AOS PROTOCOL AND PROCEDURE: ZOOPLANKTON SAMPLING IN LAKES

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1 DESCRIPTION

1.1 Purpose

The primary purpose of this document is to provide a change-controlled version of Observatory protocols and procedures. This document provides the content for training and field-based materials for NEON staff and contractors. Documentation of content changes (i.e. changes in particular tasks or safety practices) will occur via this change-controlled document, not through field manuals or training materials.

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

1.2 Scope

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

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

It does identify procedure-specific safety hazards and associated safety requirements.

1.3 Acknowledgements

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

AD [01]	NEON.DOC.004300	EHS Safety Policy and Program Manual
AD [02]	NEON.DOC.004316	Operations Field Safety and Security Plan
AD [03]	NEON.DOC.000724	Domain Chemical Hygiene Plan and Biosafety Manual
AD [04]	NEON.DOC.001155	NEON Training Plan
AD [05]	NEON.DOC.050005	Field Operations Job Instruction Training Plan
AD [06]	NEON.DOC.001197	Bathymetric Sampling of Lakes and Non-Wadeable Streams

2.2 Reference Documents

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

RD[01]	NEON.DOC.000008	NEON Acronym List
RD[02]	NEON.DOC.004300	EHS Safety Policy and Program Manual
RD[03]	NEON.DOC.005003	NEON Scientific Data Products Catalog
RD[04]	NEON.DOC.014051	Field Audit Plan
RD[05]	NEON.DOC.001152	NEON Aquatic Sample Strategy Document
RD[06]	NEON.DOC.000824	Data and Data Product Quality Assurance and Control Plan
RD [07]	NEON.DOC.001154	NEON Aquatic Decontamination Protocol
RD [08]	NEON.DOC.001646	NEON General AQU Field Metadata Sheet

2.3 External References

External references contain information pertinent to this document, but are not NEON configuration-controlled. Examples include manuals, brochures, technical notes, and external websites.

ER [01]	
ER [02]	
ER [03]	

2.4 Acronyms

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

2.5 Definitions

A protocol is a formal summary description of a procedure and its related rationale, and includes information on knowledge and resources needed to implement the procedure. A procedure is a set of prescribed actions that must take place to achieve a certain result, and can also be called a method. It differs from a science design in that science designs provide a more complete description of the rationale for selecting specific protocols. It differs from a training manual in that training manuals provide materials in support of skills acquisition in the topic areas including information on how to best train staff rather than detailing only the steps of the procedure.

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

Eutrophic : The ecosystem having 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 hyplolimnion 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 BACKGROUND AND OBJECTIVES

3.1 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 (Fig. 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 whilst also being the primary source of food for planktivorous fish. 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, whilst 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 (Fig. 1a-d). The planktonic protozoa in particular, have limited locomotion and are dominated by the meroplanktonic pelagial 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 (Fig. 2). Most rotifers are nonpredatory, and omnivorously feed on bacteria, small algae, and detrital particulate organic matter. The majority of cladoceran 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.

