

AOS PROTOCOL AND PROCEDURE: DEP – SECCHI DISK AND DEPTH PROFILE SAMPLING IN LAKES AND NON-WADEABLE STREAMS

PREPARED BY	ORGANIZATION	DATE
Stephanie Parker	SCI	09/17/2021

APPROVALS	ORGANIZATION	APPROVAL DATE
Kate Thibault	SCI	12/15/2021

RELEASED BY	ORGANIZATION	RELEASE DATE
Tanisha Waters	СМ	12/15/2021

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	
А	05/28/2015	ECO-02892	Initial release	
В	02/08/2017	ECO-04359	Update NEON templateRevisions to under ice sampling	
с	01/19/2018	ECO-05293	 Add snow depth measurement Clarify auto-stabilize for handheld YSI Move datasheets to appendix 	
D	12/19/2018	ECO-05968	Update ice-cover and stratification and high flow in rivers	
E	12/15/2021	ECO-06698	 Updated to new template Clarified scheduling and dependencies on other protocols Add alternate location 	



TABLE OF CONTENTS

1	OV	ERVIEW 1				
	1.1	Background1				
	1.2	Scope 1				
	1.2.	1 NEON Science Requirements and Data Products				
	1.3	Acknowledgments 2				
2	REL	ATED DOCUMENTS AND ACRONYMS				
	2.1	Applicable Documents				
	2.2	Reference Documents 3				
	2.3	Acronyms 3				
	2.4	Definitions				
3	ME	THOD5				
4	SAN	MPLING SCHEDULE				
	4.1	Sampling Frequency and Timing7				
	4.2	Criteria for Determining Onset and Cessation of Sampling7				
	4.3	Timing for Laboratory Processing and Analysis7				
	4.4	Sampling Timing Contingencies				
	4.5	Missed or Incomplete Sampling8				
	4.6	Estimated Time10				
5	SAF	ETY11				
6	PER	SONNEL12				
	6.1	Training Requirements12				
	6.2	Specialized Skills12				
7	STA	NDARD OPERATING PROCEDURES				
SC	ра	PREPARING FOR SAMPLING14				
SC	ЭРВ	FIELD SAMPLING15				
SC	OPC	POST-FIELD SAMPLING TASKS20				
SC	SOP D DATA ENTRY AND VERIFICATION					
8	8 REFERENCES					
A	APPENDIX A QUICK REFERENCES					
AI	APPENDIX B EQUIPMENT					



LIST OF TABLES AND FIGURES

Table 1. Sampling frequency for Secchi Disk and Depth Profile procedures on a per SOP per plot type
basis
Table 2. Contingent decisions. 8
Table 3. Protocol-specific Sampling Impractical reasons entered in the Fulcrum application. In the event
that more than one is applicable, choose the dominant reason sampling was missed
Table 4. Estimated staff and labor hours required for implementation of the Secchi and Depth Profile
protocol
Table 5. Equipment list – Secchi Depth and Vertical Profile Sampling
Figure 1. Diagram representing the zones in a thermally stratified and non-stratified lake. Deep, clear
lakes may have a double thermocline 1
Figure 2. A generic site layout for lakes and rivers with Secchi depth and vertical profile sampling
locations (red box). 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
Figure 3 . The documentation to account for a Missed Sampling event depends on the situation for each sampling unit not sampled per bout that is not sampled. Diamonds represent decision points and boxes
describe the required action. Required actions may include: a) Submitting a ServiceNow incident, b)
creating a Sampling Impractical record, c) creating a data Flag, d) creating a Site Management record, or
e) some combination of (a) – (d)
Figure 4. A high level workflow diagram that visually shows how the separate SOPs are sequentially
connected13
Figure 5. Workflow for Secchi Depth16
Figure 6. Example of a Secchi disc underwater. 17
Figure 7. Workflow for Depth Profile



1 OVERVIEW

1.1 Background

Operated by Battelle

Aquatic communities and water quality are highly dependent on water clarity and temperature. Secchi depth is often used as a quick measurement of productivity as it measures the depth to which light penetrates and can indicate whether a lake is oligotrophic (low nutrient) or eutrophic (high nutrient), but will also vary with turbidity and watershed CDOM inputs. In lakes, light penetration in the water column is typically limited by phytoplankton growth, although turbidity and CDOM also limit light penetration in some lakes.

