United States Environmental Protection Agency
Office of Water
Washington, DC
EPA 841-B-21-011

National Lakes Assessment 2022

Field Operations Manual

Version 1.2, May 2022

Version History

Version	Date	Revisions and Comments
0.0	December 2021	Internal EPA version for project QAC review and comments
0.0	February 2022	• Updated introduction to be consistent with other NLA 2022 manuals • Corrected typos, formatting, numbering of tables and figures • Updated field data and tracking form names to be consistent with the NLA App • Updated sample bottle types • Added ESA conservation measures (Section 6.4, Section 10 and Appendix C)
1.0	February 2022	Final approved document • Corrected NLA App entry inconsistencies • Clarified whole fish sample storage and shipping (Section 7.3) • Revised ESA conservation measures (Section 6.4, Section 10 and Appendix C) • Updated base kit list
1.1	March 2022	• Added a project identifier (NLA2022) for bloomWatch reports (Section2.2.4.5) • Clarified DO probe calibration requirements (Section 5.2.2.3) • Added direction to homogenize last integrated sample if the last pull does not fit in container (Section 5.5.3) • Updated Figure 5-4 with attachment bridle length • Corrected shipping timeframe for T-2 samples in Table B-1 • Corrected cross reference errors, formatting and typos
1.2	May 2022	• Corrected atrazine preservation and shipping. Atrazine samples are to be kept chilled until analysis (Section 5.5.3.2, Section 8.4 and Appendix B (T-2 Frozen Batched Samples)) • Added Appendix D: NLA Handpicked Sites: Resampling of the National Eutrophication Study Lakes

NOTICE

The intention of the National Lakes Assessment 2022 (NLA 2022) project is to provide a comprehensive "State of the Lakes" assessment for lakes, ponds, and reservoirs across the United States. The complete documentation of overall project management, design, methods, and standards and Quality Assurance/Quality Control (QA/QC) measures is contained in this document and companion documents, including:

National Lakes Assessment 2022: Quality Assurance Project Plan (QAPP) (EPA 841-B-21-009)

National Lakes Assessment 2022: Site Evaluation Guidelines (SEG) (EPA 841-B-16-008)

National Lakes Assessment 2022: Laboratory Operations Manual (LOM) (EPA 841-B-16-010)

These documents together comprise the integrated set of QAPP documents. This document (*Field Operations Manual [FOM]*) contains a brief introduction and procedures to follow at the base location and on-site, including methods for sampling water chemistry (grabs and *in situ*), phytoplankton, zooplankton, *Enterococci*, environmental DNA (eDNA), algal toxins, benthic macroinvertebrates, physical habitat, and contaminants in fish tissue. These methods are based on both the guidelines developed and followed in the Western Environmental Monitoring and Assessment Program (Baker, et. al., 1997), methods employed by several key states that were involved in the planning phase of this project and prior National Lakes Assessments. Methods described in this document are to be used specifically in work relating to the NLA 2022. All Project Cooperators should follow these guidelines. Mention of trade names or commercial products in this document does not constitute endorsement or recommendation for use. Details on specific methods for site evaluation and sample processing can be found in the appropriate companion document.

The suggested citation for this document is:

USEPA. 2022. National Lakes Assessment 2022. Field Operations Manual. Version 1.2. EPA 841-B-16-011. U.S. Environmental Protection Agency, Washington, DC.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ACRONYMS/ABBREVIATIONS

ANC	Acid neutralizing capacity
CPR	Cardiopulmonary resuscitation
DBH	Diameter at breast height
DI	Deionized
DO	Dissolved oxygen
DOC	Dissolved organic carbon
ESA	Endangered Species Act
FLC	Field Logistics Coordinator
FOM	Field Operations Manual
GIS	Geographic information system
GPS	Global positioning system
HAB	Harmful Algal Bloom
HDPE	High density polyethylene
HQ	Headquarters
IM	Information Management
LOM	Laboratory Operations Manual
MPCA	Minnesota Pollution Control Agency
NARS	National Aquatic Resource Surveys
NH_4	Ammonium
NHD	National Hydrography Dataset
NIST	National Institute of Standards and Technology
NLA	National Lakes Assessment
NO_3	Nitrate
OSHA	Occupational Safety and Health Administration
PBS	Phosphate buffered saline
PCBs	Polychlorinated biphenyls
PDOP	Position Dilution of Precision
PFAS	Polyfluoroalkyl substances
PFD	Personal Flotation Device
PHab	Physical habitat
QA	Quality assurance
QAPP	Quality Assurance Project Plan
QA/QC	Quality assurance/quality control
QCS	Quality control check solution
QRG	Quick Reference Guide
SEG	Site Evaluation Guidelines
SOPs	Standard Operating Procedures
TSS	Total suspended solids
UL	Underwriters Laboratory

USGS	United States Geological Survey
UTM	Universal Transverse Mercator

1.0 BACKGROUND

This manual describes field protocols and daily operations for crews to use in the National Lakes Assessment 2022 (NLA 2022). The NLA 2022 is a statistical assessment of the condition of our nation's lakes, ponds, and reservoirs (subsequently referred to in this manual as "lakes"). The survey is designed to address three key questions about the quality of the nation's lakes:

1. What percent of the nation's lakes are least, moderately, and most disturbed for key indicators of trophic state, ecological health, and human use (recreation)?
2. What is the relative importance of key stressors such as nutrients and pathogens?
3. What changes are occurring in the condition of the nation's lakes?

The surveys are also designed to help expand and enhance state and tribal monitoring programs. Through these surveys, states and tribes have the opportunity to collect data that can be used to supplement their existing monitoring programs or to begin development of new programs.

The NLA 2022 is one of a series of water surveys being conducted by states, tribes, the U.S. Environmental Protection Agency (EPA), and other partners. In addition to lakes, partners will also study coastal waters, wadeable streams, rivers, and wetlands in a revolving sequence. The purpose of these surveys is to generate statistically-valid reports on the condition of our nation's water resources and identify key stressors to these systems.

The NLA 2022 is designed to be completed during the summer growing season before fall lake turnover (June through September[a]). Field crews will collect a variety of measurements and indicators from an "index site" located at the deepest point of the lake up to 50 meters (or near the middle of the lake if the lake is a reservoir), and document conditions of the littoral zone and shoreline from stations around the lake.

1.1 Selection of Sampling Locations

EPA selected sampling locations using a probability-based survey design (Stevens and Olsen, 2004). Sample surveys have been used in a variety of fields (e.g., election polls, monthly labor estimates, and forest inventory analysis) to determine the status of populations or resources of interest using a representative sample of relatively few members or sites. Using this survey design allows data from the subset of sampled lakes to be applied to the larger target population and assessments with known confidence bounds to be made.

With input from the states and other partners, EPA used the following framework to guide the site selection process:

1. The National Hydrography Dataset Plus High Resolution (NHDplusHR) data layer was used to derive a list of lakes for potential inclusion in the NLA 2022.
2. For purposes of this survey, "lakes" refers to natural and man-made freshwater lakes, ponds,

[a] The NLA index period is June through September. Sampling in May could be approved for lakes in areas where stratification is expected earlier in the year. Please coordinate these requests with your Regional EPA Coordinator and the NLA Technical Lead.

and reservoirs greater than one hectare (approximately 2.5 acres) in the conterminous U.S., excluding the Great Lakes.

Mine ponds, retention basins, cooling ponds, and tidally-influenced lakes were excluded from this study. For more information on the site exclusion criteria refer to the National Lakes Assessment 2022: Site Evaluation Guidelines (EPA 841-B-21-008).

3. The sample size was set to include 1,000 lake sampling events.

EPA used an unequal probability design to select 904 lakes and reservoirs greater than 1 hectare (ha) in size (note: in NLA 2007, the lower size limit was 4 ha) in the continental United States. The design includes 2 revisits in each state resulting in the target total of 1,000 site visits. Revisit samples are collected for quality assurance purposes including evaluation of the ability of an indicator to distinguish *among* sites from differences *within* individual sites. Of the 904 lakes, approximately 50% of the lakes are new lakes selected for 2022 and 50% are previously sampled lakes as part of the NLA 2017. The NLA 2017 lakes are referred to as *resample lakes*. Also, of the 904 lakes, approximately 70% of the lakes (i.e., 636 lakes) are designated for whole fish composite sample collection for human health. An "oversample" list of additional lakes was also generated to allow for replacement of non-target or otherwise unsampleable sites. The oversample list will also accommodate any state wishing to conduct a state scale survey.

Lakes selected for the NLA 2022 are distributed among five size class categories and are spatially distributed across the lower 48 states and nine aggregated Omernik Level 3 ecoregions (USEPA, 2013).

Related NLA 2022 documents include the following:

National Lakes Assessment 2022: Quality Assurance Project Plan (EPA 841-B-21-009)

National Lakes Assessment 2022: Site Evaluation Guidelines (EPA 841-B- 21-008)

National Lakes Assessment 2022: Laboratory Operations Manual (EPA 841- 21-010)

These documents are available at: https://www.epa.gov/national-aquatic-resource-surveys/manuals-used-national-aquatic-resource-surveys.

1.2 Selection and Description of Survey Indicators

As part of the indicator selection process, EPA and the NLA 2022 Steering Committee evaluated indicators used in prior NLAs, refined methodologies, and identified new indicators for NLA 2022. The Steering Committee, comprised of state representatives from each of the EPA regions, provided advice and recommendations to the Agency on matters related to the NLA 2022. Key screening and evaluation criteria included indicator applicability on a national scale, the ability of an indicator to reflect various aspects of ecological condition, and cost-effectiveness (e.g., Kurtz et al., 2001). EPA used the Committee's recommendations to refine methods and develop final documents.

The remainder of this section briefly describes the indicators that the NLA 2022 will use to assess trophic status, ecological integrity, human use value, and lake characteristics (Table 1.1). Some indicators provide a basis for evaluating more than one category. For example, an assessment of zooplankton allows for an examination of ecological integrity and trophic status, and to a certain extent, human use.

1.2.1 **Trophic Status and Water Quality Indicators**

Lakes are classified according to their trophic state. “Trophic” means nutrition or growth. A eutrophic (“well-nourished”) lake has high nutrients and high plant growth. An oligotrophic lake has low nutrient concentrations and low plant growth. Mesotrophic lakes fall somewhere in between eutrophic and oligotrophic lakes.

Chlorophyll-a, total phosphorus, and Secchi disk transparency are most often used to estimate biomass and define the trophic state of a particular lake. Other variables are measured in conjunction with the trophic state variables to supplement and enhance understanding of lake processes that affect primary productivity.

1.2.1.1 Chlorophyll-a

Chlorophyll is the pigment that allows plants (including algae) to use sunlight to convert simple molecules into organic compounds via the process of photosynthesis. Of the several kinds of chlorophyll, chlorophyll-*a* is the predominant type found in green plants and algae. Measuring chlorophyll-*a* concentrations in water is a surrogate for actually measuring algae biomass and it is used to estimate trophic status.

1.2.1.2 Secchi Disk Transparency

A Secchi disk is a black and white patterned disk commonly used to measure the clarity of water based on the distance the disk can be seen when it is lowered into the water column. The Secchi disk measurement is used to estimate the euphotic zone depth in the field which is generally defined as two-times the Secchi disk depth.

1.2.1.3 Vertical Profile Measurements

Depth profiles for temperature, pH, and dissolved oxygen (DO) are taken with a calibrated water quality probe meter or multi-parameter probe sonde from the index site in each lake. This information is used to determine the extent of stratification and the availability of the appropriate temperature range and level of DO necessary to support aquatic life.

1.2.1.4 Water Chemistry and Associated Measurements

Water chemistry measurements are used to determine the acidic conditions, trophic state and nutrient enrichment, and water chemistry type.

1.2.1.5 Atrazine Pesticide Screen

Atrazine pesticides are herbicides used to control the growth of weeds. Although applied to the land, these chemicals can enter lakes via transport in water (e.g., runoff, groundwater, etc.) or atmospheric transport. This screen will provide information about the occurrence and concentration of atrazine pesticides in water samples from lakes across the nation.

1.2.2 **Biological Indicators**

Ecological integrity describes the ecological condition of a lake based on different assemblages of the aquatic community and their physical habitat (PHab). The indicators include zooplankton, benthic macroinvertebrates, and the physical habitat of the shoreline and littoral zones.

1.2.2.1 Benthic Macroinvertebrate Assemblage

Benthic macroinvertebrates are bottom-dwelling animals without backbones ("invertebrates") that are large enough to be seen with the naked eye ("macro"). Examples of macroinvertebrates include crayfish, snails, clams, aquatic worms, leeches, and the larval and nymph stages of many insects, including dragonflies, mosquitoes, and mayflies. Populations in the benthic assemblage respond to a wide array of stressors in different ways so that it is often possible to determine the type of stress that has affected a macroinvertebrate assemblage (e.g., Klemm et al., 1990). Because many macroinvertebrates have relatively long life cycles of a year or more and are relatively immobile, the structure and function of the macroinvertebrate assemblage is a response to exposure of present or past conditions. For the NLA, the benthic macroinvertebrate assemblage occupying the littoral zone will be assessed, rather than the profundal assemblage occupying the deeper regions of lakes.

1.2.2.2 Zooplankton Assemblage

Zooplankton are animal microorganisms that consist of crustaceans (e.g., copepods and cladocerans), rotifers ("wheel-animals"), pelagic insect larvae (e.g., phantom midges), and aquatic mites. The zooplankton assemblage constitutes an important element of the food web, where zooplankton transfer energy from algae (primary producers) to larger invertebrate predators and fish. The zooplankton assemblage responds to environmental stressors such as nutrient enrichment and acidification (e.g., Stemberger and Lazorchak 1994, Dodson et al. 2005). The effects of these environmental stressors on zooplankton can be detected through changes in species composition, abundance, and body size distribution.

1.2.3 **Physical Habitat Characterization**

The characterization of shoreline and littoral zone (the nearshore areas of a lake) physical habitat (PHab) conditions serves three purposes. First, habitat information is essential to the interpretation of expected lake ecological condition in the absence of human disturbance (anthropogenic impacts). Second, the habitat evaluation is a reproducible, quantified estimate of habitat condition, serving as a benchmark against which to compare future habitat changes that might result from anthropogenic activities. Third, the specific selections of habitat information collected aid in the diagnosis of probable causes of ecological degradation in lakes.

In addition to information collected in the field by the shoreline and littoral surveys, the physical habitat description of each lake includes many map-derived variables such as lake surface area, shoreline length, and shoreline complexity. Furthermore, an array of information, including watershed topography and land use, supplements the physical habitat information. The shoreline and littoral characterizations concentrate on information best derived "on the ground". As such, these results provide the linkage between large watershed-scale influences and those influences that directly affect aquatic organisms day to day. Together with water chemistry, the habitat measurements and observations describe the variety of physical and chemical conditions that are necessary to support biological diversity and foster long-term ecosystem stability. These characteristics of lakes and their shorelines are the very aspects that are often changed as a result of anthropogenic activities.

1.2.4 **Human Health and Recreational Use Indicators**

Human health indicators address the ability of the lake population to support recreational uses such as swimming, fishing, and boating. The protection of these uses is one of the requirements of the Clean Water Act. The NLA 2022 human health indicators include the extent of cyanobacterial harmful algal bloom (cyanoHAB), algal toxins (microcystins and cylindrospermopsin), fecal indicator (*enterococci*) and fish fillet contaminants.

1.2.4.1 CyanoHAB Visual Observations and Real-time Reporting

Cyanobacteria are microscopic organisms found naturally at low concentrations in freshwater systems. Under optimal conditions (such as high light and calm weather, usually in summer), cyanobacteria occasionally form harmful algal blooms (HABs), or dense aggregation of cells, that float on the surface of the water. At higher concentrations, cyanoHAB events may be so dense that they resemble bright green paint that has been spilled in the water. These blooms potentially affect water quality, human health (e.g., *Microcystis* can produce microcystin, a liver toxin), and natural resources. Decomposition of large blooms can lower the concentration of DO in the water, resulting in hypoxia (low oxygen) or anoxia (no oxygen) which may result in fish kills. Field crews will be submitting real-time reports of a potential cyanoHAB events to the state and tribal organizations that monitor water quality for recreational use support and swimming advisories. These organizations may use this information to determine if follow up monitoring and reporting is needed.

1.2.4.2 Algal toxins (microcystins and cylindrospermopsin)

Microcystins and cylindrospermopsin are two types of toxins produced by cyanobacteria. During a cyanoHABs event, the toxin concentration can rapidly increase and may become elevated before a visible bloom is observed. Elevated cyanotoxin concentrations in surface waters can persist after the bloom fades, so human exposures can occur even after the visible signs of a bloom are gone or have moved downstream. Exposure to elevated-levels of microcystins can potentially lead to liver damage; the kidneys and liver appear to be the primary target organs for cylindrospermopsin toxicity.

1.2.4.3 Fecal indicator (Enterococci)

Enterococci are bacteria whose presence indicates that water may be contaminanted by human or animal wastes. Microbes in these wastes can cause short term effects, such as diarrhea, cramps, nausea, headaches, or other symptoms. They may pose a special health risk for infants, young children, and people with severely compromised immune systems.

1.2.4.4 Fish Fillet Contaminants Indicator

Fish are important integrators of toxic contaminants that are bioavailable in the water column and in sediment. EPA monitors the occurrence of toxic chemicals in fish fillet samples to assess the potential health impacts for people who consume fish. Collecting whole fish composite samples and submitting them to the laboratory for filleting and homogenization during the NLA 2022 provides sufficient tissue for analysis of multiple chemical contaminants of concern (e.g., mercury, polychlorinated biphenyls or PCBs, and per- and polyfluoroalkyl substances or PFAS).

1.2.5 **Other Indicators**

1.2.5.1 Lake Characterizations

Observations and impressions about the lake and its surrounding catchment by field crews will be useful for ecological value assessment, development of associations and stressor indicators, and data verification and validation.

1.2.5.0 Environmental DNA

Two water samples will be collected and analyzed for environemtnal DNA (eDNA). Consistent with NLA 2017, one sample will be collected from the index site. The second sample will be a composite sample from 10 littoral stations. This research measure will be used to explore indicator development for future NLAs.

Table 1-1 Summary table of indicators and sampling location.

Indicator Type	Indicator	Specifications/Location in Lake			
		Desktop Evaluation	**Boat Launch**	**Index Site**	**Littoral Site**
Trophic and Chemical Indicators	Vertical profile measurements (DO, Temperature, pH)			X	
	Secchi Disk transparency			X	
	Water chemistry (NH_4, NO_3), major anions and cations, alkalinity (ANC), DOC, TSS, silica, conductivity, nutrients (total and dissolved TN and TP)			Integrated water sample	
	Chlorophyll-*a*			Integrated water sample	
	Atrazine pesticide screen			Integrated water sample	
Biological	Benthic macroinvertebrate assemblage				10 stations
	Zooplankton assemblage (composition, structure, and size distribution)			Vertical tow (2 mesh sizes) through water column	
Physical Habitat	Physical habitat characterization				10-12 stations
Human Health	Fecal indicator (*Enterococci*)		Grab sample; lakes >10,000 ha		Grab sample; last station, lakes < 10,000 ha
	Phytoplankton (cyanobacteria)			Integrated water sample	
	CyanoHAB visual observations		X	X	X
	Algal toxins (microcystins and cylindrospermopsin)			Integrated water sample	
	Fish fillet contaminants		Whole fish composite sample (for fillet analysis) collected lake wide		
Other Indicators	eDNA		1L Grab sample; lakes >10,000 ha	1L Grab sample	10 sample composite; 1L total
	Lake area, basin morphometry, and characteristics of watershed	Using GIS			

2.0 LOGISTICS

2.1 Roles and Contact Information

Effective communication between field crews, EPA coordinators, and NLA 2022 contractor support staff is essential for the survey to proceed with maximum efficiency and to ensure collection of high quality data. This section provides:

- A general description of the roles of key NLA 2022 personnel in providing logistical and technical support to the field crews;
- Flow of communication between Field Crews and these individuals (i.e., *who to call for specific types of questions or support needs*); and
- Contact information.

The **EPA Headquarters (HQ) Project Management Team** consists of the Project Leader, Logistics Leader, and Project QA Lead, along with the EPA Technical Lead for the Fish Fillet Contaminants Indicator. The Team is responsible for overseeing all aspects of the project and ensuring technical and QA requirements are properly carried out. The Team is the final authority on all decisions regarding field sampling, site evaluation, site replacement, and laboratory analysis.

The **EPA Regional Coordinators** are the primary EPA point of contact for Field Crews operating in their Region. Field Crews should direct all technical and logistical questions to their EPA Regional Coordinator, who will work with the EPA HQ Team to resolve the issue. Field Crews should also work with their EPA Regional Coordinator to schedule an **Assistance Visit** to occur within the first two weeks of field sampling. An Assistance Visit is part of the QA component of the NLA 2022 QAPP. To meet the requirements of the QAPP, each Field Crew will allow an EPA employee or contractor to observe that crew sampling for one day. The Assistance Visit is used to confirm the protocols are implemented as intended and to suggest corrective actions, if needed, to the Field Crew's sampling approach.

The **Information Management (IM) Coordinator** provides the Field Crews with packets of forms and labels for each site scheduled to be sampled. Crews will request these packets through a fillable PDF Request form. The IM Team also tracks the transition of each NLA 2022 sample from the field to the laboratory.

The Contract **Field Logistics Coordinator (FLC)** is responsible for tracking the Field Crew's sampling activities and overall progress throughout the field season, ensuring that requests for supplies and equipment are filled, and assisting Field Crews with questions concerning field logistics, equipment, and supplies as they arise during the field season. The FLC will also review submitted status and tracking forms to ensure that the correct samples have been taken and that those samples are being sent to the laboratories in an appropriate timeframe.

Table 2-1 Personnel to call for specific types of questions and support needs.

Personnel	Call
EPA Regional Coordinators	First, to ask any questions about NLA, including questions on field protocols Grant questions Schedule Field Assistance Visit
EPA HQ Project Management Team	Ask questions about site access, site evaluation, and site replacement Ask questions about shipping locations and sample handling procedures Ask questions about Field Methods Ask questions about Target Fish species Ask questions about Survey Design Ask questions about QA procedures Ask questions about Laboratory Methods If you can't reach Regional Coordinator, IM Coordinator, or Field Logistics Coordinator If you are unsure who to call
Personnel	**ONLY Call**
Information Management (IM) Coordinator	Order field forms or site kits Submit a status report Notify EPA about change in sampling schedule Ask questions about submitting data packet If EPA Regional Coordinator directs you to them
Contract Logistics Coordinator	Order replacement items for site kits, base kits, or miscellaneous supplies Ask questions about shipping contract, or to order more shipping forms If EPA Coordinator directs you to them If you can't reach an EPA HQ or Regional Coordinator and it is an urgent question

Table 2-2 Contact information

Title	Name	Contact Information
EPA HQ Project Lead	Lareina Guenzel, OW	guenzel.lareina@epa.gov 202-566-0455
EPA HQ Project QA Lead	Sarah Lehmann, OW	lehmann.sarah@epa.gov 202-566-1379
EPA HQ Logistics Lead	Brian Hasty, OW	hasty.brian@epa.gov 202-564-2236
Contract Field Logistics Coordinator	Chris Turner, Great Lakes Environmental Center, Inc.	cturner@glec.com 715-829-3737

Title	Name	Contact Information
EPA HQ Fish Fillet Contaminants Indicator Leads	Leanne Stahl, OW/OST	stahl.leanne@epa.gov 202-566-0404
	John Healey, OW/OST	Healey.john@epa.gov 202-566-0176
Contract Fish Fillet Contaminants Indicator Trainer	Blaine Snyder	Blaine.snyder@tetratech.com 410-902-3158
Information Management (IM) Coordinator	Michelle Gover, GDIT	gover.michelle@epa.gov 541-754-4793
Regional EPA Coordinators	Hilary Snook, Region 1	snook.hilary@epa.gov 617-918-8670
	Emily Nering, Region 2	nering.emily@epa.gov 732-321-6764
	Frank Borsuk, Region 3	borsuk.frank@epa.gov 304-234-0241
	Leah Ettema, Region 3	ettema.leah@epa.gov 304-234-0245
	Chris McArthur, Region 4	mcarthur.christopher@epa.gov 404-562-9391
	Mari Nord, Region 5	nord.mari@epa.gov 312-886-3017
	Rob Cook, Region 6	cook.robert@epa.gov 214-665-7141
	Gary Welker, Region 7	welker.gary@epa.gov 913-551-7177
	Liz Rogers, Region 8	Rogers.liz@epa.gov 303-312-6974
	Tom Johnson, Region 8	Johnson.tom@epa.gov 303-312-6226
	Tina Yin, Region 9	yin.christina@epa.gov 415-972-3579
	Matthew Bolt, Region 9	Bolt.matthew@epa.gov 415-972-3578
	Lil Herger, Region 10	herger.lillian@epa.gov 206-553-1074

2.2 Key Information and Materials

2.2.1 Site Maps

Geospatial files in the form of geographic information system (GIS) design point and polygon files and state leaflet maps have been provided on the NLA SharePoint sites to assist in the site evaluation process. From these files, crews should generate their own site maps with relevant information displayed. The site maps will be helpful in the planning and preparation for visiting and sampling a

particular NLA 2022 site. These maps should become part of your site packet. See more information on the site packet in Section 4.1.

2.2.2 **Forms**

Forms are the key to data collection and tracking for the NLA 2022. For NLA 2022 we have developed electronic forms which will be accessed via the NLA App. Paper forms will only be provided as backups to the App forms.

2.2.2.1 Field Forms

The NLA App is the primary way crews will record measures, observations, and collection information during the course of the field day. Additional information regarding specifics of data entry is contained in Section 3.2.

- **Electronic Field Forms:** This form of data collection will be collected through an Apple iPad which will be provided for all state, tribal, and EPA crews. Each of the field forms are separated into sections for easier data entry. It is important for a field crew to familiarize themselves with the NLA App prior to field sampling. Field crews should note that each individual field form must be submitted by only one device. For example, if there are 5 field forms (A,B,C,D,E) and iPad 1 submits forms A, B, and D, then iPad 2 should not submit those 3 forms or data will be overwritten. In this example, iPad 2 could still submit forms C and E with no issues. While a data or Wi-Fi connection is required to submit the data, no data connection is required for the data collection process.

- **Paper Field Forms:** Extra paper field forms will only be provided to field crews to serve as backup copies in case of problems with electronic field forms. As soon as possible, the completed paper field forms should be transcribed to the NLA App for data submission. The original completed version of the forms must also be scanned, emailed to the EPA Logistic Lead and stored by the field crew for two years.

2.2.2.2 Tracking Form

The **Tracking** form in the App describes the status and location of all samples and specimens collected during the sampling of an NLA site and is transmitted electronically to the IM Team at specified times. When samples are shipped to the lab, a packing slip is included in the shipping container to convey to the lab which samples are included in the shipment. These packing slips (which are pre-printed with the same sample IDs as the individual sample labels) are included in the Label Packet with each Site Kit.

The **Tracking** form is divided into several shipping groups, each labelled with a shipping group number (e.g., T-1, T-2, T-3, etc.). Sample labels, packing slips, and FedEx shipping labels also carry these shipping group numbers to help Field Crews group correct items together for shipping. See APPENDIX B: SHIPPING GUIDELINES for more information.

2.2.3 **Equipment and Supplies**

2.2.3.1 Request Form

Field Crews will submit requests for site kits, whole fish composite sample kits, and other needed supplies via an electronic **Request** form provided by the FLC. This form will be submitted to the NARS IM Coordinator who will ensure that the request reaches the appropriate entity. Crews must submit basic

sampling information (i.e., tentative start date and number of sites crews are planning to sample) to the FLC at or before the time of submitting request forms. Crews should submit the **Request** form at least two weeks prior to their desired sampling date. The **Request** form will be in fillable PDF format. Users must enter the required information, save a copy of the form to their computer or device and attach the updated copy to an email to sampletracking@epa.gov. The IM Team will send email notification that the request has been received.

2.2.3.2 Base Kit

The Base Kit is comprised of the subset of durable equipment and supplies needed for NLA 2022 sampling and is provided by EPA through the FLC. Typically one Base Kit is provided to each Field Crew and contains some of the equipment that is used throughout the field season. SeeAPPENDIX A: EQUIPMENT & SUPPLIES for a list of the items provided by EPA in the Base Kit. EPA anticipates that Base Kitequipment will be available for use in future NLA efforts.

2.2.3.3 Site Kit

A Site Kit contains the subset of consumable supplies (i.e., items used up during sampling or requiring replacement after use) provided by EPA through the FLC. The site kit will contain all the sample bottles necessary for sampling a single lake. A new Site Kit should be requested for each site sampled, and crews should consider having at least one additional site kit available as a spare should any supplies be lost See APPENDIX A: EQUIPMENT & SUPPLIES for the consumable items that will be provided by EPA.

2.2.3.4 Whole Fish Composite Sample Kit

A sampling kit for the whole fish composite sample will be provided for all designated fish sampling sites. In the survey design, “FT” in the “Panel_Use” identifies lakes designated for fish sampling. This sampling kit contains consumable supplies provided by EPA. Field crews should request a new whole fish sampling kit for each designated fish site to be sampled. See APPENDIX A: Equipment & Supplies for the consumable items that will be provided by EPA.

2.2.3.5 Field Crew Supplied Items

The field crew will also supply particular items for the field sampling day. These items might include supplies from the NLA 2007, NLA 2012, or NLA 2017, typical field equipment (like a global position system (GPS) receiver or multi-paramter probe), or boat equipment. See APPENDIX A: EQUIPMENT & SUPPLIES for the items that the field crew will need to provide.

2.2.4 **Other Resources**

The complete documentation of overall project management, design, methods and standards, and QA/QC measures is contained in this document and companion documents (listed in NOTICE and described below). The NLA 2022 participants must agree to follow the QAPP, including the protocols and design, and the associated documents – the NLA 2022 FOM, LOM, and SEG.

2.2.4.1 Quick Reference Guide

Field crews will NOT receive a NLA 2022 QRG. All components of the QRG will be contained in the FOM and as “i” buttons in the NLA App. The electronic version of the FOM will be provided on the EPA issued Apple iPads and will be a searchable document. Detailed steps to complete protocols can be found in the FOM. Many of the tables and figures in the FOM will also be included in the App.

2.2.4.2 Site Evaluation Guidelines

The NLA 2022 SEG (EPA 841-B-16-001) outlines the process to compile the final list of candidate lakes for sampling. The process includes locating a candidate lake, evaluating the lake to determine if it meets the criteria for inclusion in the target population and is accessible for sampling, and if not, replacing it with an alternate candidate lake.

2.2.4.3 Quality Assurance Project Plan

Large-scale and/or long-term monitoring programs such as those envisioned for national surveys and assessments require a rigorous QA program that can be implemented consistently by all participants throughout the duration of the monitoring period. QA is a required element of all EPA-sponsored studies that involve the collection of environmental data (USEPA 2000a, 2000b). Field crews will be provided a copy of the NLA 2022 QAPP (EPA 841-B-16-003) and the field crew leader is required to sign the QAPP signature page prior to beginning field sampling activities. The QAPP contains more detailed information regarding QA/QC activities and procedures associated with general field operations, sample collection, measurement data collection for specific indicators, and data reporting activities. For more information on the project level QA procedures, refer to the NLA 2022 QAPP.

2.2.4.4 Laboratory Operations Manual

The methods used for the laboratory sample analysis is available in the NLA 2022 Laboratory Operations Manual (LOM) (EPA 841-B-21-010).

2.2.4.5 Realtime cyanoHAB Reports

Field crew leads are to report a cyanoHAB event if a bloom is occuring at the time of sampling. The Apple iPads are preloaded with the bloomWatch application to support realtime reporting of a bloom to the appropriate state, tribal and/or watershed organization authority. Alternatively, field crews are encouraged to download state-specific monitroing and reporting tools that will be in use for the 2022 recreation season. Additional information on state-specific HABs monitoring programs can be found here (https://www.epa.gov/cyanohabs/state-habs-monitoring-programs-and-resources). To submit a report to bloomWatch or a state-specific tool, the field crews should should be prepared with emails for state officals that are to receive these report (i.e., officals that monitor and report on cyanobacterial bloom events). Field crews must select the preferred reporting approach and be familiar with its input needs (e.g., extent, photos, email contacts etc.) prior to initiating field work. All reports submitted via the bloomWatch app should add the project identifier 'NLA2022' in the comment section in the app.

3.0 DAILY FIELD ACTIVITIES SUMMARY

This section presents a general overview of the activities that a field crew conducts during a typical 1-day sampling visit to a lake. The following sections include general guidelines for health and safety, recording data, and using standardized field data forms and sample labels.

3.1 Health and Safety

Collection and analysis of samples can involve risks to personal safety and health, and **the safety of the field crew must always be the primary consideration during sampling**. This section describes general safety considerations, some safety equipment, and safety guidelines for field operations.

