AOS PROTOCOL AND PROCEDURE: ZOOPLANKTON SAMPLING IN LAKES

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See configuration management system for approval history.

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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.

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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 filter feeders that feed primarily on algae while also being the primary 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 (U.S. Environmental Protection Agency, 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), dominated 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 (higher 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.

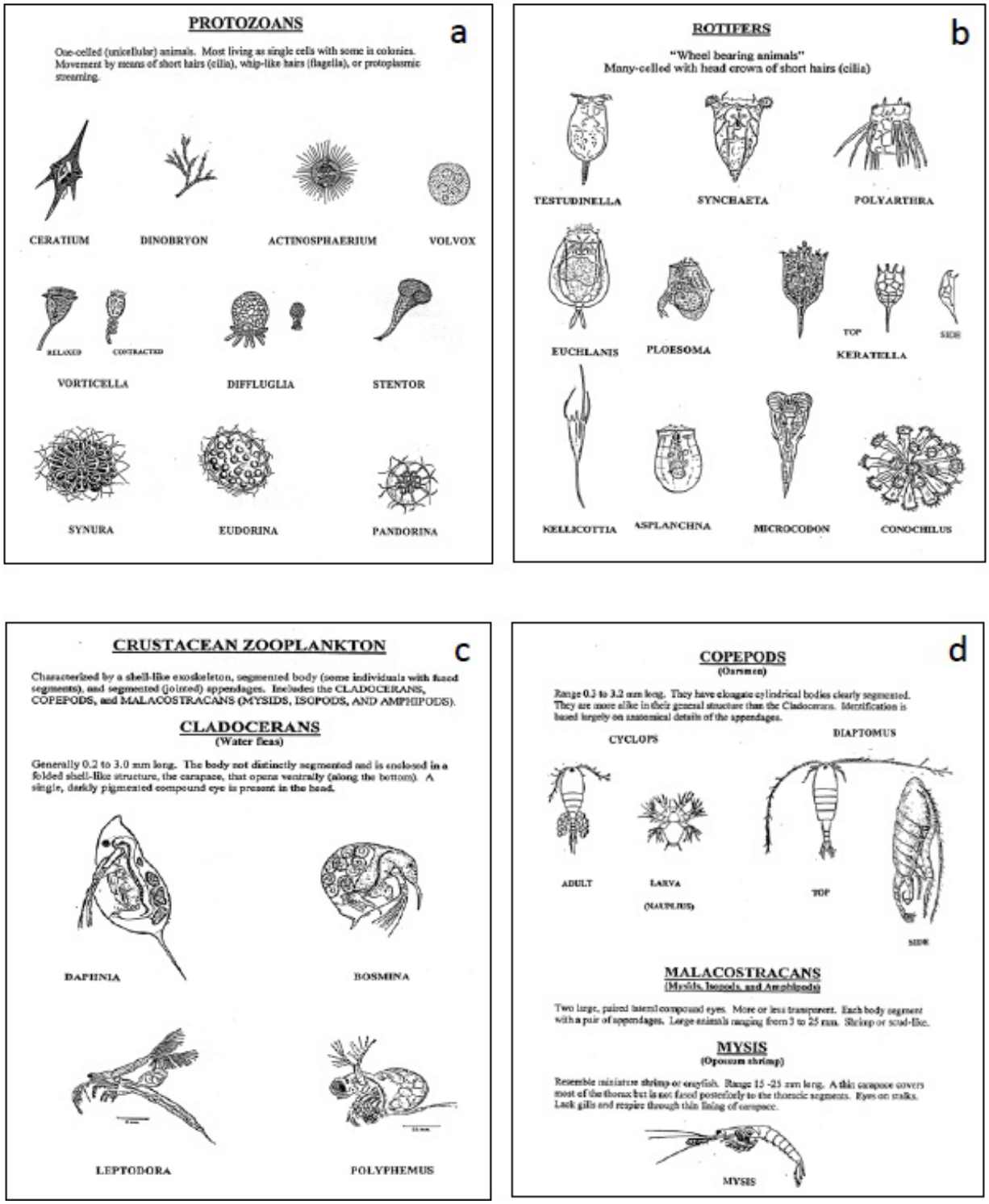

Figure 1. Classes of zooplankton (from Tharp).