Depth or vertical temperature profiles indicate whether the lake or river is thermally stratified, where the body of water is separated into two or more layers based on temperature. Typically, in a thermally stratified system, the top layer of water is warmer (epilimnion) while the lower layer is colder (hypolimnion). The area separating the two layers is known as the thermocline. The thermocline occurs when the rate of decreasing temperature with increasing depth is greatest, where there is a change of >1°C per 1.0 m change in depth (USEPA 2012). Thermal stratification can dramatically change the water chemistry and biology of each layer, and can capture organisms that are weak swimmers between the boundaries. If the lake or river is thermally stratified, samples may be taken at multiple depths to capture the conditions or community of each layer in the associated protocols (RD[08-11]).



Figure 1. Diagram representing the zones in a thermally stratified and non-stratified lake. Deep, clear lakes may have a double thermocline.

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 document is based on the protocols of the US Environmental Protection Agency (USEPA) National Lakes Assessment program (USEPA 2012) and the USEPA Sampling Procedures of the Great Lakes (USEPA 2010).



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.001155	NEON Training Plan	
AD[05]	NEON.DOC.050005	Field Operations Job Instruction Training Plan	
AD[06]	NEON.DOC.004104	NEON Science Data Quality Plan	

2.2 Reference Documents

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

RD[01]	NEON.DOC.000008	NEON Acronym List	
RD[02]	NEON.DOC.000243	NEON Glossary of Terms	
RD[03]	NEON.DOC.002652	NEON Level 1, Level 2, Level3 Data Products Catalog	
RD[04]	NEON.DOC.001271	NEON Protocol and Procedure: Manual Data Transcription	
RD[05]	NEON.DOC.001646	General AQU Field Metadata Sheet	
RD[06]	NEON.DOC.002191	Datasheets for Secchi Depth and Depth Profile Sampling	
RD[07]	NEON.DOC.004257	NEON Standard Operating Procedure (SOP): Decontamination of	
		sensors, field equipment and field vehicles	
RD[08]	NEON.DOC.003045	AOS Protocol and Procedure: Periphyton and Phytoplankton	
		Sampling	
RD[09]	NEON.DOC.001194	AOS Protocol and Procedure: Zooplankton Sampling in Lakes	
RD[10]	NEON.DOC.003044	AOS Protocol and Procedure: Aquatic Microbial Sampling	
RD[11]	NEON.DOC.0025905	AOS Protocol and Procedure: Water Chemistry Sampling in Surface	
		and Groundwater	

2.3 Acronyms

Acronym	Definition		
DO	Dissolved oxygen		
NLA	National Lakes Assessment		
PFD	Personal flotation device		
USEPA	U.S. Environmental Protection Agency		

2.4 Definitions

Benthic: The region in or near the sediments or bed of a body of water (e.g., bottom of the lake).



Epilimnion: Top layer of water of a thermally stratified lake, denoted by highest temperatures and least dense water in the summer (**Figure 1**).

Euphotic zone (or "Photic zone"): The upper layer of lake water where sunlight penetrates, and photosynthesis can occur.

Eutrophic: Having high primary production. In lakes, this is often a response to nutrient enrichment leading to increased production of algae or algal blooms.

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

Hypolimnion: The dense bottom layer of a thermally stratified lake that sits below the thermocline (**Figure 1**). This layer is characterized by cooler summer temperatures and slightly warmer winter temperatures relative to the epilimnion.

Metalimnion: The layer of water in a thermally stratified lake that sits between the hypolimnion and the epilimnion. The metalimnion is often equated with the thermocline (**Figure 1**).