This section does not substitute for an official Health and Safety Plan. The crew MUST ALWAYS carefully follow the protocols in their Health and Safety Plan for the NLA field work that was approved by the state, tribe, or other organization with which the field crew is affiliated. The crew should carry a copy of this approved Health and Safety plan in the field.

3.1.1 General Considerations

It is the responsibility of the group safety officer or project leader to ensure that the necessary safety courses are taken by all field personnel and that all safety policies and procedures are followed. Each state, tribe, or other organization must have a specific safety plan for the sampling the NLA sites, including a communications plan that addresses safety and emergency situations. The plan should have a daily check-in procedure for field personnel, and emergency contacts for police, ambulance, fire departments, hospitals, and search and rescue personnel. Important considerations related to field safety are listed below. It is the responsibility of the group safety officer or project leader to ensure that the necessary safety courses are taken by all field personnel and that all safety policies and procedures are followed. Sources of information regarding health and safety considerations and/or safety-related training include the American Red Cross (http://www.redcross.org/m/phssmrd/take-a-class), the National Institute for Occupational Safety and Health (1981) (see the most recent revisions at https://www.cdc.gov/niosh/docs/81-123/, and the U.S. Coast Guard (http://www.uscgboating.org/recreational-boaters/boating-safety-courses.php).

3.1.1.1 Recommended Training

- First aid;
- Cardiopulmonary resuscitation (CPR);
- Vehicle safety (e.g., operation of 4-wheel drive vehicles, trailer towing and maneuvering);
- Boating and water safety;
- Field safety (weather, personal safety, orienteering, site reconnaissance prior to sampling);
- Equipment design, operation, and maintenance; and
- Handling of chemicals and other hazardous materials.

3.1.1.2 Communications

A communications plan to address safety and emergency situations is essential. All field personnel need to be fully aware of all lines of communication. Field personnel should have a daily check-in procedure

with their supervisor for safety. An emergency communications plan should include contacts for police, ambulance, fire departments, hospitals, and search and rescue personnel. Below are some items to address:

- Check-in schedule;
- Sampling itinerary (vehicle used & description, time of departure & return, and travel route);
- Contacts for police, ambulance, hospitals, fire departments, and search and rescue personnel;
- Emergency services available near each sampling site and base location; and
- Cell (or satellite) phone number, if possible.

3.1.1.3 Personal Safety

Proper field clothing should be worn to prevent hypothermia, heat exhaustion, sunstroke, drowning, or other dangers. Field personnel should be able to swim, and a personal flotation device (PFD) must be used. Chest waders made of rubberized or neoprene material and suitable footwear must always be worn with a belt to prevent them from filling with water in case of a fall. Below are some personal safety items to address:

- Field clothing and other protective gear including lifejackets for all crew members;
- Medical and personal information (allergies, personal health conditions, and required medications);
- Personal contacts (family, telephone numbers, etc.); and
- Physical exams and immunizations.

Many hazards lie out of sight in the bottoms of lakes, rivers, and streams. Broken glass or sharp pieces of metal embedded in the substrate can cause serious injury if care is not exercised when walking or working with the hands in such environments. Infectious agents and toxic substances that can be absorbed through the skin or inhaled may also be present in the water or sediment. Personnel who may be exposed to water known or suspected to contain human or animal wastes that carry causative agents or pathogens must be immunized against tetanus, hepatitis, typhoid fever, and polio. Biological wastes can also be a threat in the form of viruses, bacteria, rickettsia, fungi, or parasites.

Lakes and surrounding landscapes can be home to dangerous organisms. Field crews should take care to minimize contact with biting insects, bees, poisonous snakes and dangerous animals. Insect repellent and protective clothing will help to limit exposure. At the end of each field day, workers should inspect their bodies for ticks. Any person allergic to bee stings, other insect bites, or plants (i.e., poison ivy, poison oak, poison sumac, etc.) should take proper precautions and have any needed medications on hand. In addition, field crew members should always be aware of their surroundings to protect themselves from dangerous animals, such as alligators, mountain lions, bears, and wolves.

3.1.1.4 Sampling Equipment

Field crew members should be familiar with hazards associated with the use of sampling equipment and establish appropriate safety practices prior to their use. They must ensure that all equipment is in safe working condition.

Because boats are used to access NLA sampling sites, personnel must be trained in operating the type of boat in use incouding appropriate state or other certifications. Personnel must consider and prepare for

hazards associated with the operation of motor vehicles, boats, winches, tools, and other incidental equipment. Boat operators should be familiar with U.S. Coast Guard rules and regulations for safe boating contained in a pamphlet, *Federal Requirements for Recreational Boats*, available from a local U.S. Coast Guard Director or Auxiliary or State Boating Official and online (U.S. Coast Guard, https://www.uscgboating.org/images/420.PDF). All boats with motors must have fire extinguishers, boat horns, life jackets or flotation cushions, and flares or communication devices.

3.1.2 **Safety Equipment**

Appropriate safety apparel such as life jackets, waders, gloves, safety glasses, etc. must be available and used when necessary. First aid kits, fire extinguishers, and blankets must be readily available in the field. Cellular or satellite telephones and/or portable radios should be provided to field crews working in remote areas for use in case of an emergency. Supplies such as anti-bacterial soap and an adequate supply of clean water or ethyl alcohol must be available for cleaning exposed body parts that may have been contaminated by pollutants in the water or sediments.

3.1.2.1 Safety Guidelines for Field Operations

General safety guidelines for field operations are presented below.

Personnel participating in field activities on a regular or infrequent basis should be in sound physical condition and have a physical examination annually or in accordance with Regional, State, or organizational requirements.

All surface waters and sediments should be considered potential health hazards due to potential toxic substances or pathogens. Persons must become familiar with the health hazards associated with using chemical fixing and/or preserving agents. Chemical wastes can be hazardous due to flammability, explosiveness, toxicity, causticity, or chemical reactivity. All chemical wastes must be discarded according to standardized health and hazards procedures (e.g., National Institute for Occupational Safety and Health [1981]; for the most recent revisions see https://www.cdc.gov/niosh/docs/81-123/;.)

During the course of field research activities, field crews may observe violations of environmental regulations, may discover improperly disposed hazardous materials, or may observe or be involved with an accidental spill or release of hazardous materials. In such cases it is important that the proper actions be taken and that field personnel do not expose themselves to something harmful. The following guidelines should be applied:

1. First and foremost, protect the health and safety of all personnel. Take any necessary steps to avoid injury or exposure to hazardous materials. If you have been trained to take action such as cleaning up a minor fuel spill during fueling of a boat, do it. However, you should always err on the side of personal safety.
2. Field personnel should never disturb or retrieve improperly disposed hazardous materials from the field to bring back to a facility for "disposal." To do so may worsen the impact, may incur personal liability or liability for the crew members and their respective organizations, may cause personal injury, or may cause unbudgeted expenditure of time and money for proper treatment and disposal of the material. However, it is important not to ignore environmental incidents. Notify the proper local, state, and/or federal authorities of any incident of this type so that they may take the necessary actions to properly respond to the incident.
3. For most environmental incidents, the following emergency telephone numbers should be

provided to all field crews: State or Tribal department of environmental quality or protection, U.S. Coast Guard, and the EPA regional office. In the event of a major environmental incident, the National Response Center may need to be notified at 1-800-424-8802.

Specific Safety Guidelines are below:

- Two persons must be present during all sample collection activities, and no one should be left alone while in the field.
- Minimize exposure to lake water and sediments as much as possible. Use gloves when necessary, and clean exposed body parts as soon as possible after contact.
- All electrical equipment must bear the approval seal of Underwriters Laboratories (UL) and must be properly grounded to protect against electric shock.
- Use heavy gloves when hands are used to agitate the substrate during collection of benthic macroinvertebrate samples.
- Use appropriate protective equipment (e.g., gloves, safety glasses, specialized garments, etc.) when handling and using hazardous chemicals.
- Persons working in areas where venomous snakes may be encountered must check with the local Drug and Poison Control Center for recommendations on what should be done in case of a bite from a venomous snake.
- Any person allergic to bee stings, other insect bites, or plants (i.e., poison ivy, oak, sumac, etc.) must take proper precautions and have any needed medications handy (e.g., an "Epi-Pen").
- Protect yourself against the bite of deer or wood ticks because of the potential risk of acquiring pathogens that can cause Rocky Mountain spotted fever, Lyme disease, and other diseases.
- Be familiar with the symptoms of hypothermia and know what to do in case symptoms occur. Hypothermia can kill a person at temperatures much above freezing (up to 10°C or 50°F) if he or she is exposed to wind or becomes wet.
- Be familiar with the symptoms of heat/sun stroke and be prepared to move a suffering individual into cooler surroundings and hydrate immediately.
- Handle and dispose of chemical wastes properly. Do not dispose of any chemicals in the field.

3.1.3 COVID Precautions

Safety is the number one concern for all personnel. In implementing the NLA, crews should follow their agencies' guidance on maintaining social distance, use of personal protective equipment, travel restrictions, sanitizing equipment, vehicles and boats, and if necessary, hotel rooms. NLA training and assistance visits will be implemented in a manner that considers Covid-19 safety requirements and restrictions.

3.2 Recording Data and Other Information

All samples need to be identified and tracked; and associated information for each sample must be recorded. It is imperative that field and sample information be recorded accurately, consistently, and legibly. The cost of a sampling visit coupled with the short index period severely limits the ability to resample a lake if the initial information recorded was inaccurate. As mentioned in Section 2.2.2, there are two forms for collecting sample data: electronic field forms and backup paper field forms. See below for important information pertaining to data entry for each of these forms.

3.2.1 Field Forms

The NLA 2022 field data and tracking forms are formatted in the NLA App so that the data you record can be electronically submitted to the NARS IM database. It is important that field data and sample information are recorded accurately and consistently. General guidelines for recording field measurements are presented in **Table 3-1.** More detailed instructions for filling out specific forms are provided in each protocol chapter of this manual.

Table 3-1 Guidelines for recording field measurements and tracking information.

ACTIVITY	GUIDELINES
Field Measurements	
Data Recording	• Record observations and measurement values only using the official NLA App (provided on EPA owned Apple iPads for all regional, state, and tribal crews). • If you make an error when recording data and changes are required, it is best to enter the new value and resubmit that electronic form. • Use the correct crew ID assigned during field training. • Use the units and formats specified on individual data forms for recording data. • For any sample or data where additional explanation is needed, use the provided comment bubble adjacent to the data
Sample Collection	
Sample Labels and Tags	• Use a writing instrument that leaves clear, dark text to record information (e.g., a No. 2 pencil on paper tags or a water or smear proof fine-point indelible marker on adhesive labels). Use the sample-type appropriate adhesive labels with preprinted Sample ID numbers for each sample. Be sure to fill in any requested information about the sample on the sample label and affix it to the outside of the sample container. Cover completed labels with clear tape. • Place a waterproof paper tag inside each benthic macroinvertebrate collection jar with the required information written with a No. 2 lead pencil.
Sample Collection Information	• Record that each sample has been collected on the appropriate data form. Be sure to cross-check the Sample ID number from labels and tags with the Sample IDs populated in the **Tracking** form .
QA and Tracking	
Before Leaving Site: Review of Data Forms and Comparison of Sample Labels and Data Forms	• Review all data forms for accuracy and completeness. • Review all sample labels for accuracy, completeness, and legibility. • Verify that the information recorded on the sample labels and tags is consistent with all Sample IDs listed on the **Tracking** form in the NLA App.
Before Shipping Samples: Review of Sample Labels and Tracking Form	• Complete all sections in the **Tracking** form required for all samples being shipped. Review the **Tracking** form for consistency and correctness. • Compare labels on samples with the Sample IDs recorded on the **Tracking** form for accuracy and completeness before shipping samples.
Review of Data Forms	• The Field Crew Leader should review the completed forms in the NLA App as soon as is practicable to ensure they are complete and all data forms are consistent and correct

ACTIVITY	GUIDELINES
	• Confirm that the forms have been reviewed by selecting the reviewed bubble in the App for each electronic data form. • If any revisions are made, re-submit the updated form(s) as soon as possible to update the IM Database. • After each submission, a data summary email will be sent to the email address which submitted the data. This data summary contains a list of the data forms and their most recent submissions date/time as well as a list of the most critical data points collected at the site. The Field Crew Leader should review this data summary to ensure that the data forms were successfully received and that critical values are present and correct.

3.3 Sampling Scenario

Field methods for the NLA 2022 are designed to be completed in one field day for most lakes. Depending on the time needed for both the sampling and traveling for that day, an additional day may be needed for pre-departure and post-sampling activities (e.g., cleaning equipment, repairing gear, shipping samples, and traveling to the next lake). Remote lakes with lengthy or difficult approaches may require more time to gain access to the lake, and field crews will need to plan accordingly.

A field crew typically will consist of at least two people. Two people are always required in the boat together to execute the sampling activities and to ensure safety. Any additional crew members may either remain on shore to provide logistical support or be deployed in a second boat to assist in data collection. Figure 3-1 and Figure 3-2 present a daily field sampling scenario showing how the workload may be split between crew members. Each field crew should define roles and responsibilities for each crew member to organize field activities efficiently. Minor modifications to the sampling scenario may be made by crews; however, the sequence of sampling events presented in Figure 3-1 cannot be changed and is based on the need to protect some types of samples from potential contamination and to minimize the possibility of holding time exceedance once samples are collected. The following sections further define the sampling sequence and the protocols for sampling activities.

***NOTE**: When sampling large lakes (lakes > 10,000 hectares), field crews may omit the physical habitat, benthic macroinvertebrate, and littoral eDNA sampling efforts altogether.*

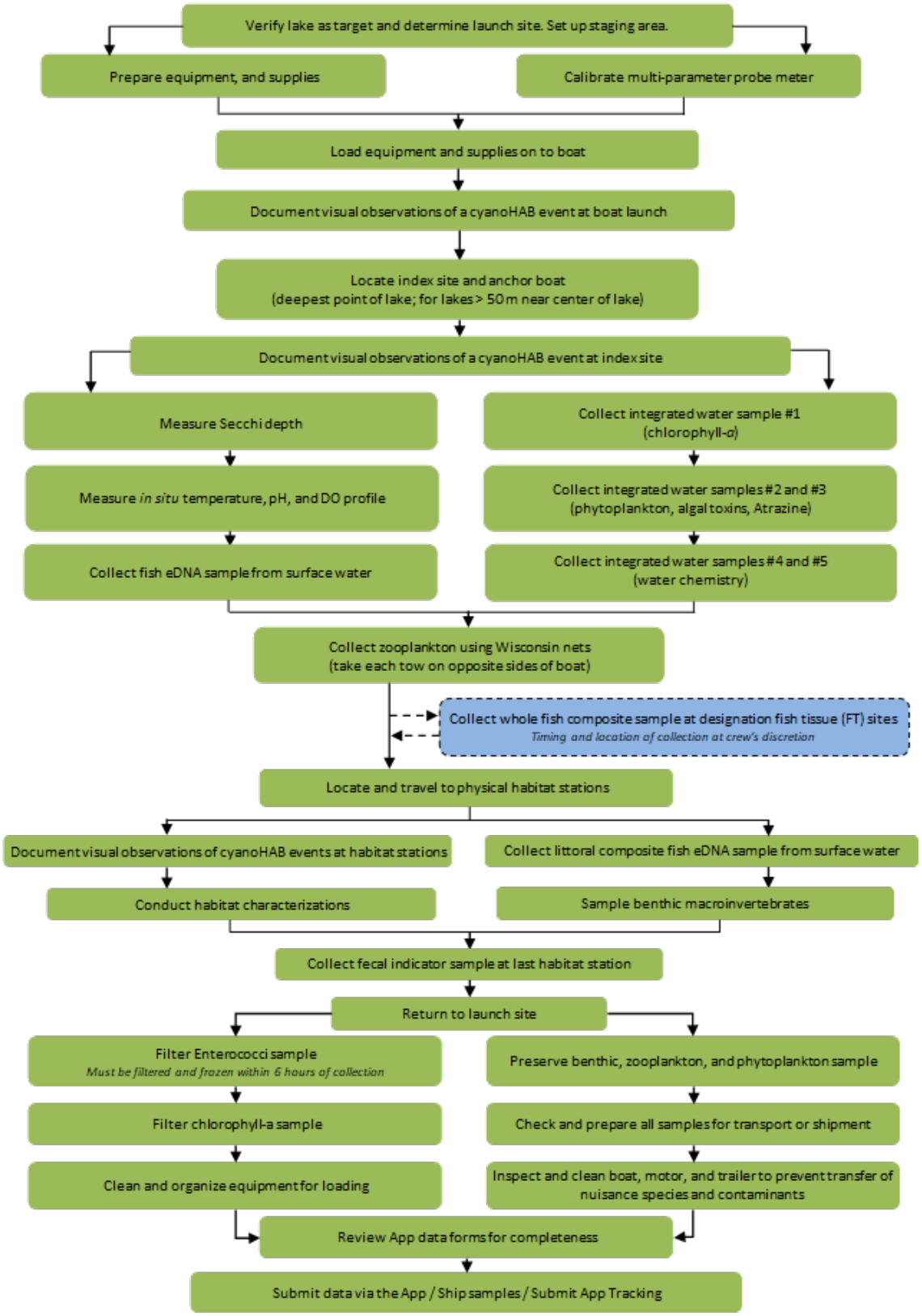


Figure 3-1 Daily operations summary

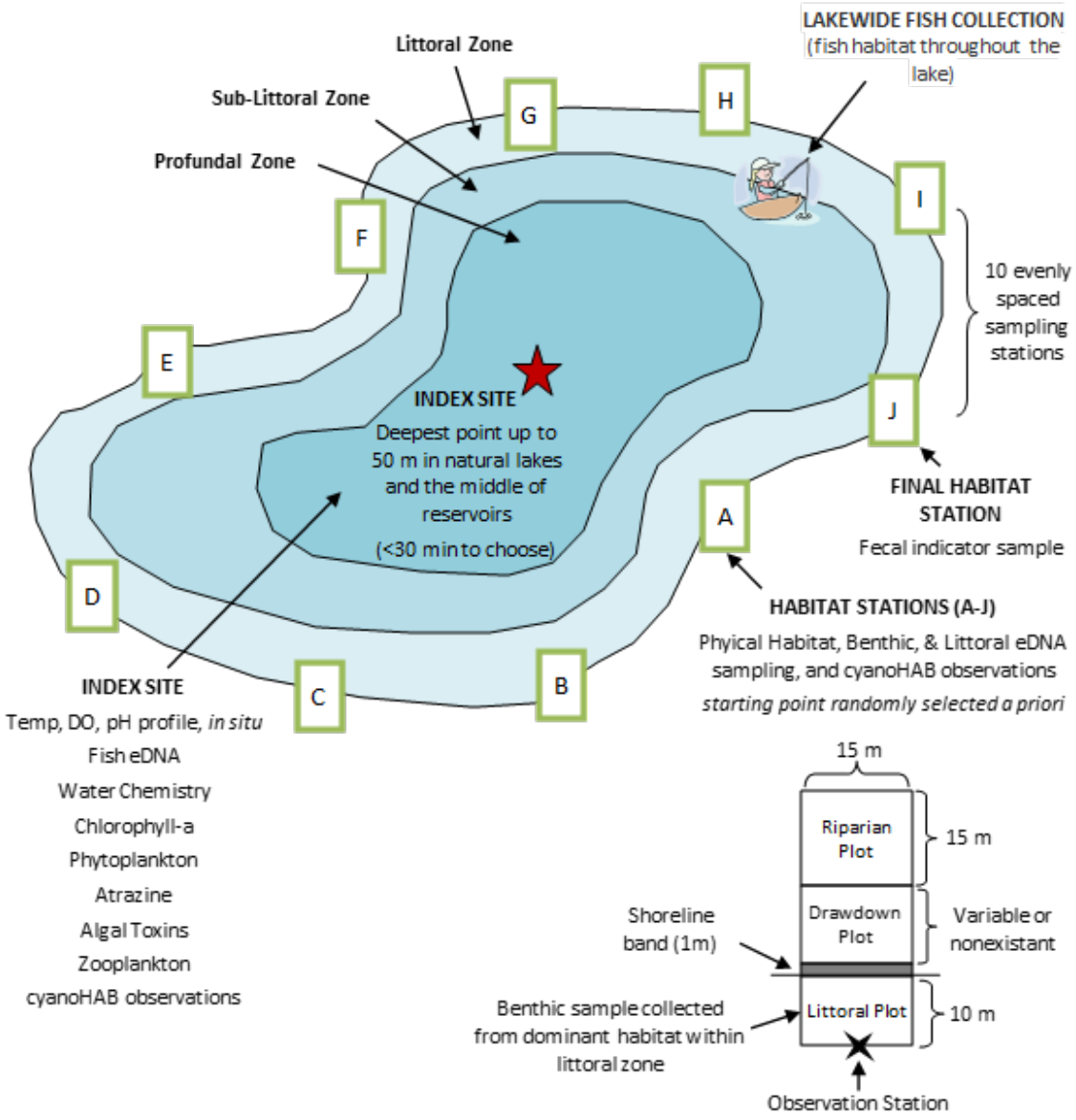


Figure 3-2 Location of sample collection points and physical habitat (PHab) stations.

The field crew arrives at the lake in the early morning to complete the sampling in a single day. The sampling sequence is to:

1. verify lake, calibrate equipment, locate, and travel to the index site;
2. document visual observations of a cyanoHAB event at boat launch and index site;
3. conduct depth profile measurements of DO, temperature, and pH;
4. take Secchi disk transparency depth measurement;
5. collect fish eDNA sample;
6. use the integrated sampler to collect water chemistry, chlorophyll-*a*, atrazine, algal toxin, and phytoplankton samples;
7. collect zooplankton samples;

8. collect fish eDNA sample for composite sample from the littoral plot at the ten PHAb stations (A,B,C,D,E,F,G,H,I,J) prior to initiating physical habitat characterization or benthic sampling;
9. document visual observations of cyanoHABs and conduct physical habitat characterization around the margin of the lake at ten PHab stations (A,B,C,D,E,F,G,H,I,J);
10. collect benthic samples at ten PHAb stations (A,B,C,D,E,F,G,H,I,J) concurrent with physical habitat characterization;
11. collect whole fish composite samples;
12. collect fecal indicator sample at last PHab station (<10,000 ha lakes) or boat launch when exiting lake (>10,000 ha lakes);
13. filter enterococci and chlorophyll-*a* samples;
14. preserve and prepare all samples for shipment;
15. review App field forms ;
16. report sampling event; and
17. ship time-sensitive samples (water chemistry, chlorophyll-*a*, eDNA, and fecal indicator samples).

4.0 BASE SITE ACTIVITIES

Field crews are to conduct a number of activities at their base site (i.e., office or laboratory, camping site, or motel). These include tasks that must be completed both before departure to the lake site and after return from the field (Figure 4-1). Close attention to these activities is required to ensure that the field crews know:

- where they are going;
- that access is permissible and possible;
- that equipment and supplies are available and in good working order to complete the sampling effort; and
- that samples are packed and shipped appropriately.

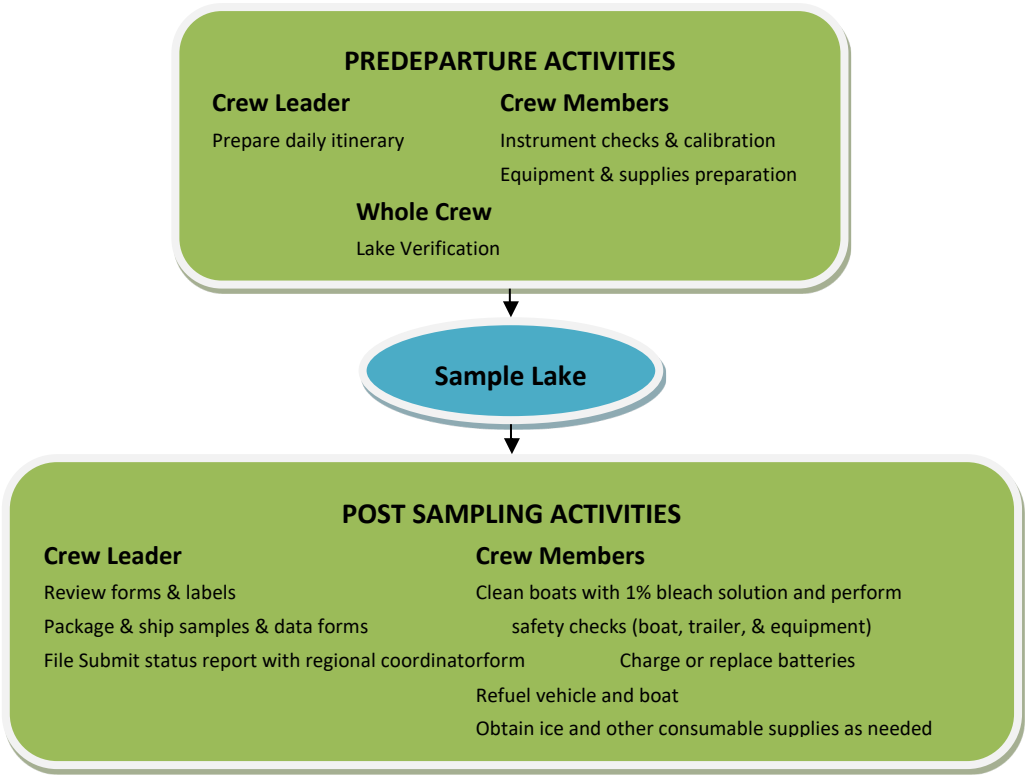


Figure 4-1 Overview of base site activities

4.1 Pre-departure Activities

Pre-departure activities include developing daily itineraries, checking and calibrating instruments, and preparing equipment and supplies. Procedures for these activities, which will take place at your office or laboratory, camping site, or motel, are described in the following sections.

4.1.1 Daily Itineraries and Site Packets

The Field Crew Leader is responsible for developing daily itineraries and a site packet. A site packet contains information key to the planning and preparation for visiting and sampling a particular NLA site.

Development of site packets should have been initiated during site evaluation and reconnaissance (See NLA 2022 Site Evaluation Guidelines); however, the field crew may need to gather additional information for the site packet during preparation for the sampling visit. Also, it is the responsibility of the field crew to obtain access permissions and any needed permits as part of developing the site packet.

Prior to a field crew traveling to a NLA site for sampling, the information for the site packet must be gathered and reviewed. Site packet development entails compiling maps, contact information, copies of permission letters, and access instructions. The Field Crew Leader must be sure to lay out the physical habitat (PHab) stations on a site map before the sampling day (see Section 6.2.3). Additional activities include confirming the best access routes, calling the landowners or local contacts, and confirming lodging plans. The site packet may include the following documents:

- **Site maps**: Generated by crew (see Section 2.2.1 Site Maps).
- **Other Maps, Imagery, or GIS Data:** Any other maps, aerial photos, GIS data, or sources of information compiled by Field Crews and/or their partners that could be helpful to sampling the NLA sites.
- **Land Ownership Status, Requirements and Permissions for Access:**
    - Landowner identity and contact information.
    - Results of communication with landowners.
    - Documentation of permission to access private land.
    - Permissions for crossing private lands to reach sites located on public lands.
    - For public land, response of relevant agency to notification that you will be accessing a site, and, if needed, permissions to do so.
- **Permits:** Any permits or documentation required for site access, or for data collection activities or sample/specimen collection.
- **Information for Accessing the Site:**
    - Contact information for landowners.
    - Notes about whether landowner(s) want to be informed when Field Crew is on site.
    - Contact information for individuals who must be available to open gates or allow entry to a site, and the time and location for meeting them.
    - Notes on locked gates, pets, livestock, or other things that could impede access.
    - Notes about active hunting, farming, mining, or other activities on or near the site.
    - Current conditions that could prevent access (e.g., high water, forest fires, road closures, etc.).
- **Site Evaluation Notes:**
    - Site Evaluation notes, annotated aerial photos, sketch map, and completed site evaluation form that can aid with planning for accessing or sampling a site.
- **Driving and Hiking Routes to the Site:**
    - Detailed driving directions may be obtained from the NLA Google Earth files.
    - Results from the Site Evaluation may include driving directions and notations about site access or logistically challenging conditions on the site, which can be useful in relocating the site or helpful in anticipating special circumstances.

- **Preliminary Plan for Establishing Physical Habitat Stations:** As part of the base location activities to prepare for field work, review aerial photos and maps of the site and make a plan for laying out the PHab stations. This plan should be included in the site packet.
- **Federally Protected Species (EPA Regional and Contract Crews ONLY):** See Section 10.0.
- Any other site specific information (e.g., road construction and road closings) useful to the Field Crew.

4.1.2 Instrument Checks and Calibration

Test and calibrate instruments prior to sampling. You can calibrate instruments and probes prior to departure for the lake site or at the lake, with the exception of the DO probe. Because of the potential influence of altitude, calibrate the DO probe at the lake site (NOTE: some newer instruments may allow for calibration independent of altitude). Field instruments include a multi-parameter probe unit for measuring temperature, DO, and pH and a GPS receiver. Field crews should have access to backup instruments if any instruments fail the manufacturer performance tests or calibrations.

4.1.2.1 Depth sounding equipment

Crews are responsible for checking the accuracy of their devices against a sounding line at the beginning of the field season to verify that the device is providing accurate depth information.

4.1.2.2 Multi-parameter probe Meter Performance Test

Test and pre-calibrate the multi-parameter probe meter prior to departure from the base site, following either the Standard Operating Procedure (SOP) developed for the instrument or the manufacturer's calibration and maintenance procedures. Field crews should perform a QC check of the pH meter calibration (and conductivity meter calibration, if this optional measurement is taken) at regular intervals designated by the manufacturer. Field crews will be responsible for preparing or purchasing their own QC solution.

4.1.2.3 Global Positioning System Use and Battery Check

A GPS unit is used to locate the launch, index site and each of the physical habitat stations. Therefore, it is imperative that the Field Crew understands how to operate their GPS unit. The Global Positioning System (GPS) uses signals sent from multiple orbiting satellites to a ground-based sensor in order to fix a position on the earth. GPS uses signals sent from multiple orbiting satellites to a ground-based sensor in order to fix a position on the earth. Position accuracy depends on the Position Dilution of Precision (PDOP) which is a measure of the geometry of the satellite spread over the location of the observer. Low PDOP values are typically conveyed to the user as a measure of accuracy or precision and represent more advantageous satellite geometry and therefore less locational error. For NLA, crews should regularly monitor the accuracy reading on their GPS and should record coordinates only after achieving the lowest amount of error possible.

GPS uses many alternative mathematical models to describe the spherical shape of the earth and each is a separate datum. Commonly used datums include NAD27 CONUS, NAD83, and WGS84. Each represents a different interpretation of the shape of the earth. The NARS standard is **NAD83**. Thus, all GPS units should be switched to this standard - prior to completing any field activities. Crews should also confirm that the NAD83 datum is being used when the GPS is turned on prior to data collection. If the GPS is not

set for NAD83 and the unit cannot be changed readily, note the datum used on the data forms for later conversion.

GPS devices use a variety of units for position designation based on an imaginary latitude and longitude coordinate grid system laid across the earth (degrees, minutes, seconds, or degrees and decimal minutes, and UTM (a metric system). The NARS standard is **decimal degrees** for reporting all GPS positions.

Refer to the GPS user's manual to provide specific instructions on setting the Datum, coordinate system, and units to NLA standards. Turn on the GPS receiver and check the batteries prior to departure. Replace batteries immediately if a battery warning is displayed.

4.1.2.4 Electronic Data Capture Device Battery Check (Apple iPad)

Charge the Apple iPad each night and check the power prior to departure. External battery packs are often available for these devices if battery life is a concern.

Table 4-1 Instrument checks and calibration

Equipment	Preparation
GPS Unit	Check the batteries prior to departure Ensure map datum is set to NAD83 Ensure locational units are set to decimal degrees • latitude xx.xxxxxx; longitude -xxx.xxxxxx Perform manufacturer checks as necessary to ensure accuracy
Multi-parameter Probe	Calibrate per manufacturer guidelines (DO to be calibrated at lake) Check the batteries prior to departure Perform QC Check as directed by manufacturer and/or laboratory protocols (field crews will supply QC check solution)
Electronic Data Capture Device (Apple iPad)	Check the power prior to departure Ensure NLA Data collection App is installed, up to date, and functioning

4.1.3 Equipment and Supply Preparation

Check your inventory of supplies and equipment prior to departure using the equipment and supplies checklists provided in the Appendix; use of the lists is strongly recommended. Pack meters, probes, and sampling gear in such a way as to minimize physical shock and vibration during transport. If necessary, prepare stock preservation solutions as described in Table 4-2. Follow the regulations of the Occupational Safety and Health Administration (OSHA).

Table 4-2 Stock solutions, uses, and methods for preparation.