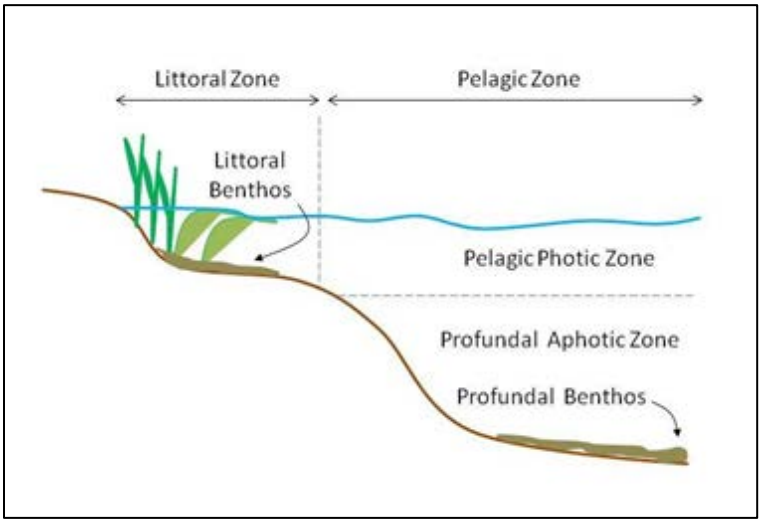

Figure 2. Zones of a lake.

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 U.S. Environmental Protection Agency (2012a, b), Baker et al. (1997), and U.S. Environmental Protection Agency (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.014051	Field Audit Plan
AD[06]	NEON.DOC.000824	Data and Data Product Quality Assurance and Control 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.005003	NEON Scientific 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.001154	AOS Protocol and Procedure: Aquatic Decontamination
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

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

Euphotic zone: The upper layer of lake water where sunlight penetrates and photosynthesis can occur. Usually demarcated by the 1% light penetration level.

Eutrophic: An ecosystem with high primary production. In lakes, this often leads to increased production of algae or algal blooms.

Epilimnion: The upper layer of the lake that overlies the thermocline. This layer tends to be less dense with a higher pH and oxygen concentrations.

Hypolimnion: The dense bottom layer of a stratified lake that sits below the thermocline. This layer is denoted by cooler summer temperatures and slightly warmer winter temperatures relative to the Epilimnion.

Metalimnion: The layer of water in a stratified lake that sits between the hypolimnion and the epilimnion. Often equated with the thermocline (Figure 2).

Oligotrophic: The ecosystem response to 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: Indicating the presence of a thermocline.

Thermocline: A distinct layer in a body of water where the change in temperature is more rapid than increasing depth. The denser and cooler layer below the thermocline is defined by 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.

This protocol is based on modified version of U.S. Environmental Protection Agency (2012), Baker et al. (1997), and U.S. Environmental Protection Agency (2009).

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 taken 3 times per year in order to capture multiple species presence and abundance.

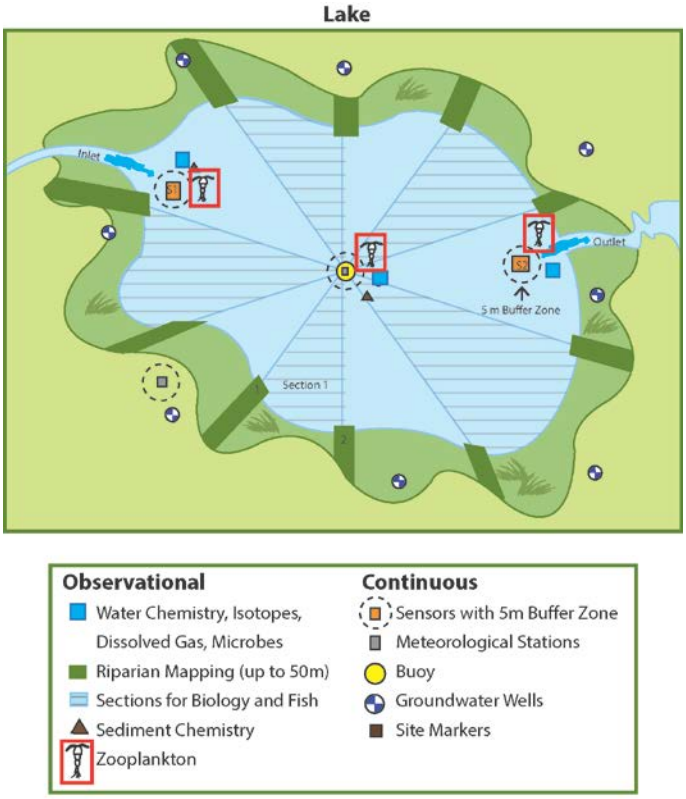

Figure 3. A generic lake site layout with zooplankton sampling locations

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.