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.

Secchi disk: 20 cm diameter disk with black and white markings used to determine water clarity.

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

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

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 (USEPA 2012). The denser and cooler layer below the thermocline is defined by the hypolimnion. The warmer upper layer is termed the epilimnion.

Thalweg: The line of least resistance to water flow in a stream or river, often the line of maximum water velocity.



3 METHOD

Secchi depth and depth (or vertical) profiles are data commonly collected during pelagic sampling (Poikane 2009, USEPA 2010, USEPA 2012). These data must be collected when collecting samples for any NEON SOP that samples the water column:

- phytoplankton (RD[08]) secchi and stratification data required to determine sample depth
- zooplankton (RD[09])
- pelagic microbes (RD[10]) stratification data required to determine sample depth
- pelagic surface water chemistry (RD[11]) stratification data required to determine sample depth

Secchi depth and vertical profile data are only collected at the deepest location of the lake at the buoy or near the river (non-wadeable stream) buoy or sensor set (**Figure 2**). These data not only provide metadata to accompany the sampling modules, but also inform sampling depths for certain protocols based on euphotic depth (phytoplankton) and thermal stratification (water chemistry, microbes, and phytoplankton).

Secchi depth measurements are only collected during ice-free periods. Depth/vertical profile measurements, including temperature, DO, and specific conductance, are collected year-round along with the associated protocols (RD[08-11]) regardless of ice cover. Data collected for this protocol applies to multiple protocols performed in a day. Collect data for this protocol once before starting sampling for other protocols scheduled in a day, you do not need to repeat Secchi and depth profile data collection for each protocol in a single day.

neon	Title: AOS Protocol and Procedure: I Non-Wadeable Streams	DEP – Secchi Disk and Depth Profile Sampling in Lakes and	Date: 12/15/2021
Operated by Battelle	<i>NEON Doc. #</i> : NEON.DOC.002792	Author: S. Parker	Revision: E



Figure 2. A generic site layout for lakes and rivers with Secchi depth and vertical profile sampling locations (red box). 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 is performed on data collected via these procedures according to the NEON Science Data Quality Plan (AD[06]).



4 SAMPLING SCHEDULE

4.1 Sampling Frequency and Timing

Data collection occurs with associated protocols (phytoplankton, zooplankton, pelagic microbes, and pelagic surface water chemistry, RD[08-11]).

- AOS Protocol and Procedure: Periphyton and Phytoplankton Sampling (RD[08])
- AOS Protocol and Procedure: Zooplankton Sampling in Lakes (RD[09])
- AOS Protocol and Procedure: Aquatic Microbial Sampling (RD[10])
- AOS Protocol and Procedure: Water Chemistry Sampling in Surface and Groundwater (RD[11])

Table 1. Sampling frequency for Secchi Disk and Depth Profile procedures on a per	SOP per plot type basis.
---	--------------------------

SOP	AOS Site Type	Location	Bout Duration	Bouts Per Year	Remarks
SOP B.2 - Secchi	Lake, River	Buoy	1 day	Variable: any day when RD[08-11] are scheduled	Data collection occurs with SOPs that sample the water column during ice-off periods
SOP B.3 – Depth Profile	Lake, River	Buoy	1 day	Variable: any day when RD[08] or RD[10-11] are scheduled	Data collection occurs with SOPs that sample the water column

Scheduling Considerations

- 1. Scheduling is dependent on any scheduling considerations detailed in the water column protocols listed above.
- 2. During ice-free periods, perform SOP B.2 and SOP B.3 first, before the water column protocols listed above.
- 3. During ice-on period, perform SOP B.3 first, before the water column protocols listed above. SOP B.2 is not done under ice cover.

4.2 Criteria for Determining Onset and Cessation of Sampling

Secchi depth will only be measured during ice-free periods due to logistical challenges. Vertical profile data will be collected year-round. Follow the associated protocols (RD[08-11]) scheduling considerations.

