



United States Environmental Protection Agency

Office of Water

Washington, DC

EPA # 841-F-19-005

# National Coastal Condition Assessment 2020 Field Operations Manual



April 2020

## NOTICE

The National Coastal Condition Assessment provides a comprehensive assessment for coastal waters across the United States. The complete documentation of overall project management, design, methods, and standards is contained in four documents:

- National Coastal Condition Assessment 2020: *Quality Assurance Project Plan* (EPA # 841-F-19-003)
- National Coastal Condition Assessment 2020: *Site Evaluation Guidelines* (EPA # 841-B-20-001)
- National Coastal Condition Assessment 2020: *Field Operations Manual* (EPA # 841-F-19-005)
- National Coastal Condition Assessment 2020: *Laboratory Operations Manual* (EPA # 841-F-19-004)

This Field Operations Manual contains a brief introduction and base and site location procedures for *in situ* measurements, sampling water (grabs for chemistry, pathogen analysis, and algal toxin analysis), benthic macroinvertebrates, sediment (for composition, contamination and toxicity), and fish tissue (for human health and ecological indicators). These methods are based on the guidelines developed and followed in the Coastal 2000 and National Coastal Assessment Monitoring and Assessment Program (USEPA, 2001). All National Coastal Condition Assessment Project Cooperators must follow the methods and guidelines in this Field Operations Manual. 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 citation for this document is:

U.S. EPA. National Coastal Condition Assessment 2020 Field Operations Manual. United States Environmental Protection Agency, Office of Water, Office of Wetlands, Oceans and Watersheds. Washington, D.C. EPA- 841-F-19-005. 2020.

Version History

<b>FOM Version</b>	<b>Date</b>	<b>Changes Made</b>
1.0	April 10, 2020	Not Applicable
1.1	April 23, 2020	Change only to update Version to 1.1. and maintain version alignment with QAPP V1.1 and LOM V1.1
1.2	February 18, 2021	Section 6.4, step 3, added text to take another reading at 0.5m from bottom as you start upcast. This is not a change in procedure, just a clarification.
1.2	February 18, 2021	Section 6.4, step 4, added text that crews should sample at least to 30m if limited by equipment.
1.2	February 18, 2021	Section 13.4, step 2, added text that crews need to record grab number in the App.
1.2	March 1, 2021	Insertion of Section 2.8.5 to reflect COVID-19 safety considerations.

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## ACRONYMS/ABBREVIATIONS

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CPR	Cardiopulmonary resuscitation
DI	Deionized
DO	Dissolved oxygen
EPA	Environmental Protection Agency
ESA	Endangered Species Act
FLC	Field Logistics Coordinator
GED	Gulf Ecology Division, U.S. EPA Office of Research and Development
GIS	Geographic information system
GL	Great Lakes
GPS	Global positioning system
GRTS	Generalized Random Tessellation Stratified survey design
HDPE	High density polyethylene
HQ	Headquarters
IM	Information Management
MED	Mid-Continent Ecology Division, U.S. EPA Office of Research and Development
NAD 83	North American Datum of 1983
NARS	National Aquatic Resource Surveys
NCA	National Coastal Assessment
NCCA	National Coastal Condition Assessment
NEP	National Estuary Program
NIST	National Institute of Standards and Technology
NM	Nautical miles
NOAA	National Oceanic and Atmospheric Administration
ORD	Office of Research and Development, U.S. EPA
OSHA	Occupational Safety and Health Administration
PAR	Photosynthetically active radiation
PBS	Phosphate Buffer Solution
PDF	Portable Document Format

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PET	polyethylene terephthalate
PETG	polyethylene terephthalate copolyester, glycol modified
PFD	Personal flotation device
PSI	Pounds per square inch
QAC	Quality Assurance Coordinator
QAPP	Quality Assurance Project Plan
QA/QC	Quality assurance/quality control
QCS	Quality Check Solution
SAV	Submerged aquatic vegetation
SOP	Standard Operating Procedure
SRM	Standard Reference Material
TOC	Total organic carbon
USDA	United States Department of Agriculture
USGS	United States Geological Survey
VHS	Viral Hemorrhagic Septicemia

## CONTACT LIST

Table 1.1 Contacts

Role	Name	Phone/Email	Address
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## 2 BACKGROUND

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The National Coastal Condition Assessment (NCCA) is one of a series of water assessments being conducted by states, tribes, the U.S. Environmental Protection Agency (EPA), and other partners. In addition to coastal waters, the National Aquatic Resource Surveys (NARS) focus on rivers and streams, lakes, and wetlands in a revolving sequence. The purpose of these assessments is to generate statistically-valid reports on the condition of our Nation's water resources and identify key stressors to these systems.

The goal of the NCCA is to address two key questions about the quality of the Nation's coastal waters:

- What percent of the Nation's coastal waters are in good, fair, and poor condition for key indicators of water quality, ecological health, and recreation?
- What is the relative importance of key stressors such as nutrients and contaminated sediments?

The NCCA is designed to be completed during the index period of June through the end of September. Field crews collect a variety of measurements and samples from preselected sampling sites that are located at predetermined coordinates.

This manual describes field protocols and daily operations for crews in the NCCA. As a probability-based survey of our Nation's coastal and estuarine waters, the NCCA is designed to:

- Assess the condition of the Nation's coastal and estuarine waters at national and regional scales, including the Great Lakes;
- Identify the relative importance of selected stressors to coastal and estuarine water quality;
- Evaluate changes in condition from previous National Coastal Assessments (NCA) starting in 2005; and
- Help build State and Tribal capacity for monitoring and assessment and promote collaboration across jurisdictional boundaries.

### 2.1 SURVEY DESIGN

EPA selected sampling locations using a probability-based survey design, allowing data from a subset of sampled sites to be applied to the larger target population, and permitting assessments with known confidence bounds.

The 2020 NCCA survey design produces:

1. National and regional estimates of the status of all coastal waters, including major estuary groups and the Great Lakes; and
2. National and regional estimates of the change in status in coastal water condition between 2005 and 2020.

With input from the states and other partners, EPA used an unequal probability, stratified design to select 1000 probabilistic sampling events, of which roughly 50% are resample sites (sites that were sampled in 2010 or 2015 and will be sampled again in 2020). Resample sites from 2010/2015 are identified as Base 10 sites; while newly drawn sites are identified as Base 20 sites. Approximately 7% of the 2010/2015 resample sites are also designated “revisit sites,” which indicates that they will be sampled twice in 2020 to assess crew sampling and temporal variability. In addition to the 1000 probabilistic sampling events, a number of intensification sites have been added to NCCA 2020, many of which were also selected using a stratified probabilistic design.

Sample site stratification is based on major estuaries using the National Oceanic and Atmospheric Administration (NOAA) Coastal Assessment framework and National Estuary Program (NEP). The Great Lakes sites are stratified based on the individual Great Lake, depth zone, and country. Only the shallow nearshore depth zone is included in the probabilistic design for NCCA Great Lakes sites. The shallow nearshore depth zone is defined as the region extending from the shoreline to a depth of 30 meters, and no more than 5 kilometers from the shoreline.

Oversample sites were drawn to provide alternate sampling sites if primary sites are rejected and to provide supplemental sampling locations for states that wish to conduct a state level or NEP-level condition assessment.

Additional details on the NCCA survey design can be found in the NCCA survey design documents.

## 2.2 TARGET POPULATION AND SAMPLE FRAME

The target population for the estuarine resources consists of all coastal waters of the conterminous United States from the head-of-salt to confluence with the ocean, including inland waterways, tidal rivers and creeks, lagoons, fjords, bays, and major embayments (see **Figure 2.1**, **Figure 2.2** and **Figure 2.3** for examples). For the purposes of this study, the head-of-salt is defined as waters with salinity less than 0.5 parts per thousand (ppt) salinity, representing the landward/upstream boundary. The seaward boundary extends out to where an imaginary straight-line intersecting two land features would fully enclose a body of coastal water. All waters within the enclosed area are defined as estuarine, regardless of depth or salinity.

The target population for the Great Lakes consists of all waters of the Great Lakes of the United States and Canada. The current target population is restricted to the shallow nearshore zones of Lake Superior, Lake Michigan, Lake Huron, Lake Erie, and Lake Ontario. The Great Lakes target population excludes embayments with connection to open water that are less than 200 meters in width. The NCCA Great Lakes sites are restricted to waters within the United States. Please refer to the Site Evaluation Guidelines and the NCCA Web site (<http://www.epa.gov/owow/monitoring/nationalsurveys.html>) for more detailed information on the target population.

The sample frame was derived from prior NCA developed by EPA Office of Research and Development (ORD) Gulf Ecology Division (GED). The prior GED sample frame was



enhanced as part of the National Coastal Monitoring Network design by including information from NOAA's Coastal Assessment Framework, boundaries of NEP and identification of major coastal systems. For the first NCCA in 2010, information on salinity zones was obtained from NOAA. For the Delaware Bay, Chesapeake Bay, Puget Sound, and the State of South Carolina, the prior NCA sample frames were replaced by geographic information system (GIS) layers provided by the organizations that manage the coastal waters in these areas, ensuring that prior areas sampled in NCA were not excluded and any differences from the previous sample frames to the current sample frame are clearly identified in this NCCA 2020 sample frame. For the Californian Province excluding San Francisco Bay, the GED sample frame was changed to match a 2004 sample frame used for the NCA 2004 study. In 2013, the sample frame was updated to include information related to 1999-2001 and 2005-2006 NCA sample frames. This update was necessary to provide the information required to estimate change between the periods of 2010 and 2015. The sample frame for the Great Lakes sites was obtained from EPA ORD Mid-Continent Ecology Division (MED).