Solution	Use	Preparation
Bleach (1%)	Clean nets, other gear, and inside of boat.	Add 40 mL bleach to 4,000 mL distilled water.
Lugol's	Preservative for phytoplankton samples.	Lugol's will be supplied with base kit. If preparation is needed: Dissolve 100 g KI in 1 L of distilled water. Dissolve 50 g iodine (crystalline) in 100 mL glacial acetic acid. Mix these two solutions. Remove any precipitates. Store in the dark.
95% Ethanol	Preservative for benthic invertebrate samples and zooplankton samples.	No preparation needed (use stock solution as is).

Refuel vehicle(s) and conduct maintenance activities the night before a sampling trip. Check trailer lights, turn signals, and brake lights before departure. In addition, inspect your vehicles, boats, and trailers every morning before departure. Pay particular attention to the trailer hitch, electrical connections, tie downs, tire pressure, and the overall condition of the boats.

Label and package as many of the sample containers as possible in the site kit prior to departure. Container labels should not be covered with clear tape until all information is completed during sampling at the lake. Store an extra kit of sampling supplies (Cubitainers®, bottles, filters, foil, gloves, forms, pencils, permanent markers, and labels) in the vehicles. Inventory these extra supply kits prior to each lake visit. Be sure to order field sampling site kits well in advance (two week minimum) by submitting the electronic **Request** form.

4.1.4 General Equipment and Supplies for all Activities

Table 4-3 indicates equipment and supplies that will be used for all activities.

Table 4-3 Equipment and supplies – all activities.

Type	Item	Quantity
Forms	NLA App pre-installed on Apple iPad	2
Reference	NLA 2022 Field Operations Manual (FOM) NLA 2022 Quality Assurance Project Plan (QAPP) NLA 2022 Site Evaluation Guidelines (SEG) NLA 2022 Fact Sheets	1 1 1 10
Documentation	Clipboard Pencils (#2, for benthos inner sample tags) Permanent markers (fine tip, for adhesive labels) Labels Field Notebook (optional) Clear tape strips (to cover sample labels)	1 1 1 1 As needed
Collection	Access permission documents/permit (if required)	1

4.2 Lake Verification

4.2.1 Equipment and Supply List

Table 4-4 is the checklist for equipment and supplies required to conduct protocols described in this section. It is similar to, but may be somewhat different from, the checklist that is used at a base site to assure that all equipment and supplies are taken to, and available at, the lake. Field crews should use the checklist presented in this section to ensure that the equipment and supplies are organized and available on the boat in order to conduct protocols correctly and efficiently.

Table 4-4 Equipment and supplies – lake verification.

Type	Item	Quantity
Form	NLA Verification Form	1
Collection	Depth Finder (hand-held or boat mounted sonar) GPS unit (with manual, reference card, extra battery) Anchor (with 75 m line or sufficient to anchor in 50 m depth)	1 1 1-2

4.2.2 Lake Verification at the Launch Site

Before sampling activities begin, you must verify that you are at the correct lake and whether it meets the criteria for sampling. Confirming that you are at the correct lake is based on map coordinates, locational data from the GPS when possible, and any other evidence such as signs or conversations with local residents. The Design Name for the site is pre-populated in the App (UNKNOWN if no name), crews should populate the Lake Name if known. Record locational coordinates for the lake on the **Verification** form. If GPS coordinates are obtained, fill in the bubble to indicate GPS and record the latitude, longitude in decimal degrees.

Determine the number of satellites being used by the GPS and select if 3 or fewer satellites or if 4 or more satellites were used. All coordinates will be recorded in the NAD83 datum. Compare the map coordinates pre-populated in the App for the lake with the GPS coordinates you record and verify that you are at the correct lake. [Note: The map coordinates in the App represent the "labeling point" in NHD and may not be near either the index site or the launch site]. Verification that you are at the target lake can be confirmed via other methods (e.g., map, landowner confirmation). If GPS coordinates are not available, do not record any information, but try to obtain the information at a later time during the visit. A GPS location may be taken at any time during a lake visit and recorded by flagging the launch site coordinates and providing a comment.

Record directions to the lake and a description of the launch site on the **Verification** form regardless of whether the site is sampled or not. This information is very important and will be used in the future if the lake is revisited by another sampling crew. Provide information about signs, road numbers, gates, landmarks, and any additional information you feel will be useful to another sampling crew in locating this lake in the future. It is also helpful to describe the distance traveled (miles) between turns. Also describe the launch site. For example: Can the boat be launched with a trailer? Are there fees? Is the launch paved or does it consist of soft sand? What landmarks are at the launch? Due to privacy concerns, do not record landowner contact information (e.g., name, address, phone, email address) on the **Verification** form.

In addition to, or in the absence of, an accurate GPS reading, use as many of the following methods as possible to verify the site:

- Obtain confirmation from a local person familiar with the area.
- Identify confirming roads and signs.
- Compare the lake shape to that shown on a topographic map (United States Geological Survey (USGS) 7.5-minute map or equivalent).
- Determine lake position relative to identifiable topographic features shown on the map.

If the lake shape on the USGS topographic map does not correspond with the actual lake shape from your site map, and you cannot verify the lake by any other means, check "Not Verified" and provide comments on the **Verification** form. At each lake, evaluate whether or not the lake meets the NLA operational definition of a "lake":

- ≥ one ha in total surface area;
- ≥ 1,000 square meters of open water;
- ≥ one meter in depth;
- Not saline (due to saltwater intrusion or tidal influence); and

- Not used for aquaculture, disposal-tailings, mine-tailings, sewage treatment, evaporation, or other unspecified disposal use.

If the lake does not meet this definition, select the "non-target" bubble in the lake sampled section on the **Verification** form and provide an explanation for not sampling the lake. Add any additional explanation as required. (For complete details on the Site Evaluation process, refer to the companion document *Site Evaluation Guidelines* [EPA 841-B-06-003]).

Record the names of each crew member on the **Verification** form. At the launch site, document visual observations of a potential cyanobacterial bloom in the NLA App and photo document the bloom according to the reporting mechanism selected by the field crew (i.e., bloomWatch or state-specific reporting mechanism).

Regardless of whether the lake is sampled or not, the field crew must fill out and submit a **Verification** form for every lake that is visited with the intent to sample.

4.2.3 Locating Index Site

When determining lake origin, i.e., lake vs. reservoir in the field, a body of water that was a stream or river and subsequently dammed to create a lake is considered a reservoir. A lake which has had its level raised because of a dam is an “enhanced” lake and will be considered a natural lake for NLA 2022.

For natural lakes, go to the deepest point in the lake to locate the index site (or middle of the lake for reservoirs). If the deepest point exceeds 50 m in depth, do not establish the index site at this location; instead, choose a point as close to the middle of the lake as you can without exceeding 50 m in depth. The procedure below outlines sonar operation and procedures for finding the index site.

For reservoirs, the index site is located near the mid-point of the reservoir rather than at the deepest point, which may be near the dam. If this would result in an index site that is very shallow or otherwise non-representative of the reservoir as a whole, choose a point near the center of a main basin, where depths and the water column will be more representative of the reservoir.

Once in the general area, use the sonar unit to locate the deepest point (≤ 50 m). When an acceptable site is located, anchor the boat. Lower the anchor slowly to minimize disturbance to the water column and sediment. Determine the coordinates of the index site by GPS (if satellite coverage is available) and record on the **Index Samples** form. In addition, select if 3 or fewer or 4 or more satellites were used. If satellite coverage is not available at that time, try again before leaving the index site. The following is the procedure to be used to locate the index site:

1. Operate sonar unit according to manufacturer's specific operating procedures. If possible, depth readings should be made and recorded in metric units (be sure to specify units on the **Index Samples** form).
2. Use the sonar in the area expected to be the deepest. Spend no more than 30 minutes searching for the deepest point; the maximum depth for the index site is 50 meters.
3. Anchor the boat.
4. Determine the coordinates using GPS. Record GPS coordinates on the **Index Samples** form.

4.3 Post Sampling Activities

Upon return to the launch site after sampling, review all labels and completed data forms for accuracy and completeness, and make a final inspection of samples. If information is missing from the forms or labels, the Field Crew Leader gathers and records the missing information. The Field Crew Leader selects the red circle at the top of each data form after review. Other post sampling activities include: sample filtering, inspection and cleaning of sampling equipment, inventory and sample preparation, sample shipment, and communications.

4.3.1 Equipment Cleanup and Check for Invasive Species

You must inspect all equipment, including nets, boat, and trailer, and clean off any plant and animal material. This effort ensures that introductions of invasive species such as Eurasian watermilfoil (*Myriophyllum spicatum*) quagga mussels (*Dreissena rostriformis* bugensis), and zebra mussels (*Dreissena polymorpha*) do not occur between lakes. Prior to leaving a lake, drain all bilge water or live wells in the boat. Inspect, clean, and handpick plant and animal remains from vehicle, boat, motor, and trailer that contact lake water. Inspect and remove any remnants of vegetation or animal life. Before moving to the next lake, if a commercial car wash facility is available, thoroughly clean vehicle, boat, and trailer (hot water pressurized rinse – no soap). Rinse equipment and boat with 1% bleach solution or other approved biological disinfectant to prevent spread of exotics. Procedures are below.

1. Clean for biological contaminants (e.g., Eurasian watermilfoil, zebra mussels, and alewife):
    a. Prior to departing from a lake, drain all bilge water from the boat.
    b. At the lake, inspect motors, boat, and the trailer for evidence of plant fragments, especially in or near the propeller and water intakes. Remove all plant fragments.
    c. At the lake or base site, dry out and inspect nets and buckets and remove any remnant vegetation or animal life. Disinfect gear with 1% bleach solution or other approved biological disinfectant.
    d. If a commercial car wash facility is available, thoroughly clean vehicle and boat (hot water pressurized rinse--no soap).
2. Clean and dry other equipment prior to storage:
    a. Rinse chlorophyll-*a* collection bottle three times with DI water after each use.
    b. Rinse graduated cylinders, integrated sampler, and other sampling devices three times with DI water after each use.
    c. Briefly soak zooplankton nets and fishing nets in a 1% bleach solution or other approved biological disinfectant and dry after each use. Do not dry in sunlight because the mesh is photosensitive.
    d. Rinse coolers with water to clean off any dirt or debris on the outside and inside.
    e. Rinse boots and waders with water to clean off any dirt, debris, or biological contaminants on the outside and inside.
3. Inventory equipment and supply needs and request supplies via the electronic **Request** form (forms or site kits) or from the FLC (other items or urgent requests).
4. Remove multi-parameter probe meter and GPS from carrying cases and set up for pre-departure checks and calibration. If present (i.e., not using optical DO sonde), examine the oxygen membranes for cracks, wrinkles, or bubbles. Replace if necessary.
5. Recharge/replace batteries as necessary.
6. Recheck field forms from the day's sampling activities. Make corrections and completions where possible, and initial each form after review.

4.3.2 Shipment of Samples and Forms

You must ship or deliver time-sensitive samples (i.e., all chilled samples) to the appropriate analytical laboratories as soon as possible after collection and no later than the day after collection. These samples will be shipped in two groups for next day delivery. Other frozen and non-chilled samples may be shipped or delivered in batches provided they can be adequately stored. Report all sample shipments to the NARS IM Coordinator (by submitting the **Tracking** form in the NLA App) as soon as possible so that and they can be tracked if they do not arrive when expected.

Field crews are to fill out the pertinent section(s) of the **Tracking** for each sample shipment. In each section of the tracking form, the following information must be recorded:

- Package tracking number
- Date sample(s) were sent
- Sample type code:
  - ENTE – Fecal Indicator (*Enterococci*)
  - BENT – Benthic macroinvertebrates
  - CHEM – Water Chemistry
  - CHLX – Chlorophyll-*a*
  - FDNA – eDNA (index)
  - LDNA - eDNA (littoral)
  - MICZ – Algal toxin (microcystins and cylindrospermopsin)
  - PHYX – Phytoplankton
  - TRIA – Atrazine Pesticide Screen
  - ZOCN – Zooplankton coarse (150-micron mesh)
  - ZOFN – Zooplankton fine (50-micron mesh)
  - FTIS - Human health fish tissue sample
- Sample ID number from preprinted sample label
- Number of containers for each sample
- Any additional shipping comments

See APPENDIX B: SHIPPING GUIDELINES for further information.

4.3.3 Communications

After the Field Crew Leader has reviewed form content at the end of the sampling day, click the SUBMIT menu button and choose the form(s) that you wish to submit. After you have chosen which form to submit, click the green submit button at the bottom of the form list. An email will pop up on your device addressed to NARSFieldData@epa.gov. Copy yourself and any other crew members or managers and click send. To ensure that the email was sent, check the SENT mailbox on your email App and look for the recent email containing the data. If the email is not in the SENT mailbox, it was not sent and you should try again after verifying an internet connection.

After each submission, a data summary email will be sent to the email address which submitted the data. This data summary contains a list of the data forms and their most recent submission date/time as well as a list of the most critical data points collected at the site. The Field Crew Leader should review this data summary to ensure that the data forms were successfully received and that critical values are present and correct.

At any point, if it is determined that data needs to be revised or updated, crews should feel free to do so in the App and re-submit any edited data forms using the steps above. Newly revised data will supersede previous data. It is not necessary to re-submit data forms that were unchanged.

Each field crew must submit the **Verification** form in the NLA App after each site visit (whether the site is sampled or not). General communications information, including contact information for the NARS IM Center, is outlined in Section 2.1.

If the field crew documented the potential occurrence of a cyanoHAB event at one or more station in the lake, the Field Crew leader is responsible for reporting the bloom to the appropriate state, tribal and/or watershed organization. See Section 2.2.4.5 for potential reporting mechanisms.

5.0 INDEX SITE ACTIVITIES

Field crews will collect several different measurements and indicators at the index site (as described in Table 1-1): cyanoHABs assessment, a temperature, DO, and pH depth profile, secchi transparency, fish eDNA, chlorophyll-*a*, phytoplankton, algal toxins, water chemistry, atrazine, and zooplankton samples. A detailed description of the individual elements is provided below.

5.1 CyanoHABs Visual Assessment

When arriving at the index site, field crew views and documents visual observations of a potential cyanobacterial bloom in the NLA App and photo documents the bloom according to the reporting mechanism selected by the field crew (i.e., bloomWatch or state-specific reporting mechanism).

5.2 Temperature, DO, and pH profile

5.2.1 Summary of Method

Use a multi-parameter water quality meter (or sonde) to measure temperature, DO, and pH at predefined depth intervals. Measurement intervals for the profile are based on the site depth (see Section 5.2.3). Once the profile is completed, make another DO measurement at the surface and compare it to the initial reading to see if the probe is functioning correctly and holding calibration. If the lake is thermally stratified, note the top and bottom of the metalimnion based on the temperature readings (observed as a change of ≥1 °C per meter of depth).

The meters and probes are delicate; take care to avoid putting the probe into contact with sediments which could foul the probes. An accurate measure of the site depth will help prevent contact between the sediment and the probes.

5.2.2 Equipment and Supplies

Table 5-1 is the checklist for equipment and supplies required to conduct protocols described in this section.

Table 5-1 Equipment and supplies – temperature, pH, and DO profiles.

Type	Item	Quantity
Forms	NLA Profile Calibration NLA Index Profile	1 1
Collection: **Water column depth**	Depth Finder (hand-held or boat mounted sonar) Sounding line (50 m, calibrated, marked in 0.5 m intervals) with clip OR Sounding rod (calibrated) for very shallow lakes	1 1
Collection: **Profile measurements & calibration**	Multi-parameter water quality meter (with temperature, pH, and DO probes) Sounding line or 50-meter tape Sounding weight Squirt bottle (1 L Nalgene) – De-ionized (DI) Squirt bottle (1 L Nalgene) – lake water Calibration cups and standards QC check solution Barometer or elevation chart to use for calibration	1 1 1 1 1

5.2.2.1 Multi-parameter Sonde

The multi-parameter sonde must be heavy enough to minimize sway and wobbling as it is lowered and raised in the water column. The instrument must be stabilized prior to taking a reading. Experiment with the sonde prior to sampling and add weight to the cable if needed.

5.2.2.2 Temperature Meter Calibration

Check the accuracy of the sensor against a thermometer (a non-mercury type is recommended) that is traceable to the National Institute of Standards and Technology (NIST) at least once per sampling season. The entire temperature range encountered in the NLA 2022 should be incorporated in the testing procedure and a record of test results kept on file.

5.2.2.3 DO Probe Calibration

Crews are to calibrate the DO probe at the lake prior to each sampling event. The probe calibration is to be completed in the field against an atmospheric standard (ambient air saturated with water, or water saturated with air for optical probes) prior to launching the boat. In addition, manufacturers typically recommend periodic comparisons with a DO chemical analysis procedure (e.g., Winkler titration) to check accuracy and linearity. Small "mini-Winkler" titration kits are suitable for this check and can be taken into the field.

5.2.2.4 pH Meter Calibration

Calibrate the pH electrode daily, prior to each sampling event in accordance with the manufacturer's instructions and your organization's existing SOP. Conduct a QC check with a different standard to verify the calibration and periodically evaluate instrument precision (see Section 2). Ideally, a quality control solution (QCS) should be used that is similar in ionic strength to the lake water samples you will be measuring. Standard buffer solutions used to calibrate electrodes may not be representative of typical lake waters.

5.2.2.5 Conductivity Calibration

A field conductivity measurement is optional for the NLA 2022. If the Field Crew opts to take conductivity measurements, the conductivity meter must be calibrated prior to each sampling event. Calibrate the meter in accordance with the manufacturer's instructions. Ensure that the conductivity meter is temperature corrected to 25 °C.

5.2.3 **Depth Profile Procedure**

Below are the step-by-step procedures for measuring temperature, pH, and DO profiles at the index site.

1. Calibrate Instrument
    a. Check meter and probes and calibrate according to manufacturer's specifications.
    b. Enter calibration information on the **Profile Calibration** form.
2. Record Site Conditions:
    a. Observe site conditions and fill out the "Site Conditions" portion of the **Index Samples** form. Conditions to be reported include:
        i. Precipitation ("None", "Light," or "Heavy")
        ii. Surface conditions ("Flat," "Ripples," "Choppy," or "Whitecaps")
    b. Presence or absence of odor or scum. If present, select "Yes" and describe the odor or scum.

3. Determine Site Depth:
    a. Accurately measure the depth using a sounding line with a weight or other means and record on the **Index Samples** form.
    b. Indicate method used.
4. Determine Measurement Intervals:
    a. The number of readings and the depth intervals taken depends on the site depth. Below is a list of rules for determining the intervals:
        i. The profile will always begin with a measurement just below the surface (e.g., approximately 10 cm or the minimum depth required to keep all probes submerged).
        ii. The last (deepest) measurements will always be at 0.5 m above the bottom.
        iii. If the site is < 3.0 m deep, record measurements beginning just below the surface and at 0.5 m intervals, until 0.5 m above the bottom.
        iv. If the depth is between 3.0-20 m (inclusive), record measurements beginning just below the surface and then at 1.0 m intervals until reaching 0.5 m above the bottom.
        v. If the depth exceeds 20 m, record measurements beginning just below the surface, then at 1.0 m intervals until you reach 20 m, then at 2.0 m intervals until 0.5 m above the bottom.
        vi. If the metalimnion is encountered (observed as a change of ≥1 °C per meter of depth), take measurements at least every meter within the metalimnion.
5. Measure Temperature, DO, and pH:
    a. Lower the sonde in the water and measure the vertical profile of temperature, DO and pH at the predetermined depth intervals. Be careful not to let the probe touch the bottom.
    b. Record the intervals and measurements on the **Profile Data** form.
    c. Use the provided comment bubble to provide extra information about any measurements that the crew feels needs further comment or when a measurement cannot be made.
6. Duplicate Surface DO Measurement
    a. When the profile is completed, take another DO measurement at the surface, record it, and compare it to the initial surface DO reading.
    b. Mark ‘Yes’ or ‘No’ on the form if the second DO reading is within 0.5 mg/L of the initial surface reading. This provides information regarding measurement precision and possible calibration drift during the profile.
        i. If measurement is not within 0.5 mg/L, verify your calibration.
        ii. If DO is found to be out of calibration, re-calibrate and re-record DO measurements.
7. Determine the Metalimnion:
    a. If the lake is thermally stratified, note the top and bottom of the metalimnion in the Metalimnion column.
    b. For standardization purposes, the metalimnion has been defined in the protocol as an area where water temperature changes at least 1 degree Celsius per meter.
    c. If you suspect that the metalimnion exists but does not change at the specified rate, estimate the top and bottom of the metalimnion to the best of your ability, use the provided comment bubble to flag the data, and explain.
    d. In deep sites where measurement intervals are 2.0 meters apart, decrease the interval

to 1.0 meters while taking measurements within the metalimnion.

5.3 Secchi Disk Transparency

5.3.1 Summary of Method

A Secchi disk is a black and white patterned disk used to measure a lake's clarity (see Figure 5-1). Take the reading on the shady side of the boat, without sunglasses, hat, or other viewing aids. Record the depths where the disk disappears when descending and reappears when retrieving.

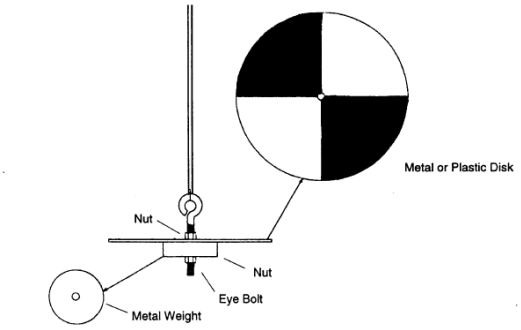


Figure 5-1 Secchi disk diagram (USEPA, 1991).

5.3.2 Equipment and Supplies

Table 5-2 is the checklist for equipment and supplies required to conduct protocols described in this section.

Table 5-2 Equipment and supplies – Secchi disk transparency.

Type	Item	Quantity
Form	NLA Index Samples	1
Collection	Metric tape measure Secchi Disk (20 cm diameter) Sounding line (50 m, calibrated, marked in 0.5 m intervals) with clip	1 1 1

5.3.3 Procedure for Determining Secchi Transparency

Because different people measuring Secchi transparency at the same site may obtain different results (due to differences in vision and interpreting disk disappearance and reappearance), it is recommended that one crew member conduct Secchi disk measurements at all lakes.

If the lake is shallow and the water clear, the Secchi disk might reach the bottom and still be visible. If this is the case, it is important to not stir up the bottom sediments while anchoring the boat. Move the boat away from the anchor before taking the reading. If the disk is visible at the bottom of the lake, indicate this by filling in the "clear to bottom" check box on the **Index Samples** form and record the water depth in both the disappearance and reappearance fields.

States that wish to take additional measurements for comparisons using a view scope are encouraged to do so after completing the Secchi disk measurements are completed following the NLA protocols.

The following procedure is to be followed when collecting NLA Secchi disk measurements:

1. Confirm that the lowering line is firmly attached to the Secchi disk.
2. Remove sunglasses and hat. Also, do not use view scopes or other visual aids. If wearing prescription sunglasses, temporarily replace them with regular clear lens prescription glasses.
3. Lower the Secchi disk over the shaded side of the boat until it disappears.
4. Read the depth indicated on the lowering line. If the disappearance depth is <1.0 meter, determine the depth to the nearest 0.05 meter by marking the line at the nearest depth marker and measuring the remaining length with a tape measure or meter stick. Otherwise, record the disappearance depth to the nearest 0.1 meter. Record the disappearance depth on the **Index Samples** form.
5. Lower the disk a bit farther and then slowly raise the disk until it reappears and record the reappearance depth, using the same level of precision as before.
6. Calculate the euphotic zone depth by multiplying the depth where the Secchi disk reappears by two. Use this calculation to determine the depth at which water samples will be taken with the integrated water sampler:
    - 6.1 If euphotic zone is less than 2 meters, water samples will be collected only within the euphotic zone.
    - 6.2 If euphotic zone is greater than 2 meters, water samples will be taken from the top 2 meters of the water column.
    - 6.3 If the Secchi is clear to the bottom and the lake is less than 2.5 m deep, water samples will be collected 0.5 m from the depth at the index site.
7. Record the depth that will be targeted for the integrated water samples.
8. Note any conditions that might affect the accuracy of the measurement in the comments field.

5.4 eDNA Sample Collection - Index

5.4.1 Summary of Method

Collect water samples for eDNA with a grab sample collected at the water surface from the boat.

5.4.2 Equipment and Supplies

Table 5-3 provides the equipment and supplies needed for field operations to collect fish eDNA samples at the index site.

Table 5-3 Equipment and supplies - index eDNA samples.

Type	Item	Quantity
Form	NLA Index Samples	1
Documentation	Label: FDNA - Index Clear tape strips (to cover sample labels)	1 As needed
Collection	Index eDNA bottle (1 L clear, square) Gloves (latex/nitrile, non-powdered)	1 1 pair
Storing and preserving	Wet ice Cooler Plastic electrical tape	As needed 1 As needed

5.4.3 Sampling Procedure

While anchored at the index site, collect a grab sample from the water surface using the 1 liter index eDNA bottle.

1. Make sure all necessary data has been written on the sample label, the label is placed on the outside of the bottle, and the label is completely covered with clear tape.
2. Put on surgical gloves (non-powdered). Do not handle any food, drink, sunscreen, or insect repellant until after the water samples have been collected, or implement measures to reduce contamination by such chemicals, if applied, such as washing, wearing long gloves, etc.
3. Remove the cap from the bottle.
4. Do not rinse the bottle and avoid touching the inside of the bottle or the inside of the cap.
5. Dip the sample container into the surface water (do not fully submerge mouth of bottle). At the surface of the water, angle the mouth of the sample container away from the boat. Collect any surface scums, if present, at the site.
6. Fill the 1 L bottle to the 1000 mL mark, leaving headspace for air. This headspace is important since the sample will be frozen.
7. If the bottle is filled above the 1000 mL mark, discard excess water.
8. Carefully replace the cap. Seal the cap with plastic electrical tape.
9. Immediately after sample is collected, place in a cooler with ice to minimize exposure to light and begin chilling the sample.
10. Upon return to the vehicle or a base location, freeze the sample. The sample will be sent frozen with dry ice.

5.5 Water Sample Collection and Preservation

5.5.1 Summary of Method

Collect water samples using an “integrated sampler”, which is based on a design by the Minnesota Pollution Control Agency (MPCA) (see

Figure 5-2). The device is a PVC tube 6.6 feet (2 meters) long with an inside diameter of 1.24 inches (3.2 centimeters) fitted with a stopper plug on one end and a valve on the other. The device allows collection

of water from the upper two meters of the water column (e.g., within the euphotic zone). If the euphotic zone is < 2.0 m deep (as calculated in the Secchi Disk Transparency section of the form), lower the integrated sampler only to the depth of the euphotic zone, and take additional grab samples as necessary to collect the total volume needed for the samples.

5.5.2 Equipment and Supplies

Table 5-4 provides the equipment and supplies needed to collect water samples at the index site.

Table 5-4 Equipment and supplies – water samples.

Type	Item	Quantity
Form	NLA Index Samples	1
Documentation	Labels: water chemistry, algal toxins, phytoplankton, atrazine, and chlorophyll-*a*	1 per sample
	Clear tape strips (to cover sample labels)	As needed
Collection	Integrated sampler device (MPCA design)	1
	Funnel	1
	Gloves (latex/nitrile, non-powdered)	1
Storing & Preservation	Cubitainer® (4L) – water chemistry	1
	HDPE bottle (60 mL, white, wide-mouth) – atrazine	1
	HDPE bottle (500 mL, white, round) – algal toxins (MICZ)	1
	HDPE bottle (1 L, white, narrow-mouth) – phytoplankton	1
	Poly bottle (2 L, brown) – chlorophyll-*a*	1
	Wet ice	As needed
	Lugol's solution (250 mL bottle)	5-10 mL
	Cooler	1
	Plastic electrical tape	As needed

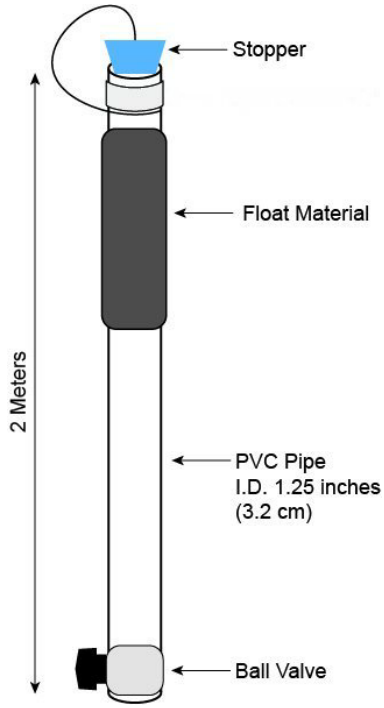


Figure 5-2 Integrated water sampler device (MPCA).

5.5.3 Sampling Procedure

Assuming the euphotic zone is ≥ 2 meters and the depth of the lakes is greater than 2.5 m, collect four integrated water samples (Figure 5-3). Sample #1 is emptied into a 2 L sample bottle for ***chlorophyll-a*** filtering. Sample #2 is to be transferred from the sampler to the 4 L Cubitainer®, mixed thoroughly, and, poured off into one 1 L sample bottle for ***phytoplankton*** processing, one 500 mL bottle for the ***algal toxins*** samples, and one 60 mL bottle for the ***atrazine*** pesticide sample. Samples #3 and #4 are to be transferred from the sampler to the 4 L Cubitainer® for the ***water chemistry*** sample. If the euphotic zone is <2 meters, only collect water from the euphotic zone and increase the number of grab samples accordingly. Additionally, if the secchi is clear to the bottom and the lake is less than 2.5 m deep, water samples will be collected 0.5 m from the depth at the index site.

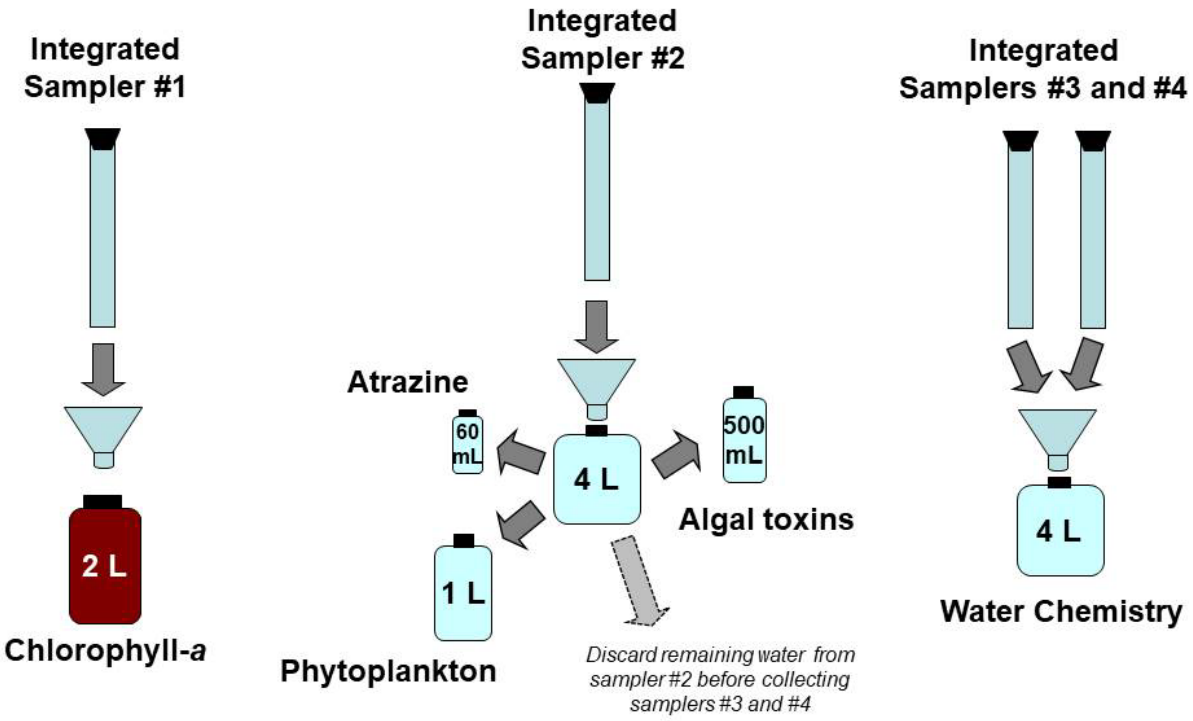


Figure 5-3 Procedure for using the integrated sampler device to collect depth integrated samples.

5.5.3.1 Sample Collection

1. Make sure all necessary data has been written on the sample labels and labels are completely covered with clear tape.
2. Put on surgical gloves (non-powdered). Do not handle any food, drink, sunscreen, or insect repellant until after samples have been collected, or implement measures to reduce contamination by such chemicals, if applied, such as washing, wearing long gloves, etc.
3. Rinse each water sample collection container and lid with surface water three times.
4. Remove the rubber stopper cap and open the valve on the bottom end of the integrated sampler. Rinse by submerging it three times in the lake and draining after each rinse. Complete rinsing on the opposite side of the boat from which you plan to sample. Do not take samples near the motor or other sources of contamination.
5. Slowly lower the sampler into the lake as vertically as possible. For a 2 m sample, stop lowering the device when the upper end is just above the surface. If the euphotic zone is < 2.0 m deep (as calculated in the Secchi Disk Transparency section of the form), the integrated sampler will be lowered only to the depth of the euphotic zone; additional

samples will be taken to collect the volume needed for the samples (8 L total). In clear shallow lakes, samples will be collected 0.5 m from the depth at the index site.