4.3 Timing for Laboratory Processing and Analysis

There is no laboratory processing associated with this protocol.



4.4 Sampling Timing Contingencies

All data collection for this protocol occurs with the associated protocols listed in RD[08-11]. Data from this protocol are necessary for determination of sample depth in RD[08], RD[10], and RD[11]. For RD[09], it is required to collect Secchi depth and profile data at the same time as sample collection, but these data do not affect zooplankton collection methods. Follow contingent decisions for each associated protocol.

 Table 2. Contingent decisions.

Delay/Situation	Action	Outcome for Data Products
Hours	If circumstances occur that impede	None as long as data are collected
	sampling (e.g., wildlife, weather), start	with the water column protocols
	over the next day that conditions permit.	(RD[08-11]).

4.5 Missed or Incomplete Sampling

Sampling according to the schedule is not always possible, and multiple factors may impede work in the field at one or more plots or sampling locations in a given bout. For example:

- Logistics e.g., insufficient staff or equipment
- Environment e.g., deep snow, flooding, inclement weather, or
- Management activities e.g., controlled burns, pesticide application

Instances such as those listed above must be documented for scheduling, tracking long-term plot suitability, and informing end users of NEON data availability. Some types of missed sampling are due to events that should be recorded in the Site Management App; refer to the Site Management and Event Reporting Protocol for more detail (RD[06]).

Missed or Incomplete Sampling Terms

Terms that inform Missed or Incomplete Sampling include:

- **Protocol Sampling Dates**: Bout-specific sampling dates. For this protocol, follow bout-specific sampling dates from the associated protocols (RD[08-11]).
- Scheduled Sampling Dates: Bout-specific sampling dates scheduled by Field Science and approved by Science. These dates coincide with or are a subset of the Protocol Sampling Dates. Follow protocol sampling dates from the associated protocols (RD[08-11]).
- **Missed Sampling**: Incidence of *scheduled sampling* that did not occur. Missed Sampling is recorded at the same resolution as data that are ordinarily recorded.
- **Sampling Impractical**: The field name associated with a controlled list of values that is included in the data product to explain a Missed Sampling event i.e., why sampling did not occur.



• **Rescheduled**: Missed Sampling is rescheduled for another time according to one of the scenarios documented in **Figure 3**, resulting in no change to the total number of sampling events per year.

The documentation that must accompany missed sampling depends on the timing, subsequent action, and the audience appropriate for numerous scenarios (**Figure 3**).



Figure 3. The documentation to account for a Missed Sampling event depends on the situation for each sampling unit not sampled per bout that is not sampled. Diamonds represent decision points and boxes describe the required action. Required actions may include: a) Submitting a ServiceNow incident, b) creating a Sampling Impractical record, c) creating a data Flag, d) creating a Site Management record, or e) some combination of (a) – (d).

To Report Missed or Incomplete Sampling:

- 1. Missed or Incomplete Sampling for this protocol are dependent on missed or incomplete sampling in RD[08-11]. Follow missed or incomplete sampling rules in those protocols, there is no separate Service Now reporting required for this protocol.
- 2. For each Missed Sampling record, the **Sampling Impractical** field must be populated in the mobile collection device if Sampling Impractical was entered in all of the SWC/AMC, ALG, and ZOO also scheduled on the same day (**Figure 3**).



Table 3. Protocol-specific Sampling Impractical reasons entered in the Fulcrum application. In the event that more than one is applicable, choose the dominant reason sampling was missed.

Sampling Impractical reason	Description
Location dry	Sampling location is dry
Location frozen	Water at sampling location is frozen such that sampling cannot occur through the ice
Location snow covered	Location is snow covered such that sampling cannot occur through the snow
High water velocity	Water velocity to fast for sampling
Logistical	Site or plot access compromised, staffing issues, errors (e.g., equipment not available in the field)
Other	Sampling location inaccessible due to other ecological reason described in the remarks

4.6 Estimated Time

The time required to implement a protocol will vary depending on a number of factors, such as skill level, 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.