Please refer to the **NCCA 2020: Site Evaluation Guidelines** for more detailed information on the target population and exclusion criteria.



Figure 2.1 Example of an estuarine system comprised of an embayment plus a complex of bays and tidal rivers and creeks



*Figure 2.2 Example of an intra-coastal estuarine system*

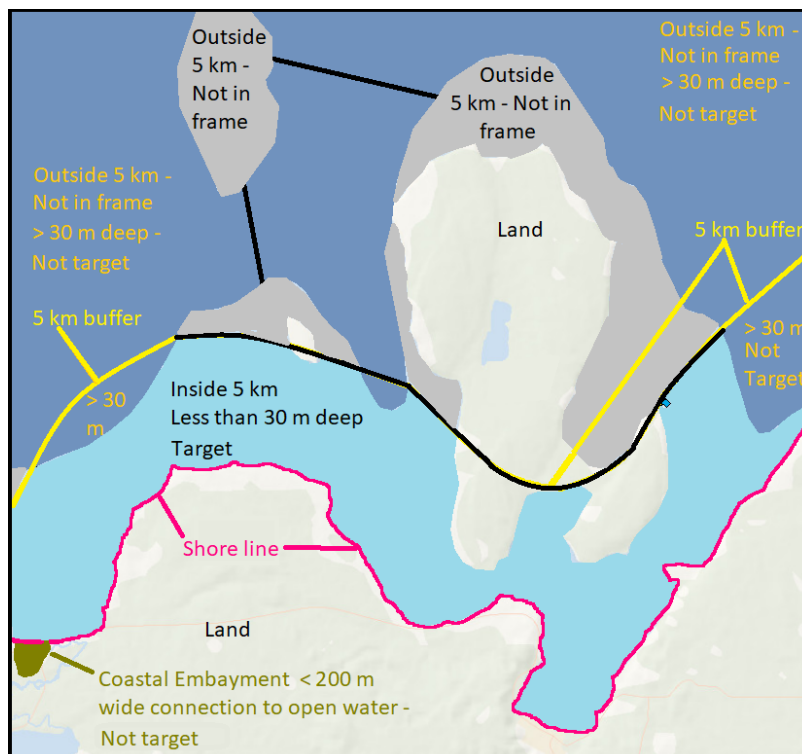


Figure 2.3 Hypothetical Great Lakes Nearshore target population.

## 2.3 SITE EVALUATION

Base site sampling points were drawn using a Generalized Random Tessellation Stratified (GRTS) survey design, a stratified design that gives all points within a target population equal probability of selection. Each point selected as a sample site is designated the “X-site” and represents the point at which sample collections are targeted.

### 2.3.1 SITE SAMPLE-ABILITY

X-sites will be found in waterbodies of varied sizes and shapes depending on coastal morphology. Site depth and salinity are considered when the initial site draw is made; therefore, those conditions should not generally be a factor when choosing to replace a planned sampling site. However, there may be instances when a field crew determines that an X-site does not meet the operational definition of an estuary in marine environments, or lacustrine and nearshore coastal waters in the Great Lakes. Sampleable sites must:

- Have access to open water;
- Be navigable using a shallow-draw boat. Typically this means that the depth of the X-site is generally  $\geq 1$  meter. Actual sampleable depths, however, may be adjusted based on the vessel and sampling equipment being used, and wave action at the site observed by the field crew.

If the specific site does not fit the definition of a sampleable site, and every attempt to relocate a site within the margin provided has been made (see Section 5.1.3), complete

the appropriate “Non-Sampleable-Permanent” category on the Verification Form in the NCCA App. Document the reason for not sampling the site in the comments section of the form. Add any additional explanation as required. (For complete details on the site evaluation process, refer to the **NCCA Site Evaluation Guidelines**).

### 2.3.2 REPLACING SITES

It is likely that some sites will be determined to be unsampleable; therefore, a number of backup sites, in the form of an oversample list, are provided to each state/organization. A site can be deemed unsampleable for any number of reasons, including being too shallow to properly operate sampling equipment or in the middle of a navigational channel where it is unsafe.

When a site is determined to be unsampleable, field crews will document the sampling status of the site and select the next oversample site within the same stratum (i.e., same state and estuary type or Great lake) and the same base year (Base 10 sites must be replaced with Base 10 oversamples sites and Base 20 sites must be replaced with Base 20 oversamples sites). This process maintains the probabilistic integrity of the survey. This process is handled through the Site Evaluation Spreadsheets that EPA Headquarters (HQ) has provided for each state/organization. These spreadsheets are available on the NARS SharePoint site. Please refer to the **NCCA Site Evaluation Guidelines** for more detailed information on determining site sampling status and completion of the Site Evaluation Spreadsheets. These updated spreadsheets will be turned in when sampling is completed, or throughout the field season should it be necessary for communicating the replacement of specific sites to EPA HQ and the Contractor Field Logistics Coordinator (FLC).

If a dropped site is designated as a revisit site (designated “RVT2” in the panel code), then the replacement site takes on the RVT2 assignment. That is the replacement site must be visited twice in 2020.

If a site is generally sampleable, but one or more indicators cannot be collected (e.g., no fish caught or site is too deep to collect sediment), the site should not be dropped. Rather, the crew will mark that indicator as not collected and document the reason why the indicator could not be collected in the comment area of the NCCA App. See **Section 13** and **Section 14** for information regarding the collection of sediment and fish samples, the two indicators which crews most likely may experience difficulty collecting.

## 2.4 DESCRIPTION OF NCCA INDICATORS

Indicators for the 2020 survey will basically remain the same as those used in 2015 and other past coastal surveys, with a few modifications. Additionally, sample collection methods and laboratory methods will reflect freshwater and saltwater matrices to account for marine and Great Lakes sampling.

### 2.4.1 IN SITU WATER COLUMN MEASUREMENTS

#### *2.4.1.1 Hydrographic Profile*

Measurements for dissolved oxygen (DO), pH, salinity (at marine sites) or conductivity (at freshwater sites), and temperature will be taken with a calibrated water quality meter or

multi-parameter sonde at each site. Measurements will be taken at specific depth intervals within 37 meters of the X-site. The specific location of the profile (and subsequently the area where several samples are collected) is referred to as the Y-location. This information will be used to detect extremes in condition that might indicate impairment.

#### *2.4.1.2 Light Attenuation*

A Photosynthetically Active Radiation (PAR) meter will be used to obtain a vertical profile of light in order to calculate the light attenuation coefficient at each station. PAR measurements are taken at the same depths as other water column indicators.

#### *2.4.1.3 Secchi Disk Transparency*

A Secchi disk is a commonly used black and white patterned disk used to measure the clarity of water within a visible distance.

### 2.4.2 WATER CHEMISTRY (CHEM) AND ASSOCIATED MEASUREMENTS

Water chemistry measurements will be used to determine nutrient enrichment, as well as classification of trophic status. Parameters measured include total and dissolved nitrogen and phosphorus.

#### *2.4.2.1 Chlorophyll-a (WCHL)*

Chlorophyll-*a* is the green pigment used in photosynthesis by plants and algae. Its measurement is used to determine algal biomass in the water.

#### *2.4.2.2 Dissolved Nutrients (NUTS)*

A portion of the filtrate produced from the processing of the chlorophyll-*a* sample will be collected in the field and processed in the laboratory for dissolved nutrients.

#### *2.4.2.3 Phytoplankton Assemblage (PHYT)*

Phytoplankton are plant microorganisms that float in the water, such as certain algae, and are the primary source of energy in most lake systems (Schriver et al. 1995). Phytoplankton are highly sensitive to environmental changes in ecosystems (e.g., turbidity and nutrient enrichment). **Phytoplankton will be collected in Great Lakes sites only.**

### 2.4.3 ALGAL TOXINS (CYLINDROSPERMOPSIN AND MICROCYSTINS [MICX] and MICROCYSTINS [MICZ])

Algae are microscopic organisms found naturally at low concentrations in freshwater and marine systems. They often form large blooms under optimal conditions, potentially affecting water quality as well as human health and natural resources. *Microcystis*, for example, is one organism that produces microcystin, a potent liver toxin. One water sample is taken to analyze for both cylindrospermopsin and microcystins, and another will be taken specifically for microcystin.

### 2.4.4 UNDERWATER VIDEO (UVID)

At Great Lakes sites only, crews will use an underwater video camera array to capture one minute of video focused on the substrate at the Y-location. Video will be used in the lab to visually document the bottom composition, and record the presence or absence of zebra mussels, *Cladophora*, or other organisms. If the benthic macroinvertebrate sample

is collected at a location other than the Y-Location, a second video focused on the substrate at the benthos collection location will be taken.

#### 2.4.5 SEDIMENT ASSESSMENT, (SEDG, SEDC, SEDX, SEDO, AND D15N)

Sediment grab samples will be obtained to measure sediment composition (e.g., grain size [SEDG] and percent moisture, organic content, etc. [SEDC]), toxicity [SEDX], and contaminant chemistry [SEDO] in order to determine sediment condition. The nitrogen stable isotope ratio [D15N] in sediment will be measured to evaluate its utility as a measure of anthropogenic development in watersheds of estuaries and will also be collected at marine sites only.