6. Cap the upper end with the rubber stopper firmly and slowly raise the sampler.
7. When the bottom of the sampler is near the surface, reach underwater and close the valve on the bottom end.
8. Lift the sampler into the boat, keeping it as vertical as possible. When possible, move the containers to a shaded area of the boat to avoid exposing the sample to direct sunlight when dispensed.
9. Carefully open the valve and dispense the contents of sample #1 (or more, as necessary to fill bottle**) into the 2 L brown bottle. This is the chlorophyll-*a* sample, which will be filtered on shore (see Section 8.2). Place the bottle on ice until filtration can be initiated.

   ***if the last sample pull does not completely fit in the container, homogenize the sample in the integrated tube prior to dispensing*
10. Repeat the collection process in steps 5-8 and dispense the contents of sample #2 (or more, as necessary) into the 4 L Cubitainer® and mix well.
11. Fill the 1 L phytoplankton bottle from the 4 L Cubitainer®, allowing enough headspace to add at least 5 mL of Lugol's preservative.
12. Fill the 500 mL bottle from the 4 L Cubitainer®, leaving headspace so that the bottle doesn't burst when frozen. This is the algal toxin sample. Seal the cap with plastic electrical tape. Place the bottle in the cooler with ice.
13. Fill the 60 mL bottle from the 4 L Cubitainer® to just below the shoulder, leaving headspace so that the bottle does not burst when frozen. This is the atrazine sample. Seal the cap with plastic electrical tape. Place the bottle in the cooler with ice.
14. Discard the residual water in the Cubitainer®.
15. Pour the contents of sample #3 and sample #4 (or more, as necessary) from the integrated sampler into the 4 L Cubitainer®, removing as much air from the Cubitainer® as possible. This is the water chemistry sample. Seal the cap with plastic electrical tape. Place the Cubitainer® in the cooler with ice.

5.5.3.2 Sample Preservation

1. All samples are to be kept in a cooler on wet ice until preservation as needed.
2. For the phytoplankton sample, add 5 mL of Lugol's solution to the 1 L phytoplankton bottle. (Fill the provided plastic transfer pipette up to the bulb with Lugol's three times to get 5 mL). Cap the bottle and invert until well mixed. The sample should resemble the color of weak tea. If needed, add additional Lugol's 2-3 mL at a time up to a maximum of 10 mL. Seal the cap with plastic electrical tape.
3. Upon return to a base location with a freezer or portable freezer, freeze the algal toxin and eDNA samples. These samples will be sent frozen with dry ice.

5.6 Zooplankton Collection

5.6.1 Summary of Method

Collect two vertical samples using a fine mesh (50 µm) and coarse mesh (150 µm) Wisconsin nets with collection bucket attached at the cod end. Each net is slowly lowered over the side of the boat into the water. The net is retrieved back to the surface at a slow, steady rate. Lift the net out of the water; rinse it from the outside to free organisms from the side of the net and to concentrate them in the collection bucket. Narcotize the organisms with an effervescent sodium bicarbonate tablet (e.g., Alka-Seltzer® tablet). Transfer the sample from the collection bucket to a 125 mL sample container and preserve each sample with 95% ethanol. You will repeat the procedure with the other net on the opposite side (or end) of the boat. The cumulative tow length for each net is 5 m. In shallow lakes, multiple tows with each net are required to achieve the cumulative tow length. The objective is to sample a sufficient volume of water to obtain at least 300 organisms per sample from all but the most oligotrophic lakes.

5.6.2 Equipment and Supplies

Table 5-5 provides the equipment and supplies needed to collect a zooplankton sample. Figure 5-4 is an illustration of the zooplankton nets and collection buckets.

Table 5-5 Equipment and supplies – zooplankton collection.

Type	Item	Quantity
Form	NLA Index Samples	1
Documentation	Labels: zooplankton course and zooplankton fine	1 per sample
	Clear tape strips (to cover sample labels)	As needed
Collection	Plankton net fine (50 µm) and collection bucket	1
	Plankton net course (150 µm) and collection bucket	1
	Sounding line (50 m, calibrated, marked in 0.5 m intervals) with clip	1
	Funnel	1
	Squirt bottle (1 L Nalgene) – de-ionized (DI)	1
	Squirt bottle (1 L Nalgene) – lake water	1
	Effervescent sodium bicarbonate (Alka seltzer) tablets	1
	Pail (narcotization and concentration chambers)	1
		2
Storing & Preservation	HDPE bottle (125 mL, white, wide-mouth)	2
	Ethanol (95%)	1
	Plastic electrical tape	As needed

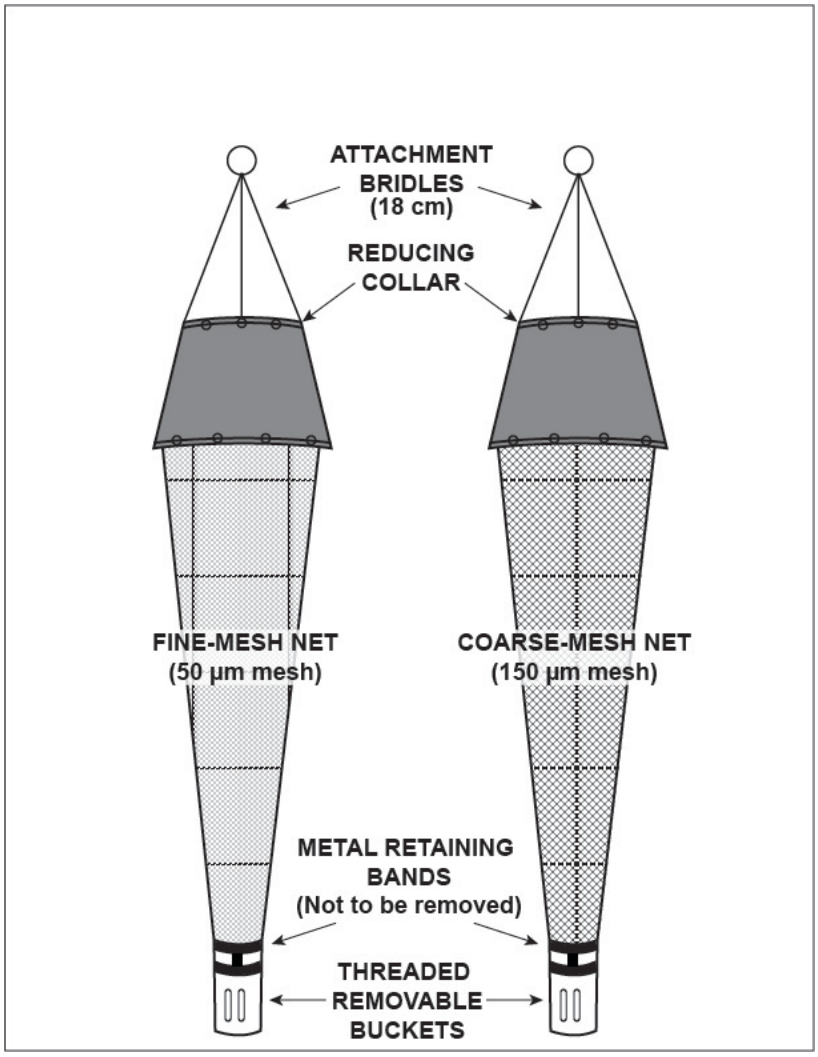


Figure 5-4 Wisconsin net and collection bucket diagram.

5.6.3 **Sampling Procedure**

The procedures for collecting and processing zooplankton samples are presented below.

5.6.3.1 Sample Collection

1. Determine and record the number of tows required to achieve the standard cumulative 5 m tow on the **Index Samples** form.
    a. For lakes deeper than 7 m, you will take a single 5 m tow with each net.
    b. For lakes with a depth less than 7 m, you will determine and record the number of tows that will be required to achieve a standard cumulative 5 m tow for each net (Table 5-6). For example, if the lake is 6 meters deep, you will take two 2.5 m tows with each net. All lakes less than 7 m deep require at least two tows because the collection bucket should never touch the sediment due to potential fouling.

Table 5-6 Lengths and numbers of zooplankton tows based on Index Site depth

Depth of lake (m)	Length of Tow	Number of Tows
7 or more	5 m	1
4 to <7	2.5 m	2
2 to <4	1 m	5
less than 2	0.5 m	10

c. The zooplankton collection methods vary slightly depending on the number of tows required to achieve a standard cumulative 5 m tow.
    i. If the number of tows = 1: follow steps 2 through 13 described below.
    ii. If the number of tows ≥ 2, follow steps 2 through 12 described below. After step 12, pour the contents of the collection bucket into a clean (i.e., DI rinsed) 1-gallon pail. Rinse the collection bucket with DI. While taking care not to tip the zooplankton sample in the pail, repeat steps 2 through 12 for the second tow. Add the contents of the collection bucket from the second tow to the pail. Continue to take zooplankton tows and add samples from the collection bucket into the pail until the target number of tows (2, 5, or 10) is reached. On the last tow, pour the contents of the pail into the collection bucket to filter the excess water. Rinse the bucket with DI water and pour the contents of this rinse into the collection bucket with the zooplankton sample. Once the zooplankton sample has been filtered down to an appropriate volume in the collection bucket, continue on to step 13.

2. Prior to each use, carefully clean and thoroughly rinse the interior of the plankton nets and buckets with DI water.
3. Carefully inspect the nets and buckets for holes or tears.
4. Attach the collection buckets to the “cod” end of the nets and secure. Make sure the correct bucket is attached to the correct net (i.e., the mesh sizes match).
5. Attach the bridled end of the plankton net to a 0.25” calibrated line with markings every 0.5 m (use the same line as was used with the Secchi disk).
6. Carefully and slowly lower the first net in a constant upright position over the side of the boat.
7. Continue lowering the net to the correct depth (remember to account for the length of the bridle). If more than one tow is needed, be sure to take additional tows from different locations around the boat.
8. Retrieve the net by pulling back to the surface at a steady rate (0.3 m or 1 ft/s) without stopping.
9. Once at the surface, slowly dip the net up and down in the water without submersing the net mouth to rinse contents into the collection bucket.
10. Complete the rinsing of the net contents by spraying lake water against the outside of the net with a squirt bottle or similar tool. Be careful not to splash or squirt lake water into the net mouth, or additional organisms may be collected.
11. If additional rinsing is needed on the interior of the net, use a squirt bottle with DI water only to avoid introducing additional organisms.

12. Once all organisms have been rinsed into the collection bucket, hold the collection bucket in a vertical position, and carefully remove the bucket from the net.
13. Repeat steps 5-12 with the second net on the opposite side (or end) of the boat.

5.6.3.2 Sample Processing

1. Set the collection buckets in a pail filled half full of lake water to which 2 CO_2 (e.g., Alka-Seltzer) tablets have been added outside of the collection buckets. Wait 30 to 60 seconds for the CO_2 tablets to dissolve before placing the collection buckets into the water. Ensure that the organisms in the collection buckets are submerged in the water, but be careful not to submerge the top of the collection buckets, or sample loss will occur. The CO_2 narcotizes the zooplankton to relax their external structure prior to preservation in 95% ethanol. This facilitates taxonomic identification. Wait until zooplankton movement has stopped (usually about one minute). Raising and lowering the collection buckets in the pail can help water exchange within the bucket.
2. Check the sample label on the bottle to verify which sample has been collected (coarse or fine mesh).
3. Use small volumes of DI water from a squirt bottle to rinse the contents of the mesh net collection bucket into the 125 mL polyethylene bottle. Rinse the collection bucket with DI water three to four times or until the majority of zooplankton have been removed without allowing the bottle to fill more than half full (~60-70 mL of sample and rinse water combined). After the zooplankton have been transferred and the sample bottle is half full with sample and rinsate, fill the bottle to the shoulder with 95% ethanol. Use a funnel, if necessary, when transferring the sample, rinsate or ethanol to the 125 mL sample bottle
4. In some cases, the volume of zooplankton collected in the collection bucket may exceed 125 mL. Under this scenario do not try to force the entire sample into a single bottle, or the preservative will not function properly and the sample may be lost. In such cases, fill the first bottle half full, and then use a second bottle to preserve the additional amount of sample. Use an “extra jar” label (i.e., one with no sample ID pre-printed on it) for identification purposes. Complete the label and print in the sample number assigned to the first container on the label of the second container. On the form, record a “2” in the “No. Jars” field.
5. On the **Index Samples** form, fill in the bubble to indicate that the sample is preserved.
6. Verify that all information on the labels and the form is complete and correctly recorded.
7. Repeat steps 2-6 for the second sample collected.

6.0 LITTORAL AND SHORELINE ACTIVITIES

To better understand near-shore habitats and their condition, travel to 10 evenly spaced physical habitat (PHab) stations around the lake and document conditions and characteristics observed within defined plot areas. The full set of measurements, observations, and sampling described in this chapter includes:

- water sample for eDNA analyses at each of the 10 stations;
- measures or observations of physical habitat cover and structure in the littoral, shoreline, draw-down, and riparian zone plots at the 10 PHab stations;
- visual assessment of a cyanobacterial bloom;
- sampling of benthic macroinvertebrates at each of the 10 stations; and
- water sample for Enterococci at the last station sampled (for lakes 10,000 ha or less) or boat launch (for lakes >10,000 ha).

For lakes with a surface area of greater than 10,000 ha (defined as large lakes), crews are **not** required to travel to the 10 PHab stations to make physical habitat measurements and collect benthic macroinvertebrate samples. The requirement was waived on large lakes because of the increased level of effort required to travel around the shorelines of these large lakes. Nevertheless, we encourage crews to complete the physical habitat characterizations and macroinvertebrate collections on large lakes, just as they are done on smaller lakes, so that large lake physical habitat information can be included in the national assessment.

Note: When large islands are present, more than 10 PHab sites will be identified and assessed for a lake.

6.1 Littoral eDNA Sample Collection

6.1.1 Summary of Method

Collect a grab sample for eDNA at each of the 10 shoreline PHab stations. Grab samples are collected at the water surface from the boat and added to a single 1L littoral composite sample. If more than 10 PHab stations are identified for the lake, littoral eDNA samples will only be collected from the 10 standard PHab stations around the perimeter of the lake. For “large lakes” (greater than 10,000 ha) the entire 1L sample is to be collected from the launch site at the end of the day with the enterococci sample.

6.1.2 Equipment and Supplies

Table 6-1 provides the equipment and supplies needed for field operations to collect the littoral eDNA sample.

Table 6-1. Equipment and supplies – fish littoral eDNA samples.

Type	Item	Quantity
Form	NLA Littoral Samples	1
Documentation	Label: LDNA - littoral Clear tape strips (to cover sample labels)	1 As needed
Collection	Littoral eDNA bottle (1 L clear, square) Littoral eDNA collection bottle (125 mL clear, square) Gloves (latex/nitrile, non-powdered)	1 1 1-2 pairs
Storing and preserving	Wet ice Cooler Plastic electrical tape	As needed 1 As needed

6.1.3 Sampling Procedure

When arriving at the PHab station, prior to the collection of PHab and benthic macroinvertebrate sample , collect a 100 mL littoral eDNA sub-sample from an undisturbed area in the littoral plot. Sub-samples from all stations are added to the 1 L littoral eDNA composite bottle.

1. At the first PHab station, make sure all necessary data has been written on the littoral eDNA label, the label is placed on the outside of the 1L littoral eDNA bottle, and the label is completely covered with clear tape. Samples from 10 physical habitat stations will be added to this bottle.
2. Put on surgical gloves (non-powdered). Do not handle any food, drink, sunscreen, or insect repellant until after the water samples have been collected, or implement measures to reduce contamination by such chemicals, if applied, such as washing, wearing long gloves, etc.
3. Identify an area in or near the littoral plot that is about 1 m deep (~waist deep) to collect the sample. Avoid sampling shallower water and water at the shoreline due to potential sediment contamination. If the entire littoral plot is less than 1 m deep, you may collect the sample outside the littoral plot (e.g., as you are approaching the plot from a location that is about 1 m deep).
4. Remove the cap from the 125 mL collection bottle. Avoid touching the inside of the bottle or the inside of the cap.
6. Dip the sample container into the surface water (do not fully submerge mouth of bottle) in an undisturbed area of the lake. At the surface of the water, angle the mouth of the sample container away from the boat or body and fill the collection bottle to the 100 mL mark. Collect any surface scums, if present, at the site. If more than 100 mL is collected, pour off excess.Remove the cap from the 1L composite bottle. Do not rinse this bottle and avoid touching the inside of the bottle or the inside of the cap.

7. Add the 100 mL sample to the 1 L composite bottle. Cap both bottles and place both bottles in a cooler with wet ice while transporting to the next station to minimize exposure to light and begin chilling the sample.
8. REPEAT steps 2-6 at the next PHab station, up to a total of ten stations (1L sample total).
9. At the last (10th) PHab station, ensure the sample does not exceed the 1000 mL mark, leaving headspace for air. This headspace is important since the sample will be frozen. If the bottle is filled above the 1000 mL mark, cap the bottle, gently mix 3 times, and discard excess water down to the 1000 mL mark.
10. Carefully replace the cap. Seal the cap with plastic electrical tape.
11. Immediately place the 1L bottle in a cooler with ice and keep the sample chilled throughout the sampling day.
12. Discard the 125 mL collection bottle after collecting all 10 subsamples. A new collection bottle will be sent in each site kit.
13. Upon return to the vehicle or a base location, freeze the sample. The sample will be sent frozen with dry ice.

**At large lakes (lakes over 10,000 hectares) when littoral sampling is not conducted, the entire 1L littoral eDNA sample will be collected at the launch site with the enterococci sample.

6.2 Physical Habitat Characterization

6.2.1 Summary of Method

Prior to the sampling visit, determine the approximate locations of the 10 PHab stations and mark them on a Site Map (see Chapter 3), if applicable. Figure 3-2 shows example placement and distribution of PHab stations around a lake. At each of the 10 PHab stations, you will set up a plot as shown in Figure 6-1 based on visually estimated dimensions. The plot measures 15 m wide and includes: a littoral plot extending 10 m out from the shoreline; a drawdown zone plot extending inland from the shoreline to the normal high-water level; a 1 m shoreline zone band at the shore just above the present water line; and a 15 m wide riparian plot that begins at the normal high water mark and extends 15 m landward. The drawdown zone plot extends a variable distance inland depending on the degree of drawdown and, if the distance from the present shoreline to the normal high water mark is negligible (i.e., <1m), there will be no drawdown zone plot.

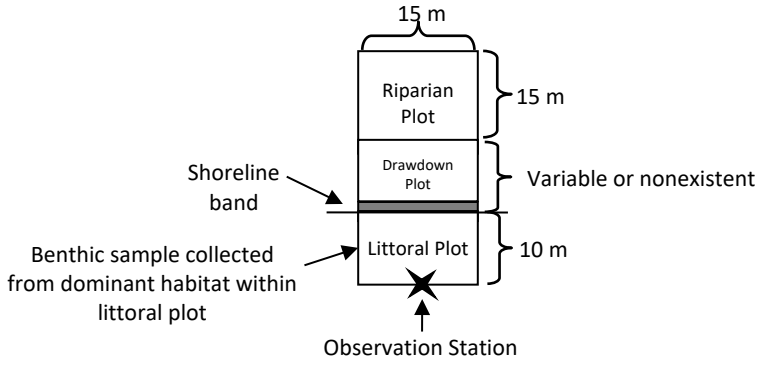


Figure 6-1 Dimensions and layout of a physical habitat station.

As described below, you will record observations from each of the zones on the **Physical Habitat** form.

6.2.2 Equipment and Supplies

Table 6-2 lists the equipment and supplies needed to locate the PHab stations and conduct the physical habitat characterization.

Table 6-2 Equipment and supplies – physical habitat assessment.

Type	Item	Quantity
Forms	NLA Verification	1
	NLA Physical Habitat	10-12
Collection	Depth Sounder (hand-held or boat mounted sonar)	1
	Sounding rod (3 m, marked in 0.1 m increments, calibrated, PVC)	1
	GPS unit (with manual, reference card, extra battery)	1
	Rangefinder (for estimating horizontal drawdown)	1 (optional)
	Clinometer (for use as a level to measure vertical drawdown)	1 (optional)
	Surveyors rod (for measuring vertical drawdown)	1 (optional)
	50-meter tape (measurements as needed)	1
	Binoculars (for making observations of distant riparian)	1
	Map wheel or string (for measuring shoreline distances on site map)	1
	Anchor (with 75 m line or sufficient to anchor in 50 m depth)	1
	Buoy (for marking observation point)	1

6.2.3 Locating the Physical Habitat Stations and Defining the Shoreline Boundary

6.2.3.1 Base Site Activities

It is important that you set up PHab stations in the office to minimize bias in site selection and to ensure efficient location of stations once at the lake.

1. Using a lake map, select a random starting point on the lake outline. Any reasonable method may be used select the starting point (e.g., toss a pencil randomly on the map, letting the sharp end point to the nearest shoreline location). This random starting point is your “A” station.
2. It is important that the remaining nine stations be located at equal distances around the lake (see Figure 3-2). These will be your “B” through “J” stations. Field crews can do this manually (by either using a string to trace the perimeter of the lake, which can then be straightened and marked in equal intervals, or by using a map wheel) or electronically (with GIS or other digital mapping tools) to measure the perimeter of the lake and divide by 10.
3. Using a GIS or other digital mapping tool application to locate the coordinates of the 10 stations that can then be entered as GPS waypoints greatly facilitates correctly locating PHab stations by boat in the field, especially on large lakes.
4. Mark the physical habitat stations on a site map, if applicable.

Note: In revisit lakes (see Section 9.1 for more information), crews will re-randomize and relocate the PHab stations. The stations are re-randomized because the revisit data is used to examine variability of the entire lake assessment.

6.2.3.2 Littoral and Shoreline Activities

Using the site maps and GPS, proceed by boat around the lake, locate, and stop at each of the 10 PHab stations. Position the boat at a distance of 10 m from shore, anchor if necessary, and make the measurements and semi-quantitative observations specified on the **Physical Habitat** form in the NLA App. Complete a separate **Physical Habitat** form for each station (see tabs A-L at the top of the form).

Make every reasonable attempt to record physical habitat observations and measurements for all 10 PHab stations. Where physical habitat observation and measurements are impossible, record comments as specified in Table 3-1.

6.2.3.3 Shoreline and Station Location Adjustments

Once in the field, you may encounter situations that require you to modify the shoreline and/or station location(s) from the intended locations marked on the site map. If this occurs, make the corrections and adjustments on the **Physical Habitat** form and note the reasons in the comments section of the form. The general guidelines for locating or modifying the location of the littoral and shoreline stations are summarized below.

1. Locate station using maps, aerial photos, or GPS units.
2. Define shore as either the current waterline OR the boundary between open water and the edge of dense vegetation (terrestrial, wetland, or emergent vegetation) or extensive very shallow water (shoreline defined by limit for navigating your boat).
3. If the shoreline observed in the field differs from the mapped shoreline, mark “Station Relocated” and enter a comment on the **Physical Habitat** form stating the apparent reason (e.g., drought, lake drawdown, flooding, dredging, limited boat access, etc.).
4. If a PHab station is inaccessible because of shoreline changes, mark “Station Relocated” at the top of the **Physical Habitat** form, and position one or more new stations at approximately equal intervals.
5. If a station is eliminated, select the check box at the top of the form.
6. If the shoreline observed in the field differs radically from the site map and you are sure you are at the correct lake, you can sketch a map of the lake. Use a string to measure the new outline, divide it into 10 equal parts, and lay out the 10 station locations.

6.2.3.3.1 Islands

Islands may be an additional source of shoreline habitat on a lake and those will be accounted for by adding island physical habitat stations. Island stations are in addition to the 10 stations (A-J). The guidelines for adding island stations follow:

- If the combined shoreline of all islands make up 10-20% of the lake’s total shoreline, add one PHab station (stations will now be labeled A through K)
- If the combined shoreline of all islands make up more than 20% of the lake’s total shoreline, add two PHab stations (stations will now be labeled stations A through L)
- Island stations are designated by marking the “Is it an island?” bubble on the form, by a new station letter (K or L), and by marking the island location and station on a site map.

Island stations, i.e., K and L, should be selected at random (e.g., toss a pencil randomly on a map, letting the sharp end point to the nearest island location, if needed, repeat to identify station L).

6.2.3.3.2 Ambiguous Shorelines

The shoreline is defined as the interface between "lake-like" conditions and riparian or wetland conditions. In most cases, the shoreline will be easily identified as the current waterline. In some instances, however, the shoreline might not be obvious. Listed below are some general situations and rules that should be applied to ambiguous shorelines.

- If there has been a significant drop in lake level due to drought, purposeful drawdown, dam repair, or other reasons, shallow areas may be exposed that are usually covered with water. In this case, consider the current waterline as shoreline for the purposes of this survey, not the normal waterline.
- If there are extensive very shallow areas or shoals, consider the shoreline to be the boundary between the shallow area and deeper open water, as defined by ease of access by a small sampling boat.
- If access to the true shoreline is prevented by an area of dense aquatic or terrestrial vegetation, consider the shoreline to be the boundary between the vegetation and deeper open water. Again, define the operational shoreline by ease of access by a small sampling boat. This may result in a riparian zone that can be more of a wetland than an upland vegetation plot.
- If a river or stream enters a lake, the shoreline begins where no flow is visible.
- If there is flooding, try to find the position of the normal high water mark. The normal high water mark may be evident by the extent of flooded trees or other terrestrial vegetation.

6.2.3.3.3 Actual shoreline is different than appears on the map

The goal of the physical habitat survey is to characterize the lakeshore based on observations of conditions at 10 evenly spaced PHab stations around the lake. Adjustments to station locations might be needed if the field crew runs into unusual conditions or problems. Below are some rules concerning modifications to the station location(s).

- If only a small portion of the shoreline differs and it does not affect, or only slightly affects, a PHab site location, sketch the lake shoreline on the site map and reposition the station (if needed).
- If the difference causes a contraction of the shoreline and a PHab station location is lost, sketch the lake shoreline on the site map and make a decision to either: (a) keep the station, relocate it on the revised shoreline map and adjust some or all other stations in order to keep stations evenly spaced around the lake (i.e., keep 10 stations), or (b) eliminate the station altogether (i.e., reduce the number of stations).
- If the Site Map does not in any way match the lake shoreline, draw a new sketch map approximating the shoreline, and re-establish the 10 PHab stations. A quick way to locate 10 evenly-spaced PHab stations is to: (a) lay a piece of string on the lake perimeter, (b) pick up the string, measure it, and mark out 10 equal parts, and (c) lay the string back on the perimeter and use the marks to locate the 10 sites on the map.

6.2.3.3.4 PHab Station is inaccessible

- If a PHab station is inaccessible, you must make a decision to either: (a) relocate the station and adjust some or all other stations so that they are evenly spaced around the lake (i.e., keep 10 stations), or (b) eliminate the station altogether (i.e., reduce the number of

stations). The size of the lake will help drive this decision.

- Draw all adjustments to the shoreline based on field observations directly on the Site Map and explain the adjustments in the comments section of the **Physical Habitat** form.

6.2.3.3.5 Identifying Relocated and New Stations on the Form

Use the following notations when recording station location modifications.

- If you relocate a station, note the new location on the Site Map and fill in the bubble corresponding to the original station letter (e.g., "C") on the **Physical Habitat** form. In addition, fill in the "Station Relocated" bubble on the form to indicate that the station has been moved from its originally intended location.
- If a station is lost and cannot be replaced, cross out the original station location on the Site Map, fill in the bubble corresponding to the original station letter, and fill in the "Dropped" bubble on the **Physical Habitat** form.

6.2.4 **Establishing the Physical Habitat Plots at each station**

Establish a plot for physical habitat characterization at each PHab station by visually estimating the plot dimensions. Most littoral, shore, and near-shore observations and measurements can be made from the boat at the observation point 10 m off-shore (estimated by eye). Limit observations at each station to the area that is within the defined plot dimensions (additional observations of human activities are made adjacent to or behind the defined plots). After setting plot dimensions, you may need to move around within the littoral plot to see or probe the bottom, or even go onto shore to make observations.

6.2.4.1 Physical Habitat Plot Dimensions

You will identify up to four distinct zones within each physical habitat plot (Figure 6-1), where you estimate the zone dimensions by eye or with the aid of a rangefinder.

6.2.4.1.1 Littoral

This within-lake zone is a fixed size that is 15 m wide along the shoreline and extending 10 m offshore into the lake.

6.2.4.1.2 Shoreline

The shoreline band is a fixed 15 m wide strip along the shore from the present water line to 1 m inland. If the horizontal drawdown distance is <1 m, the shoreline band is within the riparian plot; if the horizontal drawdown is ≥1 m, the shoreline band is within the drawdown plot. The shoreline boundary is defined as the approximate interface between "lake-like" conditions and riparian or wetland conditions. In cases where the lake shoreline is not obvious (e.g., where there is evidence of large seasonal change in lake level), define the shoreline as the current waterline. In cases where the lake shoreline is not visible, define the lake shoreline as the approximate boundary between open water and swamp or marsh conditions into which your boat could not easily move.

6.2.4.1.3 Drawdown

Under all circumstances, vertical height and horizontal distance of drawdown or water level fluctuations are measured or estimated. When horizontal drawdown is ≥1 m, establish a drawdown zone plot with a fixed width (15 m) but with a variable extent inland. The inland extent of the drawdown plot is equal to the horizontal extent of drawdown and may differ among the 10 PHab stations, depending on the topography at each station. It is determined by your judgment and measurement of the horizontal

drawdown distance from the shore to the normal high water mark at each station.

The vertical height can be visually estimated or measured using a clinometer as a level in combination with a survey rod or metric tape measure. Similarly, the horizontal distance up the bank between the current lake level and the evidence of the normal high water level can be estimated visually, or measured with with a laser range finder, survey rod or metric tape measure when distances are greater than approximately 15m.

The intent of the drawdown assessment is to capture and track changes in the magnitude of lake level fluctuation or progressive decline or drawdown of lake levels relative to the normal high water mark. The normal high water mark is best indicated by the terrestrial vegetation and substrate present. In most regions of the US, summertime lake levels will be below the high water mark, so the NLA assessment may register a small amount of drawdown for most lakes. Negligible drawdown depends, to some extent, on the size of the lake. For NLA, an assessment of the vegetation and potential fish cover in the drawdown plot is not required when horizontal exposure of the littoral bottom is <1m. The vertical and horizontal drawdown must still be measured and recorded in order to establish the range of natural variability in lake level fluctuations. If no drawdown is present at the lake, enter zeros for both the vertical and horizontal drawdown distances.

6.2.4.1.4 Riparian

At stations that have <1 m horizontal distance of lake-level fluctuations or anthropogenic drawdown, there will be no drawdown zone and you will therefore define the riparian plot as a 15 m x 15 m square located at the water's edge, as presented in Figure 6-1, extending 15 m inland. When a drawdown plot is defined (i.e., stations that have ≥1m horizontal drawdown), the riparian zone plot is a 15m x 15m square just inland of the drawdown plot (i.e., a fixed size 15 m wide along the shoreline and extending 15m inland from the normal high-water mark).

6.2.4.2 Physical Habitat Station Layout Procedures

6.2.4.2.1 Normal High Water

1. Using the present shoreline, place the littoral plots lakeward from the current water's edge or the operational shoreline as defined in previous sections.
2. Draw or sight a straight line inland perpendicular to the shoreline. If this line does not intersect with the normal high water mark, move the littoral plot laterally in either direction until the perpendicular line intersects the normal high water mark. Choose to move the plot left or right based on the direction which results in the least distance moved.
3. Establish a 1 m shoreline band at the current water's edge or the operational shoreline.
4. Establish the riparian plot inland from the current water's edge or the operational shoreline identified above. If using an operational shoreline, the riparian plot may include shallow water and/ or impenetrable wetland.
5. The left and right edges of the riparian plot, the shoreline band, and the littoral plot should all align with one another.

6.2.4.2.2 Below Normal High Water (Drawdown)

1. Using the present shoreline, place the littoral plots from the current water's edge or the operational shroreline lakeward as defined in previous sections.

2. Draw or sight a straight line inland perpendicular to the shoreline. If this line does not intersect with the normal high water mark, move the littoral plot laterally in either direction until the perpendicular line intersects the normal high water mark. Choose to move the plot left or right based on the direction which results in the least distance moved.
3. Establish a 1 m shoreline band at the current water's edge or the operational shoreline.
4. Establish the riparian plot inland from the normal high water mark identified above.
5. Establish a drawdown plot between the littoral and riparian plots. If the drawdown plot is greater than 1 m, cover estimates will be assessed in this plot.
6. The left and right edges of the riparian plot, the shoreline band, the littoral plot, and the drawdown plot should all align with one another.