Data collection requires two field ecologists for 30 minutes per site, plus additional time for the associated protocols. There is no lab processing for this protocol.

Table 4. Estimated staff and labor hours required for implementation of the Secchi and Depth Profile protocol.

SOP	Estimated time	Suggested staff	Total person hours
SOP B.2: Secchi and Euphotic Zone Depth	10 mins	1-2	10 mins
SOP B.3: Vertical Profile and Thermal Stratification	20 mins	1-2	20 mins



5 SAFETY

Operated by Battelle

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.

See Section 9 in the NEON Operations Field Safety and Security Plan (AD[02]) for aquatic-specific field safety requirements. In addition, the following safety requirements are sought:

- 1. Due to site-specific hazards that may be encountered, sampling may be conducted 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 prior to entering the boat.
- 3. All personnel shall have access to a form of communication with other team members such as a two-way radio.
- 4. Be aware of any site-specific hazards and to the waters of that particular location (i.e. current status, tidal charts, etc.).



6 PERSONNEL

6.1 Training Requirements

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

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.

6.2 Specialized Skills

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



7 STANDARD OPERATING PROCEDURES

SOP Overview



Figure 4. A high level workflow diagram that visually shows how the separate SOPs are sequentially connected.

- SOP A: Preparing for Sampling
- SOP B: Field Sampling
- SOP C: Post-Field Sampling Tasks
- SOP D: Data Entry and Verification



SOP A Preparing for Sampling

A.1 Preparing for Data Capture

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

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

A.2 Preparing for Field Sampling

- This SOP shall be implemented prior to any other sampling with associated protocols (RD[08-11]).
- 2. Calibrate the handheld meter to the manufacturer's specifications. Dissolved oxygen (DO) must be calibrated on site each day that the meter is used to account for local barometric pressure.
 - a. Enable Auto Stable for specific conductance or DO on the handheld meter if it helps with data collection (see YSI Pro2030 user manual and NEON Training Center presentation 'Using and Maintaining the Handheld YSI' for maintenance information).
 - b. During winter, calibration may be done in the truck to prevent ice crystals from forming in the sensor chamber.
 - c. Note that data collection includes specific conductance (reference temperature 25 °C), dissolved oxygen saturation, and dissolved oxygen percent.
- 3. Collect Secchi depth and vertical profile data at the buoy location.
 - a. If you are not able to collect data within 10 m of the buoy, select an alternate sampling location and record in the mobile app.
- 4. Navigate the boat to the sampling location and turn motor off.
- 5. Gently lower anchors at the bow and stern so as not to suspend sediments.
 - a. Anchor ropes must be 2x the water column depth to allow the boat to drift into position and not affect sampling procedures.
 - b. Allow ~5 minutes for sediments to settle after lowering the anchor; you can use this time to prepare the sampling equipment.
 - c. The boat must be anchored at the bow and stern to prevent the boat from rotating in order to collect representative water column samples.
 - d. Always sample near the bow of the boat to minimize any prior effects of the motor on the water column.



SOP B Field Sampling

B.1 Spatially and Temporally Linked Protocols

Periphyton and Phytoplankton Sampling (RD[08])

- Periphyton and phytoplankton sampling takes place three times per year in lakes and rivers.
- Secchi disk and depth profile sampling occurs prior to, but on the same day as phytoplankton sampling.
- Euphotic depth and stratification data needed to determine phytoplankton sampling depth

Zooplankton Sampling in Lakes (RD[09])

- Zooplankton sampling takes place three times per year in lakes.
- Secchi disk and depth profile sampling occurs on the same day as zooplankton sampling.

Aquatic Microbial Sampling (RD[10])

- Surface water aquatic microbial sampling occurs six times per year in lakes and rivers.
- Secchi disk and depth profile sampling occurs prior to, but on the same day as microbial sampling during ice-free periods.
- Depth profile sampling occurs prior to, but on the same day as microbial sampling during ice-on periods.
- Stratification data needed to determine sampling depth.