#### 2.4.6 BENTHIC MACROINVERTEBRATE ASSEMBLAGE (BENT)

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: aquatic worms, mollusks, and crustaceans. 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 (Klemm et al., 1990). Because many macroinvertebrates have relatively long life cycles of a year or more and are relatively immobile, the structure of the macroinvertebrate assemblage is a response to exposure of present and/or past conditions. The benthic macroinvertebrate data will serve as the basis for assessing aquatic community health.

#### 2.4.7 ENTEROCOCCI FECAL INDICATOR (ENTE)

Enterococci are bacteria that are endemic to the guts of warm blooded creatures. These bacteria, by themselves, are not considered harmful to humans but often occur in the presence of potential human pathogens (the definition of an indicator organism). Epidemiological studies of marine and fresh water bathing beaches have established a direct relationship between the density of Enterococci in water and the occurrence of swimming-associated gastroenteritis.

#### 2.4.8 FISH TISSUE (FTIS, FPLG, HTIS)

The fish tissue indicator [FTIS], which measures bioaccumulation of persistent toxics and is also referred to as the ecofish sample, is used to estimate the ecological risks associated with fish consumption by wildlife. In this study fish will be collected and whole body tissue will be homogenized and analyzed to estimate concentrations of target contaminants. Various studies have been conducted on contaminants in different tissues of the fish (e.g., whole fish, fillets, or livers). For this study the focus will be on analyzing whole fish [FTIS] for contaminants to generate data for ecological purposes. At revisit sites, ecofish samples will only be targeted during visit 1. If a successful collection is not possible at visit 1, crews should attempt to collect ecofish at visit 2.

Crews will also collect fish tissue plugs [FPLG] at all NCCA Sites. The plugs will be sent to the lab for analysis of mercury contamination levels to assess the risk to humans of consuming fish tissue. If the fish plug sample is taken from fish other than those being collected for ecological analysis, the fish will be released back into the waters from which they were collected. At revisit sites, fish plug samples will only be targeted during visit 1.

If a successful collection is not possible at visit 1, crews should attempt to collect fish plugs at visit 2.

In the Great Lakes only, additional fish composite samples will be collected at all of the 225 probabilistic nearshore Great Lakes sites (prefix = NGL20), all 38 Great Lakes island sites (prefix = ISA20), and all 12 Great Lakes park sites (prefix = NPA20) for a combined total of 275 sites. Fillet tissue from these samples will be homogenized in the lab and analyzed to generate fish contamination data related to human health [HTIS]. Fish submitted in the human health fish tissue sample should remain intact and fish plugs are not to be taken from these fish. At Great Lakes revisit sites crews that are unsuccessful at collecting the human health fish tissue sample during visit 1 are expected to attempt the collection of that sample during visit 2, but HTIS will only be collected at one of the two visits to a revisit site. Note that human health fish tissue samples will **NOT** be collected at Great Lakes enhancement sites other than those listed above.

## 2.4.9 OCEAN AND COASTAL ACIDIFICATION RESEARCH INDICATOR

### 2.4.9.1 *Total Alkalinity (ALKT)*

At marine sites only, crews will collect a water sample in two bottles for the measurement of total alkalinity. Total alkalinity (TA) is a characteristic of seawater that, in combination with other measurements, can be used to calculate total pH (i.e., coastal acidification) and the availability of carbonate ions used by marine organisms to produce structural materials such as corals and shells. TA is also used to calculate the fate of carbon that enters coastal waters in various forms and is useful as a direct indicator of seawater buffering capacity. TA is defined differently from the alkalinity measurements typically used in freshwater monitoring. In addition, the above seawater calculations are sensitive to tiny errors in TA determination, so monitoring programs aim for extreme care in the collection, handling, and analysis of TA samples.

## 2.5 SUPPLEMENTAL MATERIAL TO THE FIELD OPERATIONS MANUAL

The Field Operations Manual describes field protocols and daily operations for crews to use in the NCCA. Following these detailed protocols will ensure consistency across regions and reproducibility for future assessments. Before sampling a site, crews should prepare a **Site Packet** for each site containing pertinent information to successfully conduct sampling. This site packet typically includes a road map or navigation chart and a set of directions to the site, topographic/bathymetric maps, land owner access forms (where applicable), sampling permits (if needed), site evaluation forms, and other information necessary to ensure an efficient and safe sampling day.

The primary means of data collection during the 2020 NCCA will be through a specifically designed application for use on iOS devices (e.g., the NCCA App). Within the NCCA App, there are a number of information (i) buttons that contain tables, figures, pictures, and other information summarizing field activities and protocols from the Field Operations Manual. Field crews are also required to keep the equipment manuals (probes, etc.) available in the field for reference and for possible protocol clarification.

Large-scale and/or long-term monitoring programs such as those envisioned for national surveys and assessments require a rigorous Quality Assurance (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 integrated **Quality Assurance Project Plan (QAPP)**. The QAPP contains more detailed information regarding QA/Quality Control (QC) activities and procedures associated with general field operations, sample collection, measurement data collection for specific indicators, data reporting activities, and the information management plan for this project. For more information on the QA procedures, refer to the *National Coastal Condition Assessment 2020: Quality Assurance Project Plan (EPA-841-R-14-003)*.

## 2.6 RECORDING DATA AND OTHER INFORMATION

Field data and sample information must be **recorded completely, accurately, and consistently**. The cost of a sampling visit coupled with the short index period severely limits the ability to resample a site if the initial records are inaccurate. Incorrect information can result in substantially increased time to process information from the electronic field forms to the **National Aquatic Resource Surveys Information Management (NARS IM)** system. Guidelines for recording field measurements are presented in **Table 2.1**.

All samples need to be identified and tracked, and associated information for each sample must be recorded. To assist with sample identification and tracking, packing slips and sample labels with sample ID numbers are preprinted and provided by EPA.

### 2.6.1 ELECTRONIC FIELD FORMS

Field crews will utilize the **NCCA App** to complete data collection. The NCCA App is available in the iTunes store and will come preloaded on iPads that will be distributed to all non-contract field crews. These iPads will be designated for crew use during the 2020 season and will be returned to EPA at the end of the field season.

The NCCA App is the required format for data submission as it reduces processing time required in scanning paper field forms, prevents data entry errors, eliminates redundant entry of common fields, eliminates issues caused by illegible entries, and provides validation checks of data entry fields. In addition, the NCCA App generates all sample IDs based on the initial entry of the CHEM sample ID and includes fish pick lists for consistent naming of fish species. If field crews are utilizing this form of data entry, they will upload site sketches of their sites to the NARS SharePoint site.

### 2.6.2 PAPER FIELD & TRACKING FORMS

Paper field forms are only to be used if the App fails and will be provided prior to the field season. Paper packing slips (provided with label packets in site kits) must be included in every cooler to maintain chain of custody.



Table 2.1 Guidelines for recording field measurements & tracking information

Activity	Guidelines
<b>Field Measurements</b>	
<b>Data Recording</b>	<ul style="list-style-type: none"> <li>• If recording using the NCCA App, populate all values in the App</li> <li>• If recording using paper forms (in the event of an App failure):               <ul style="list-style-type: none"> <li>• Record measurement values and observations on data forms preprinted on water-resistant paper.</li> <li>• Use No. 2 pencil only (fine-point indelible markers can be used if necessary) to record information on forms.</li> <li>• Record data and information using correct format as provided on data forms.</li> <li>• Be sure to <b>accurately</b> record site and sample IDs.</li> <li>• For all primary sampling visits indicate the event as Visit 1. For revisit sites use Visit 2 to indicate the second sampling event during the same season.</li> <li>• Print legibly (and as large as possible). Clearly distinguish letters from numbers (e.g., 0 versus O, 2 versus Z, 7 versus T or F, etc.), but do not use slashes.</li> <li>• When recording comments, print or write <b>legibly</b>. Make notations in comments field only; avoid marginal notes. Be concise, but avoid using abbreviations or “shorthand” notations. If you run out of space, attach a sheet of paper with the additional information, rather than trying to squeeze everything into the space provided on the form.</li> </ul> </li> </ul>
<b>Data Comments</b>	<ul style="list-style-type: none"> <li>• Comment fields are found throughout the App and associated with every sample and all key data points.</li> <li>• Use the provided areas to make comments about any data or sample that will explain any deviation for normal protocol or will otherwise assist data reviewers in better understanding the data.</li> <li>• Be as clear as possible in your comments to convey all necessary information.</li> </ul>
<b>Sample Labels</b>	<ul style="list-style-type: none"> <li>• Use adhesive labels with preprinted sample IDs and follow the standard recording format for each type of sample.</li> <li>• Use a fine tipped permanent marker to record information on label. Cover the completed label with clear tape.</li> <li>• Record sample ID from label and associated collection information in Sample Collection form in the App</li> </ul>
<b>Sample Collection and Tracking</b>	
<b>Sample Comments</b>	<ul style="list-style-type: none"> <li>• Comment fields are found throughout the App and associated with every sample and all key data points.</li> <li>• Use the provided areas to make comments about any data or sample that will explain any deviation for normal protocol or will otherwise assist data reviewers in better understanding the data.</li> <li>• Be as clear as possible in your comments to convey all necessary information.</li> </ul>
<b>Review of Labels and Data Collection Forms</b>	<ul style="list-style-type: none"> <li>• Before leaving site, compare information recorded on labels and sample collection form to ensure agreement and accuracy.</li> <li>• Before leaving site, review labels and App data for accuracy, completeness, and legibility.</li> <li>• The Field Crew Leader must review all data on the App. Submission of data to NARS IM confirms review.</li> </ul>

## 2.7 DATA MANAGEMENT

All field crews will be given access to the **NARS SharePoint** site. This site will be a resource for field crews to access important NCCA documentation as well as for facilitating document transfer to and from field crews.