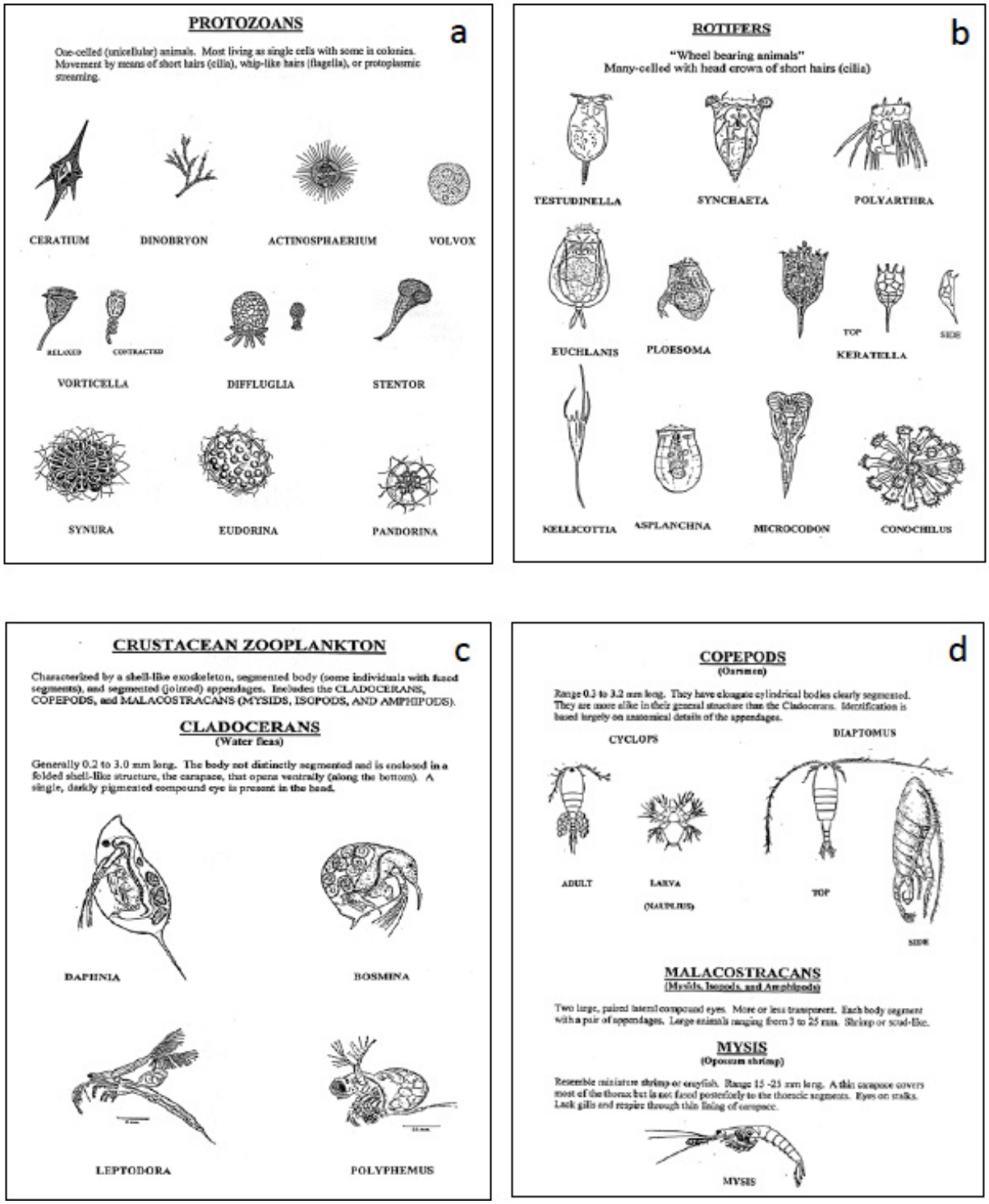


Figure 1. Classes of zooplankton (from Tharp).

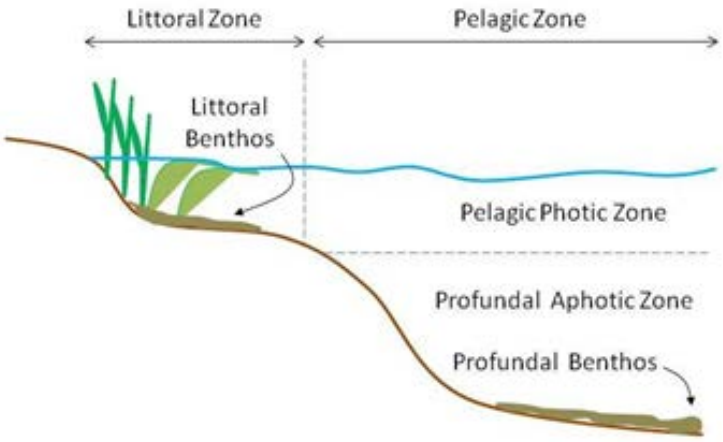


Figure 2. Zones of a lake.

Seasonality plays an important role in zooplankton species presence and abundance, with cyclopoid and calanoid copepods dominate 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).

3.2 NEON Science Requirements

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.

3.3 NEON Data Products

Execution of this protocol procures samples and/or generates raw data satisfying NEON Observatory scientific requirements. These data and samples are used to create NEON data products, which are documented in the NEON Scientific Data Products Catalog, available on the NEON website.

4 PROTOCOL

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.

5 QUALITY ASSURANCE AND CONTROL

The procedures associated with this protocol will be audited according to the Field Audit Plan (RD[04]). Additional quality assurance will be performed on data collected via these procedures according to the NEON Data and Data Product Quality Assurance and Control Plan (RD[06]).

If conditions become too unsafe to continue working before all samples have been collected, discard collected samples and return to the shore. Rinse all equipment thoroughly. Return to sample after conditions have stabilized.