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

4 SAMPLING SCHEDULE

4.1 Sampling Frequency and Timing

Lake zooplankton sampling occurs three times per year at each site, roughly spring, summer, and autumn. Sampling must occur within 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.

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.

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 Sampling Timing Contingencies

All samples from one sampling bout must be collected within one day (i.e., all samples per lake/non-wadeable stream as detailed in this protocol). A minimum of 2 weeks between sample periods shall be observed.

Table 1. Contingent decisions

Delay/ Situation	Action	Outcome for Data Products
Hours	If circumstances occur that impede sampling (e.g., wildlife, weather), start sampling again the next day that conditions permit.	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 circumstances occur that delay sampling (e.g., lightning), but sampling can be continued the same day while still meeting the weather requirements below, continue to collect samples.	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 weather conditions deteriorate and the lake/non-wadeable stream becomes too windy (>9 km hr^{-1}) to hold the boat stationary over a sampling point, return to shore and wait in a safe location for 30 minutes. If wind subsides, 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 lake/non-wadeable stream 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 lake/non-wadeable stream 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.
5 or More Days	Samples shall be taken a minimum of 5 days following a major flow even (>25% change in flow within 15 minutes and/or turbidity levels are double the monthly average), allowing for recolonization before sampling occurs.	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.

4.5 Sampling-specific Concerns

- 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.
- Samples must be preserved within 30 minutes in the field.

5 SAFETY

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

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

Safety Data Sheets (SDS) shall be readily available and reviewed for all chemicals used during this task.

See Section 10 in the NEON Operations Field Safety and Security Plan (AD [01]) for aquatic-specific field safety requirements. In addition, the following safety requirement must be followed:

1. Due to site-specific hazards that may be encountered, technicians may conduct sampling from the boat, without dismounting from the vessel. In addition, technicians are required to use extra caution in waters where alligators are present and to make sure a safe distance from hazards is maintained.
2. All personnel must be wearing a personal flotation device (PFD) prior to entering the boat.
3. All employees shall have access to a form of communication with other team members such as a two-way radio.
4. Technicians should 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.	R/S	Description	Purpose	Quantity	Special Handling
Durable items					
	R	Site-specific Bathymetry Map (RD[10])	Determining sampling locations	1	N
	R	Work gloves	Used with the samplers for safe handling of the rope	1 pair	N
	R	Calculator	Calculating sample volume	1	N
	R	Cooler with ice packs	Keeping samples cool	1	N
Consumable items					
	R	Aquatic Field Metadata Sheet, RD[06] (all-weather paper)	Recording metadata	1	N
	R	Field data sheets (all-weather paper, RD[05])	Recording data	2	N

Item No.	R/S	Description	Purpose	Quantity	Special Handling
	R	Pre-printed adhesive labels	Labeling sample bottles	1 sheet	N
	R	Laboratory nitrile gloves	Preventing preservative contact with skin	3 pair	N
	R	Pencils	Recording data	4	N
	R	Permanent markers	Labeling samples	4	N
	R	Tape (roll)	Taping sample bottles lids shut	1	N

R/S=Required/Suggested

Table 3. Equipment list – Secchi depth and Depth Profile equipment

Item No.	R/S	Description	Purpose	Quantity	Special Handling
Durable items					
MX100453	R	GPS and Sounder Unit (Humminbird)	Navigating to sampling locations	1	N
MX100447	R	Secchi disk and weight	Determining the depth of the euphotic zone	1	N

Item No.	R/S	Description	Purpose	Quantity	Special Handling
	R	Braided polyester line, calibrated	Determining the depth of the euphotic zone	1	N
MX100514	R	Handheld meter for conductivity, temperature, and depth	Measuring water temperature for temperature profile	1	N
Consumable items					
	R	Sounder Battery	Powering the Sounder Unit (Humminbird)	1	Y