## 2.8 SAFETY AND HEALTH

Sample collection and analysis can pose significant risks to personal safety and health. This section describes recommended training, communications, safety considerations, safety equipment and facilities, and safety guidelines for field operations.

### 2.8.1 GENERAL CONSIDERATIONS

Important considerations related to field safety are presented in **Table 2.2**. The Field Crew Leader is responsible for ensuring that all field personnel have successfully completed the necessary safety courses and follow all safety policies and procedures. Please follow your own agency's health and safety protocols. Additional sources of information regarding safety-related training include the American Red Cross (2006), the National Institute for Occupational Safety and Health (1981), and U.S. Coast Guard (1989).

Field crew members should become familiar with the hazards involved with sampling equipment and establish appropriate safety practices prior to their use. Make sure all equipment is in safe working condition. Personnel must consider and prepare for hazards associated with the operation of motor vehicles, boats, winches, tools, and other incidental equipment. Boat operators must meet any state requirements for boat operation and be familiar with U.S. Coast Guard rules and regulations for safe boating contained in the pamphlet, "*Federal Requirements for Recreational Boats*," available from a local U.S. Coast Guard Director or Auxiliary or State Boating Official (U.S. Coast Guard, 1989). While on the water, all crew members must wear Personal Flotation Devices (PFD). All boats with motors must be equipped with fire extinguishers, boat horns, PFDs, and flares or other U.S. Coast Guard approved signaling devices.

*Table 2.2 General health & safety considerations*

<b>Recommended Training</b>	<ul style="list-style-type: none"> <li>• First aid and cardiopulmonary resuscitation (CPR)</li> <li>• Vehicle safety (e.g., operation of 4-wheel drive vehicles, trailering boats, etc.)</li> <li>• Field safety (weather, personal safety, navigation, site reconnaissance prior to sampling)</li> <li>• Equipment design, operation, and maintenance</li> <li>• Handling of chemicals and other hazardous materials</li> </ul>
<b>Communications</b>	<ul style="list-style-type: none"> <li>• Check-in schedule</li> <li>• Sampling itinerary (vehicle used &amp; description, time of departure &amp; return, travel route and destination)</li> <li>• Contacts for police, ambulance, hospitals, fire departments, search and rescue personnel</li> <li>• Emergency services available near each sampling site and base location</li> <li>• Cell (or satellite) phone and VHF radio.</li> </ul>
<b>Personal Safety</b>	<ul style="list-style-type: none"> <li>• Field clothing and other protective gear including PFDs for all crew members</li> <li>• Medical and personal information (allergies, personal health conditions)</li> <li>• Personal contacts (family, telephone numbers, etc.)</li> <li>• Physical exams and immunizations</li> </ul>

Prior to beginning a sampling day, each field crew must develop an **Emergency Communications Plan**. This plan will include contacts for police, fire departments, emergency medical services, hospitals, and search and rescue personnel. In addition, the plan must include daily check-in procedures with personnel who will not be in the field. A copy of the plan should be filed with a supervisor, safety specialist, or other staff member who is not in the field. All field personnel must be fully aware of all lines of communication and able to initiate emergency communications if needed. Field crew members must carry clothing and equipment to protect from exposure to different weather conditions. Inadequate clothing could lead to hypothermia, heat exhaustion, or heat stroke. Field personnel must be able to swim. A PFD and suitable footwear must be worn at **all** times while on board a boat.

### 2.8.2 SAFETY EQUIPMENT

Crews may face many hazards when working in coastal areas. Broken glass or other sharp objects may be embedded in the substrate. Infectious agents and toxic substances may be present in the water or sediment. Dangerous weather may approach with little warning. Vessels can lose power and navigation.

Field crews must stock appropriate safety apparel such as gloves, foul weather gear, safety glasses, etc., and use them when necessary. All vessels must have first aid kits, fire extinguishers, and blankets available in the field, and crew members must be trained in how to use them. All crews must carry cellular or satellite telephones and all crew members must be proficient in how to use them. Crews must carry supplies such as clean water, anti-bacterial soap, and ethyl alcohol for cleaning exposed body parts that may have been contaminated by pollutants in the water.

### 2.8.3 SAFETY GUIDELINES FOR FIELD OPERATIONS

Personnel participating in field activities must be in sound physical condition and have a physical examination annually or in accordance with organizational requirements.

Field crew members must become familiar with the health hazards associated with collecting, preserving, and storing field samples. All surface waters and sediments are considered potential health hazards due to the potential presence of toxic substances or pathogens, and chemical fixing and/or preserving agents are often comprised of hazardous materials. In addition, chemical wastes can be flammable, explosive, toxic, caustic, or chemically reactive. Therefore, all chemical wastes must be discarded according to standardized health and hazards procedures (e.g., National Institute for Occupational Safety and Health [1981]; U.S. EPA [1986]).

During the course of field research activities, field crews may observe violations of environmental regulations, discover improperly disposed hazardous materials, or observe or be involved with an accidental spill or release of hazardous materials. In such cases proper actions must be taken and field personnel must not expose themselves to something harmful.

The following safety guidelines should be applied:

First and foremost, protect the health and safety of all personnel. Take 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.

Field personnel should never disturb or retrieve improperly disposed hazardous materials from the field to bring them back to a facility for “disposal”. To do so may worsen the impact, incur personal liability for the crew members and/or their respective organizations, cause personal injury, or cause unbudgeted expenditure of time and money for proper treatment and disposal of the material. Notify the appropriate authorities so they may properly respond to the incident. 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 U.S. 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.

#### 2.8.4 GENERAL SAFETY GUIDELINES FOR FIELD OPERATIONS

- At least two crew members must be present during all sample collection activities, and no one should be left alone while out on the water.
- Use caution and wear a suitable PFD.
- Use caution using the Ponar-type samplers. The jaws are sharp and may close unexpectedly.
- Exposure to water and sediments should be minimized as much as possible. Use gloves if necessary, and clean exposed body parts as soon as possible after contact.
- All electrical equipment must bear the approval seal of Underwriters Laboratories and must be properly grounded to protect against electric shock.
- Use appropriate protective equipment (e.g., gloves, safety glasses) when handling and using hazardous chemicals.
- Crews working in areas with venomous snakes 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.
- Field personnel should 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. Immersion in the cool waters experienced during the summer along most of the marine coasts and Great Lakes can also rapidly result in hypothermia.
- Field personnel should 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.

#### 2.8.5 COVID-19 SAFETY CONSIDERATIONS

Safety is the number one concern for all personnel. In implementing the NCCA, 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. NCCA training and assistance visits will be implemented in a manner that takes into account Covid-19 safety requirements and restrictions.

## 3 INTRODUCTION TO SAMPLING

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This Field Operations Manual describes procedures for collecting samples for the NCCA 2020. Overall, the same indicators will be collected at both estuarine and coastal freshwater Great Lakes sites, though some of the sampling will be conducted using different equipment. Field crews at all Great Lakes sites will collect additional water samples to be analyzed for phytoplankton, whole fish composite samples to analyze fillets for human health risks, and will record underwater video of the bottom substrate.

This section presents a general overview of the field activities and guidelines for field operations, recording data, and labeling samples. This section also describes field crew makeup and other sampling considerations.

### 3.1 SITE VISIT DURATION

NCCA field methods are designed to be completed in one field day. Depending on the time needed for sampling and travel, crews may require an additional day to complete sampling, pre-departure and post-sampling activities (e.g., cleaning equipment, repairing gear, shipping samples, and traveling to the next site). Remote sites with lengthy or difficult approaches may require more time, and field crews must plan accordingly. Conversely, some sites may be in relatively close proximity to each other, allowing multiple sites to be sampled in a single day.

### 3.2 FIELD CREW MAKEUP

A field crew typically consists of three to four people. However, a minimum of two people may be able to properly execute sampling activities. To ensure safety, at least two people are always required in a boat when conducting field work for the NCCA. In order to organize field activities efficiently, each field crew should define roles and responsibilities for each crew member prior to beginning field activities. One crew member is primarily responsible for boat operation and navigation. Additional crew members assist with sample collection/processing and/or provide logistical support.

### 3.3 SAMPLING SEQUENCE

The field crew arrives at the site in the early morning to complete the sampling in a single day. The typical sampling scenarios are shown in **Figure 3.1** and **Figure 3.2**.

### 3.4 SAMPLING CONSIDERATIONS

#### 3.4.1 CONSIDERATIONS FOR FISH TISSUE COLLECTION

The sequence of daily field activities may differ depending on whether the field crew is collecting fish that day or another day, or using active (trawling, seining, hook and line, etc.) or passive (gill net, hoop net, long-lines, etc.) fish collection methods. Other minor modifications to the sampling scenario may be made by crews; however, the sequence of sampling events presented in the following figures (depending on the type and timing of

fish collection) should be adhered to and is based on the need to protect some types of samples from contamination and to minimize holding times once samples are collected.