6.2.4.2.3 Above Normal High Water (Flooding)

1. Establish the littoral plots lakeward from the normal high water mark, which may be evident by the extent of flooded trees or other terrestrial vegetation, lakeward.
2. Establish the riparian plot inland from the normal high water mark. In flooded situations, the riparian zone might be dry, partly flooded, or completely flooded with lake water.
3. Establish a 1 m shoreline band at the current water's edge. In flooded situations, the shoreline band might be within or inland from the riparian plot.
4. The left and right edges of the riparian plot, shoreline band, and the littoral plot should all align with one another.

6.2.5 **General Observations**

Begin the physical habitat characterization with general observations.

1. Set up your plots within your physical habitat station.
2. Measure and record the lake depth 10 m from the shore at each PHab station (observation point)
3. Note on the **Physical Habitat** form whether there is shoreline flooding (i.e., observed water level presently above the normal high water mark) by checking the box.
    a. If flooding is present, try to find the position of the normal high water mark, which may be evident by the extent of flooded trees or other terrestrial vegetation. Measure the depth at this point; record this as the "depth of flooding." Measure or estimate the distance from this normal high water position landward towards the margin of flooding; this establishes the location of the 1m shoreline band and the horizontal distance of flooding. The riparian plot is located as a 15 x 15 meter square abutting the normal high water mark, regardless of whether it is dry, partly flooded, or completely flooded with lake water.
    b. If the current water level is not above normal high water, enter zeros for height and horizontal distance of flooding.
4. Note on the **Physical Habitat** form whether the horizontal drawdown distance is greater than 1m by checking the box. **Regardless of the amount of drawdown of lake level fluctuation, measure or estimate it by recording the vertical height and the horizontal distance between the present lake level and the normal high water line**. Note that these measurements or estimates may be zeros if the water level is at or above the normal high-water mark, but should

never be left blank. If the horizontal drawdown distance is <1m, the drawdown zone cover and human influence estimates are left blank in the form.

5. Record the bank angle description that best reflects the current shoreline that is dominant within your field of vision in the 1 m shoreline band:
    a. Near vertical/undercut (>75 degrees);
    b. steep (>30 to 75 degrees; need hands to climb up);
    c. gradual, (5 to 30 degrees; can walk up); or
    d. flat (< 5 degrees).

    NOTE: Complete this estimate even if there is no drawdown.
6. Record the presence or absence of water surface scums, algal mats, or oil slicks within the littoral zone.

6.2.6 Estimate Substrate Characteristics

You will estimate and assign percentage areal cover for substrate types (e.g. bedrock, boulders, cobble, gravel, sand, silt/clay/muck, woody debris, organic matter, and vegetation) and also for fish habitat cover, aquatic macrophytes, and terrestrial vegetation. The categories are as follows:

- 0 = absent (0% cover)
- 1 = sparse (<10% cover)
- 2 = moderate (10 – 40% cover)
- 3 = heavy (>40 – 75% cover)
- 4 = very heavy (>75% cover)

When estimating cover combinations in the substrate section of the **Physical Habitat** form, combinations consider that the combined cover of the various types of substrate should add up to approximately 100%. Because you are assigning cover percentage categories, look at various combinations of the high and low end of each class. Accordingly, more than one class might be given sparse (1), moderate (2), or heavy (3) ratings. One dominant class with no clear subdominant class might be ranked very heavy (4) with all the remaining classes either sparse (1) or absent (0). Two dominant classes with more than 40 percent cover can both be given a 3.

Estimate the areal cover of bottom substrate types and particle size classes observed within the littoral and the shoreline zones. Cover categories range from absent to very heavy. Record all observations by filling in the appropriate bubbles on the **Physical Habitat** form. In most cases these estimates can be made from the boat or can be made while wading, if preferred. Substrate types not present should be assigned zero cover.

- If the bottom substrate is not visible, you should probe the bottom beneath the boat with the sounding rod (you may have to move closer to shore if you are too deep to use the rod). Soft sediment can be brought to the surface for examination. Hard sediments can be "felt" with the sounding rod. Sandy substrate can be "felt" or "heard" by twisting the sounding rod and detecting grittiness. Estimating cover of various substrate types will typically require multiple probes within the littoral plot. If you have to move into shallow water to use the sounding rod to observe sediment characteristics, use the provided comment bubble to flag the observation and record the depth where you observed the sediment.
- If the bottom is covered with materials other than mineral substrates, choose “Woody Debris”, “Organic (leaf pack, detritus)”, or “Vegetation/Other”.

- If the substrate is concealed and remote sampling is not possible, identify that the substrate is “not observed” in a comment bubble associated with each zone.

Record the color of sediment within the littoral plot. Select "None” or “Other" if the sediment does not match one of the color categories options on the **Physical Habitat** form.

Record sediment odor within the littoral zone. For sediment odor, the choices are "H_2S" (sulfurous, rotten egg), "Anoxic" (sewage odor), "Chemical" (strong odor like turpentine, paint, etc.), "Oil", or “Other" (including musty, organic, and fishy odors). If "Other" is indicated, explain the observation in the comment section of the form.

6.2.7 Estimate Aquatic Macrophyte Cover

Note and record whether macrophytes extend lake-ward from the observation point (e.g., further than 10 meters from the shoreline).

Estimate the areal cover of submerged, emergent (i.e., has erect portions above the water surface), floating/floating leafed (either rooted or non-rooted vegetation), and total macrophytes within the littoral zone. Cover categories range from absent to very heavy, as described in 6.2.6. Each of the three types of aquatic macrophyte (submergent, emergent, floating) can have cover ranging from 0 – 100%. They are evaluated independently, so the sum of their separate covers is theoretically 0% to 100% times the number of types. So, in contrast to substrate cover percentages, the combined cover of submergent, floating, and emergent aquatic macrophytes could theoretically add up to 300% due to the overlap of plant types within the water column. The fourth question on the form about aquatic macrophyte cover asks how much areal cover is there if you ignore the types and just estimate how much of the littoral plot has aquatic macrophyte cover of any type, where this value is constrained to 0-100%.

As with substrate, estimating aquatic macrophyte cover may require multiple probes within the littoral plot. Record all observations by filling in the appropriate bubbles on the **Physical Habitat** form. These estimates can be made from the boat or while wading.

- If you cannot see or probe the bottom, move closer to shore, use the provided comment bubble to flag your observation and note your new location and depth in the comments field on the form.

6.2.8 Estimate Fish Habitat Cover

Estimate the areal cover of potential fish habitat observed within the littoral plot and, when present, in the separate drawdown zone plot. Littoral fish habitat cover features are within or partially within the water and conceal fish from aquatic and terrestrial predators such as large fish, otters, kingfishers, and osprey. When evaluating cover of potential fish habitat in the drawdown zone, however, estimate the percentage cover that would be present at normal high water conditions, when these features would be inundated. Cover categories range from absent to very heavy, as specified in Section 6.2.6. Record all observations by filling in the appropriate bubbles on the **Physical Habitat** form. In most cases these estimates can be made from the boat. Estimating fish habitat cover may require multiple probes within the littoral plot. If certain cover types are not present, mark absent (0% cover) as your entry. In contrast to the cover of substrate types, the sum of the various types of fish habitat features can exceed 100%, sometimes summing to several hundred percent. Each type of fish cover can have cover ranging from 0 to 100%. They are evaluated independently, so the sum of their covers is theoretically 0 to 100% times the number of cover types.

Estimate and record cover for the following fish habitat types:

- Aquatic macrophytes and Inundated Herbaceous Vegetation: Submerged, floating, or emergent live aquatic or non-woody herbaceous plants
- Woody Debris/Snags: Inundated or partially inundated dead trees, branches, or rootwads with diameter >0.3 m (1 ft)
- Woody brush/woody debris: Inundated dead or living woody vegetation <0.3 m diameter.
- Inundated Live Trees: Inundated portions of trees >0.3 m in diameter
- Overhanging Vegetation: <1 m from the water surface (do not include higher overhanging vegetation, which might provide perches for birds such as kingfishers)
- Ledges or Sharp Drop-offs: Overhanging banks, submerged rock shelves, and steep sloping rock walls
- Boulders: Larger than basketball size
- Human Structures: Docks, barges, houseboats, swimming platforms, tires, car bodies, and habitat enhancement structures (e.g., log rafts)

Note: In the drawdown zone you will estimate the potential fish cover. The potential fish cover estimates are made only if there is a visible drawdown zone extending ≥1 m from the shoreline. For these observations, the question is “What cover would there be if the drawdown zone were inundated – i.e., it were to become part of the littoral zone? “ Then, for example, a bunch of dried aquatic macrophtes would be “Aquatic and Inundated Herbaceous Vegetation”, as would newly-grown terrestrial grasses. Cyprus trees left “high and dry” would qualify as “Inundated Live Trees >0.3m diameter” and overhanging vegetation rooted above the drawdown zone could be “Overhanging Vegetation within 1m of the Surface”.

6.2.9 **Estimate the Cover and Type of Riparian and Drawdown Zone Vegetation**

Three independent layers of riparian vegetation will be examined, each of which can range in cover from 0 to 100%. The ground layer components must add up to 100% because this layer includes bare ground and water. The mid-layer and upper layer do not include ground or water, so vegetation within these layers do not have to add up to 100%.

The areal cover of different types of vegetation will be estimated in the riparian plot and, when present, in the drawdown zone. Vegetation cover is divided into three layers, which are described below. Remember that individual plants can contribute cover to more than one layer and cumulatively the three layers can potentially add up to 300% cover (up to 100% per layer). Also note that some things other than vegetation are possible entries for the "Ground Cover" layer (e.g., water or barren ground). Within each layer, the summed cover cannot exceed 100%, but, the ground layer covers must add up to 100%. As with the other visual cover estimates, you are assigning cover percentage categories, so the summed cover is estimated by looking at various combinations of the high and low end of each percentage cover class.

6.2.9.1 Canopy Vegetation (greater than 5 m high)

Record the type of vegetation in the canopy as deciduous, coniferous (needle-leafed evergreen), broadleaf evergreen, or mixed, where mixed is defined as a segment where chosen if there is more than one of these types of vegetation that has at least 10% areal coverage. If no canopy exists in the plot, do not mark any of the bubbles.

Estimate the areal cover of big (trunk >0.3 m diameter at breast height [dbh]) and small (trunk <0.3 m dbh) trees. Cover categories range from absent to very heavy, as described in Section 6.2.6. Record all observations by filling in the appropriate bubbles on the **Physical Habitat** form.

Total cover in the canopy layer can range anywhere from 0% to 100% depending on conditions present.

6.2.9.2 Understory Vegetation (5m to 0.5m high)

Record the type of vegetation in the understory as deciduous, coniferous, broadleaf evergreen, or mixed, where mixed is defined as above. If no understory exists in the plot, do not mark any of the bubbles.

Estimate the areal cover of all woody vegetation (which includes both the trunks and branches of trees and shrubs, woody stems of perennial plants, etc.) and tall herbs, grasses, and forbs. Cover categories range from absent to very heavy, as specified in Section 6.2.6. Record all observations by filling in the appropriate bubbles on the **Physical Habitat** form. Total cover in the understory layer can range anywhere from 0% to 100% depending on conditions present.

6.2.9.3 Ground Cover (lower than 0.5m high)

Estimate the areal cover of woody vegetation; tall herbs, grasses, and forbs; standing water or inundated vegetation; and barren, bare dirt, or buildings. Areas of exposed rock or bedrock should be considered 'barren'. Cover categories range from absent to very heavy, as specified in Section 6.2.6. Record all observations by filling in the appropriate bubbles on the **Physical Habitat** form.

Ground cover is the only layer in which cover estimates should add up to roughly 100% (as opposed to ranging from 0-100% in the other two layers). Certain ground cover types should be considered mutually exclusive, i.e., if ground layer vegetation overlays barren ground, record the vegetation cover even though there is barren ground beneath it.

6.2.9.4 Considerations for Drawdown conditions

Drawdown Zone vegetation entries are located to the right of Riparian Zone Vegetation on the **Physical Habitat** form. They are filled out only if there is a drawdown zone extending ≥1 m from shore. Unlike the case with potential fish cover, record these vegetation estimates just as you see them --- i.e., do not in this case imagine that the drawdown zone is under water. For example: there must be water on the ground (e.g., puddles) to have an entry for "standing water or inundated vegetation" in the drawdown zone. Large trees rooted above the drawdown zone can contribute cover over the drawdown zone. Dried aquatic macrophyte vegetation cover is entered under "Herbs, Grasses, & Forbs" with comment that it is dried aquatic macrophytes. There may also be a lot of zeros for vegetation in the drawdown zone (especially if the drawdown is fairly recent).

6.2.10 **Record Evidence of Human influence**

You will record any observations of human influences within the littoral, riparian, and, when present, drawdown zone plots. Human influences within the littoral plot will be recorded in different locations on the form depending on whether or not there is a drawdown plot.

- When drawdown or lake level fluctuations are minimal, i.e., <1 m horizontal distance, there is no drawdown zone plot, and the drawdown zone human influence field will be left blank. Human influences in the littoral plot are recorded along with the influences in riparian zone plot and recorded in the riparian zone portion of the form.

- When a drawdown zone extending ≥1 m from shore is present, a drawdown plot is defined and human influences within both the drawdown and littoral zone are recorded in the drawdown portion of the **Physical Habitat** form.

Within each zone, observations are recorded as not present (0), present outside and/or adjacent to (P), or contained within (C) the plot area (Figure 6-2). Record all observations by filling in the appropriate bubbles on the **Physical Habitat** form. The proximity "P" zones have a defined width of 15 m as shown in Figure 6-2. If there is no drawdown zone plot, all C and P human disturbances in the littoral plot are recorded on the riparian part of the form along with human influences present in the riparian zone, and the drawdown portion of the human influences section will be left blank. In contast, if there is a drawdown zone, all C and P human disturbances in the littoral plot are recorded in the drawdown part of the form along with human influences present in the drawdown zone.

For each zone and influence, indicate the presence only of the influence 'closest' to the plot itself. Do not mark "P" for a particular influence type if it is already marked "C" in that zone (use the more influential proximity code). Human Disturbances absent (0) and within-plot (C) are straightforward. For 'Present but outside or adjacent to the plots' (P), use these guidelines:

- A disturbance is marked "P" if the disturbance is seen entirely outside of any of the plots, but is adjacent to (i.e., within 15 meters left or right hand side of the entire Littoral-Drawdown-Riparian plot), or behind, the riparian plot within the defined areas.
- A disturbance is also marked "P" if the disturbance is seen behind, but entirely outside of all of the plots but is visible looking on-shore through the three plot zones (littoral, drawdown, and riparian)
- As a result, a single disturbance that is adjacent to both plots would be marked "P" in both the Riparian-Littoral and the Drawdown zones.
- If there is a drawdown plot, the presence of a human influence item WITHIN THE LITTORAL PLOT is recorded as "C" in the DRAWDOWN portion of the form (e.g., consider the littoral and drawdown zones to be a single plot when drawdown is present).
- If there is NO DRAWDOWN PLOT, i.e., the riparian plot abuts the shoreline, then human disturbances in the littoral plot are recorded by entering "C" in the Riparian portion of the form (e.g., consider the littoral and riparian zones to be a single plot when no drawdown is present).

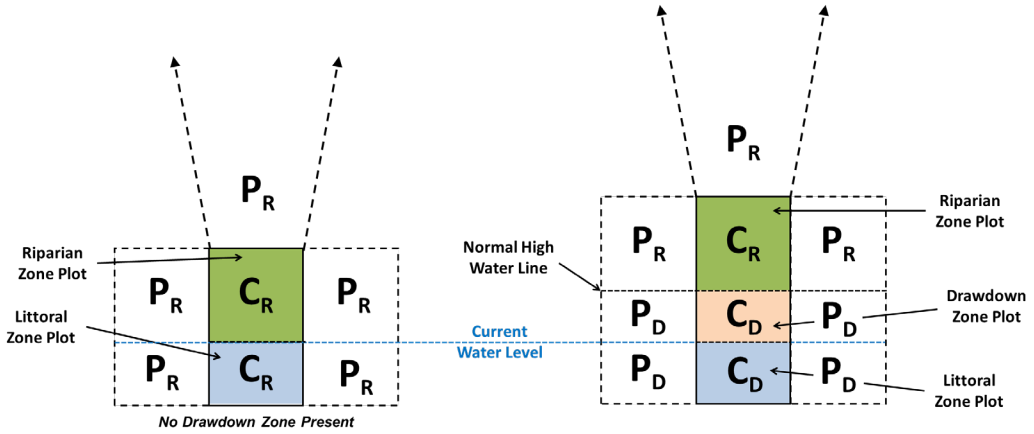


Figure 6-2 Human disturbance and proximity determinations

C_R = Marked on form as contained within riparian plot
P_R = Marked on form as adjacent to riparian plot
C_D = Marked on form as contained within drawdown zone
P_D = Marked on form as adjacent to drawdown zone

6.3 CyanoHABs Visual Assessment

Field crew views and documents visual observations of a potential cyanobacterial bloom in the NLA App and photo documents the bloom according to the reporting mechanism selected by the field crew (i.e., bloomWatch or state-specific reporting mechanism).

6.4 Benthic Macroinvertebrate Sampling

6.4.1 Summary of Method

Benthic macroinvertebrates are collected using a semi-quantitative sampling of multiple habitats in the littoral zone of lakes using a 500 µm mesh D-frame dip net (Figure 6-3). Sample collection is stratified on the following specific habitat types: rocky/cobble/large woody debris; macrophyte beds; fines (including mud, sand, or silt); and leaf packs.

Figure 6-3 D-frame net (500 µm mesh) used for collecting benthic macroinvertebrates.

6.4.2 Equipment and Supplies

Table 6-3 provides the equipment and supplies needed for field operations to collect benthic macroinvertebrates.

Table 6-3 Equipment and supplies – benthic macroinvertebrate collection.

Type	Item	Quantity
Form	NLA Littoral Samples	1
Documentation	Inner and outer labels: Benthic samples	1
	Labels: Benthic extra jar	As needed
	Scissors	1
	Clear tape strips (to cover sample labels)	As needed
Collection	Kick net (500 µm D-shaped, modified) with 4-foot handle	1
	Spare net(s) and/or spare bucket assembly for end of net	As needed
	Bucket (5-gallon capacity, plastic)	1
	Sieve bucket (500 µm)	1
	Watchmakers' forceps	1
	Squirt bottle (1 L Nalgene) – lake water	1
	Spoon (stainless steel)	1
	Funnel	1
	HDPE bottle (1 L, white, wide-mouth)	1 or more
	Ethanol (95%)	2 gal
	Gloves (latex/nitrile, non-powdered, box)	2 pair
Storing and preserving	Cooler	1
	Plastic electrical tape	As needed

6.4.3 Sampling Procedure

6.4.3.1 Site Selection and Sample Collection

The process for selecting the PHab stations is described in Section 6.1. All benthic samples should be collected from the dominant habitat type within the 10 m x 15 m littoral zone component of each of the PHab stations (Figure 3-2). The sampling process is described below. As part of the 2022 survey, new conservation measures for native freshwater mussels were added to the benthic sampling protocol[b].

6.4.3.2 Sample Processing in the Field

Use a 500-mm mesh sieve bucket placed inside a larger bucket full of lake water while sampling to carry the composite sample as you travel around the lake.

6.4.3.2.1 Benthic macroinvertebrate sampling

1. After locating the sample site according to procedures described in the physical habitat section, identify the dominant habitat type within the plot from the classifiers below:

[b] For this protocol, native freshwater mussels only refer to bivalves in the families Unionidae and Margaritiferidae. See Appendix C for examples of freshwater mussels. The conservation measures do not apply to the invasive quagga and zebra mussels, nor do they apply to any other mussel or clam (e.g., native fingernail clams, invasive Asiatic clam).

- Rocky/cobble/large woody debris;
- Macrophyte beds;
- Fines (including mud, sand or silt); or
- Leaf pack.

2. Prior to collecting the sample and when the water clarity allows, inspect the targeted substrate for the presence of native freshwater mussels. If native freshwater mussels are present, move to an area within the same habitat type where native mussels are absent and the previously identified mussels will not be exposed to sampling pressures. Check the box in the NLA App that identifies native mussels were observed and the collection location was shifted.
3. Use the D-frame dip net (equipped with 500-µm mesh) to sweep through one linear meter of the dominant habitat type at a single location within the 10 m x 15 m littoral zone sampling area, making sure to disturb the substrate enough to dislodge organisms.
    - When safe to do so, it is preferable that you exit the boat and disturb the substrate (e.g., overturn rocks, logs) using your feet while sweeping the net through the disturbed area.
    - Because a dip-net is being used for sampling, the maximum depth for sampling will be approximately 1 m (the length of the dip-net handle); therefore, in cases in which the depth of the lake quickly drops off, it may be necessary to sample in the nearest several meters to the shore.
4. After completing the 1 m sweep, remove all organisms and debris from the net and place them in a bucket following sample processing procedures described in the following section.
5. Inspect the sample for native freshwater mussels after each sweep. If one or more native mussels were unintentionally collected:
    a. In the bucket, gently rise each mussel with lake water to remove invertebrates that may be on its shell.
    b. Carefully return the native mussel to the same habitat it was sampled from and away from further sampling pressures. They are NOT to be pushed into the substrate but to be placed gently on their side on top of the same substrate type or as instructed by local permit.
    c. Check the box in the NLA App that native freshwater mussels were removed from the sample and returned to the lake.
    d. <u>For EPA contractor and regional crews:</u> when sampling a lake where ESA-listed freshwater mussels have the potential to be present, crews must report the encounter in the **Federal ESA** form in the NLA App.
    e. Never intentionally collect and retain a native freshwater mussel that has the potential to be an ESA-listed species.
6. Proceed to the next sampling station and repeat steps 1-5. The organisms and detritus collected at each station on the lake should be combined in a single bucket to create a single composite sample for the lake. After sampling at all PHab stations is complete, process the composite sample in the bucket according to procedures described in the following section. One to five bottles should be sufficient to hold the composited sample from each lake.

- If there is a large amount of debris (rocks, sticks, etc.) accumulating in the composite sample, remove debris between sampling stations, after the debris is inspected, picked, and/or washed to ensure no organisms are lost.
- If your first collection at a sampling station results in too much debris, discard it, move the location within the same habitat station, and take another sample.
- It is recommended that crews carry a sample bottle containing a small amount of ethanol with them to enable them to immediately preserve larger predaceous invertebrates such as hellgrammites and water beetles. Doing so will help reduce the chance that other specimens will be consumed or damaged prior to the end of the field day. Crews should NEVER, however, attempt to 'field-pick' the samples.

6.4.3.2.2 Preparing composite samples for benthic macroinvertebrates

1. Pour the entire contents of the bucket through a sieve (or into a sieve bucket) with 500 µm mesh size. Remove any large objects and wash off any clinging organisms back into the sieve before discarding. Once again review the sample for native freshwater mussels and follow the steps identified above in the sampling process.
2. Using a wash bottle filled with clean lake water, rinse all the organisms from the bucket into the sieve. This is the composite sample for the lake.
3. Estimate the total volume of the sample in the sieve and determine how many jars (1 L jars, each no more than half-full with sample) will be required.
4. Fill in a sample label with the Site ID and date of collection. Attach the completed label to the jar and cover it with a strip of clear tape. Record the sample ID number for the composite sample on the **Littoral Samples** form. For each composite sample, make sure the number on the form matches the number on the label.
5. Wash the contents of the sieve to one side by gently agitating the sieve in the water. Wash the sample into a jar using as little water from the wash bottle as possible. Use a large-bore funnel if necessary. If the jar is too full, pour off some water through the sieve until the jar is not more than half full, or use a second jar if necessary. Carefully examine the sieve for any remaining organisms and use watchmakers' forceps to place them into the sample jar.
6. If additional jars are needed, use a pre-printed benthos extra jar label or, if needed, fill in a blank sample label that does not have a pre-printed ID number on it. Record the ID number from the pre-printed label prepared in Step 4 in the "SAMPLE ID" field of the label. Attach the label to the additional jars and cover them with a strip of clear tape. Record the number of jars required for the sample on the **Littoral Sample**s form. **Make sure the number you record matches the actual number of jars used.** Write "Jar *N* of *X*" on each sample label using a waterproof marker ("*N*" is the individual jar number, and "*X*" is the total number of jars for the sample).
7. Place a waterproof label inside each jar with the following information written with a number 2 lead pencil:
   - Site ID;
   - Site name;
   - Date of collection;
   - Sample type;
   - Collector(s) names or initials; and
   - Sample ID.

8. Completely fill the jar with 95% ethanol (no headspace). It is very important that sufficient ethanol be used, or the organisms will not be properly preserved. Existing water in the jar should not dilute the concentration of ethanol below 70%. **NOTE:** Prepared composite samples can be transported back to the vehicle before adding ethanol if necessary. In this case, fill the jar with lake water, which is then drained using the net (or sieve) across the opening to prevent loss of organisms, and replaced with ethanol at the vehicle.
9. Replace the cap on each jar. Slowly tip the jar to a horizontal position, then gently rotate the jar to mix the preservative. Do not invert or shake the jar. After mixing, seal each jar with plastic electrical tape.
10. Store labeled composite samples in a container with absorbent material that is suitable for use with 70% ethanol until transport or shipment to the laboratory.

6.5 Fecal Indicator Sample (Enterococci)

6.5.1 Summary of Method

Field teams are to collect a water sample within or near the littoral zone of the **last PHab station** where the water is about waist deep (1 meter). The water sample should be collected when the crew first arrives at the station (i.e., prior to substrate assessment and collection of the benthic macroinvertebrate sample). For "large lakes" (greater than 10,000 ha) the sample is to be collected from the launch site at the end of the day. **Filters must be frozen within six hours of collection.** Teams are to use a pre-sterilized, 250 ml bottle and collect the sample at about 0.3 meter (12 inches) below the water. Following collection, samples are placed in coolers and maintained on ice prior to filtration of two 50 mL volumes. Again, samples must be filtered and frozen on dry ice within six hours of collection.

6.5.2 Equipment and Supplies

Table 6.4 provides the equipment and supplies needed to collect the fecal indicator sample.

Table 6-4 Equipment and supplies: fecal indicator sample

Type	Item
Form	NLA Littoral Samples
Collection	Nitrile gloves Pre-sterilized, 250 ml sample bottle Sodium thiosulfate tablet Wet ice Cooler

6.5.3 Sampling Procedure

The procedure for collecting the fecal indicator sample is presented in Table 6-5.

Table 6-5 Procedure: fecal indicator (enterococci) sample collection

Enterococci Sample
1. **Put on nitrile gloves**.
2. Identify an undisturbed area within or near the littoral zone of the final habitat station where the water is about waist deep (1 meter). For "large lakes" (greater than 10,000 ha) the sample is to be collected from the launch site at the end of the day.
3. Lower the uncapped, inverted 250 ml sample bottle to a **depth of 1 foot (0.3 m) below the water surface,** avoiding surface scum, vegetation, and substrates.
4. Point the mouth of the container away from the body or boat. Right the bottle and raise it through the water column, allowing bottle to fill completely.
5. After removing the container from the water, discard a small portion of the sample to allow for proper mixing before filtering (down to the 250 mL mark on the bottle).
6. **Add the sodium thiosulfate tablet, cap, and gently shake the bottle 25 times**.
Storage
7. Store the sample in a cooler on ice to chill (do not freeze immediately). Chill for at least 15 minutes before filtering.
8. Sample must be filtered and all filters frozen within six hours of collection.

6.5.4 Sample Processing in the Field

You will need to process two separate filters for the *Enterococci* sample. All the filters required for an individual site should be sealed in plastic bags until use to avoid external sources of contamination. Please refer to Section 8.2 for more information regarding prcessing the *Enterococci* samples.

7.0 LAKE WIDE FISH SAMPLE COLLECTION

7.1 Summary of Method

Procedures for the whole fish composite sample are described in detail in Section 7.3**.**The objective is to collect one whole fish composite sample from the 636 designated lakes. This subsample is approximately 70% of the survey design (904 lakes total). Sites with "FT" in the design panel (i.e. Panel_Use) identify lakes designated for fish sampling. The two revisit sites in each state are also designated as fish sampling sites. Crews should collect fish during the *first* site visit for a revisit site, to allow for a second opportunity, if fish cannot be collected during the first visit.

The focus is on obtaining predator fish species that:

- are commonly consumed by humans;
- satisfy legal requirements of harvestable size for each lake site (or at least consumable size if no legal harvest requirements exist); and
- are sufficiently abundant within the lake.

Each fish sample will be a **composite of five adult whole fish** of the same species that are similar in size (i.e., the smallest individual in the sample is no less than 75% of the total length of the largest individual). See the last paragraph in 7.1 for clarification about the acceptable number of predator fish in a composite sample.

Collection of the whole fish composite sample is a lakewide activity. It does not need to be associated with the index site or PHab stations. Field crews may target areas in the lake with high quality habitat for the target fish species, except for areas near a lake inlet or adjacent to riverine habitat. Crews are to avoid these areas (a minimum of a 5-meter buffer zone) since they are more likely to contain nonresident fish. Additionally, when sampling run-of- the river reservoirs, crews should focus their sampling efforts in lacustrine habitats and avoid the river-reservoir transtion zone (i.e., do not sample in habitats with flowing water). Crews should make a reasonable effort to collect a whole fish composite sample before determining a fish sample cannot be collected. This may take *up to* three hours, depending on the waterbody.

If a lake designated for fish sampling is determined target and sampleable, but the crew was unable to collect a whole fish composite sample, no site replacement is needed. If a lake designated for fish sampling is dropped (i.e., determined non-target or not accessible), a replacement site is identified following procedures described in the NLA 2022 SEG and a whole fish composite sample must be collected at the replacement site. At revisit sites, this sample is only collected at one of the two site visits; however, crews that are unsuccessful at collecting the whole fish composite sample during visit 1 are expected to attempt the collection of that sample during visit 2. Whole fish composite samples are shipped to the laboratory designated for interim storage of the samples.

This section contains the sampling procedures and target predator fish species for the whole fish composite sample collection. Note that the target fish species list (Table 7-2) includes 12 primary target predator fish species and 10 secondary predator fish species. Field crews must attempt to collect a primary target predator fish species wherever possible. If primary target predator species are not available at a particular site, then the field crew collects a composite of one of the secondary predator fish species. In the event that a crew is unable to collect fish which are on either of the predator species

lists, then the on-site biologist can select an appropriate **pelagic predator**. If the field crew has any questions they should contact the NLA Fish Fillet Indicator Trainer or the EPA Technical Lead for the Fish Fillet Contaminants Indicator (Table 2.2).

Field crews are encouraged to collect fish for the composite sample using hook and line or electrofishing. Crews may also seine or use gill nets when this would be an efficient approach to sample the target fish species and when allowed by the sampling permit. Crews are not to use trawling to collect the fish. **Crews may not purchase fish for the whole fish composite sample**.

The whole fish composite sample should consist of five similarly sized (i.e., the total length of the smallest specimen is no less than 75% of the total length of the largest specimen) adult predator fish of the same species. The minimum acceptable length for a fish in any composite sample is 190 mm. **Field crews should make every effort to consistently obtain five fish for the composite sample; however, a sample of fewer than five fish is acceptable. Conversely, for the exceptions where field crews collect five fish that are small, they should collect up to five additional fish (for an overall composite of up to 10 fish)** to provide adequate tissue for analysis. Fish retained as the fish composite sample should remain intact and be submitted as whole specimens.

7.2 Equipment and Supplies

Table 7-1 lists the equipment and supplies necessary for field crews to collect whole fish composite samples. A human health fish sampling kit will be provided to field crews for whole fish sampling sites (separately from site kits) as requested by the crew (see Appendix A). Additional fish collection supplies can be ordered through the **Request** form. A list of frequently asked questions and responses will be provided with the fish sampling supplies to clarify situations that field crews may encounter while collecting whole fish composite samples. Detailed procedures for collecting and processing the whole fish composite samples are presented below.

Table 7-1. Equipment & supplies: whole fish composite sample collection for human health

Type	Item
Form	NLA Whole Fish Sample in NLA App
Collection	scientific collection permit electofisher, hook and line, trap nets (or other device allowed in the sample collection permit) sampling vessel (including boat, motor, trailer, oars, gas, and safety equipment) nitrile gloves* Coast Guard-approved personal floatation devices Global Positioning System (GPS) livewell and/or buckets measuring board (millimeters)
Storing and preserving	aluminum foil (solvent rinsed)* polyethylene tubing (food-grade)* large plastic (composite) bags*

	plastic cable ties* human health fish sample coolers* dry ice (for preservation) or wet ice (for temporary transport)
Documentation	human health fish sample labels (individual fish labels and composite bag labels)** fine-tipped indelible markers (for labels) Tyvek label tag with grommet (for composite bag)* clear tape

*Provided by EPA in FTIS kits.