R/S=Required/Suggested

Table 4. Equipment list – Sampling equipment

Item No.	R/S	Description	Purpose	Conditions Used	Quantity	Special Handling
Durable items						
MX100397	R	Plankton tow net, 12” mouth, 63 µm mesh	Collecting samples	Water depth is > 2	1	N
	R	Nylon safety line (10-50 m)	Used with tow net	Water depth is > 2	1	N
MX105580	R	Schindler-Patalas sampler, 12 L, 63 µm mesh size	Collecting samples	Water depth is < 2	1	N

Item No.	R/S	Description	Purpose	Conditions Used	Quantity	Special Handling
	R	500 mL Wash bottle	Rinsing the sampler net	All	1	N
	S	Plastic sampling tray	Removing debris from the sample	All	1	N
	S	Forceps	Removing debris from the sample	All	1	N
Consumable items						
	R	500 mL Nalgene sample bottles	Sample container	All	3	N
	S	1 L Nalgene sample bottles	Sample container for large samples	Large samples	3	N
	R	70% Ethanol	Preservative	All	1	Y
	R	DI water	Rinsing	Schindler-Patalas	2L	N
	R	Resealable bags (gallon)	Container for sample bottles	All	3	N

R/S=Required/Suggested

Table 5. Equipment list – General boating equipment

Item No.	R/S	Description	Purpose	Quantity	Special Handling
Durable items					
	R	Boat		1	Y
	R	Anchor with rope		2	N
	R	Oars		2	N
	R	Trolling Electric Motor		1	Y
	R	Battery (12 volt)		1	Y
	R	Safety kit for boat (e.g., flares, bailer, float with rope)		1	Y
	R	First Aid Kit		1	N
	R	Personal Flotation Devices (PFDs)		1 per person	N
Consumable items					
		(None)			

R/S=Required/Suggested

Table 6. Equipment list – Shipping supplies

Item No.	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
	R	Vermiculite, Grade 2	Absorbing liquid leaks and cushioning shipment	TBD	N
	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
	R	Shipping inventory (RD[11])	Provides sample information to external lab	1	N

6.2 Training Requirements

Additionally, technicians 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 the field protocols associated with this document, and trained in safe working practices for lake- and river-based field work.

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.

Field sampling requires two technicians 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

1. Collect and prepare all equipment, including sample bottles and labels.
   a. Load GPS sampling coordinates in GPS.
2. Pre-print adhesive labels (Figure 4) for sample bottles and data sheets on all-weather paper.
3. 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.
4. Have ice or ice packs frozen and ready for cooler.
5. Check that all equipment is in good condition and all batteries are charged.

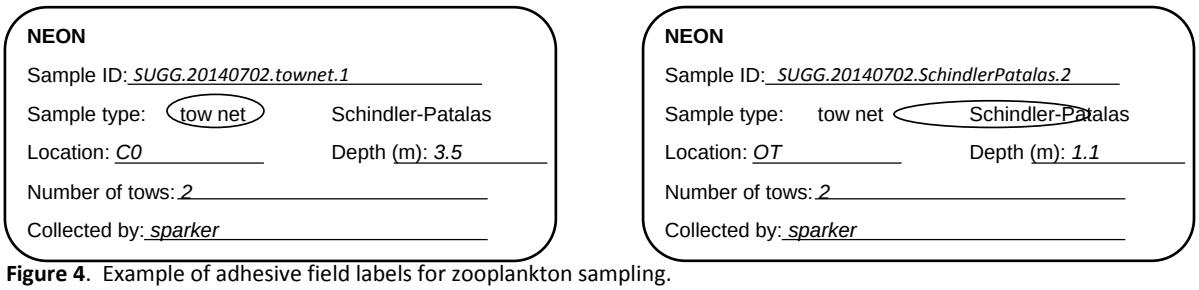

Figure 4. Example of adhesive field labels for zooplankton sampling.

SOP B Field Sampling

B.1 Locating Sampling Locations

1. In the deepest point in the lake, determined by site map and pre-loaded GPS coordinates, 1 integrated sample.
   a. Location ID = "C0"
2. Near the major inlet in the littoral zone, 1 integrated sample.
   a. Location ID = "IN"
3. Near the outlet in the littoral zone, 1 integrated sample.
   a. Location ID = "OT"

B.2 Collecting Samples

1. Ensure the General AQU Field Metadata Sheet (RD[06]) is completed.
2. Navigate the boat to the central sampling location as defined by bathymetric maps of the lake (RD[10]).
3. Fill in the location information on the field sheet (RD[05]) and note the GPS position of the sampling location.
4. Gently lower anchors at the bow and stern to the bottom of the lake so as not to suspend sediments.
   a. Allow ~5 minutes for sediments to settle after lowering the anchor; you can use this time to prepare the sampling equipment.
   b. The boat must be anchored at the bow and stern in order to collect representative water column samples.
5. 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.
6. Determine the total water depth from the sonar readings.