### 3.4.2 LISTED SPECIES CONSIDERATIONS

Field crews have the potential to encounter federally listed species and critical habitats that are protected under the Endangered Species Act (ESA) while conducting field sampling. Field crew leads are expected to have an understanding of the federally listed species and their 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. Crew leads 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<sup>a</sup> of listed species while implementing the NCCA field protocols. For example, crews are expected to:

- abide by all boating speed regulations, including “No Wake” and “Minimum Wake” zones;
- remain a respectful distance from marine mammals and sea turtles<sup>b</sup>;
- designate a marine animal spotter for when the boat is in motion;
- understand the circumstances when it would be necessary to shut down a vessel due to the presence of a listed species;
- allow a listed species to naturally move away from the sampling area (do not herd or harass);
- immediately release listed taxa if they are unintentionally collected while implementing the sediment, benthic macroinvertebrate, or fish tissue sampling protocols (do not preserve); and
- implement additional limitations that may be established in the scientific sampling permits.

These best practices are not an exhaustive list of requirements for field crews. Regulations and guidelines that have been developed for marine life viewing provide useful risk minimization practices when boating in area that may support listed manatee, whales, turtles, sea lions, and sharks. Field crews are expected to be aware of the recommendations and guidelines that apply in a given state and for a given species. Additional information on boating best practices is available on the NOAA Fisheries [Marine Life Viewing](#) page and provided by the [Florida Fish and Wildlife Conservation Commission](#).

### 3.4.3 CONSIDERATIONS FOR ENTEROCOCCI COLLECTION

Enterococci levels tend to be highest in the morning prior to high levels of solar irradiation; therefore, these samples must be collected as early in the day and with as

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<sup>a</sup> “Take” means to harass, harm, pursue, hunt, shoot, wound, kill, trap, capture, or collect, or to attempt to engage in any such conduct.

<sup>b</sup> For whales, remain at least 100 yards away unless other restrictions apply (e.g., 200 yards from killer whales in Washington State inland waters). For seals, sea lions or turtles in the water, or on shore, remain at least 50 yards away. To learn more, visit the Marine Mammal Viewing Guidelines and Distances page, as well as 50 CFR 216.3 and 50 CFR 224.103

little water and sediment disturbance as possible. Regardless of when the Enterococci samples are collected, **crews must complete filtration within six hours of collection. Enterococci samples not filtered within six hours of collection must be discarded, recollected, and filtered.**

#### 3.4.4 OTHER CONSIDERATIONS

Crew members responsible for collecting water chemistry, sediment grabs, and fish tissue must remember to **not** apply sunscreen or other chemical contaminants until after each of these samples is collected to avoid compromising the integrity of the sample (or implement measures to reduce contamination by such chemicals if applied such as washing, wearing long gloves, etc.).



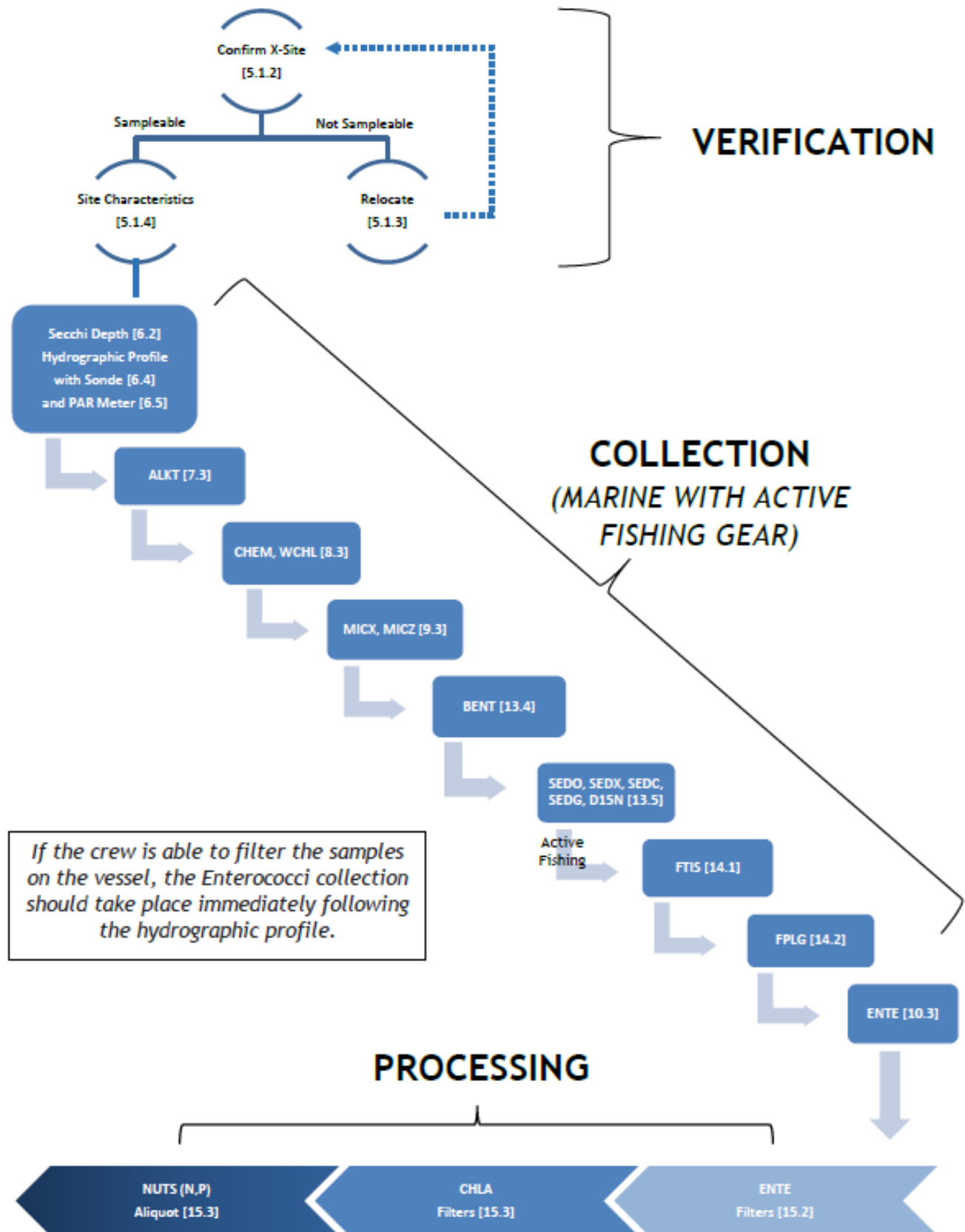
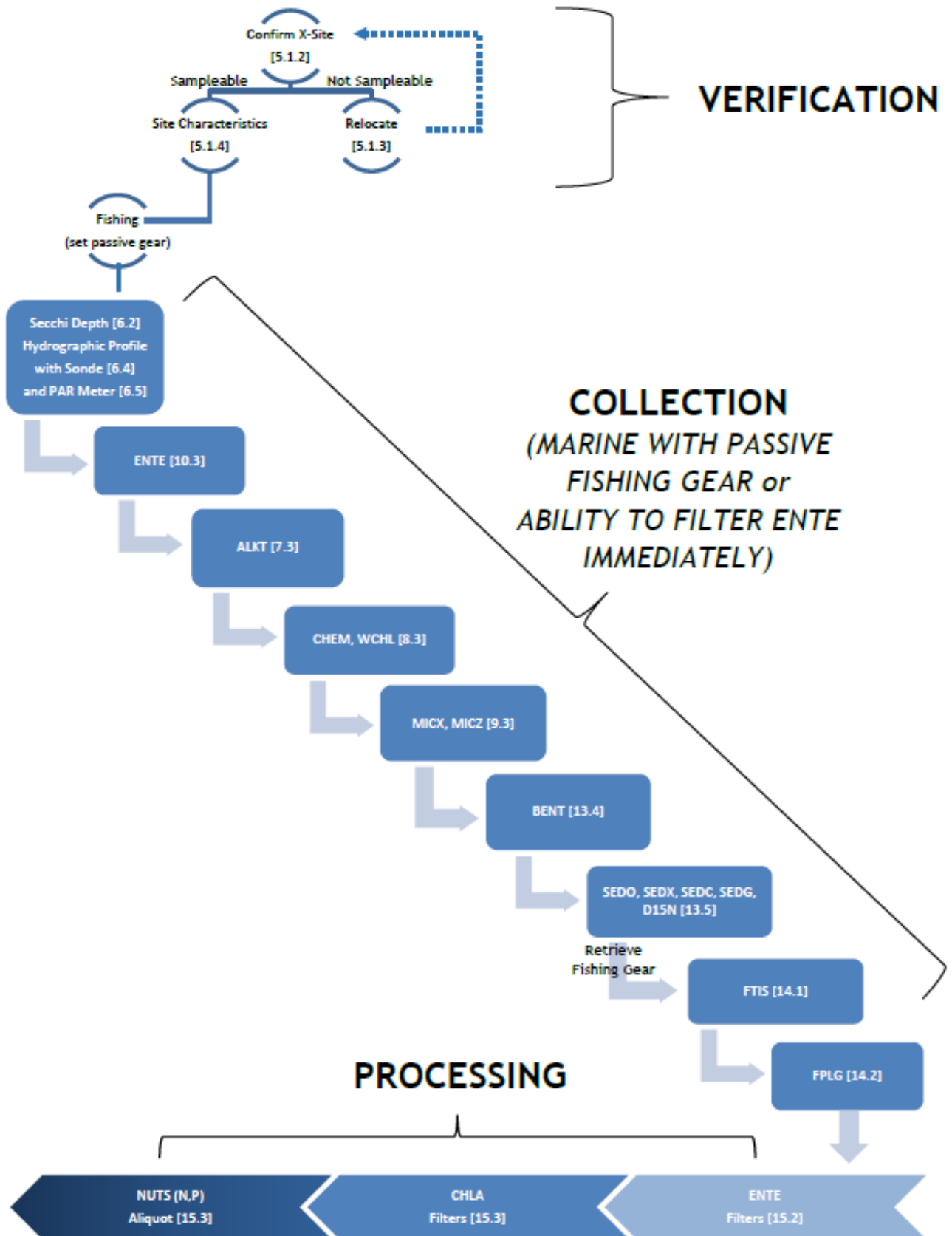