When unexpected field conditions require deviations from this protocol, the following field implementation guidance must be followed to ensure quality standards are met. Should the 2 week window for sampling not be met, the data shall be flagged.

6 SAFETY

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

In addition, the following safety requirements are sought:

- Due to site-specific hazards that may be encountered, sampling may have to take place from the boat, without dismounting from the vessel. In addition, technicians are required not to put hands and feet in waters where alligators are present and to make sure a safe distance from hazards is maintained.
- All personnel must be wearing a personal flotation device (PFD) prior to entering the boat.
- Technicians should be aware of any site-specific hazards and to the waters of that particular location (i.e. current status, tidal charts, etc.)

7 PERSONNEL REQUIREMENTS

We estimate sampling for zooplankton in lakes requires 2 technicians for 4 hours each sampling day plus travel to and from the site.

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

8 TRAINING REQUIREMENTS

All technicians must complete required safety training as defined in the NEON Training Plan (AD[04]) and EHS Safety Policy and Program Manual(AD[01]). Additionally technicians complete protocol specific training for safety and implementation of protocol as required in Field Operations Job Instruction Training Plan (AD[05]).

Personnel are to be trained in lake zooplankton sampling and safe working practices for lake-based fieldwork.

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

9 FIELD STANDARD OPERATING PROCEDURE

9.1 Sampling Frequency and Timing

Lake zooplankton sampling will occur three times per year (spring, summer and autumn). Replicate samples will be taken at each location, resulting in 3 sampling locations and a total of 6 samples per collection date. Dates for each site will be detailed in the NEON Aquatic Sample Strategy Document (RD [05]).

9.1.1 Criteria for Determining Sampling Dates

Sample timing will be site-specific and based on historical data such as water temperature, light and stratification. These sample times reflect the seasonal dynamics of zooplankton species as well as the seasonal cycle of phytoplankton, nutrient and light availability. The peak biomass of zooplankton is found shortly following the peak phytoplankton, when nutrients have become more depleted and light availability is at its maximum. In northern systems this window is relatively short with only a couple of months between the rise and decline of populations between July and September. In tropical systems these periods fluctuate throughout the year with broad peaks occurring both near the end of the year (November-December) and another peak occurring in the early spring in February- March. Sample timing will be defined as a function of growing degree days and light availability.

9.1.2 Sampling Frequency

Sampling occurs three times per year at the determined sampling dates.

9.1.3 Sampling Timing Parameters

Sampling must occur within two weeks of determined sampling dates. Lake zooplankton samples shall be taken a minimum of 5 days following a major flow event (>25% change in flow within 15 minutes and/or when turbidity levels are double the monthly average). Because zooplankton typically migrate vertically during the day and night, collections should be made at roughly (+/- 1 hours) the same time daily to maintain consistency.