**Provided by EPA in site kits.

7.3 Sampling Procedure

Note: *Do not handle any food, drink, sunscreen, or insect repellant until after the composite sample has been collected, measured, and wrapped (or implement measures to reduce contamination by such chemicals if applied such as washing, wearing long gloves, etc.).*

1. Put on clean nitrile gloves before handling the fish.
2. Rinse potential target species/individuals in ambient water to remove foreign material from the external surface and place them in clean holding containers (e.g., livewells, buckets).
3. For each human health fish composite sample, select five whole fish. Criteria for inclusion in the human health fish composite sample:
    a. All fish are of the same primary target species or secondary fish species (See Table 7-2) ***Note:*** *It is essential that field crews accurately identify the organisms submitted for analysis. Do not submit organisms from different species in a single sample.*
    b. All fish are adult fish; and
    c. All fish are of similar size, so that the smallest individual in a composite is no less than 75% of the total length of the largest individual. The minimum acceptable fish length is 190 mm.
4. Measure each fish selected for the composite from the anterior-most part of the fish to the tip of the longest caudal fin ray (when the lobes of the caudal fin are depressed dorsoventrally) to determine total body length in millimeters.
5. On the **Whole Fish Sample** form in the NLA App:
    - Verify the date of the fish collection on the top of the form. The date in this field will be automatically entered based on the date recorded on the **Verification** form. If you have not filled out the Verification Form or if the date of the fish collection is different, you can edit the date here. Note however that changing the value on this form will not change the value on the Verification Form.
    - Ensure the sample identification number is entered.
    - Check the boxes verifying that all samples are of similar length and the same species.
    - Record species selected for analysis, individual specimen lengths (total length in mm), and any relevant comments. Extra rows are provided in the App in the event that additional specimens are collected to ensure adequate tissue for analysis (refer to Frequently Asked Questions for further clarification).

- Make sure the sample ID and specimen numbers recorded in the App match those on the sample labels.

6. Wearing clean nitrile gloves, remove each fish selected for analysis from the clean holding container(s). Dispatch each fish using the most humane method available.
7. Wrap each fish specimen in extra heavy-duty aluminum foil, with the dull side in contact with the fish (foil is solvent rinsed and baked and will be provided by EPA).
8. Prepare a sample label for each sample specimen, ensuring that the label information matches the information recorded on the **Whole Fish Sample** form in the App. **Be sure to record the common name and specimen length on each label.**
9. Cut separate lengths of food grade tubing (provided by EPA) long enough to contain each individual fish, allowing extra length on each end to seal with cable ties. Place each foil-wrapped specimen into the appropriate length of tubing. Seal the ends of each tube with a plastic cable tie. Attach the appropriate sample label to the plastic tubing by wrapping clear tape around the label and then completely around the wrapped fish (so that the clear tape wraps over itself).
10. Double-bag the entire set of specimens in the composite by placing all fish composited from the site inside a large plastic bag (provided by EPA). If additional bags are required for large fish specimens or fish samples, please use plastic bags of similar thickness as those provided by EPA.
11. Fill out a Sample Identification Label for the outer bag, ensuring that the label information matches the information recorded on the **Whole Fish Sample** form in the App. **Be sure to record the common name and specimen length range on the label.**
12. Affix the sample label to a composite bag tag (Tyvek tag) and cover with clear plastic tape. Thread a cable tie through the grommet in the tag and seal the outer bag with the cable tie.

SAMPLE STORAGE AND SHIPPING PREPARATION

1. After the fish sample is packaged, immediately place the fish sample in a cooler of dry ice and use either of the following options: (1) replenish dry ice at least daily until the sample can be properly frozen at ≤-20°C in a laboratory or other interim facility or (2) pack cooler with 50 pounds of block dry ice and ship to Microbac Laboratories (Baltimore, MD) before the end of the day.
   - If a fish sample is held on dry ice in the field, the field crew should replenish the supply of dry ice at least daily until the sample can be properly frozen or shipped.
   - Packaged fish samples may be placed on wet ice in coolers if they will be immediately transported to a nearby laboratory or other interim facility to be frozen before shipment (wet ice should be replenished frequently before it melts).
   - Keep all specimens in a particular fish composite sample in the same cooler for transport. Ship only one fish composite sample in a cooler.
2. Crews have two options for freezing and shipping fish composite samples, depending on site logistics:
   a. Ship the samples via priority overnight delivery service (i.e., Federal Express), packed on dry ice, so that they arrive at Microbac Laboratories (Baltimore, MD) within 24 hours from the time of sample collection. Do NOT ship on Fridays, Saturdays, or the day before federal holidays. Fish samples must be packed on sufficient dry ice (**50 pounds minimum**, with blocks of dry ice layered to ensure direct contact between fish and dry

ice) to keep them frozen for up to 48 hours. **Do not use dry ice pellets for shipping the whole fish composite sample.** Remember to record the tracking number on the **Tracking** form in the App before submitting it to NARS IM.

b. Add fish samples to a freezer capable of maintaining temperatures of ≤-20°C within 24 hours of collection, and store the frozen samples until shipment within two weeks of sample collection. If fish samples cannot be stored in a freezer within 24 hours of collection, the field crew should replenish the supply of dry ice in the cooler containing the samples, at least daily, until the samples can be properly frozen or shipped. Frozen fish samples will subsequently be packed on at least 50 pounds of layered blocks of dry ice and shipped to Microbac Laboratories (Baltimore. MD) via priority overnight delivery service. Refer to reminders in option 2a (above) about not shipping on Fridays, Saturdays, or the day before federal holidays and about including sample tracking numbers on App tracking forms.

Table 7-2. Primary and secondary NLA target species for human health fish collection

PRIMARY PREDATOR HUMAN HEALTH FISH TARGET SPECIES		
FAMILY	SCIENTIFIC NAME	COMMON NAME
Centrarchidae	*Micropterus salmoides*	Largemouth Bass
	Micropterus dolomieu	Smallmouth Bass
	Pomoxis nigromaculatus	Black Crappie
	Pomoxis annularis	White Crappie
Percidae	*Sander vitreus*	Walleye
	Perca flavescens	Yellow Perch
Moronidae	*Morone chrysops*	White Bass
Esocidae	*Esox lucius*	Northern Pike
Salmonidae	*Salvelinus namaycush*	Lake Trout
	Salmo trutta	Brown Trout
	Oncorhynchus mykiss	Rainbow Trout
	Salvelinus fontinalis	Brook Trout
SECONDARY PREDATOR HUMAN HEALTH FISH SPECIES		
FAMILY	SCIENTIFIC NAME	COMMON NAME
Centrarchidae	*Lepomis macrochirus*	Bluegill
	Ambloplites rupestris	Rock Bass
	Micropterus punctulatus	Spotted Bass
Percidae	*Sander canadensis*	Sauger
Moronidae	*Morone saxatilis*	Striped Bass
	Morone americana	White Perch
Esocidae	*Esox niger*	Chain Pickerel
Salmonidae	*Oncorhynchus clarkii*	Cutthroat Trout
	Coregonus clupeaformis	Lake Whitefish
	Prosopium williamsoni	Mountain Whitefish

8.0 FINAL LAKE ACTIVITIES

Prior to leaving the lake, make a general visual assessment of the lake and its surrounding catchment. This assessment is based on the collective observations of all crew members. The objective of the lake assessment is to record field crew observations of catchment and lake characteristics that are useful for future data interpretation, ecological value assessment, development of associations, and verification of stressor data. While often subjective, these observations and impressions are extremely valuable.

In addition, review all data forms and sample labels for completeness, accuracy, and legibility. Make sure all samples are labeled, sealed, and properly preserved. Activities described in this section are summarized in Figure 8-1.

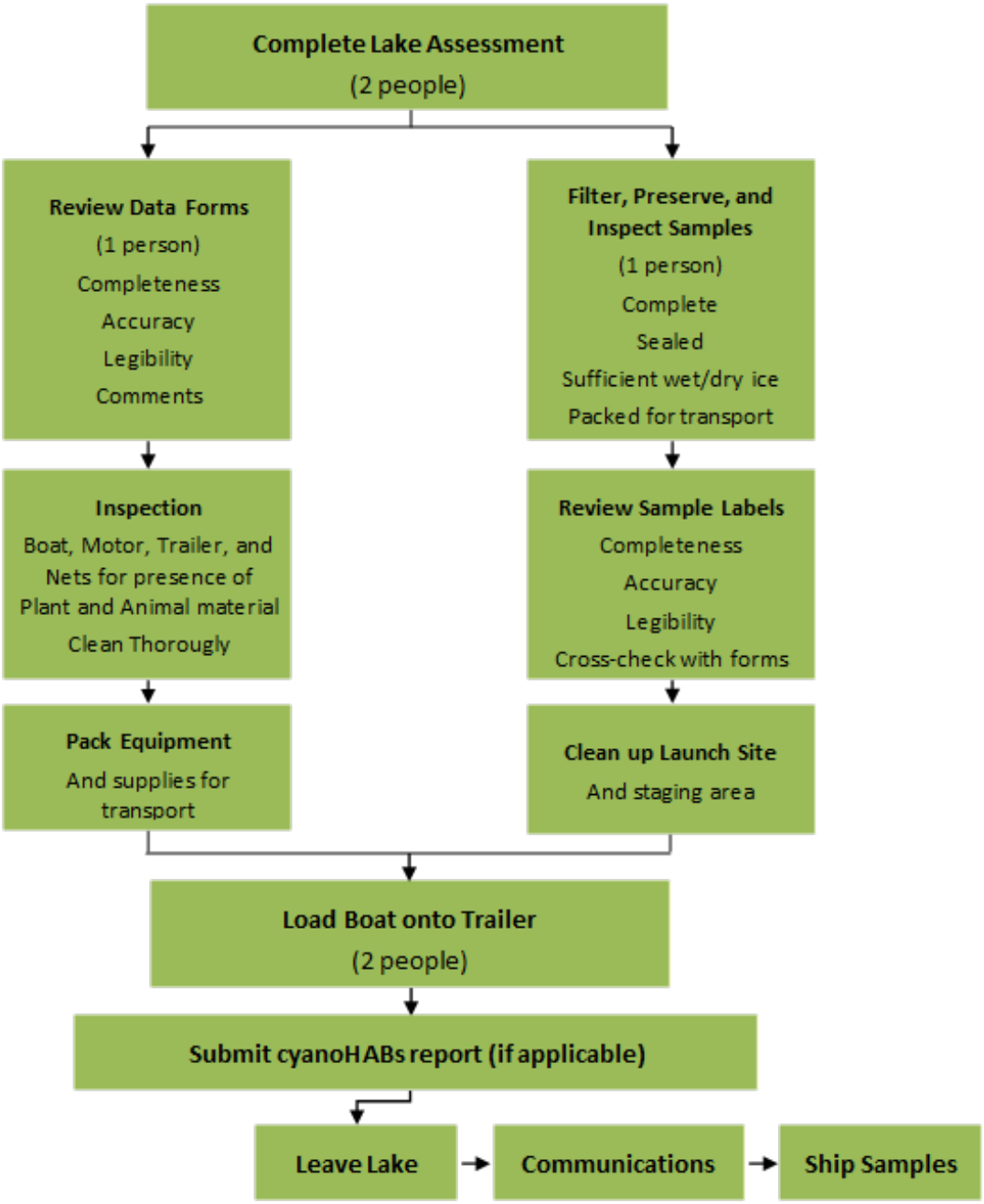


Figure 8-1 Final lake activities summary

8.1 General Lake Assessment

Complete the **Assessment** form at the end of lake sampling, recording all observations from the lake that were noted by all crew members during the course of the visit. This form is designed as a template for recording pertinent field observations. It is by no means comprehensive, and any additional observations should be recorded in the comments section. The form consists of six major sections: 1) Lake/Catchment Site Activities and Disturbances Observed, 2) General Lake Information, 3) Shoreline

Characteristics, 4) Qualitative Macrophyte Survey, 5) Waterbody Character, and 6) Qualitative Assessment of Environmental Values. In 2022, new questions were added to assist the determination of natural and human-made lakes.

8.1.1 Lake/Catchment Site Activities and Disturbances Observed

Record any of the sources of potential stressors listed in Table 8-1 on the **Assessment** form. These potential stressors may have been observed while on the lake, while driving or walking through the lake catchment, or while flying over the lake and catchment. For activities and stressors that you observe, rate their abundance or influence as low (L), moderate (M), or heavy (H) by filling in the correct bubble next to each disturbance listed. Leave the line blank for any disturbance not observed. The distinction between low, moderate, and heavy will be subjective. For example, if there are two to three houses on a lake, fill in the "L" bubble for low next to "Houses." If the lake is ringed with houses, rate it as heavy (H). Similarly, a small patch of clear-cut logging on a hill overlooking the lake would rate a low ranking. Logging activity right on the lake shore, however, would get a heavy disturbance ranking. The section for “Lake Site Activities and Disturbances Observed” includes residential, recreational, agricultural, industrial, and lake management categories.

Table 8-1 Site activities and disturbances observed during final lake assessment.

Observe lake activities or disturbances listed and record as L (low), M (moderate), or H (heavy) intensity on the Assessment form (except as noted below):	
Residences	Presence of any houses and residential buildings around the lake.
Maintained Lawns	Presence of any maintained lawns around the lake.
Construction	Presence of any recent construction in the immediate area around the lake or signs of recent sedimentation events (depositional fans).
Pipes/Drain	Presence of any pipes or drains feeding into or out of the lake. If known, record the type of activity with which the pipe is associated (e.g., storm sewer, plant intake) in the "Comments" section of the form.
Dumping	Any evidence of landfill or dumping around the lake, including garbage pits and informal dumping of large amounts of trash or cars and appliances along roads or lakeshore. This does not include small amounts of litter. If informal dumping areas exist, note that they are informal sites in the "Comments" section of the form.
Roads	Presence of any maintained roads in the immediate area around the lake.
Bridges/Causeways	Presence of any bridges or causeways across or in the immediate vicinity of the lake.
Sewage Treatment	Presence of sewage treatment facility.
Hiking Trails	Presence of formal hiking trails around the lake.
Parks, Campgrounds	Presence of organized public or private parks, campgrounds, beaches or other recreational areas around the lake.
Primitive Parks, Camping	Presence of informal or primitive parks, camping areas, beaches or other recreational areas (e.g., swimming holes) around the lake.
Resorts	Level of resort activity; this could include motels, resorts, golf courses, and stores.
Marinas	Presence of any marinas.
Trash/Litter	Relative abundance of trash or litter around the lake.
Surface Films, Scum or Slicks	Relative abundance of surface films, scum, or slicks on the lake.
Cropland	Presence of cropland.

Observe lake activities or disturbances listed and record as L (low), M (moderate), or H (heavy) intensity on the Assessment form (except as noted below):	
Pasture	Presence of pastures.
Livestock Use	Presence of livestock use.
Orchards	Presence of orchards.
Poultry	Presence of poultry operations.
Feedlot	Presence of feedlot or concentrated animal feeding operations.
Water Withdrawal	Any evidence of water withdrawal from the lake.
Industrial Plants	Any industrial activity (e.g., canning, chemical, pulp) around the lake or in the catchment. Describe the type of industry in the "Comments" section of the form.
Mines/Quarries	Any evidence of mining or quarrying activity in the catchment or around the lake.
Oil/Gas Wells	Any evidence of oil or gas wells in the catchment or around the lake.
Power Plants	Presence of any power plants.
Logging	Any evidence of logging or fire removal of trees in the lake area.
Evidence of Fire	Any evidence of forest fires in the lake area.
Odors	Presence of any strong odors.
Commercial	Any commercial activity (e.g., convenient stores, shopping centers, restaurants) around the lake or in the catchment.
Liming	Any evidence of liming activities.
Chemical Treatment	Presence of any chemical treatment facilities.
Angling Pressure	Estimate of the intensity of fishing activity in the lake.
Drinking Water Treatment	Presence of any drinking water treatment facilities.
Macrophyte Control	Any evidence of dredging or other activities to control macrophyte growth; describe these in the "Comments" section of the form.
Water Level Fluctuations	Any evidence of water level fluctuations due to lake management.
Fish Stocking	Any evidence of fish stocking in the lake.
Record any other oddities observed or additional information for any specific activity in the "Comments" section of the form.	

8.1.2 General Lake Information

Observations regarding the general characteristics of the lake are described in Table 8-2. Record these observations on the **Assessment** form. The hydrologic lake type is a very important variable for defining subpopulations for acidic deposition effects. Note if there are any stream outlets from the lake, even if they are not flowing. If no lake outlets are observed, record the lake as a seepage lake. If the lake was created by a manmade dam (not that a dam is present just to raise the water level), record the lake as a reservoir. Otherwise record the lake as a drainage lake. Note any flight hazards that might interfere with either low-altitude fly-overs by aircraft (for future aerial photography or videography) or landing on the lake for sampling purposes (either by float plane or helicopter). When estimating the intensity of motor boat usage, in addition to the actual number of boats observed on the lake during the visit, use other observations such as the presence of boat houses, docks, and idle craft**.** Note your opinion as to the swimmability of the lake in general. Observe any regular change in the lake levels and estimate the typical elevation change.

Table 8-2 General lake information observed during final lake assessment.

Record general information about the lake as a whole	
Hydrologic Lake Type	Note if there are any stream outlets from the lake, even if they are not flowing. If no lake outlets are observed, record the lake as a seepage lake. If the lake was created by a manmade dam (not that a dam is present just to raise the water level), record the lake as a reservoir. Otherwise record the lake as a drainage lake.
Outlet Dams	Note the presence of any dams (or other flow control structures) on the lake outlet(s). Differentiate between artificial (man-made) structures and natural structures (beaver dams).
Motor Boat Density	Record your impression of the density of motor boat usage on this lake (high or low). If there is a restriction on the size of motor boat engines, check "Restricted." If motor boats are banned, check "Banned." Consider the day of the week and weather in your assessment as well as the number of boathouses, idle craft. Count jet skis and any other motorized craft, which could stir up the lake, as motor boats.
Swimmability	Record a subjective impression about the aesthetics of swimming in this lake (swimmability) along the range of "good" to "not swimmable."
Lake Level Changes	Examine the lake shoreline for evidence of lake level changes (e.g., bathtub ring). If there are none, check "zero;" otherwise try to estimate the extent of vertical changes in lake level from the present conditions based on other shoreline signs. Estimates should be made to the nearest 0.1 m.

8.1.3 Shoreline Characteristics

Shoreline characteristics of interest during the final lake assessment are described in Table 8-3. Record observations related to this portion of the assessment on the **Assessment** form. To estimate the extent of major vegetation types, limit the assessment to the immediate lake shoreline (i.e., within 20 m of the water). Also estimate the percentage of the immediate shoreline that has been developed or modified by humans.

Table 8-3 Shoreline characteristics observed during final lake assessment.

Record percent of shoreline characteristics (within 20 meters of water):	
Forest	Deciduous, coniferous, or mixed forest, including sapling vegetation.
Grass	Meadows, lawns, or other open vegetation.
Shrub	Shrub vegetation
Wetland	Forested and non-forested wetlands (submerged terrestrial vegetation).
Bare Ground	Non-vegetated areas such as beaches, sandy areas, paved areas, and exposed rock.
Agriculture	Cropland, orchard, feedlot, pastureland, or other horticultural activity.
Shoreline Modifications	Actual shoreline that has been modified by the installation of riprap, revetments, piers, or other human modifications.
Development	Immediate shoreline area developed by human activity; include lawns, houses, stores, malls, marinas, golf courses, or any other human-built land use.

8.1.4 Qualitative Macrophyte Survey

Aquatic macrophytes (aquatic plants large enough to be seen without magnification) are important indicators of lake trophic status. The most important indicator for this survey is the percentage of the entire lake area (not just near the shore) covered with macrophytes, as perceived by observers. For both "emergent/floating" and "submergent" coverage, choose one of the four percentage groupings (<5%, 5-25%, 26-75%, >75%) on the **Assessment** form. In some cases, it will be fairly easy to estimate the percentage from observations made at the PHab stations. In other cases, it will be an educated guess, especially if the water is turbid or the lake is deep. After recording the areal percentage of macrophyte coverage, record the typical density of the plants in the observed macrophyte beds as absent, sparse, moderate, or high. Record your estimates on the **Assessment** form.

8.1.5 Waterbody Character

Rate the ***waterbody character*** which is the physical habitat integrity of the waterbody. Waterbody character is largely a function of riparian and littoral habitat structure, volume change, trash, turbidity, slicks, scums, color, and odor. The NLA 2022 attempts to define waterbody character through two attributes: degree of human disturbance and aesthetics. Rate each of these attributes on a scale of 1 to 5. For development, give the lake a "5" if it is pristine, with no signs of any human disturbance. A "1" would indicate that a lake is highly disturbed; for example, the entire lake is ringed with houses, seawalls, docks, etc. For aesthetics (whether the lake is appealing or not) base the decision on any factors about the lake that disturb you (trash, algal growth, weed abundance, overcrowding). Fill in the bubble next to the number that best describes your opinion about how suitable the lake is for recreation and aesthetic enjoyment today:

1. Enjoyment is nearly impossible;
2. Level of enjoyment is substantially reduced;
3. Enjoyment is slightly impaired;
4. There are very minor aesthetic problems; it is otherwise excellent for swimming, boating, and enjoyment; or
5. It is beautiful and could not be any nicer.

8.1.6 Qualitative Assessment of Environmental Values

The primary goal of this study is to assess three major ecological values with respect to lakes: trophic state, ecological integrity, and human health (through use of the lake). Based on your field experience, record your own assessment of these values on the **Assessment** form. Write comments on these values in this section.

1. ***Ecological integrity*** is the ability to support and maintain a balanced, integrated, adaptive community with a biological diversity, composition, and functional organization comparable to natural lakes of the region. Record your overall impression of the "health" of the biota in the lake. Note any possible causes of impairment. The presence of higher order consumers (fish-eating birds and mammals) is an indication of a healthy food web and should be noted here. Similarly, the absence of an organism that you might expect to see is an important observation.
2. ***Trophic state*** is the rate or amount of phytoplankton and macrophytes produced or present in a lake. Give your visual impression of the trophic status as oligotrophic (little or no biomass in the lake water), mesotrophic (intermediate amounts of biomass in the lake water), eutrophic (large

amounts of biomass in the lake water), or hypereutrophic (choked lake, with more biomass than water). Give your overall impression of algal abundance and general type (e.g., filamentous). List any observed potential nutrient sources to the lake (e.g., septic tanks and agricultural runoff).

3. ***Recreational value*** is the ability to support recreational uses such as swimming, fishing, and boating. Record your overall impression of the lake as a site for recreation. Note any possible causes of impairment or risk to human health. Note the presence or absence of people using the lake for recreational activities.

Use the comments section on the **Assessment** form to note any other pertinent information about the lake or its catchment. Here the field crew can record any observations that may be useful for future data interpretation, especially data that was not captured during other sampling or data collection activities.

8.1.7 CyanoHAB assessment and report

In the **Assessment** form, field crews document the presence of potential cyanobacterial blooms that were observed in areas of the lake that were not associated with the launch site, index site or physical habitat stations (i.e., not previously reported the NLA App). Crews are to photo document the bloom according to the reporting mechanism selected by the field crew (i.e., bloomWatch or state-specific reporting mechanism).

If the field crew documented the potential occurrence of a cyanoHAB event at one or more station in the lake, the Field Crew leader is responsible for reporting the bloom to the appropriate state, tribal or local organization. See Section 2.2.4.5 for potential reporting mechanisms. A cyanoHAB report will only be submitted when a potential bloom was observed. Reports are not needed to document the lack of a bloom. All reports submitted via the bloomWatch app should add the project identifier ‘NLA2022’ in the comment section in the app.

8.2 Filtering: Processing the Fecal Indicator (Enterococci) and Chlorophyll-*a* Samples

8.2.1 Equipment and Supplies: Fecal Indicator

Table 8-4 provides the equipment and supplies needed for field crews to collect the fecal indicator sample.

Table 8-4 Equipment and Supplies: Fecal Indicator (Enterococci) Sample

Type	Item	Quantity
Form	NLA Littoral Samples form	1
For recording measurements	Fine-tipped indelible markers for filling out sample labels	1
	Fecal Indicator sample labels: vial	
	Fecal Indicator sample labels: bag label)	2
	Filter blank label if collecting filter blank	1
For processing samples	Nitrile gloves	1
	Sterile centrifuge tube (50 mL, screw top) in zip top bag	1
	Filtration apparatus with collection flask	1
	Sterile filter holder, Nalgene 145/147	1
	Vacuum pump (electric pump may be used if available)	1
	Sterile phosphate buffered saline (PBS)	1
	Osmotics 47 mm polycarbonate sterile filters	1+
	Sterile disposable forceps	1
	Petri dishes (60 x 15, disposable)	1
	Sterile microcentrifuge tubes containing sterile glass beads	2
	Additional sterile microcentrifuge tube if collecting filter blank	1
	Bubble bag	1
	Zip-top bag	1
	Dry ice	As needed
	Cooler	1

8.2.2 Procedures for Processing the Fecal Indicator (*Enterococci*) Sample

The fecal indicator sample **must be filtered before the chlorophyll-*a*** sample since the filtering apparatus needs to be sterile. The procedures for processing the fecal indicator sample are presented in **Table 8-5**. The sample must be filtered and frozen within six hours of collection.

Table 8-5 Procedure: Processing Fecal Indicator (*Enterococci*) Sample

Filtering for the fecal indicator (Enterococci) Sample

Prior to beginning the filtering process, chill the Enterococci sample and PBS on WET ice for at least 15 minutes and chill the microcentrifuge tubes on DRY ice.

1. Put on nitrile gloves.
2. Set up sample filtration apparatus on flat surface and attach vacuum pump. Set out 50 mL sterile PP tube, sterile 60 mm Petri dish, chilled phosphate buffered saline (PBS), Osmotics 47 mm polycarbonate sterile filter box, and two sterile filter forceps.
3. Chill Filter Extraction tubes with beads on dry ice.
4. Aseptically transfer two polycarbonate filters from filter box to base of opened Petri dish. Close filter box and set aside (this step is to prevent wind from disturbing the remaining filters in the box but is optional in calm/controlled environments).
5. Remove the pre-loaded cellulose nitrate (CN) filter (the filter with grid design on it) from funnel and discard. Be sure to leave the support pad in the filter funnel.
6. Load filtration funnel with sterile polycarbonate filter on support pad (shiny side up).
7. Gently shake sample collection bottle 25 times to mix well.
8. Measure 25 mL of the mixed water sample in the sterile graduated sterile PP tube and pour into filter funnel.
9. Replace cover on filter funnel and pump to generate a vacuum (do not generate more than 7 inches of Hg of vacuum [3.44 psig]). Keep pumping until all liquid is in filtrate collection flask.
10. If the first 25 mL volume passes readily through the filter, measure and add another 25 mL and continue filtration. If it was very difficult to filter the first 25 mL, proceed to step 11. If the filter clogs before completely filtering the first or second 25 mL volume, discard the filter and repeat the filtration using a lesser volume.
11. Pour approx. 10 mL of the chilled phosphate buffered saline (PBS) into the graduated PP tube used for the sample. Cap the tube and shake 5 times. Remove the cap and pour rinsate into filter funnel to rinse filter.
12. Filter the rinsate and repeat with another 10 mL of PBS, ensuring all PBS is filtered through.
13. Remove filter funnel from base without disturbing filter. Using sterile disposable forceps remove the filter (touching only the filter edges) and fold it in half, in quarters, in eighths, and then in sixteenths (filter will be folded four times).
14. Insert filter into chilled filter extraction tube (with beads). Filter should be inserted open end down (pointed side up) into the tube. Replace and tighten the screw cap.
15. Record the volume of sample filtered through the filter on the small yellow label (marked as FILTER 1) and apply the label to the extraction tube (DO NOT cover with clear tape).
16. Record the volume of sample filtered through the filter on the outer bag label and apply the label to the bubble bag (DO NOT cover with clear tape).
17. Insert tube(s) into bubble bag and zip-top bag on dry ice for preservation during transport and shipping.

18. Record the volume of water sample filtered through each filter and the volume of buffer rinsate each filter was rinsed with on the **Littoral Samples** form. Record the filtration start time and finish time for the sample as well as the time the filters were frozen.
19. Repeat steps 6 to 15 for the remaining 50 mL sub-sample volume to be filtered. Make every effort to filter the same volume of sample through each of the two filters.

Processing procedure—fecal indicator (*Enterococci*) filter blank

Enterococci filter blanks will be prepared at all revisit sites during the first visit. Prepare the filter blanks **before** filtering the lake sample.

1. Set up sample filtration apparatus using same procedure as used for the lake sample. Chill Filter Extraction tubes with beads on dry ice.
2. Aseptically transfer 1 polycarbonate filter from filter box to base of opened Petri dish. Close filter box and set aside (this step is to prevent wind from disturbing the remaining filters in the box but is optional in calm/controlled environments).
3. Remove the pre-loaded cellulose nitrate (CN) filter (the filter with grid design on it) from funnel and discard. Be sure to leave the support pad in the filter funnel.
4. Load filtration funnel with sterile polycarbonate filter on support pad (shiny side up).
5. Measure 10 mL of the chilled phosphate buffered saline (PBS) in the sterile graduated PP tube and pour into filter funnel.
6. Replace cover on filter funnel and pump to generate a vacuum (do not generate more than 7 inches of Hg of vacuum [3.44 psig]). Keep pumping until all liquid is in filtrate collection flask.
7. Remove filter funnel from base without disturbing filter. Using sterile disposable forceps remove the filter (touching only the filter edges) and fold it in half, in quarters, in eighths, and then in sixteenths (filter will be folded 4 times).
8. Insert filter into chilled filter extraction tube (with beads). Filter should be inserted open end down (pointed side up) into the tube. Replace and tighten the screw cap.
9. Record the volume of PBS filtered through the filter on the small yellow label (marked as BLANK) and apply the label to the extraction tube (DO NOT cover with clear tape). Note that there is a specific label for the blank sample. At sites where a blank is not collected, this label will be discarded.
10. Insert tube(s) into bubble bag and zip-top bag on dry ice for preservation during transport and shipping.
11. Package and submit this sample to the lab with the standard samples.
12. Indicate that you have collected a filter blank by filling in the “Blank Collected” button on the **Littoral Samples** form.

8.2.3 Equipment and Supplies: Chlorophyll-*a*

Table 8-6 provides the equipment and supplies needed to process the chlorophyll-*a* sample. Much of this equipment will have been used previously for the filtering of the Enterococci sample.

Table 8-6 Equipment and supplies – chlorophyll-*a* processing.

Type	Item	Quantity
Form	NLA Index Samples	1
Documentation	Labels: Chlorophyll-*a* sample label	1
	Chlorophyll-*a* outer bag label	1
	Clear tape strips (to cover sample labels)	As needed
Processing	Poly bottle (2 L, brown)	1
	Centrifuge tube (50 mL, screw top) in zip top bag	1
	Sterile disposable forceps	1
	Filtration chamber (with filter holder)	1
	Filtration flask (with silicone stopper and adapter)	1
	Filtration pump (hand vacuum)	1
	Graduated cylinder (250 mL)	1
	Squirt bottle (1 L Nalgene) – de-ionized (DI)	1
	Test tube holder	1
	Whatman 0.7 µm GF/F glass fiber filter	1
Storing and preserving	Cooler	1
	Plastic electrical tape	As needed
	Foil squares	1
	Zip top bag	1
	Wet ice	As needed

8.2.4 Procedures for Processing the Chlorophyll-*a* Samples

The procedure for processing the chlorophyll-*a* sample is presented below. Whenever possible, sample processing should be done in subdued light, out of direct sunlight.

1. Place a glass fiber filter in the filter holder apparatus with the grid side down. Do not handle the filter with bare hands; use clean forceps.
2. Gently shake the chlorophyll-*a* sample collection bottle to homogenize the sample, measure and pour 250 mL of water into the filter holder using the graduated cylinder, replace the cap of the filter holder, and pump the sample through the filter. Take care not to exceed 7 inches of Hg (approximately 3.4 psi) in the vacuum gauge on the filtration pump. If 250 mL of lake water will not pass through the filter, discard the filter, rinse the apparatus with DI water, and repeat the procedures using a new filter and 100 mL of lake water. **NOTE: If the water is green or turbid, use a smaller volume to start.**
3. Observe the filter for visible color. If no visible color is present, repeat step 3 until color is visible on the filter or until a maximum of 2,000 mL have been filtered.
4. Once visible color is present and/or 2,000 mL of lake water has been filtered, record the actual sample volume filtered on the **Index Sample**s form and on the sample label. Rinse the graduated cylinder and upper portion of the filtration apparatus thoroughly with DI water to

include any remaining cells adhering to the sides and pump through the filter. Monitor the level of water in the lower chamber to ensure that it does not contact the filter or flow into the pump.