Figure 3.1 Marine Field Sampling Scenario - Active Fishing Methods



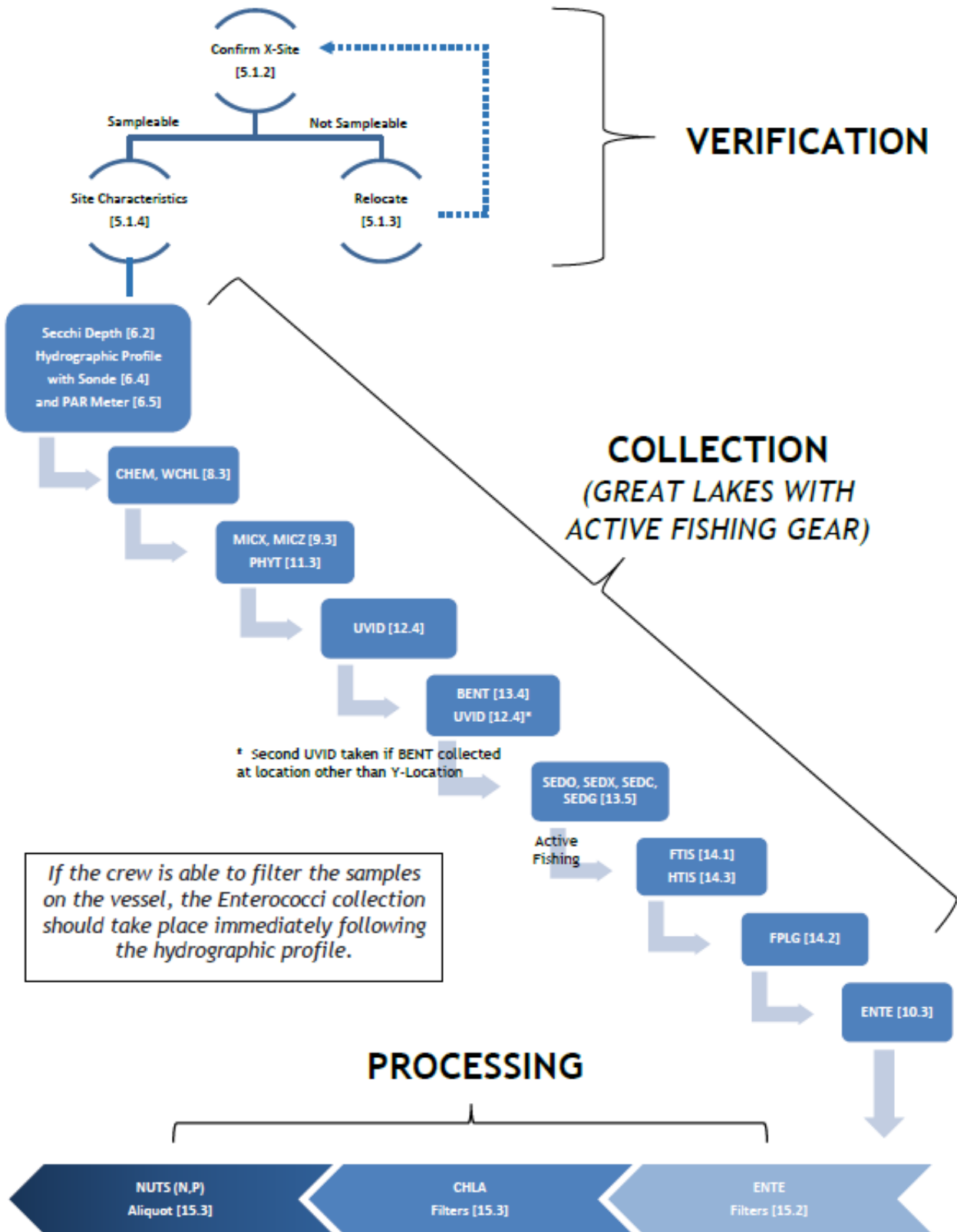


Figure 3.3 Great Lakes Field Sampling Scenario - Active Fishing Methods

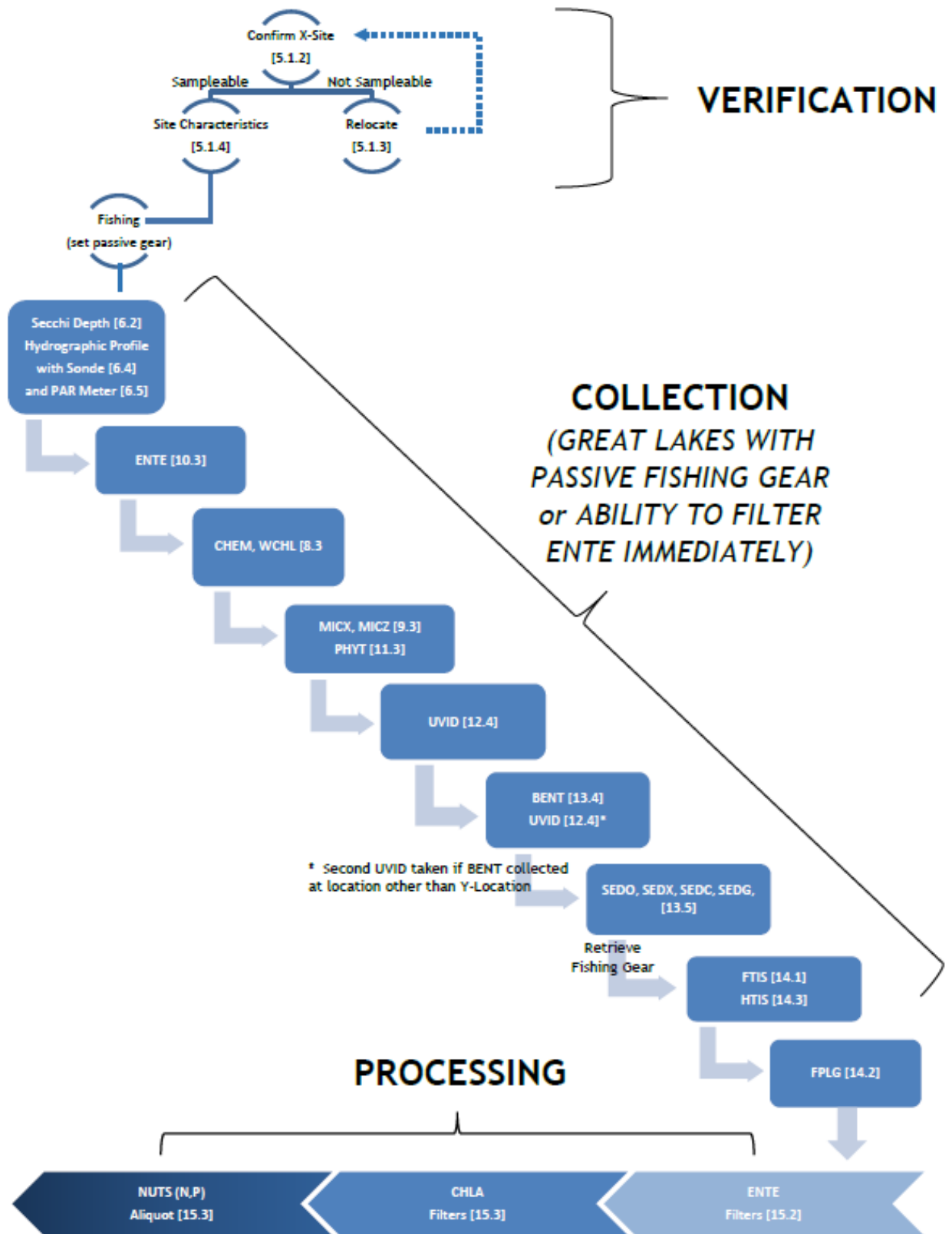


Figure 3.4 Great Lakes Field Sampling Scenario - Passive Fishing Methods or Ability to Filter Samples Immediately

## 4 PRE-DEPARTURE ACTIVITIES

Field crews conduct a number of activities at their base site (i.e., office or laboratory, camping site, or hotel) before departure to the site and after returning from the field (Figure 4.1). Before leaving the base site, the crews must know: (1) where they are going; (2) that the site is accessible and that, if necessary, they have permission to sample it; (3) that equipment and supplies needed to complete the sampling effort are available and in good working order; and (4) any and all federally listed species that have the potential to occur at the sites. After sampling, crews must ensure that: (1) samples are labeled, packed, and shipped appropriately; (2) the sampling event is communicated to EPA via the NCCA App submissions; and (3) equipment and supplies are cleaned and replenished as necessary.

