*	*1.1*	*NA*	*SUGG.20170926.schindler.2*
in		*Schindler*	*Y*	*2*	*2*	*0.5*	*1.1*	*NA*	*SUGG.20170926.schindler.2.DNA*
ot		*Schindler*	*N*	*3*	*2*	*0.5*	*1.3*	*NA*	*SUGG.20170926.schindler.3*
ot		*Schindler*	*Y*	*3*	*2*	*0.5*	*1.3*	*NA*	*SUGG.20170926.schindler.3.DNA*