9.2 Equipment and Materials

Table 1. Field equipment list

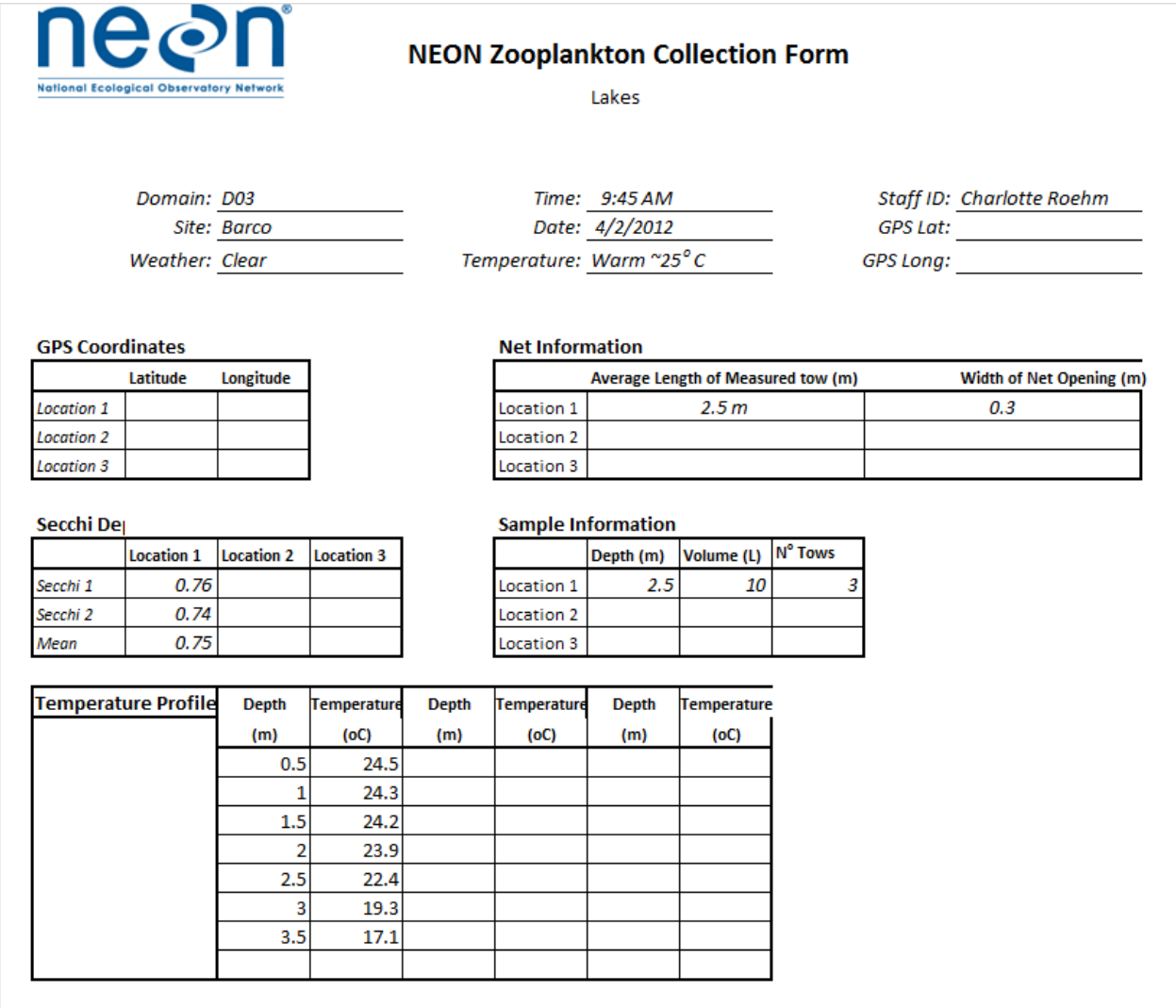
Maximo Item No.	Item Description	Quantity	Habitat-Specific	Special Handling
MX100397	Plankton tow net 63 μm pore size with collection bottle	1		
MX105580	Schindler-Patalas sampler 64 µm pore size	1		
	Nylon safety line for net (10-50 m)	1		
	500 mL Nalgene sample bottles	6		
MX100450	Secchi disk and attached rope	1		
MX100512	Multisonde for temperature and depth	1		
	500 mL Wash bottle	1		
	70% Ethanol	1		Y
	De-ionized water (mL)	500		
	Metric ruler	1		
	Calculator	1		
	Tape (roll)	1		
	50-100 mL graduated cylinder	1		
	Plastic sampling tray	1		
	Forceps	1		
	Laboratory nitrile gloves	3		
	Pre-printed adhesive labels	10		
	Field data sheets (printed on waterproof paper)	3		
	Resealable bags (gallon)	3		
	Pencils	4		
	Permanent markers	4		
	Cooler with ice packs	1		
	Field notebook	1		
	Site Map	1		
	Boat, Oars and Electric Motor	1		
	Anchor and line	1		
MX100453	GPS and Sounder Unit (Humminbird)	1		
	Sounder Battery	1		Y
	Personal flotation devices (PFDs), 1 per person	2		
	Safety kit for boat	1		
	First aid kit	1		

9.3 Preparation

1) Know which site(s) are to be visited, and where the sample points are located. Ensure GPS locations are loaded into the GPS unit.
2) Pre-print adhesive labels for sample bottles and data sheets
3) Check that all necessary equipment is present, clean, and in good working condition (see NEON Aquatic Decontamination Protocol (RD[12]).

9.4 Sample Collection in the Field

1) Locations for sampling:
   a) In the deepest point in the lake, determined by site map and pre-loaded GPS coordinates, 2 integrated samples.
   b) Near the major inlet in the littoral zone, 2 integrated samples.
   c) Near the outlet in the littoral zone, 2 integrated samples.
2) Ensure the General AQU Field Metadata Sheet (RD [08]) is completed.
3) Navigate the boat to the central sampling location as defined by bathymetric maps of the lake [AD (06)]
4) Fill in the metadata section of the field sheet (Appendix A) and note the GPS position of the sampling location.
5) Gently lower the anchor to the bottom of the lake so as not to suspend sediments.
6) 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.
7) Determine the total water depth from the sonar readings.
8) Determine the depth of the thermocline (if present) at the sampling location using the multisonde. Record the depth and water temperature every 0.5 m on the field data sheet (Figure 3, Appendix A).