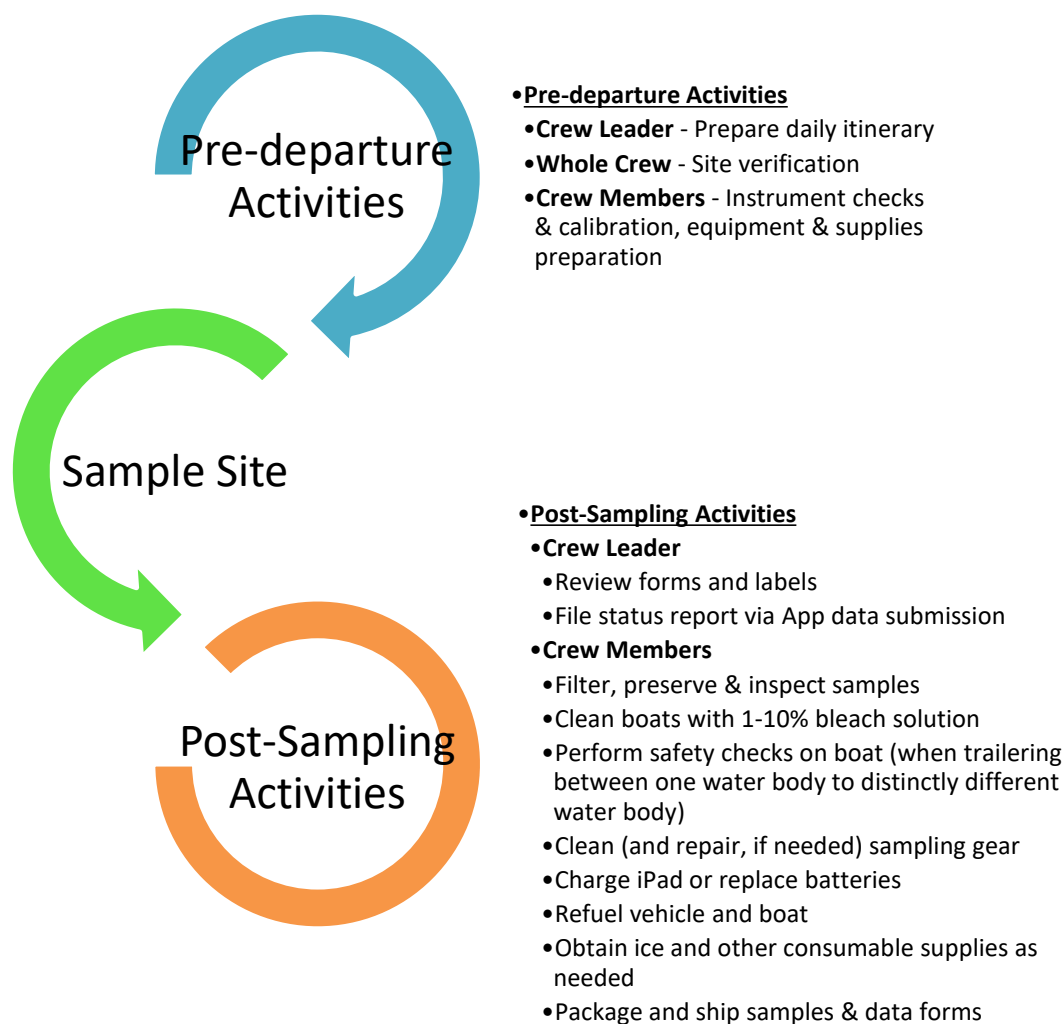


Figure 4.1 Overview of base site activities

Pre-departure activities are included here, while post-sampling activities are also discussed in **Section 15: Final Site Activities** and **Section 16: Post-Sampling Activities**.

Pre-departure activities include the development of a daily itineraries, instrument checks and calibration, and equipment and supply preparation.

#### 4.1 DAILY ITINERARIES

Field Crew Leaders are responsible for developing daily itineraries and site information, which are compiled as a **Site Packet**. This site packet typically includes maps, navigational charts, contact information, copies of permission letters, permits, access instructions, location of FedEx offices, and location and contact information of hospitals or other emergency services. If applicable and per field crew's standard operating procedures, Site Packets should include information on federally listed species that may occur at the site, how to avoid them, and actions to be taken if they are encountered. Additional pre-sampling activities include confirming the best access routes, calling the landowners or local contacts, confirming lodging plans, and coordinating rendezvous locations with individuals who must meet with field crews prior to accessing a site.

Also, the Field Crew Leader must identify appropriate boat ramps or marinas and gas docks. If the crew is planning a multiple day/multiple site trip, information for each day and site must be developed and compiled into separate site packets.

#### 4.2 INSTRUMENT CHECKS AND CALIBRATION

Each field crew must test and calibrate instruments prior to sampling. Equipment can be calibrated either prior to departure for the site or at the site. However, due to variations in elevation, DO probes must be calibrated at the site. The field crew will verify site location using a global positioning system (GPS) receiver. They will collect measurements using a Photosynthetically Active Radiation (PAR) meter and a multi-parameter unit for measuring DO, pH, temperature, salinity (recorded at marine sites) and conductivity (measured at freshwater sites). Field crews must have access to backup instruments if any instruments fail the manufacturer performance tests or calibrations. Prior to departure, field crews must perform the following checks and calibrations:

- If using a hand-held GPS unit, turn on the GPS receiver and check the batteries. Replace batteries immediately if a battery warning is displayed. Boat-mounted GPS units run off of the boat electrical system.
- Test and calibrate the multi-parameter meter (or sonde). Each field crew must refer to and follow the manufacturer's calibration and maintenance procedures to calibrate multi-parameter meters according to manufacturer specifications. Once each week, crews must verify that the meter is functioning properly by performing manufacturer recommended internal diagnostic readouts (e.g., pH millivolts, cell constants, and/or other diagnostic readings). Records of these checks should be saved in a logbook or other documentation. For those meters that do not have internal check capabilities, crews will need to verify on a weekly basis that the meter is measuring pH and conductivity properly by measuring a commercially available Quality Check Solution (QCS) with properties similar to YSI 5580 confidence solution.

- Ensure that the PAR meter's handheld display unit has fresh batteries, that the unit is functioning properly, and that the correct calibration factors are entered for each probe.  
*Note: Calibration factors are supplied by the manufacturer and are specific to each individual probe. PAR sensors require no field calibration; however, they should be returned to the manufacturer at least every two years for calibration. Field crews must use the procedures for the initial setup of the LI-COR Datalogger (Section 4.2.1) to verify the setup of the unit or to enter coefficient values should a new sensor need to be installed.*
- Crews operating in the Great Lakes must ensure that batteries of the underwater cameras and lights are charged and all components are correctly attached to the frame.

#### 4.2.1 INITIAL ASSEMBLY AND SETUP PROCEDURES FOR LI-COR FRAME, SENSOR AND DATALOGGER

Field crews must use a pre-configured LI-COR system. Use the following instructions to assemble the system if needed and the following section to reconfigure the LI-COR if needed.

##### *4.2.1.1 Assembly of the LI-COR lowering frame and sensor (from LI-COR 2006)*

For NCCA, crews will need to attach one LI-192 Underwater Quantum Sensor to the LI-COR lowering frame. **IMPORTANT:** Do not use the LI-COR underwater cable to support the sensor and lowering frame, as damage to the cable can result. The lowering line provided in your base kit should be used to support the lowering frame and sensor by attaching the in-line clip to the suspension ring at the top of the lowering frame. In addition, the cable should not be bent sharply near the sensor.

The lowering frame provides for the placement of two sensors, however, NCCA crews will only attach a single underwater sensor. Each LI-COR underwater sensor has three 6-32 tapped mounting holes on the underside of the sensor for connection to the mounting ring (**Figure 4.2**). Corrosion resistant mounting screws are used with each sensor.

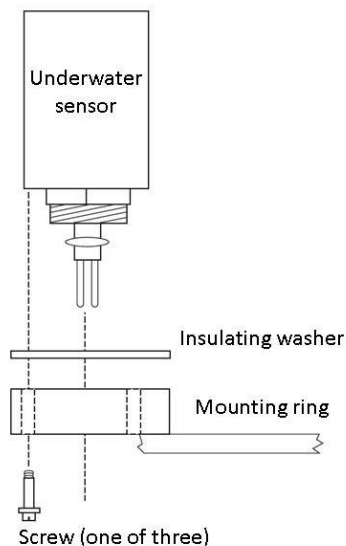


Figure 4.2 Attachment of the underwater sensor to the mounting rings (adapted from LI-COR, 2006)

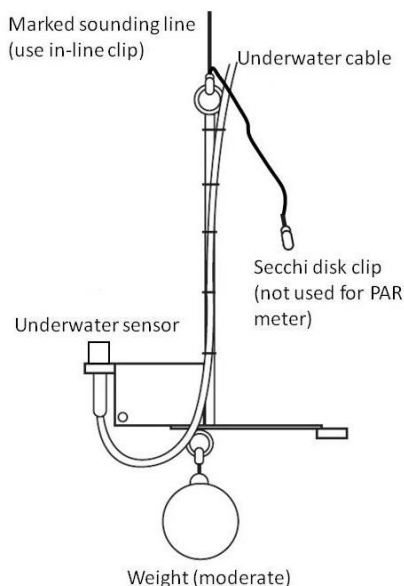


Figure 4.3 Lowering frame assembly with sensor, weight, and lowering line (adapted from LI-COR, 2006)

The underwater sensor will be attached using the mounting ring on the fin of the lowering frame (**Figure 4.3**). To accommodate for any tilting of the frame and to ensure a straight downward direction, a compact weight should be attached to the weight ring at the bottom of the frame. Depending upon the speed of the current, moderate weights will often suffice (4 kg). Weights over 25 kg should be avoided.

Once the sensor is installed to the mounting ring using the three screws and insulating washer, plug the underwater cable into the sensor by aligning the sensor pins and tightening the threaded connection. There is a yellow etched mark on the sensor bottom that should be aligned with the raised nub on the cable. If the underwater sensor begins reading negative values at startup, this likely indicates that the plug on the bottom of the underwater sensor is plugged in backwards.