NEON Zooplankton Collection

Lakes

Site (4-letter code): *SUGG*	**Recorded by:** *sparker*
Date (YYYYMMDD): *20140702*	**Collected by:** *jstewart*
Local time (HH:MM): *14:30*	**Sampling protocol & Rev.:** *NEON.DOC.001194vB*

Sampling locations

Location ID	Latitude	Longitude
C0	*29.676473°*	*-82.009089°*
IN	*29.675629°*	*-82.008206°*
OT	*29.676423°*	*-82.007893°*

Location ID	Sample type	Replicate	Sample ID	Sample depth (m)	Number of tows
C0	*tow net*	*1*	*SUGG.20140702.townet.1*	*3.6*	*2*
IN	*Schindler-Patalas*	*2*	*SUGG.20140702.SchindlerPatalas.2*	*1.1*	*2*
OT	*Schindler-Patalas*	*3*	*SUGG.20140702.SchindlerPatalas.3*	*1.3*	*2*

Figure 5. Example field sheet for zooplankton collection. (Blank sheet in RD[05]).

7. Determine the Secchi depth (Figure 6, RD[07]).
   a. Lower the Secchi disk slowly into the water on the shady side of the boat (or cut the glare from the sun using your hand or other object) until the white quadrants disappear from view. NOTE: Do not wear sunglasses as this will interfere with the readings.
   b. Record depth read from the lines on the Secchi rope to the nearest 0.1 m on the Secchi depth field data sheet (RD[07]).
   c. Lower the Secchi disk so it is about 0.5 m below the first reading.
   d. Slowly pull the disk up until the white quadrants reappear, record depth to nearest 0.1 m on field data sheet as “Secchi 2”.
   e. Take the mean of the two depths and record on the data sheet (RD[07]).
   f. Repeat at each sampling location.

Figure 6. Taking a Secchi Disk measurement on the shaded side of the boat.

8. Determine the depth of the thermocline (if present) at the sampling location using the handheld meter. Record on the field data sheet (RD[07]).
   a. Put the handheld meter into the water and slowly lower through the water column, stopping at 0.5 m intervals to note depth and water temperature.
   b. Note the depth and water temperature every 0.5 m on the field data sheet.
   c. Thermal stratification occurs where the rate of decrease in temperature with increasing depth is greatest (usually >1 ^{o}C per 0.5 m depth change).
9. Determine which zooplankton sampler to use.
   a. If water depth is < 2 meters use a Schindler-Patalas sampler.
      1) If < 2 but > 1 m take samples at 0.5 m and 1.5 m depths and integrate.
      2) If water depth is < 1 m, integrate 2 samples from 0.5 m.
   b. If water is > 2 meters use a plankton tow net sampler to take an integrated sample.
   c. Note the sample type field data sheet (RD[05]).
10. If using a tow net (Figure 7).
   a. Attach the net to a nylon rope marked at 0.5 m intervals.
   b. Lower the net to where the bottom tubing is 0.5 m above the bottom of the lake. If conditions are windy or conditions necessitate, add a small weight at the bottom of the net to help lower the netting.
   c. Pull the nylon rope vertically at a rate of 0.5 m/s (net tow).
   d. Inspect the sample for organic and inorganic debris. If sediments are 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.
   e. 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.

1) If rinsing inside the net, use DI water so additional zooplankton are not added to the sample in the rinse water.

f. 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 within the net into the collection cup. *Take care not to submerse the top of the net opening below the water surface*. Repeat several times until the net is fully rinsed.

Figure 7. Zooplankton Tow Net

g. Complete the rinsing of the net contents by spraying water against the outside of the net with a wash bottle filled with lake water.