5. Disconnect the upper portion of the filter apparatus from the lower portion. Remove the filter from the holder with clean forceps. Avoid touching the colored portion of the filter. Fold the filter in half, with the colored side folded in on itself.
6. Place the folded filter into a 50 mL screw-top centrifuge tube and replace the cap. Tighten the cap as tightly as possible. The cap will seal tightly after an additional ¼ turn past the point at which initial resistance is met. Failure to tighten the lid completely could allow water to infiltrate into the sample and may compromise its integrity. Seal the cap of the centrifuge tube with plastic electrical tape
7. Record the sample volume filtered on a chlorophyll-*a* label and attach it to the centrifuge tube. Ensure that all written information is complete and legible. Cover with a strip of clear tape. Double check that the "total volume of water filtered" on the **Index Samples** form matches the total volume recorded on the sample label.
8. Wrap the tube in aluminum foil and place in a zip top bag. Place the completed outer label on the outside of the bag. Place this bag on ice in a cooler.
9. Remove the filter holder silicone stopper and adapter from the filtration flask. Pour off water from the filtration flask.
10. Discard filter chamber, forceps and filter holder. New sterile items will be used at each site.
11. Retain the filter flask, graduated cylinder, silicone stopper, and adapter. Rinse these items with DI water between sites. After returning to the office or lab: thoroughly rinse the graduated cylinder, the brown sample collection bottle and cap with tap water, complete a final rinse with DI water, and store for next sample event.

8.3 Preservation of Samples

Preserve the samples as specified in the specific protocol sections. Record the preservation information on the index and littoral sample collection forms.

8.4 Preparation of Samples for Shipping

General information regarding the preparation and shipment of samples is available in Section 4.3.2. General steps that apply to samples are the following:

- Purge the Cubitainer® of any air bubbles, seal the cap tightly and wrap plastic electrical tape clockwise around the cap. Place the Cubitainer® in a cooler with wet ice.
- Seal all pertinent caps tightly.
- Wrap plastic electrical tape clockwise around the caps, and then place the bottles in the cooler with wet ice.
- Keep chilled samples (water chemistry and chlorophyll-*a* filter) on wet ice until shipment (daily).
- Keep the atrazine samples chilled on wet ice in the field and then refrigerate until shipment (batched weekly).

- Keep the algal toxins, eDNA, and fecal indicator samples chilled on wet ice in the field and FREEZE as soon as practicable. Keep frozen until shipping (batched weekly).

**** The enterococci sample does not get tape around the cap and the sample label is not placed on the sample bottle. This sample will be shipped on dry ice.**

8.5 Data Forms and Sample Inspection

After the **Assessment** form is completed, the Field Crew Leader reviews all of the data forms and sample labels for accuracy, completeness, and legibility. The other crew member inspects all sample containers and package them in preparation for transport, storage, or shipment.

Ensure that all required data forms for the lake have been completed. Confirm that the Site ID, crew ID, and date of visit are correct. On each form, verify that all information has been recorded accurately and comments are used to explain data as needed. After reviewing each form, select the red circle at the top, which will turn green after selection. This is an indication to the crew that the form has been reviewed, but forms can be submitted before final review and amended at a later date if needed.

Ensure that all samples are labeled, all labels are completely filled in, and each label is covered with clear tape. NOTE: Do not tape over the enterococci labels. Make sure that all sample containers are properly sealed.

8.6 Launch Site Cleanup

Load the boat on the trailer and inspect the boat, motor, and trailer for evidence of plant material. Clean the boat, motor, and trailer as completely as possible before leaving the launch site. Inspect all nets for pieces of macrophyte or other organisms and remove as much as possible before packing the nets for transport. Pack all equipment and supplies in the vehicle and trailer for transport; keep them organized as presented in the equipment checklists (APPENDIX A: EQUIPMENT & SUPPLIES). Lastly, be sure to clean up all waste material at the launch site and dispose of or transport it out of the site if a trash can is not available. See Section 4.3 for additional information and follow appropriate state, tribal or other applicable protocols.

9.0 FIELD QUALITY CONTROL

Standardized training and data forms provide the foundation to help assure that data quality standards for field sampling are met. These methods for field sampling and data collection are the primary guidelines for all cooperators and field crews. In addition, repeat sampling and field evaluation and assistance visits will address specific aspects of the data quality standards for the NLA 2022.

9.1 Revisit Site

Approximately 10% of the target sites visited (96 lakes) will be revisited during the same field season by the same field crew that initially sampled the lake. If a site selected for repeat sampling is dropped, then the alternate site assigned to replace it should be revisited. The NLA 2022 Site Evaluation Guidelines provides further information regarding the replacement of revisit sites. The primary purpose of this "revisit" set of sites is to provide variance estimates that can be used to evaluate the survey design for its potential to estimate status and detect trends in the target population of lakes. The time period between the initial (Visit 1) and repeat visit (Visit 2) to a lake must be at least two weeks and should be as long as possible.

Visit 2 will include the full set of indicators and associated parameters with two notable exceptions. A fecal indicator blank will be collected at Visit 1 only and human health fish tissue will only be collected at only one of the two site visits. Crews should collect fish during Visit 1 for a revisit site, to allow for a second opportunity if fish cannot be collected during the first visit. If the crew collects fish on Visit 1, they do not need to collect fish on Visit 2. If they are unable to collect fish on Visit 1, the crew should collect fish on Visit 2.

9.2 Field Evaluation and Assistance Visits

No national program of accreditation for field work currently exists. For this reason, a rigorous program of field evaluation and assistance visits has been developed to support the NLA 2022.

9.2.1 General Information

Evaluation and assistance visits will be conducted with each field crew early in the sampling and data collection process, if possible, and corrective actions will be conducted in real time. These visits provide both a quality check for the uniform evaluation of the data collection methods and an opportunity to conduct procedural reviews, as required, minimizing data loss due to improper technique or interpretation of field procedures and guidance. Through uniform training of field crews and review cycles conducted early in the data collection process, sampling variability associated with specific implementation or interpretation of the protocols will be significantly reduced. The assistance visit also provides the field crews with an opportunity to clarify procedures and offer suggestions for future improvements based on their sampling experience preceding the visit. The field evaluations, while performed by a number of different supporting collaborator agencies and participants, will be based on the uniform training, plans, and checklists. This review and assistance task will be conducted for each unique field crew collecting and contributing data under this program; hence no data will be recorded to the project database that was produced by an 'unaudited' process or individual. The field evaluations will be based on the evaluation plan and field evaluation checklist. The checklist will be made available to all parties associated with NLA.

One or more designated EPA, state, or contractor staff members who are qualified in the procedures of the NLA 2022 field sampling operations will visit trained state, tribal, contractor, and EPA field sampling crews during sampling operations on site. If membership of a field crew changes, and at least two of the members have not been evaluated previously, the field crew must be evaluated again during sampling operations as soon as possible to ensure that all members of the field crew understand and can perform the procedures.

The purpose of this on-site visit will be to identify and correct deficiencies during field sampling operations. The process will involve the following preparation steps and field day activities .Additionally, conference calls with crews may be held approximately every two weeks to discuss issues and clarifications as they come up throughout the sampling season.

9.2.2 Preparation Activities

1. Each Field Crew Evaluator will schedule an assistance visit with their designated crews in consultation with the QA Officer, Regional NLA Coordinator, and respective Field Sampling Crew Leader. **Evaluators should be prepared to spend additional time in the field if needed (see below).** Ideally, each Field Crew will be evaluated within the first two weeks of beginning sampling operations, so that procedures can be corrected or additional training provided, if needed.
2. Each Field Crew Evaluator will ensure that field crews are aware of their visit plans and all capacity and safety equipment will be provided for the Field Crew Evaluator.
3. Each Field Crew Evaluator will need to bring along the following in Table 9-1.

Table 9-1 Equipment and supplies – field evaluation and assistance visits.

Type	Item	Quantity
Form	Field Evaluation and Assistance Visit Checklist (sent from EPA)	1
Documentation	NLA 2022 Field Operations Manual	1
	NLA 2022 Quality Assurance Project Plan	1
	Clipboard	1
	Pencils (#2, for data forms)/Pen	1
	Field notebook (optional)	1
Gear	Field gear (e.g., protective clothing, sunscreen, insect repellent, hat, water, food, backpack, cell phone)	As needed

9.2.3 Field Day Activities

1. The Field Crew Evaluator will review the Field Evaluation and Assistance Visit Checklist with each crew during the field sampling day and establish and plan and schedule for their evaluation activities for the day.
2. The Field Crew Evaluator will view the performance of a field crew through one complete set of sampling activities as detailed on the checklist.
    - Scheduling might necessitate starting the evaluation midway on the list of tasks at a site, instead of at the beginning. In that case, the Field Crew Evaluator will follow the crew to the next site to complete the evaluation of the first activities on the list.

- If the field crew misses or incorrectly performs a procedure, the Field Crew Evaluator will note this on the checklist and *immediately point this out so the mistake can be corrected on the spot*. The role of the Field Crew Evaluator is to provide additional training and guidance so that the procedures are being performed consistently with the FOM, all data are recorded correctly, and paperwork is properly completed at the site.

3. When the sampling operation has been completed, the Field Crew Evaluator will review the results of the evaluation with the field crew before leaving the site (if practicable), noting positive practices and problems (i.e., weaknesses [might affect data quality]; deficiencies [would adversely affect data quality]). The Field Crew Evaluator will ensure that the field crew understands the findings and will be able to perform the procedures properly in the future.
    - The Field Crew Evaluator will review the list and record responses or concerns from the field crew, if any; on the checklist (this may happen throughout the field day).
    - The Field Crew Leader will sign the checklist after this review.

9.2.4 **Post Field Day Activities**

1. The Field Crew Evaluator will review the checklist that evening and provide a summary of findings, including lessons learned and concerns.
    - If the Field Crew Evaluator finds major deficiencies in the field crew operations (e.g., less than two members, equipment, or performance problems) the Field Crew Evaluator must contact the EPA NLA 2022 Project Lead. The EPA NLA 2022 Project Lead will contact the EPA Technical Lead for the Human Health Fish Fillet Contaminants Indicator in OST and the EPA NARS QA Project Officer to determine the appropriate course of action.
2. The Field Crew Evaluator will retain a copy of the checklist and submit to the NLA logistics lead.
3. The EPA NLA 2022 Project Lead and EPA NARS QA Project Officer or authorized designee will review the returned Field Evaluation and Assistance Visit Checklist, note any issues, and check off the completion of the evaluation for each field crew.

9.2.5 **Summary**

Table 9-2 summarizes the plan, checklist, and corrective action procedures.

Table 9-2 Summary of field evaluation and assistance visit information.

Field Evaluation Plan	The Field Crew Evaluator: • Arranges the field evaluation visit in consultation with the QA Officer, Regional NLA Coordinator, and respective Field Sampling Crew Leader, ideally within the first two weeks of sampling • Observes the performance of a crew through one complete set of sampling activities • Takes note of errors the field crew makes on the checklist and immediately point these out to correct the mistake • Reviews the results of the evaluation with the field crew before leaving the site, noting positive practices, lessons learned, and any concerns
Field Evaluation Checklist	The Field Crew Evaluator: • Observes all pre-sampling activities and verifies that equipment is properly calibrated and in good working order, and protocols are followed • Checks the sample containers to verify that they are the correct type and size, and checks the labels to be sure they are correctly and completely filled out

	• Confirms that the field crew has followed NLA protocols for locating the lake and determining the index site on the lake • Observes the index site sampling, confirming that all protocols are followed • Observes the littoral sampling and habitat characterization, confirming that all protocols are followed • Records responses or concerns, if any, on the Field Evaluation and Assistance Checklist
Corrective Action Procedures	• If the Field Crew Evaluator's findings indicate that the Field Crew is not performing the procedures correctly, safely, or thoroughly, the Evaluator must continue working with this Field Crew until certain of the crew's ability to conduct the sampling properly so that data quality is not adversely affected. • If the Field Crew Evaluator finds major deficiencies in the Field Crew operations the Evaluator must contact the EPA NLA 2022 Project Lead who, in turn, must contact the EPA Technical Lead for the Human Health Fish Fillet Contaminants indicator if any of the major deficiencies relate to this indicator.

10.0 EPA Regional and Contracted Crews Only: Endangered Species Act Conservation Measures

A generally applicable element of all NLA sampling is that field crews make every effort not to disrupt nontarget habitats. NLA protocols are specifically designed to sample target lakes, ponds and reservoirs and to avoid non-target habitats (e.g., rivers, marine, riparian, and wetlands). This section identifies conservation and mitigations measures that are to be performed by EPA regional and contracted crews (EPA crews) at all sites where federally listed species under the Endangered Species Act (ESA) are possible, as appropriate. Before sampling or accessing an NLA site, EPA field crews will receive a list of their sites screened for ESA-listed species possible at the sites. Crews will use this information to familiarize themselves with the ESA species and to implement appropriate conservation measures. Any additional ESA documentation used to supplement the EPA provided ESA information, such as contact between the crew and local U.S. Fish and Wildlife Service office ("FWS" or "Service"), will be retained and provided electronically to the NLA ESA lead periodically throughout the field season.

The below mitigations are not an exhaustive list of requirements for EPA crews. Crews are expected to implement any additional conservation measures required by local permit. Crews should also be aware of any relevant and reasonable state conservation measures, these may be completed at the discretion of the crew lead. Field crew leads are expected to be cognizant of the federally listed species, listed species critical habitats, and state species of concern that have the potential to occur at or near a given sampling site, including habitats that will be used to access the sampling site. They are responsible for making their crew members aware of potential occurrences of listed species and their critical habitat. Efforts should be made to minimize risks to listed species and their critical habitats and avoid the take of listed species while implementing the NLA field protocols. For sites without ESA listed species possible, the following mitigations are not required.

10.1 Always Applicable

- Crews refrain from intentionally targeting known ESA species in any way (handling, harassing, feeding etc.).
- If a known or suspected ESA-listed species is accidently encountered while sampling, it will be returned as quickly as possible to the appropriate habitat and away from further sampling pressures.
- Any samples taken as part of the NLA survey protocol will be inspected carefully for the presence of ESA organisms or other resources from listed taxa groups that could be associated with collecting a non-listed species (such as glochidia on fish gills on a collected sample). Any samples collected must be carefully inspected for their presence and, if found, removed prior to sample preservation.
- Crews will wait to allow a listed species to naturally move away from the sampling area (do not herd or harass).
- All encounters with any ESA species are to be recorded in the **Federal ESA** form in the NLA App, which documents the species, number of individuals, the encounter type, and the disposition of the individual after the encounter.

10.2 When Entering or Leaving a Site and Shorline Activities

- Be aware of the habitat requirements for each life cycle stage and avoid unnecessary activity within that habitat. Crews are to limit damaging ESA species habitat when accessing or working a site.
    - Whenever available crews will use existing roads, pathways, or trails to the sampling site for activities including transporting equipment and boats. Crews will adhere to a "stay on trail" rule and refrain from roaming from designated pathways. If a trail is not available, crews will attempt to navigate to and from the site on durable surfaces which can tolerate repeated trampling. If no areas can be found that would tolerate repeated trampling without notable impacts, then crews are to walk in a single file line to and from the site whenever possible.
- When walking through or at a site, avoid stepping on vegetation mounds or holes which may contain listed reptiles, nests, or burrowing species. Whenever possible, avoid areas where listed species are congregating.
- Crews will utilize public boat docks whenever possible. Private boat docks can also be used with owner permission.
- Before putting a vessel or sampling equipment in the water, visually investigate the area for ESA species and avoid areas where observed ESA are present.
- Special Consideration: Shoreline, burrowing and sub-surface nesting ESA species possible
    - Piping Plover and other listed birds which utilize ground nesting in sandy areas: in addition to the applicable species-specific mitigations listed in FWS documentations, crews are to avoid traversing across a beach or sandy area if such species may be present. If an area is cordoned off, crews are not to enter a closed area.
    - When walking through or at a site, avoid stepping on vegetation mounds or holes which may contain listed reptiles, nests, or burrowing species (see below for more information).

10.3 Index Site Activities

Listed species that may be encountered are fish, birds, mammals, invertebrates, amphibians, and reptiles.

- Prior to introducing any equipment that disturbs the water column or aquatic habitat, the area should be carefully inspected for the presence of listed species. Avoid collecting or disturbing listed species.

10.4 Littoral Activities

Listed species that may be encountered are fish, freshwater mussels (Unionidae and Margaritiferidae), birds, mammals, invertebrates, amphibians, and reptiles.

- Prior to introducing any equipment that disturbs the water column or aquatic habitat, the area should be carefully inspected for the presence of listed species. Avoid collecting or disturbing listed species.
- Follow all freshwater mussel handling and documentation protocols in Section 6.4.

10.5 Lake Wide Activites: Fish Sample Collection

10.5.1 Sites with ESA fish possible

- When identifying a suspected fish ESA-listed species, each individual of the species is to be kept in their own bucket of fresh water. The water in the bucket is to be obtained from the collection site. The species will only be kept in the bucket for the time it takes to quickly and urgently identify that species and to provide recovery time as needed. During identification, each individual may not be continually handled for more than 1-minutes.
- Crews must not perform active electrofishing for longer than 3 hours.
    - If performing fish sampling at sites where Bull Trout (*Salvelinus confluentus*) are possible, no electrofishing is allowed.
- No gillnetting, trawling, or seining are allowed.
- All other allowable fishing methods should not exceed 4 hours.
- Crews will not perform fishing methods before sunrise or after sunset at sites where encounters with ESA-listed species are possible.
- Crews are to return any ESA-listed fish back into the lake (alive) as quickly as feasible. No ESA fish may be kept for collection.

10.5.2 Sites with ESA freshwater mussles possible (indirect impacts to lake habitat-based bivalves' host fishes)

- Crews must not perform active electrofishing for longer than 3 hours.
- No gillnetting, trawling, or seining are allowed.
- All other allowable fishing methods should not exceed 4 hours.
- Any collected target fish must be inspected for glochidia. If glochidia are found, the fish must be returned alive to the site away from further sampling pressures. All fish with glochidia must be reported in the **Federal ESA** form the app.
    - If glochidia on gills are found, crews are to be very aware of the likelihood of mussels present and make sure to properly execute the mussel conservation methods.

11.0 LITERATURE CITED

Baker, J.R., D.V. Peck, and D.W. Sutton (Eds.) 1997. Environmental Monitoring and Assessment Program -Surface Waters: Field Operations Manual for Lakes. EPA/620/R-97/001. U.S. Environmental Protection Agency, Washington, D.C.

Dodson, S.L., R.A. Lillie, and S. Will-Wolf. 2005 Land use, water chemistry, aquatic vegetation, and zooplankton community structure of shallow lakes. Ecological Applications 15:1191-1198.

Grabarkiewicz, J. and W. Davis. 2008. An introduction to freshwater mussels as biological indicators. EPA-260-R08-015. U.S. Environmental Protection Agency, Office of Environmental Information, Washington, DC. Available at: https://www.waterboards.ca.gov/water_issues/programs/swamp/docs/cwt/guidance/445.pdf

Kurtz, J. C., L. E. Jackson, and W. S. Fisher. 2001. Strategies for evaluating indicators based on guidelines from the Environmental Protection Agency's Office of Research and Development. Ecological Indicators 1:49-60.

Klemm, D. J., P. A. Lewis, F. Fulk, and J. M. Lazorchak. 1990. *Macroinvertebrate Field and Laboratory Methods for Evaluating the Biological Integrity of Surface Waters*. EPA 600/4-90/030. U.S. Environmental Protection Agency, Cincinnati, Ohio.

National Institute for Occupational Safety and Health. 1981. *Occupational Health Guidelines for Chemical Hazards* (Two Volumes). NIOSH/OSHA Publication No. 81-123. U.S. Government Printing Office, Washington, D.C. https://www.cdc.gov/niosh/docs/81-123/

Stemberger, R. S. and J. M. Lazorchak. 1994. Zooplankton assemblage responses to disturbance gradients. Canadian Journal of Fisheries and Aquatic Sciences 51:2435-2447.

Stevens, D. L., Jr. and A. R. Olsen. 2004. Spatially balanced sampling of natural resources. Journal of the American Statistical Association 99:262-278.

U.S. Coast Guard. *A Boaters Guide to the Federal Requirements for Recreational Boats and Safety Tips.* U.S. Department of Transportation, United States Coast Guard, Washington, D.C. https://www.uscgboating.org/images/420.PDF

USEPA. 2013. Level III ecoregions of the continental United States: Corvallis, Oregon, U.S. EPA https://www.epa.gov/eco-research/level-iii-and-iv-ecoregions-continental-united-states

USEPA. 2000a. EPA Quality Manual for Environmental Programs 5360A1. May 2000. http://www.epa.gov/quality/qs-docs/5360.pdf

USEPA. 2000b. EPA Order 5360.1 A2 CHG2, Policy and Program Requirements for Mandatory Agency-wide Quality System, May 5, 2000. http://www.epa.gov/quality/qs-docs/5360-1.pdf

APPENDIX A: EQUIPMENT & SUPPLIES

Base Kit

A Base Kit will be provided to the field crews for all sampling sites that they will go to. Some items are sent in the base kit as extra supplies to be used as needed.

Base Kit Item	Quantity	Protocol
Centrifuge tube (50 mL, screw top) - extras	2	Chlorophyll-*a*
Centrifuge tube stand	1	Enterococci, Chlorophyll-*a*
Electrical tape*	2	General
Filter forceps (flat blade) - extras	6	Enterococci, Chlorophyll-*a*
Filters - Millipore 47mm polycarbonate 0.4 µ (Box of 50)	1	Enterococci
Filters - Whatman 0.7 µm GF/F glass fiber (box of 100)	1	Chlorophyll-*a*
Filtration chamber (with filter holder) - extras	5	Enterococci, Chlorophyll-*a*
Filtration chamber adapter	3	Enterococci, Chlorophyll-*a*
Filtration flask (side arm, 500 mL)	1	Enterococci, Chlorophyll-*a*
Filtration pump (hand vacuum)	1	Enterococci, Chlorophyll-*a*
Filtration flask stopper (silicone)	2	Enterococci, Chlorophyll-*a*
Foil squares (package of 25)*	1	Chlorophyll-*a*
Funnel	1	Water samples Zooplankton Benthics
Gloves (latex/nitrile, non-powdered, box of 100)	1	General
Graduated cylinder (250 mL)	1	Chlorophyll-*a*
HDPE bottle (1 L, white, wide-mouth) – extras	6	Benthics
HDPE bottle (125 mL, white, wide-mouth) – extras	6	Zooplankton
Littoral eDNA collection bottle (125 mL, clear, square) - extras	3	Littoral eDNA collection
Integrated water sampler device (MPCA design)†	1	Water Samples
Kick net (500 µm D-shaped, modified) with 4 foot handle†	1	Benthics
Lugol's solution (250 mL bottle)	1	Phytoplankton
Metric tape measure (8 meter)	1	Secchi Physical Habitat
Microcentrifuge tubes (extras)	5	Enterococci
Packing tape (extra rolls)*	2	General
Packing tape (on holder)*	1	General
Pail (narcotizing/concentrating chamber)	2	Zooplankton
Petri dishes (60x15, disposable)	20	Enterococci
Plankton net course (150 µm; 30 cm diameter net with a 20 cm reducing collar)	1	Zooplankton

Base Kit Item	Quantity	Protocol
Plankton net fine (50 µm; 30 cm diameter net with a 20 cm reducing collar)	1	Zooplankton
Plankton net carry case for 2 nets	1	Zooplankton
Plastic tranfer pipette for Lugol's	5	Phytoplankton
Poly bottle (2 L, brown)	1	Chlorophyll-*a*
Rubbermaid action packer	1	General
Secchi disk (20 cm diameter) with weight†	1	Secchi
Sieve bucket (500 µm)†	1	Benthics
Small tote with lid	1	General
Sodium Thiosulfate (25 tablets in vial)	1	Enterococci
Sounding line (50 m, marked in 0.5 m intervals) with clip	1	Depth Secchi Zooplankton
Sounding rod (3 m , marked in 0.1 m increments, PVC, 2-section)†	1	Index Site Depth Physical Habitat
Sounding weight with clip	1	Depth
Spoon (stainless steel)	1	Benthics
Squirt bottle (1 L Nalgene) – for de-ionized (DI) water	1	General
Squirt bottle (1 L Nalgene) – for lake water	1	General
Surveyor's tape (50m)†	1	Depth Physical Habitat
Tape strips (3M, pad of 25)*	6	General
Watchmaker's forceps	1	Benthics

*Items may need to be replenished by field crews during field season
† Item supplied if needed

Site Kit

A Site Kit will be provided upon request to the field crews for each sampling site. Please submit an electronic **Request** form well in advance of field sampling to request the Site Kits. Each site kit will also include necessary coolers and shipping supplies for all samples collected.

Site Kit Item	Quantity Per Site Kit	Protocol(s)
Centrifuge tube (50 mL, screw top) in bag	1	Chlorophyll-*a*
CO2 (Alka seltzer) tablets (packet of 2)	1	Zooplankton
Cooler liners (1 per cooler)	-	General
Cubitainer® (4L)	1	Water Chemistry
FedEx Express shipping labels (1 per cooler)	-	Sample shipping
Gloves (latex/nitrile, non-powdered)	4 pair	Sample collection
Filtration unit (sterile 250 mL filter funnel, cap, and filter holder)	1	Enterococci Chlorophyll-*a*
Filter forceps (sterile, flat blade)	2	Enterococci Chlorophyll-a
HDPE bottle (1 L, white, narrow mouth)	1	Phytoplankton
HDPE bottle (60 mL, white, wide-mouth)	1	Atrazine
HDPE bottle (125 mL, white, wide-mouth)	2	Zooplankton
HDPE bottle (1 L, white, wide-mouth)	2	Benthics
Littoral eDNA collection bottle (125 mL, clear, square)	1	Littoral eDNA collection
Enterococci bottle (250 mL, sterile, clear, narrow-mouth)	1	Enterococci collection
HDPE bottle (500 mL, white, wide-mouth)	1	Algal Toxins (MICZ)
eDNA bottle (1 L, clear, square, narrow-mouth)	2	eDNA (Index and Littoral)
Phosphate buffered saline (sterile PBS)	1	Enterococci
Microcentrifuge tubes w/glass beads (in bubble & Zip bags)	2	Enterococci

Human Health Fish Sampling Kit

A **human health fish sampling kit** will be provided to the field crews for designated human health fish sampling sites (separately from site kits). Please submit an electronic **Request** form **well in advance** of field sampling. Kits must be requested at least two weeks before sampling is to take place. Prior to sampling, inspect each site kit to ensure all supplies are included. These human health fish sampling kits include:

Human Health Fish Sampling Kit Item	Quantity
Aluminum foil (solvent-rinsed and oven dried)	5 packs
Cable ties	24
Cooler	1
Dry ice Shipping Label	1
FedEx airbill (Pre-addressed)	1
Frequently Asked Questions handout	1
Heavy-duty food grade polyethylene tubing	1 roll
Large plastic composite bag	1
Nitrile gloves	10
NLA 2022 Human Health Fish Fillet Contaminants Site List	1
Tyvek tag	1

Electronic Forms & Labels

Apple iPads Labels will be supplied by the NARS IM Center upon request.

Item	Quantity	Protocol
Apple iPads: Field forms packet (paper backup copies as needed): NLA 2022 Verification NLA 2022 Profile Calibration NLA 2022 Profile Data NLA 2022 Index Samples NLA 2022 Littoral Samples NLA 2022 Physical Habitat NLA 2022 Assessment NLA 2022 Tracking NLA 2022 Whole Fish Sample NLA 2022 Federal ESA	2 1	General General
Labels packet (for samples)	1	General

Field Crew Supplied Equipment

This equipment will need to be supplied by the field crew. Some items are optional.

Field Crew Supplied Item	Quantity	Protocol(s)
Access instructions	1	Site Evaluation
Access permission documents/permit (if required)	1	Site Evaluation
Barometer or elevation chart to use for calibration	1	Calibration
Binoculars	1	Physical Habitat
Bleach (or bleach alternative)	1	General
Buckets (5 gallon capacity, plastic)	2	Benthic Macroinvertebrates
Buoy (for marking observation point)	1	Physical Habitat
Calibration cups and standards (for multi-parameter meter)	1	Calibration
Calibration QC check solution (for multi parameter meter, pH and conductivity)	1	Calibration
Clinometer	1	Physical Habitat
Clipboard	1	General
Depth Finder (hand-held or boat mounted sonar)	1	Index Site Profile Physical Habitat
Electronic data capture devices (tablet/phone/computer) with NARS App and extra battery pack (if needed)	1-2 (optional)	General
Ethanol (95%)		Benthic Macroinvertebrates Zooplankton
Field gear (e.g., protective clothing, sunscreen, insect repellent, hat, water, food, backpack, cell phone)		General
Field notebook - optional	1	General

Field Crew Supplied Item	Quantity	Protocol(s)
Field thermometer (not mercury)	1	Calibration
Fishing gear (e.g., electrofishing unit, fishing rods, nets, livewell and/or buckets, measuring board etc.)		Whole Fish Sample
GPS unit (with manual, reference card, extra battery)	1	Lake Verification Index Site Coordinates Physical Habitat
Kick net (500 µm D-shaped) with 4 foot handle (spare)	1	Benthic Macroinvertebrates
Laser rangefinder (for estimating drawdown) - optional	1	Physical Habitat
Map wheel or string (for measuring shoreline distances on site map)	1	Physical Habitat
Multi-parameter water quality meter (with temperature, pH, and DO probes)	1	Index Site Profile
Multi-parameter communication cable (50 m)	1	Index Site Profile
Net(s) and/or bucket assembly for end of net (spares)	1	Zooplankton
NLA 2022 Fact Sheets	20	General
Pen	1	General
Pencils (#2, for data forms)	2	General
Permanent marker (fine tip, for labels)	2	General
Scissors	1	General
Shipping tape	1	Shipping
Surveyors rod - optional	1	Physical Habitat
Water (deionized)		General

Boat Equipment List

This is suggested boat equipment.

Item
Anchor (with 75 m line or sufficient to anchor in 50 m depth)
Boat horn
Boat plug (extra)
Bow/stern lights
Emergency tool kit
Fire extinguisher
First aid kit
Gas Can
Hand bilge pump
Life jackets
Motor
Oars or paddles
Second anchor for windy conditions and littoral sampling (w/ 75m line)
Sonar unit

Spare prop shear pin
Type IV PFD (throwable life saving device)

APPENDIX B: SHIPPING GUIDELINES

General Shipping Guidelines

Samples will be shipped by the field crews according to the chart below. The Field Crew Leader will complete the appropriate section(s) of the **Tracking** form for the samples and will submit tracking via the NLA App. The Field Crew Leader will place the samples and the packing slip (in a waterproof bag or plastic sleeve) in the shipment cooler, and then the Field Crew Leader will attach the appropriate pre-addressed FedEx label from the site kit or cooler marked for the appropriate lab. The field crew will either drop off the cooler for shipment at a local FedEx location or arrange for a pick up at the hotel or other appropriate facility. If the field crew has chosen a pick up, they must follow up with the facility at which it has been left and/or track the package through FedEx tracking tools to ensure that the shipment cooler has actually been picked up by FedEx. Once the package is in the possession of FedEx, the IM Team and Field Logistics Coordinator (FLC) will track the package to its destination and take steps necessary to ensure timely delivery.

A packing slip must be filled out to accompany each sample shipment. Be very careful to fill in the information correctly and legibly, especially the Site ID. When using a packing slip that was paired with a sample label sheet, the Sample ID numbers will be pre-printed on the packing slips. The packing slip is to be placed in a resealable plastic bag/pouch secured to the inside of the cooler lid. Seal the shipping container with clear packing tape. In the NLA App, fill in the shipping details in the pertinent section(s) of the **Tracking** form and submit the **Tracking** form to the NARS IM Center to indicate that samples will be in transit to the laboratory. The **Tracking** form must be submitted the same day that the samples are shipped.

Before shipping, it is very important to preserve each sample as directed in the sample collection portion of the appropriate chapter in the NLA 2022 FOM. General directions for sample processing, shipping and tracking are found below:

- Preserve the samples as specified for each indicator before shipping.
- Be aware of the holding times for each type of sample.
- Always line the cooler with a heavy duty plastic bag (cooler liner) when shipping preserved samples or when shipping wet ice.
- When shipping frozen batched samples, use the foil-backed dry ice liner and foam pad inside the cooler.
- When shipping with dry ice, it's best to layer or intermix dry ice and samples to increase the contact between samples and dry ice.
- When shipping samples preserved with ethanol, surround the jars with crumpled newspaper or other absorbent material.

When wet ice is used for shipment:

- Ensure that the ice is fresh before shipment and use adequate amounts of ice to ensure samples will remain cold for up to 48 hours.
- Place samples and all ice inside the cooler liner and seal the cooler liner to avoid leakage of melted ice.

- Secure the cooler with clear packing tape.