NEON Zooplankton Collection Form

Lakes

Domain: D03 | Time: 9:45 AM | Staff ID: Charlotte Roehm
Site: Barco | Date: 4/2/2012 | GPS Lat:
Weather: Clear | Temperature: Warm ~25° C | GPS Long:

GPS Coordinates

	Latitude	Longitude
Location 1		
Location 2		
Location 3		

Net Information

	Average Length of Measured tow (m)	Width of Net Opening (m)
Location 1	2.5 m	0.3
Location 2		
Location 3		

Secchi De

	Location 1	Location 2	Location 3
Secchi 1	0.76		
Secchi 2	0.74		
Mean	0.75		

Sample Information

	Depth (m)	Volume (L)	N° Tows
Location 1	2.5	10	3
Location 2			
Location 3			

Temperature Profile	Depth (m)	Temperature (oC)	Depth (m)	Temperature (oC)	Depth (m)	Temperature (oC)
	0.5	24.5				
	1	24.3				
	1.5	24.2				
	2	23.9				
	2.5	22.4				
	3	19.3				
	3.5	17.1				

Figure 3. Example field sheet for zooplankton collection. (Blank sheet in Appendix A).

9) Determine the depth of the euphotic zone (depth to which 1% light penetrates) using the Secchi disk (Fig. 4).
   a) Take the Secchi reading from the shady side of the boat. If there is no shade use a hand or any object that can produce enough shade to cut down the sun glare. DO NOT WEAR SUNGLASSES WHEN UNDERTAKING THIS MEASUREMENT.
   b) Lower the Secchi disk slowly until the white quadrants disappear, and determine the depth to the nearest tenth of a meter.
   c) Lower the Secchi disk so it is about 0.5 m below the first reading. Slowly pull the disk up until it reappears, and determine this depth to the nearest tenth of a meter.
   d) Average the two depths to get the Secchi depth. Record all readings on the data sheet.
   e) Repeat at each sampling location.

Figure 4. Taking a Secchi Disk measurement on the shaded side of the boat.

10) Collect Samples with zooplankton nets:
    a) Determine which sampler is to be used.
        i) 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) < 1 m take samples from 0.5 m.
        ii) If water is > 2 meters use a plankton tow net sampler to take an integrated sample.
    b) Note the width of the tow net opening (Figure 5) or Schindler-Patalas (Figure 6) volume on the field sheet.
11) If using a tow net (Figure 5).
    a) Attach the net to a nylon rope marked at 0.5 m intervals.
    b) Lower the net to where the tubing is 0.5 m above the bottom of the lake. If conditions are windy or conditions necessitate, add a small weight around 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 (leaves, sediments etc.). If excessive debris is noted, discard sample and start again.
    e) 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*.

Figure 5. Zooplankton Tow Net

f) Repeat several times until the net is fully rinsed.
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 collection bucket in a vertical position, carefully open the spigot on the hose and pour into the 500 mL sample bottle.
i) If debris is present either discard the whole sample (i.e. lots of particulate debris) or pour the sample into the plastic tray and remove the large debris with forceps.
j) Rinse the concentrated zooplankton in the cup and the net using a squirt bottle filled with lake water.
k) Drain the remaining filtrate into the sample bottle.
l) The total sample in the bottle should be 1/3 full with zooplankton and water.
m) Note the tow length (distance in meters of water column through which the collecting unit was retrieved) on the sample bottle and the data sheet (Figures 6 and 7).
n) Rinse the net, bottle and retrieval rope thoroughly (2 to 3 times) with water from the site. Then spray with de-ionized water. Dry if possible.
o) Repeat steps e – p until the sample bottle is ¾ full (allow enough space for 40 mL of preservative solution).
p) In shallow lakes, multiple tows with each net are required to achieve the cumulative tow length representative of the water column.
q) Calculate the volume of water that was filtered through the tow net and note this on the sample label and in the field book.

r) Place sample bottle in cooler.
s) Repeat steps 1) through 10).
t) Proceed to the next sample location (inlet or outlet) 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.