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

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

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

j. The total sample in the bottle should be ~1/3 full with zooplankton and water.

k. Note the tow depth (distance in meters of water column through which the collecting unit was retrieved) on the field data sheet (Figure 8, RD[05]) so sample volume can be calculated.

l. Rinse the net, bottle and retrieval rope thoroughly (2 to 3 times) with water from the site.

m. Collect two or more tows (Steps b-l), sampling until the sample bottle is ¾ full (allow enough space for preservative).

n. Note the number of tows on the data sheet (RD[05]) in order to calculate total sample volume)
o. Preserve according to Sample Preservation (Section B.3) and place sample bottle in cooler.
p. 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.

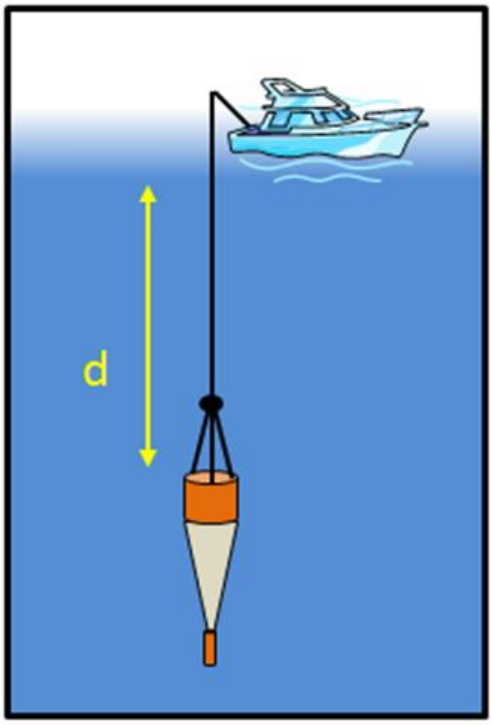

Figure 8. Tow Volume Calculation

11. If a Schindler-Patalas sampler is used (Figure 9):
    a. Ensure the dolphin bucket is attached.
    b. Lower sampler to appropriate depth.
    c. When the depth is reached the trap doors will close automatically.
    d. Bring the sampler to the surface. The water will drain through the net.
        1) When filled with water, this sampler is heavy. Work gloves may be worn to protect hands.
    e. Rinse the sampler and net with the 500 mL wash bottle filled with lake water until all samples is inside the dolphin bucket.
        1) When rinsing inside the Schindler-Patalas sampler, use DI water so zooplankton from rinse water are not 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.

g. Repeat steps above to integrate two Schindler-Patalas samplers.
h. Preserve according to Sample Preservation (Section B.3) and place sample bottle in cooler.
i. Proceed to next sampling location and repeat steps above.

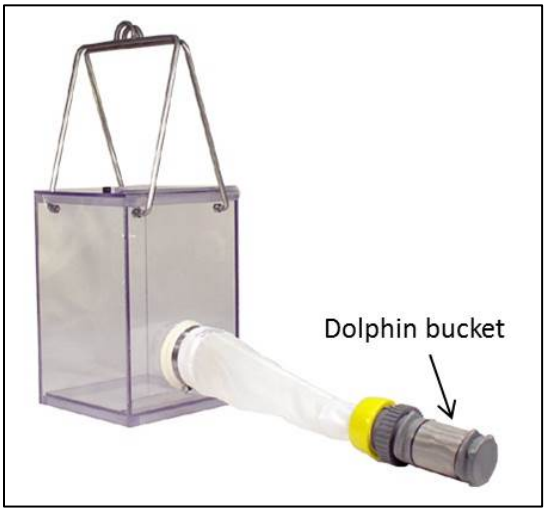

Figure 9. Example of a Schindler-Patalas sampler

B.3 Preserving Samples

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 and noted in the comments section.

1. Add ~150-200 mL of 95% ETOH solution to the 500 mL sample bottle within 5 minutes of collection (final concentration ~30-40% ETOH).
   a. The sample can be <500 mL.
   b. Consider using a larger sample bottle (1 L) if sample fills ≥1/2 of the bottle so there is enough room to add ethanol to reach a final concentration of 30-40%.
   c. Use C1V1=C2V2 to calculate preservative volume, where:
      1) C1=original concentration of ETOH
      2) V1=volume of ETOH added to sample
      3) C2=final concentration of ETOH
      4) V2=final volume of 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.

B.4 Ending the Processing Day

1. Refreshing 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 08)].
   b. Dry all equipment thoroughly before storage.
   c. Check all nets for holes and patch if necessary.
   d. Check length calibration on Secchi and zooplankton tow lines.