When dry ice is used for shipment:

Note: Not all FedEx locations will accept shipments containing dry ice. Dry ice shipments can be shipped from "FedEx staffed" locations, which is how these locations are referred to online. You can also arrange for a pick-up from your lab or hotel. Dry ice shipments usually cannot be shipped from FedEx Office® locations, FedEx Retail locations such as Walgreens/Walmart/OfficeMax, or at FedEx Authorized ShipCenter® locations. These types of locations are differentiated on FedEx.com in the "Find FedEx Locations" feature. Please be sure to call in advance to ensure your location will accept the package for shipment.

- Label the cooler with a Class 9 Dangerous Goods label
    - Place the label on the front side of the cooler, not the top.
    - If it is not already completed, fill out the upper corners of the label with the shipper and recipient information as on the FedEx airbill.
    - Declare the weight (in kg) of the dry ice in the lower right hand corner of the label, ensuring it is the same weight listed on the airbill.
- Ensure that the dry ice is fresh before shipment, and use adequate amounts of dry ice to ensure samples will remain frozen for up to 48 hours.
- Secure the cooler with clear packing tape. Do not completely seal the entire edge of the cooler such that the pressure inside the cooler could build.
- Place the provided FedEx airbill on the top of the cooler or on a handle tag secured to one of the cooler's handles.
    - Ensure the label indicates the amount of dry ice in the package and matches the weight listed on the dry ice label.

Tracking Forms and Shipment Types

Whenever NLA samples are shipped, one or more sections of the **Tracking** form is completed to relay important shipping information to the IM Team, FLC and the destination lab. The **Tracking** form is submitted electronically at the time of shipping and a packing slip is included as a hard copy in the shipping container with the samples.

Each section for the **Tracking** form has been assigned a "T" number to help crews identify the correct section of the form to use when sending samples. This "T" number is located on the top of each tracking form section). Crews will also find reference to the same "T" numbers on the individual sample labels and on the top of the pre-printed FedEx return labels provided in the site kits or with batch coolers. The FedEx return labels are pre-paid and allow crews to ship samples to any of the nationally contracted laboratories. States using their own labs for certain samples will need to arrange for shipping on their own.

When crews order site kits (via the **Request** form), a set of packing slips will accompany each set of labels in the site kit. Sample IDs for the suite of samples collected at a single site will be pre-populated

on both the labels and the packing slips. By entering the water chemistry sample ID in the **Tracking** form, the rest of the sample IDs will auto-populate in the **Tracking** form. It is important to keep the labels and packing slips organized so the sample IDs will match when shipping occurs.

Crews include the pertinent packing(s) slip in the cooler (placed in the plastic sleeve affixed to the inside of the cooler lid) when they send samples to the labs, and they also must submit the completed section of the **Tracking** form that is associated with that sample shipment via the NLA App. If a cooler contains samples from more than one site, then multiple packing slips must be placed in the cooler and the **Tracking** form for each site must be submitted.

When a crew visits a site with the intent to sample, they complete and submit the **Verification** form via the NLA App. It is very important to submit this form as soon as possible after every attempted sampling event to provide key items such as the date of the event, the crew ID, and whether the site wa ssampled. Prompt status reports allow the FLC to closely track sampling progress. More importantly, it enables NARS IM to track samples that were collected at each successfully sampled site versus those that were not, and to immediately track the shipment of the time-sensitive samples after each sampling event. Submitting the **Verification** form is crucial to report the date of a completed sampling event and must be submitted (along with sample tracking information) on the same day that samples are shipped.

Procedure for filling out and submitting tracking via the App

1. After ensuring all of the samples to be shipped are properly preserved and prepared for shipment, access the **Tracking** form in the App.
2. Ensure the correct water chemistry sample ID has been entered at the top of the form. Doing so will populate the sample IDs of all other collected samples. Samples that were not collected will display a blank sample ID field and the not collected bubble will be transferred from the individual sample collection forms. The not collected bubbles are not editable in the **Tracking** form; to change the collection status of a sample, access the pertinent sample collection forms (e.g., Index Samples, Littoral Samples, and/or Whole Fish Sample forms).
3. In the pertinent section of the **Tracking** form , check the box under the 'To Ship' column for each sample being sent in the shipment.
4. Click the 'Enter Shipping Details' button and fill out the resulting popup window with the destination lab, date shipped, tracking number, sender and sender's phone number.
5. Click the 'save shipping info' button to save the details and the **Tracking** form .
6. Once the shipping details have been saved in the App, a date will appear in the shipped column of the **Tracking** form . If the shipping details for a sample need to be edited, click the date in the shipped column to access the saved shipping details. Editing the details in this manner changes ONLY one sample at a time. The only way to enter shipping details for an entire group of samples is during the initial details entry.
    a. If the status of the sample needs to change from shipped to **not** shipped, click the date in the shipped column to access the saved shipping details and delete all the shipping info. Click the "save shipping info" button after deleting all the shipping information.

The sample will no longer be marked as shipped and the "to ship" checkbox will reappear.

7. After all pertinent shipping details have been saved, click the SUBMIT menu button and select the button next to 'Tracking' and any other the forms that you wish to submit. Click the green submit button at the bottom of the form list. An email will pop up on your device addressed to NARSFieldData@epa.gov. Copy yourself, any other crew members or managers and click send. To ensure that he email was sent, check the SENT mailbox on your email app and look for the recent email containing the data. If the email is not in the SENT mailbox, it was not sent and you should try again after verifying an internet connection.
8. At any point, if it is determined that data needs to be revised or updated, crews should feel free to do so in the App and re-submit any edited data or tracking forms using the steps above. Newly revised data will automatically take the place of previous data. It is not necessary to re-submit data or tracking forms that were unchanged however.
9. After submission, a data summary will be automatically emailed back to the email address from which the submission was received. The Field Crew Leader or his/her designee should review this data summary for accuracy and make any corrections necessary and re-submit the pertinent form(s).

Shipping Groups:

T-1 – Daily Water Chemistry Samples

- Complete the T-1 section of the **Tracking** form for the samples that are shipped immediately after each sampling event (e.g., same day as sampling or the next day):
  - water chemistry
  - chlorophyll-*a*
- These samples are shipped together in the site kit cooler with a heavy duty liner bag and ample wet ice.
- Samples from two sites may be shipped together in a single cooler if they were collected on the same day.
- Send the **Tracking** form and all data forms from the site to the IM Team via the NLA App.
- Ship all of the samples to the lab in the same cooler with the packing slip that was provided with the label packet. If any sample listed on the packing slip is not being shipped, line out the sample ID to indicate that the sample is not in the cooler. If samples from multiple sites are shipped together, then multiple packing slips must be used.
- Samples need to be shipped on as much fresh wet ice as will fit in the cooler liner. Water chemistry and chlorophyll-*a,* samples should be shipped within 24 hours of collection (i.e., the same day as sampling or the following day).

T-2 – Frozen Batched Samples

- Complete this section of the **Tracking** form when shipping the frozen batched samples:
  - Enterococci (frozen)
  - eDNA (index and littoral; frozen)

- algal toxins (frozen)
- atrazine (chilled)

- These samples are shipped together in a cooler (ordered via the **Request** form) with a two-piece dry ice liner and 20 pounds of dry ice.
- 2-3 site's worth of samples may be shipped together in a single cooler.
- Ship all of the samples to the lab in the same cooler with the packing slip that was provided with the label packet. If any sample listed on the packing slip is not being shipped, line out the sample ID to indicate that the sample is not in the cooler. If samples from multiple sites are shipped together, then multiple packing slips must be used.
- To pack the T2 cooler:
    1. Place the foam pad in the bottom of the T2 cooler and line the cooler with the foil-backed dry ice liner.
    2. Place frozen samples from up to three sites and dry ice (approximately 20 lbs) inside the dry ice liner, intermixing samples and dry ice.
    3. Please the enterococci samples on top of the frozen bottles to prevent damage during shipping.
    4. Place the foil-backed separator pad on top of the frozen samples and dry ice creating a thermal barrier within the cooler.
    5. Place the chilled atrazine samples (up to 3) inside the atrazine sleeve and insert the foam plug into the end of the sleeve.
    6. Place the sleeve ON TOP of the second foam pad inside the dry ice liner (such that the foam pad is used as a separator between the dry ice and the atrazine samples).
    7. Close the "lid" portion of the dry ice liner.
    8. Insert the T2 packing slip(s) into the provided pouch inside the cooler lid.
    9. Close the cooler lid, taping and applying FedEx shipping label as usual.
- Frozen batched samples should be shipped at least every week.
- In cases where a state lab is processing some, but not all of the samples, first select “To Ship” checkboxes for the samples being sent to the national lab and select the appropriate lab in the shipping details popup window. The T-2 packing slip should be placed in the cooler being shipped to the national lab and should only indicate the sample(s) being shipped in that cooler. Line out the sample IDs to indicate which samples are not in the cooler. Select “To Ship” checkboxes for the remaining samples being sent to the state lab and select the appropriate lab in the shipping details popup window.
- Verify the sample IDs for the sample(s) being shipped.
- Submit the **Tracking** form to NARS IM in the NLA App using the steps above.

T-3 – Non-Chilled Batched Samples

- Complete this section of the **Tracking** form for shipping batches of non-chilled samples:
  - Benthic macroinvertebrates
  - Zooplankton (course and fine)
  - Phytoplankton
- These samples are shipped together in a cooler (ordered via the **Request** form) with a heavy duty liner bag and no ice.
- The number of samples that will fit in a cooler will depend largely on the number of benthos bottles collected from each site. In most cases, samples from three to six site visits will fit in a cooler together. NEVER split samples from one site into more than one cooler.
- Ship all of the samples to the lab in the same cooler with the packing slip that was provided with the label packet. If samples from multiple sites are shipped together, then multiple packing slips must be used.
- Place 1-liter benthos bottles upright in the lined cooler and use newspaper or cardboard to fill any empty space between benthos bottles to keep them in an upright position during shipping.
- Place bagged zooplankton bottles on top of the upright benthos bottles.
- Non-chilled batched shipped samples should be shipped within two weeks of collection.
- In cases where a state lab is processing some, but not all of the samples, first select "To Ship" checkboxes for the samples being sent to the national lab and select the appropriate lab in the shipping details popup window. The T-3 packing slip should be placed in the cooler being shipped to the national lab and should only indicate the sample(s) being shipped in that cooler. Line out the sample IDs to indicate which samples are not in the cooler. Select "To Ship" checkboxes for the remaining samples being sent to the state lab and select the appropriate lab in the shipping details popup window.
- Verify the sample IDs for the sample(s) being shipped.
- Submit the **Tracking** form to NARS IM in the NLA App using the steps above.

***NOTE**: Federal regulations and FedEx rules allow for ground shipping of certain quantities of flammable liquids WITHOUT the need for special certifications and labeling. Flammable liquids may NOT be shipped via air carrier unless shipper is trained and qualified to do so and specific documentation and labeling requirements are met.*

The Code of Federal Regulations (49 CFR Section 173.150) lists the exceptions which allow shipping of flammable liquids via ground carrier without labeling or special certifications. Ethanol and formalin can be considered to be in either Packaging Group 2 or 3, so we use the more stringent PG 2 as our guideline. The limited quantity exclusion allows ground shipping of PG 2 flammable liquids provided that the individual containers inside the package are not over 1.0 liters each, that the gross weight of the package does not exceed 66 pounds, and that the outer packaging is a sturdy container. Please ensure that your shipment meets these criteria to ensure the legal ground shipment of these samples.

1T4 – Whole Fish Composite Sample – *designated sites only*

- Complete this section of the App tracking form for shipping frozen whole fish tissue samples (FTIS).
- Only one fish composite sample may be shipped in a single cooler.
- Ship the sample to the lab in the whole fish tissue kit cooler with the packing slip that was provided with the label packet.
- Samples need to be shipped with a minimum of 50 pounds of dry ice (blocks of dry ice only).
- Human health fish composite samples should be shipped within 1 week of collection.
- Verify the sample ID for the sample being shipped.
- Submit the **Tracking** form to NARS IM in the NLA App using the steps above.

Shipping Addresses

USEPA Laboratory, Corvallis, Oregon (Water Chemistry, Chlorophyll-*a*)
Attn: Judy Greydanus, CSS
c/o U.S. EPA
Willamette Research Station
3080 SE Clearwater Dr.
Corvallis, OR 97333

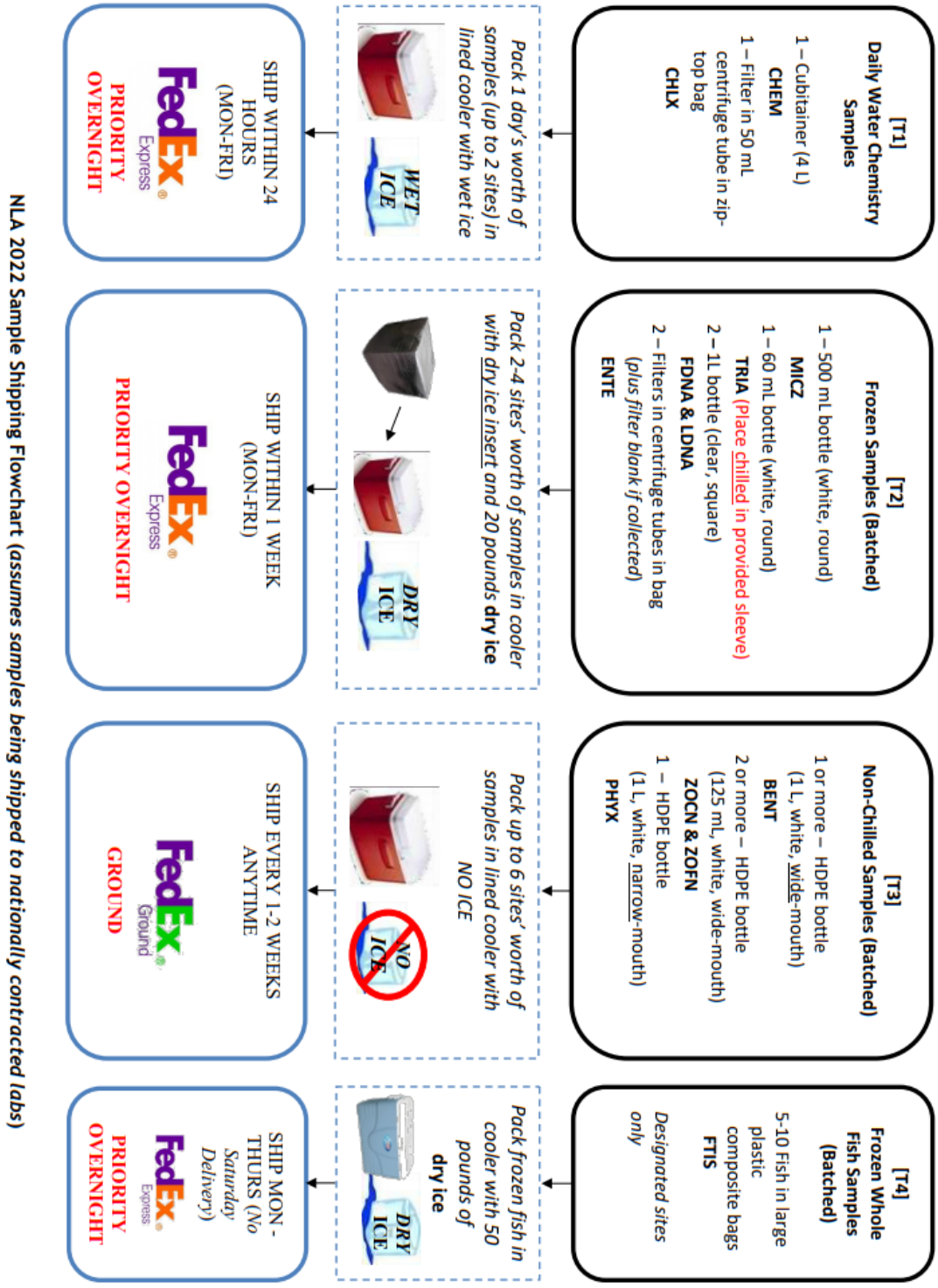
Microbac Laboratories, Inc. (Whole Fish Composite Samples)
Attn: Sample Receiving
2101 Van Deman Street
Baltimore, MD 21224

Great Lakes Environmental Center, Inc. (All other samples)
Attn: Mike Dunlop
739 Hastings Street
Traverse City, MI 49686

Table B-1. Sample preservation, packaging, and holding times

Sample Group & Lab	Sample Type	Sample Code	Location	Sample Target Volume	Container	Preparation/ Preservation	Shipping Time Frame	Packaging for Shipping
T-1 – Daily Water Chemistry Samples **WRS Laboratory - Corvallis, OR**	Water chemistry (raw, unfiltered site water)	CHEM	Index	4 L	Cubitainer (4 L)	Wet ice in field	Immediate (ship within 24 hours of sampling)	**Water Chemistry Cooler** with wet ice PRIORITY OVERNIGHT
	Chlorophyll-*a*	CHLX	Index Collection	2 L	Poly bottle (2 L, brown)	Wet ice in field		
			Processing	Stain on filter – max 2 L filtration	centrifuge tube (50 mL), in zip-top bag	Wet ice in field (after filtration)		
T2 – Frozen Batched Samples **GLEC – Traverse City, MI**	Fecal indicator (*Enterococci*)	ENTE	Last habitat station *	200 mL	Sterile 250 mL bottle (clear, square)	Dry Ice	Batch up to one week maximum	**Frozen Batched Cooler** with 2-piece dry ice liner and 20 pounds dry ice PRIORITY OVERNIGHT
	eDNA	FDNA	Index	1 L	Clear square bottle (1 L)	Wet ice in field, freeze ASAP		
		LDNA	Littoral Stations (10) *	1 L	Clear square bottle (1 L)	Wet ice in field, freeze ASAP		
	Algal toxins	MICZ	Index	500 mL	HDPE bottle (500 mL, white, wide-mouth)	Wet ice in field, freeze ASAP		
	Atrazine	TRIA	Index	50 mL	HDPE bottle (60 mL, white, wide-mouth)	Wet ice in field, keep chilled Place chilled in provided sleeve		
T3 – Non-Chilled Batched Samples **GLEC – Traverse City, MI**	Phytoplankton	PHYX	Index	1 L	HDPE bottle (1 L, white narrow mouth)	Lugol's added in field Wet ice in field	Batch up to two weeks maximum	**Non-Chilled Batched Cooler** with absorbent material No Ice GROUND
	Zooplankton (coarse – 150 µm) (fine – 50 µm	ZOCN ZOFN	Index	Vertical tow(s) 5-meter total length	HDPE bottle (125 mL, white, wide-mouth)	95% ethanol added in field		
	Benthic invertebrates	BENT	Littoral Stations (10+) *	All organisms in grabs	HDPE bottle (1 L, white, wide-mouth)	95% ethanol added in field (at least 500 mL per bottle)		
T4 - Whole Fish Composite Sample **Microbac Laboratories, Inc. – Baltimore, MD**	Whole fish	FTIS	Lakewide	5-10 whole fish (minimum fish length 190 mm)	Wrapped individually in solvent rinsed foil Sealed in poly tubing Large outer plastic bag	Dry ice in field, hold in freezer	Ship weekly (except on Fridays, Saturdays, or the day before Federal holidays) to FTIS lab. Only ship one sample per cooler.	**Ship in provided FTIS cooler with 50 pounds of DRY ICE** PRIORITY OVERNIGHT

** At large lakes (lakes over 10,000 hectares) littoral sampling is encouraged but not required. If littoral stations are not visited, BENT will NOT be collected; ENTE and LDNA will be collected at the launch site; LDNA will then be a single 1L grab sample*



NLA 2022 Sample Shipping Flowchart (*assumes samples being shipped to nationally contracted labs*)

Figure B-1. Sample shipping flowchart

APPENDIX C: NATIVE FRESHWATER MUSSEL EXAMPLES

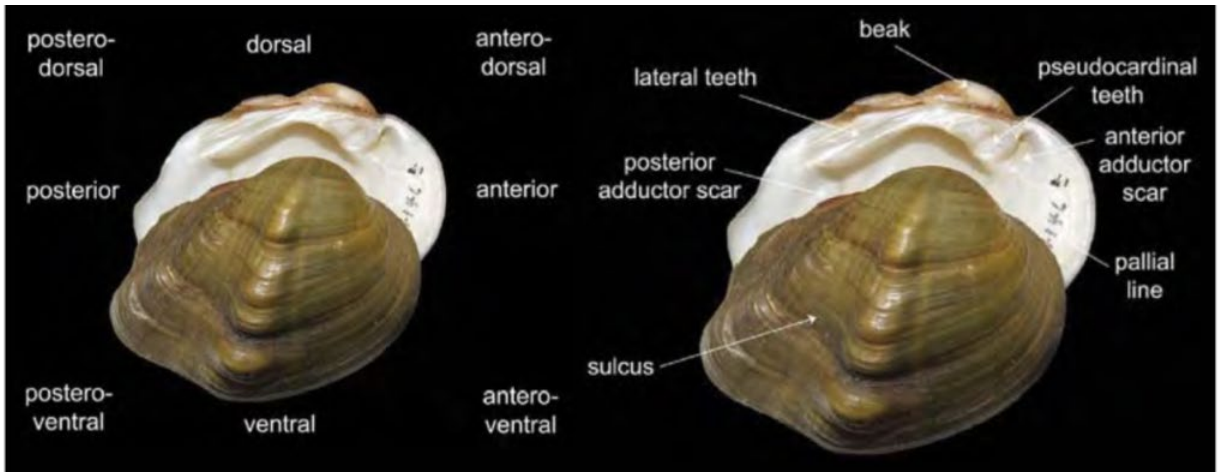


Figure C-1. Bivalve anatomy and orientation (Grabarkiewicz, J. and W. Davis 2008).

Narrow Pigtoe (*Fusconaia escambia*)[c]

Up to 3in (7.5cm) in length

Pink Mucket (*Lampsilis abrupta*)

Up to 4.1in (10.5cm) in length

Rayed Bean (*Villosa fabalis*)

Up to 1.5in (3.8 cm)in lnegth

Sheepnose (*Plethobasus cyphyus*)

Up to 5in (12.7cm) in length

Snuffbox (*Epioblasma triquetra*)[d]

Up to 2.8in (7.1 cm) in length

[c] Most photos, unless stated otherwise, were obtained through the ECOS website maintained by the U.S. Fish and Wildlife Service.

[d] Photo by G. Thomas Watters, Ohio State University

APPENDIX D: NLA 2022 HANDPICKED SITES: RESAMPLING OF THE NATIONAL EUTROPHICATION STUDY LAKES

Background

In 1972, the US Congress passed the landmark environmental legislation that became known as the Clean Water Act (CWA). The CWA has become a model for countries around the world and is heralded as the gold standard of environmental legislation. This year, 2022, marks the 50th Anniversary of the CWA, which coincides with the 4th iteration of the National Lakes Assessment (NLA). One hallmark of the CWA was inclusion of monitoring and measuring progress as an integral component to restoration and protection efforts. To that effect, the U.S. initiated a survey in 1972 to measure and report on the acceleration of eutrophication in lakes by assessing nutrient pollution known as the National Eutrophication Survey (NES). Importantly, the year 2022 also represents the 50th Anniversary of this landmark national survey.

Today, the NARS program is an EPA, State and Tribal partnership designed to address the need for monitoring the long-term progress toward the water quality objective and goals of the CWA. Through NARS, EPA and our partners are providing information on the condition and trends in coastal waters, lakes, rivers and streams, and wetlands. As a part of the NLA starting in 2007, ~200 of the original ~800 NES lakes were included as a subpopulation in the survey design and resampled. These ~200 lakes were selected using a probability-based design, similar to the broader NLA, which allowed change estimates to be made for the broader population of ~800 NES lakes. This ~200 lake subpopulation was used to report on the changes in trophic condition in the 2007 report.

As part of the celebration of the 50th Anniversary of the CWA, a resurvey of the NES lakes is taking place in conjunction with the NLA 2022. The statistically valid sampling design for NES sampling since the NLA 2007 survey will allow EPA to track environmental progress by assessing the change in condition of the NES lakes in the intervening 50 years (1972, 2007, 2012, 2017, and 2022). In additions to reporting on the condition of the NES lakes, the NES 2022 resampling will combine field data with watershed information to identify the progress we have made, the challenges we still face, and highlight the importance of water quality in our lives.

Field Crews and Coordination

The NES 2022 will rely on many of the key contacts and coordinators for NLA 2022 (See **Table 2-2**). All ten EPA Regions are supporting the NES field work in addition to the NLA field sampling contractors. In some regions, the field crew leads are the same as the regional NLA coordinator (**Table D-1**).

Table D-1 NES EPA Regional Crew Contact information

Title	Name	Contact Information
Regional EPA NLA Coordinators	Hilary Snook*, Region 1	snook.hilary@epa.gov 617-918-8670
	Emily Nering*, Region 2	nering.emily@epa.gov 732-321-6764
	Frank Borsuk*, Region 3	borsuk.frank@epa.gov 304-234-0241
	Leah Ettema, Region 3	ettema.leah@epa.gov 304-234-0245
	Chris McArthur, Region 4	mcarthur.christopher@epa.gov 404-562-9391
	Mari Nord*, Region 5	nord.mari@epa.gov 312-886-3017
	Rob Cook*, Region 6	cook.robert@epa.gov 214-665-7141
	Gary Welker*, Region 7	welker.gary@epa.gov 913-551-7177
	Liz Rogers, Region 8	Rogers.liz@epa.gov 303-312-6974
	Tom Johnson, Region 8	Johnson.tom@epa.gov 303-312-6226
	Tina Yin, Region 9	yin.christina@epa.gov 415-972-3579
	Matthew Bolt, Region 9	Bolt.matthew@epa.gov 415-972-3578
	Lil Herger*, Region 10	herger.lillian@epa.gov 206-553-1074
Regional EPA Field Crew Leads and/or Alternate Leads		
	Tom Faber, Region 1	Faber.tom@epa.gov 617-918-8672
	Raffaela Marano, Region 3	Marano.raffaela@epa.gov 215-814-2397
	Jerry Ackerman, Region 4	ackerman.jerry@epa.gov 706-355-8721
	Sue Dye, Region 4	dye.sue@epa.gov 706-355-8628
	Jonathan Burian, Region 5	Burian.Jonathan@epa.gov
	Laura Webb, Region 7	Webb.Laura@epa.gov
	Bill Schroder, Region 8	Schroeder.william@epa.gov 303-462-9472
	Ryan Monahan, Region 8	Monahan.ryan@epa.gov 303-462-9477
	Peter Husby, Region 9	Husby.peter@epa.gov

Title	Name	Contact Information
		510-412-2311
	Bob Hopeman, Region 9	Hopeman.Bob@epa.gov 510-415-2388
	Peter Leinenbach, Region 10	leinenbach.peter@epa.gov

*Regional NLA coordinator and NES field crew lead

Project and Data Quality Objectives

NES dataset provides a unique opportunity to assess change in lake condition in the intervening 50 years (1972, 2007, 2012, 2017, and 2022). In addition to the analysis of the filed collected data, analyses will include information on the types of management practices implemented at and near the lakes (wastewater treatment, etc.), population changes and other stressors to evaluate water quality changes in lakes with restoration and protection activities.

The NES 2022 resampling study is designed to address the following objectives:

1. How as the trophic condition of the NES lakes changed in the last 50 years (1972, 2007, 2012, 2017, and 2022). Are conditions better or worse?
2. Have stressors within the watersheds changed since the 1970s?
3. What stressors contribute to the current trophic condition of the NES lakes?

NES Indicators

The original NES study included the collection of lake profile data, water chemistry, chlorophyll -a, and secchi. The NES 2022 resurvey will continue to focus on measurements of trophic condition and will include the NLA indicators identified in **Table D-2.**

Table D-2 NLA 2022 NES site water quality indicators.

Indicator Type	Indicator	NES 1970s	NLA/NES 2007	NLA/NES 2022
Trophic and Chemical Indicators	*Vertical profile measurements* (DO, Temperature, pH)	x	x	X
	Secchi disk transparency	x	x	X
	Water chemistry (NH_4, NO_3), major anions and cations, alkalinity (ANC), DOC, TSS, silica, conductivity, nutrients (total and dissolved TN and TP)	x	x	x
	Chlorophyll-*a*	x	x	x
Human Health	CyanoHAB visual observations at index site and boat launch			x

Indicator Type	Indicator	NES 1970s	NLA/NES 2007	NLA/NES 2022
	Algal toxins (microcystins and cylindrospermopsin)			x
	Phytoplankton (cyanobacteria cell count)		x	x
Other Indicators	eDNA			Water chemistry filter only
	Lake area, basin morphometry, and characteristics of watershed	x	x	x

Study Design

In NLA 2007, 210 NES lakes were included in the survey design and resampled. This subpopulation was used to report on the changes in trophic condition in the 2007 report. The NES 2022 design is a random design of the NES 2007 lakes stratified by EPA Region. Due to resource limitation, all 210 lakes cannot be sampled, therefore we identified the target number of lakes in each Region (**Table D-3**). In Regions with 12 or less lakes, all NES lakes will be targeted for sampling. In Regions with greater than 12 sites, 60% of the lakes will be targeted for sampling. Note that 3 lakes will likely be sampled as part of the NLA probabilistic sampling efforts. The selection of target lakes must happen in the order of the site ID; however, sampling may happen in any order.

Table D-3 Target number of hand-picked lakes for sampling by EPA Region.

Row Labels	NES Lakes per Region	Target # of Sites	Lakes in the NLA 2022 Design	Total # of Target Lakes by Region	Regional sites	Contractor Sites
Region_1	5	5-6	1 (likely)	6	5	0
Region_2	6	6		6	6	0
Region_3	9	9		9	10**	0
Region_4	35	24*	1	25	24*	0
Region_5	48	29		29	28**	0
Region_6	32	19		19	5	14
Region_7	10	10		10	10	0
Region_8	38	23		23	9	14
Region_9	13	13		13	8	5
Region_10	12	11	1	12	6	5
Total	206	146	3	152	82	38

* Region 4 will sample the first 3 oversample lakes given there proximity to the target lakes, increasing the total number of lakes sampled by R4 to 24.

**One OH lake in Region 5 will be sampled by R3.

Field Methods and Procedures

Field Crew Training

The NES field crew leads and crew members are encouraged to participate in all portions of the NLA 2022 training. In the event crew members are not able to complete the full training program, below is the required list of training material for NES sampling teams:

1. Review the NLA QAPP, SEG and FOM (see Daily Field Activities below for FOM details)
2. Review the following training videos and take the associated quizzes:
    a. Block 1 – Background (all 4 videos)
    b. Block 2 – Launch sites activities and index sampling (videos 2-1 through 2-5; no zooplankton)
    c. Block 5 – Final lake activities (all 3 videos)
3. Participate in a Q&A session
4. Attend a regional in-person training (requirement for field crew leads only)

The NLA also includes Endangered Species Act (ESA) training for federal field crews. Although the NES sampling does not include the NLA biological indicators that are most likely to effect ESA listed species (i.e., benthic macroinvertebrates and fishing), all NES crew leads are to participate in the training in the event listed species are encountered while in the field.

Daily Field Activities

The NES sampling will include the following field activities:

1. All base site activities (see **Section 4.0**).
2. The following index site activities (see **Section 5.0**):
    a. CyanoHABS Visual Assessment
    b. Temperature, DO and pH profile
    c. Secchi Dish Transparency
    d. Water sample collection for chlorophyll-a, phytoplankton, algal toxins and water chemistry
        i. NES sampling <u>will not include</u> the collection of eDNA, atrazine, and zooplankton samples
3. All final lake activities
    a. Filtering only includes the chlorophyll-a sample (see **Sections 8.2.3** and **8.2.4**)

Field Forms and Sample Shipment

Table D-4 identifies the NLA field forms that will be used for the NES sampling. Many forms will include samples that are not part of NES field work. For the samples that are not collected at NES lakes (i.e., atrazine, all littoral samples, eDNA, zooplankton), the field crew is to identify that no sample was collected in the assoiceated forms. The Physical Habitat form <u>will not</u> be submitted.

Table D-4 NES field forms.

Form	Fill out	Submit?
Verification	Entire form	Yes
Calibration	Entire form	Yes
Index Samples	Entire form; mark FDNA, TRIA, ZOCN, and ZOFN as not collected	Yes
Profile	Entire form	Yes
Littoral Samples	Bottom of form only; mark BENT, LDNA, and ENTE as not collected	Yes
Physical Habitat	None	No
Assessment	Entire form	Yes
Whole Fish Sample	Top of form only; mark 'No'	Yes
Federal ESA	Fill in a pertinent section if an ESA-listed species is encountered	If data are entered
Tracking	T1, T2, and T3 sections when shipping samples from those groups	Yes

Sample shipments will include:

- T-1 (daily water chemistry samples);
- T-2 (frozen batched samples – algal toxins only); and
- T-3 (non-chilled batched samples – phytoplankton only).

See **APPENDIX B: SHIPPING GUIDELINES** for additional details.