Water Chemistry Sampling in Surface and Groundwater (RD[11])

- Surface water chemistry sampling occurs 12 times per year in lakes and rivers.
- Secchi disk and depth profile sampling occurs prior to, but on the same day as microbial sampling during ice-free periods.
- Depth profile sampling occurs prior to, but on the same day as with microbial sampling during ice-on periods.
- Stratification data needed to determine sampling depth.



B.2 Secchi and Euphotic Zone Depth



Figure 5. Workflow for Secchi Depth

- 1. Determine and record the total water depth from the depth finder or sonar readings (Figure 5).
- 2. Lower the Secchi disk slowly into the water on the shady side of the boat until the white quadrants disappear from view (**Figure 6**).
 - a. NOTE: Do not wear sunglasses as this will interfere with the readings. Hats are ok.







Figure 6. Example of a Secchi disc underwater.

- At river sites, high flow may interfere with Secchi readings. You may attach a weight to the probe if necessary. If you are unable to keep the line perpendicular to the water surface, you may select Sampling impractical = "high water velocity" in the mobile app and stop sampling.
- 3. Record depth read from the lines on the Secchi rope to the nearest 0.1 m in the mobile app as "Secchi Depth 1".
 - a. You may also attach a meter tape directly to the Secchi disk
- 4. Lower the Secchi disk approximately 0.5 m deeper than the first reading.
- 5. Slowly pull the disk up until the white quadrants reappear, record depth to nearest 0.1 m "Secchi Depth 2".
- 6. Mean Secchi depth: The mobile app will calculate the mean of the two depths.
- 7. Euphotic depth: The mobile app will multiply the mean Secchi depth by 2.5 to determine depth of the euphotic zone (Poikane 2009)
 - a. Use this measurement for phytoplankton sampling (RD[08]).



B.3 Vertical Profile and Thermal Stratification



Figure 7. Workflow for Depth Profile

- 1. Put probe of the handheld meter into the water. If lake is iced over, go to #2. If lake/river is ice-free, skip to #3 (Figure 7).
- 2. Lake/river with ice-cover: Drill a hole through the ice with the auger and measure the ice thickness. Record ice thickness in the mobile app.
 - a. If there is snow on top of the ice, measure the snow thickness and record.
 - b. Measure the maximum depth from the bottom of the ice layer.
 - c. When lakes are iced over, stratification is not expected. Sites with deep lakes can complete the depth profile by collecting data at 5 depths spanning the height of the water column, from top to bottom under the ice.
 - 1) When collecting data under the ice, you may collect data at >0.5 m intervals as long as a minimum of 5 data points are collected.

SOP B



- d. When collecting data under the ice, start measurements from the bottom of the ice layer (top of the water).
- e. When air temperatures are below freezing, extra care must be taken to prevent YSI membranes from freezing, such as storing the probe in a warm water bottle.
- 3. Starting at the top of the water column, slowly lower the YSI through the water column until the probe reaches the bottom, stopping at 0.5 m intervals to record depth, water temperature, DO, and specific conductance in the mobile app.
 - a. In deep, thermally stratified lakes, once the temperature measurements in the hypolimnion have stabilized (isothermal conditions, or 3 temperature readings in a row that are within 0.1 °C of each other), measurements may be taken every 1 m in the hypolimnion until the lake bottom is reached.
 - b. At river sites, high flow may interfere with depth profile readings. You may attach a weight to the probe if necessary. If you are unable to keep the cable perpendicular to the water surface, you may select Sampling impractical = "high water velocity' in the mobile app and stop sampling.
- 4. Determine whether or not the lake or river is thermally stratified by the presence or absence of a thermocline. The thermocline occurs where the rate of decrease in temperature with increasing depth is greatest (>1°C per 1.0 m depth change). This will be calculated by the mobile app.
 - a. NOTE: There may be two thermoclines present in deep lakes.
 - b. Use thermocline depth and stratification data to determine sampling depths for surface water chemistry and microbes (RD[10-11) and phytoplankton RD[08].