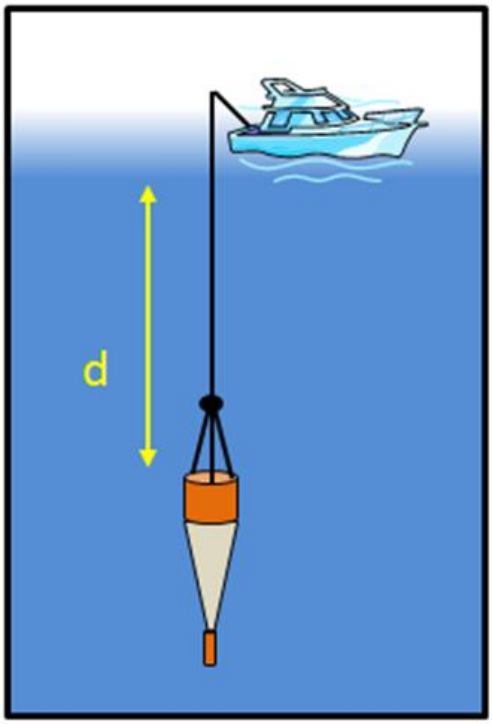


Figure 6. Tow Volume Calculation

Volume of Water through a Zooplankton Tow $=\pi r^2 \times d$ Equ. 1

Where: r = radius of net mouth (cm)

d = depth or length of tow (cm) (distance from net mouth to lake surface)

π = 3.1416

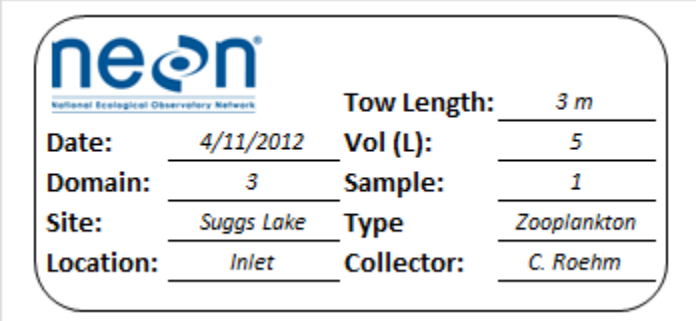


Figure 7. Example of field labels for zooplankton sample bottles.

12) If a Schindler-Patalas sampler is used (Fig. 8):
   a) Ensure the cup 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.
   e) Collect the sample as per steps 10) h-m then o-r.

Figure 8. Example of a Schindler-Patalas sampler

9.5 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 and noted in the comments section.

1) Put on Nitrile gloves
2) Add 40 mL of 70% ethanol solution to the sample within 5 minutes of collection.
3) Carefully re-cap bottle, ensuring that no sample escapes and gently invert bottle to mix.
4) Tape the bottle lids prior to shipping.
5) Store in a cooler.

9.6 Sample Shipping

Ship all bottles to zooplankton taxonomist. Samples shall be shipped in a cooler with ice packs and maintained at 4^{o}C from collection in the field to receipt by the taxonomist.

9.7 Data Handling

1) Scan in field data sheets and email to Lake Ecologist at NEON Headquarters.
2) Enter all field data into the database.
3) All data and notes shall be transcribed into the database within 72 hours of sampling.

9.8 Refreshing the Sampling Kit

1) Replace sample bottles
2) Print new field labels and field data sheets

9.9 Equipment Maintenance, Cleaning, and Storage

1) Wash all equipment that has been submerged in water with a 2% household bleach solution for 1-5 minutes (e.g., boots, nets, etc.) (see NEON Aquatic Decontamination Protocol (RD[12]).
2) Rinse thoroughly and dry all equipment thoroughly before storage.

10 REFERENCES

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APPENDIX A FIELD DATA SHEETS

The following field data sheets serve as a backup procedure for times when electronic data collection devices (PDA) are not available.

NEON Zooplankton Collection Form

Lakes

Domain: _____ *Time:* _____ *Staff ID:* _____
Site: _____ *Date:* _____ *GPS Lat:* _____
Weather: _____ *Temperature:* _____ *GPS Long:* _____

GPS Coordinates

	Latitude	Longitude
Location 1		
Location 2		
Location 3		

Net Information

	Average Length of Measured tow (m)	Width of Net Opening (m)
Location 1		
Location 2		
Location 3		

Secchi Dep

	Location 1	Location 2	Location 3
Secchi 1			
Secchi 2			
Mean			

Sample Information

	Depth (m)	Volume (L)	N° Tows
Location 1			
Location 2			
Location 3			

Temperature Profile	Depth (m)	Temperature (oC)	Depth (m)	Temperature (oC)	Depth (m)	Temperature (oC)

APPENDIX B SAMPLE BOTTLE LABELS