SOP C Data Entry and Verification

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.

Enter all data from the Lab Data Sheets into Excel workbooks “lake_zooplankton data entry”.

SOP D Sample Shipment

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

Shipments are to include a hardcopy of the "per sample" tab of the shipping inventory 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. The shipment tracking number (shipment ID) must be included in the electronic version of the shipping inventory as well as the email, but is not necessary on the hard copy.

D.1 Zooplankton Sample Shipping

1. Place sealed sample bottles into one or several gallon-sized resealable zip-top bags, grouped by site. Sample jars are acceptable "inner containers" required for shipping.
2. Line a Group II cardboard box with a heavy-duty trash bag.
3. Place all sample bottles 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.
4. Include shipping inventory/manifest in an additional zip-top bag.
5. Follow instructions for shipping ETOH in limited quantity ground shipments in AD[03]

D.2 Handling Hazardous Material

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

D.3 Supplies/Containers

See section D.1 and Table 6.

D.4 Timelines

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.

D.5 Conditions

Samples may be stored at the domain support facility at 4 ^{o}C until shipping. Ship samples at ambient temperature.

D.6 Grouping/Splitting Samples

Group samples by site per bout.

D.7 Return of Materials or Containers

Include return shipping label if any shipping materials that need to be returned to the domain support facility (e.g., cooler).

D.8 Shipping Inventory

Shipments are to have a hardcopy 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. Laboratory Contact Information and Shipping/Receipt Days

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

8 REFERENCES

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APPENDIX A DATASHEETS

The following datasheets are associated with this protocol:

Table 7. Datasheets associated with this protocol

NEON Doc. #	Title
NEON.DOC. 002302	Datasheets for AOS Protocol and Procedure: Zooplankton Sampling in Lakes
NEON.DOC.001646	General AQU Field Metadata Sheet
NEON.DOC.002191	Datasheets for Secchi Depth and Depth Profile Sampling

These datasheets can be found in Agile or the NEON Document Warehouse.

APPENDIX B QUICK REFERENCES

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

Step 2 – Prepare labels (2" * 4").

NEON

Sample ID: *SUGG.20140702.townet.1*

Sample type: (tow net) Schindler-Patalas

Location: *C0* Depth (m): *3.5*

Number of tows: *2*

Collected by: *sparker*

Step 3 – Ensure the General AQU Field Metadata Sheet (RD[06]) is completed per field site visit.

Step 4 – Determine water depth, depth of the thermocline (depth profile), and depth of the euphotic zone (Secchi disk).

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

1. If water depth is < 2 meters use a Schindler-Patalas sampler.
   a. If <2 but > 1 m take samples at 0.5 m and 1.5 m depths and integrate.
   b. < 1 m take 2 samples from 0.5 m.
2. If water is > 2 meters use a plankton tow net sampler to take an integrated sample.

Step 6 – Collect 1 integrated sample at:

1. In the deepest point in the lake, 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.

Step 8 – Ship samples to zooplankton taxonomy lab.

APPENDIX C REMINDERS

Before heading into the field: Make sure you…

- ☑ Collect and prepare all equipment including labels.
- ☑ Pre-print labels on waterproof paper.
- ☑ 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.
- ☑ Check that all equipment is in good condition and all batteries are charged.

Sample collection: Be sure to…

- ☑ Determine water depth, depth of the thermocline, and depth of the euphotic zone.
- ☑ Do not wear sunglasses when taking the Secchi depth measurement.
- ☑ 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.
- ☑ Take care not to submerse the top of the net opening below the water surface.

Sample processing: Be sure to…

- ☑ Complete sample processing 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 and noted in the comments section.

APPENDIX D ESTIMATED DATES FOR ONSET AND CESSATION OF SAMPLING

Preliminary date ranges for biological sampling bouts in lakes . Also see the Site Specific Sampling Strategy Document on AQU's NEON 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
D09	Prairie Lake	18Apr-16May	5Jul-2Aug	11Sep-9Oct
D09	Prairie Pothole	20Apr-18May	5Jul-2Aug	11Sep-9Oct
D11	South Pond at Klemme	9Feb-9Mar	27Jun-25Jul	29Oct-26Nov
D18	Toolik Lake	21May-18Jun	29Jun-27Jul	6Aug-3Sep

APPENDIX E SITE-SPECIFIC INFORMATION

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