SOP C Post-Field Sampling Tasks

- 1. Decontaminate all equipment that has come in contact with lake/river water according to the NEON Aquatic Decontamination Protocol (RD[07]). Dry all equipment thoroughly before storage.
- 2. Perform data QA/QC to check the following:
 - a. Check that stratification or no stratification has been recorded properly
 - b. Site ID, Collect Date, Sampling Protocol version
 - c. Maximum and minimum depths
 - d. Ranges of YSI readings are they what you expect at this site and time of year?

C.1 Document Incomplete Sampling Within a Site

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

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

Secchi and depth profile data collection in lakes and rivers would only be relocated if the buoy relocates, as sampling always occurs near the sensor infrastructure.



SOP D Data Entry and Verification

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

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



8 REFERENCES

- Poikane, S. 2009. Water Framework Directive intercalibration technical report. Part 2: Lakes. EUR 28838 EN/2, Office for Official Publications of the European Communities, Luxembourg.
- U.S. Environmental Protection Agency (USEPA). 2012. National Lakes Assessment Field Operations Manual. EPA 841-B-11-003. U.S. Environmental Protection Agency, Washington, DC.
- U.S. Environmental Protection Agency (USEPA). 2010. Sampling and analytical procedures for GLNPO's Open Lake Water Quality Survey of the Great Lakes. http://www.epa.gov/greatlakes/monitoring/sop/ (17 Mar 2015)



APPENDIX A QUICK REFERENCES

A.1 Steps for Secchi Depth Data Collection

Step 1 – Anchor boat at bow and stern at sampling location.

Step 2 – Lower Secchi disk on the shady side of the boat to the point where it disappears, record. Ensure that Secchi line is perpendicular to the water surface. No sunglasses!

Step 3 – Raise Secchi disk to the point where it reappears, record.

Step 4 – Mobile app calculates mean Secchi depth and multiplies by 2.5 to get euphotic depth.

A.2 Steps for Vertical Profile Data Collection

Step 1 – Lower handheld probe into water. Ensure that Secchi line is perpendicular to the water surface.

Step 2 – Record depth, temperature, DO, and specific conductance every 0.5 m to the bottom of the lake or river.

Step 3 – Determine whether the lake is thermally stratified or not (if temperature change is >1 °C per 1.0 m).



APPENDIX B 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 5. Equipment list – Secchi Depth and Vertical Profile Sampling.

Supplier/Item No.	Exact Brand	Description	Purpose	Quan- tity				
CDW-G/4452963	Y	Mobile data entry tablet	Field data entry	1				
Grainger, W.W./9CJR8	N	Secchi disk	Determiningthe depth of the euphotic zone	1				
Grainger, W.W./8X278	Ν	Weighted braided polyester line, calibrated	Determiningthe depth of the euphotic zone	1				
Fisher Scientific Company, LLC/15177622 Thomas Scientific Company, Inc./1185K52	Y	Handheld meter (Temperature, Specific Conductance)	Measuring vertical profile	1				
Fondriest Environmental, Inc./605202 Fisher Scientific Company, LLC/15177458	Y	DO Galvanic Probe and Replacement Tips	Measuring vertical profile	1				
Forestry Suppliers, Inc./39481 Cabela's Inc./IK-270217 Recreational Equipment Inc./895022	N	Handheld GPS unit (with batteries, ± 1m accuracy) or Humminbird	Navigation to sampling location	1				
Amazon Capital Services, Inc./B00X0WT8MQ	Y	Depth finder, hand-held or Humminbird	Measuring total depth of sampling location	1				
Consumable Items								
RD[05]	Y	Aquatic Field Metadata Sheet	Recording metadata	1				
RD[06]	Y	Secchi Disk and Depth Profile Datasheet	Recording data	1				