The underwater cable should be attached to the frame such that approximately 25 cm of cable forms a smooth arc to the underwater sensor connector and is restrained from being flexed or supporting any weight. Additionally, the cable must be securely attached to the shaft of the lowering frame at multiple points so that the cable does not slip and put strain on the sensor connector. However, the cable cannot be clamped so tightly as to damage it. Possible methods to use are numerous nylon cable ties along the length of the shaft, or a tight wrap of lightweight cord around the shaft and cable, starting at the suspension ring and extending downward at least 25 cm.

#### 4.2.1.2 Setup Procedures for LI-COR LI-1400 Datalogger

The following example demonstrates the process for configuring the LI-1400 (with the instrument keypad) to view or log instantaneous data from a single LI-190SA Quantum Sensor.



*Example 1a. Configure channel I1 for a LI-COR LI-190SA Quantum Sensor with calibration multiplier of  $-125.0\mu\text{mols}\cdot\text{m}^{-2}/\mu\text{Amp}$  (Each sensor has a unique multiplier value supplied from the factory)*

1. Connect the Quantum LI-190 ambient light sensor to the BNC connector on top of the LI-1400 labeled I1.
2. Turn on the LI-1400 meter.
3. Press the [Setup] key.
4. Use the left ([←]) or right ([→]) arrow keys to navigate to “SETUP CHANNELS”.
5. Press the [Enter] key to begin the sensor setup.
6. Use the left ([←]) or right ([→]) arrow keys to navigate to “I1=Light”, press Enter”.
7. Using the [Shift] key and the number/ letter keys, type a description for this channel. This description could describe the type of sensor (i.e., “QUANTUM”), or describe what the reading will be used for in the NCCA sampling (i.e., “AMB”).
8. Press the down ([↓]) arrow key to enter the multiplier. The multiplier value is found on the Certificate of Calibration provided with the sensors. Each sensor must have a unique certificate and calibration multiplier value.
9. Press the down ([↓]) arrow key; enter “AMB” for the unit label.
10. Press the down ([↓]) arrow key; select “1 sec” to display instantaneous values. The running average parameter will not be used, but could be set here to any desired value.
11. Press the down ([↓]) arrow key; select “Log Routin=none”
12. The remaining options do not need to be set as they apply only when using a Log Routine.
13. Repeat this entire procedure for channel I2 to setup the underwater sensor (“I2=Light”) using “UW” as the label for the channel.

### 4.3 EQUIPMENT AND SUPPLY PREPARATION

Field crews must check the inventory of forms, supplies, and equipment prior to departure using **Appendix A**; use of the lists is mandatory. Inventory extra site kits prior to each site visit to ensure sufficient back-up supplies are available. Store extra site kits in the vehicle and/or boat so that replacement supplies will be readily available in case of loss or damage while at the sampling site.

- Obtain sufficient wet and dry ice for sample preservation and storage.
- Pack meters, probes, and sampling gear, taking care to do so in a way that minimizes physical shock and vibration during transport.
- Pack stock solutions as described in **Table 4.1** below. Follow the regulations of the Occupational Safety and Health Administration (OSHA).

Field crews must request site kits through the supply request form at least two weeks prior to sampling. Site kits will include sample labels, packing slips, and necessary shipping labels for sampling one site and are specific to either a marine or Great Lakes

site. Great Lakes crews sampling at designated human health whole fish tissue sites will also need to request a whole fish tissue sampling kit along with the site kit. Crews will automatically receive extra labels and paper form packets as a backup to electronic data collection prior to sampling, and can request additional as needed. **Field Crew Leaders MUST provide a general schedule to the EPA and the Contractor Field Logistics Coordinator two weeks prior to initiating sampling for the season.**

**Note:** Site kits for all sites to be sampled in 2020 cannot be provided at the beginning of the field season. Consequently, site kits will be provided to crews as requested throughout the index period.

The site kit includes sample jars, bottles, and other supplies (see complete list in **Appendix A: Equipment and Supplies Lists**). After receipt, please inventory the site kit against these lists. If items are missing, damaged, or incorrect, please request replacement supplies using the supply request form or by contacting the Contractor Field Logistics Coordinator. The Contractor Field Logistics Coordinator will send replacement supplies as quickly as possible.

Table 4.1 Stock solutions, uses & methods for preparation

Solution	Use	Preparation
<b>Bleach (1-10%)</b>	Clean nets, gear, and inside of boat	Add 10 - 100 mL bleach to 1 L distilled water.
<b>Quality Check Solution for multi-parameter sonde</b>	Weekly check of meter calibration <i>In place of weekly internal meter checks</i>	No preparation needed (if purchased as ready-to-use solution)
<b>Buffered Formalin</b>	Preserve benthic samples	Add 8 tablespoons Borax to 2 gallons 100% Formalin (37% formaldehyde) solution. FOR USE AT ALL SITES: Add ¼ teaspoon Rose Bengal crystals to above solution.
<b>Lugol's Solution</b>	Preserve phytoplankton samples (Great Lakes sites only)	None (included in GL base kits); Lugol's Iodine solution is light sensitive. Take care to avoid exposure to direct light.

## 5 INITIAL SITE PROCEDURES

Upon arriving at the site, the field crew must confirm that it is the correct site and determine if the site meets the criteria for sampling and data collection activities. The crew verifies site access, safety, and general conditions to determine if the site can be sampled within the swing of the anchored boat.

*Note: Inability to collect samples for sediment, benthic, or fish indicators does not disqualify a site from meeting sample criteria. See Section 2.3.1 to determine site sampleability.*

### 5.1 SITE VERIFICATION

#### 5.1.1 EQUIPMENT & SUPPLIES

Table 5.1 Equipment & supplies: site verification

<b>For locating and verifying site</b>	sampling permit and landowner access (if required) site packet, including access information, site spreadsheet with map coordinates, navigational charts with “X-site” marked NCCA Fact Sheets for public outreach GPS unit (preferably one capable of recording waypoints) with manual, reference card, extra battery pack
<b>For recording measurements</b>	Verification form in App fine-tipped indelible markers (for labels) clipboard

#### 5.1.2 SITE VERIFICATION PROCEDURES

1. Create a waypoint in the GPS unit that corresponds to the target X-site coordinates provided by EPA in the Site Evaluation Spreadsheet. This process can be completed in the office.
2. Navigate the sampling vessel as close as possible to the target X-site using GPS (you must be no more than 0.02 nautical miles (nm) or 37 meters from the target X-site). Compare the target X-site coordinates with the GPS coordinates displayed at the sampling site.
  - Sampling may start when the sampling vessel is within 37 meters of the X-site. This distance provides the desired level of precision which is approximately equal to that of the GPS receiver without differential fix correction.
  - With the exception of fish tissue and sediment samples (see Section 5.4) crews are expected to collect all samples within a circle of 0.02 nm radius around the X-site. This allowable deviation distance accounts for typical “anchor swing” of the sampling vessel.
3. Anchor the sampling vessel in such a way as to minimize the possibility of the anchor(s) dragging or becoming dislodged.
4. Once the anchor has been set and the vessel is essentially stationary, verify that the X-site is still within 0.02 nm or 37 meters. This location (where

sampling will begin) is referred to as the Y-location. If the X-site is not within 0.02 nm or 37 meters, reposition the vessel by following the steps outlined above.

5. Determine if the site is sampleable. See **Section 2.3** for specific guidelines.
  - If not sampleable, proceed to **Section 5.1.3**.
  - If sampleable, proceed to the steps below and then to **Section 5.1.4**. Record the time of arrival to the Y-location on the Verification Form in the App.
6. Record the coordinates of the Y-location on the Verification Form in the App form in decimal degrees in the NAD 83 datum.
7. Record the number of satellites fixed as  $\leq 3$  or  $\geq 4$ .
8. After anchoring, and throughout all subsequent sampling efforts, monitor the GPS to ensure that the sampling vessel stays within the proper X-site radius.
9. Indicate any and all methods that were used to verify that you are at the correct location.
10. Measure and record the water depth at the Y-location on the Verification Form in the App. Make sure an accurate depth reading is taken at the site to ensure the depth is adequate to conduct sampling.

#### 5.1.3 SITE RELOCATION

Every attempt should be made to sample within a 0.02 nm (~37 m) radius of the X-site. If the proposed initial sampling location is not sampleable, then relocate using the following guidelines:

1. The Field Crew Leader should choose a specific compass heading (e.g., north, south, east, west) and slowly motor the vessel in that direction.
2. After moving approximately 15-20 m, assess the relocated area using the Site Verification guidelines given above.
3. Should the relocated area fail to meet the “sampleable” definition, then this process may be continued using the same heading out to 37 meters from the X-site.
4. If no suitable sampling location is found along the first chosen heading, return to the X-site and follow a new heading until an acceptable sampling location is found.
5. If after a sufficient amount of effort is expended and no suitable sampling location is found, then the determination may be made that the site is unsampleable.
6. If the site is non-sampleable or inaccessible and cannot be relocated within the designated area, indicate the reason on the Verification Form in the App. No further sampling activities are conducted at this site.
7. Replace the original site with the next oversample site on the estuary/state list.
8. Return to **Section 5.1.2**.

#### 5.1.4 SITE CHARACTERISTICS

1. If the site is sampleable, record the sampling status and method being used (marine or Great Lakes).
2. Record the general habitat type and the dominant bottom type present at the sampling site.

- At many sites, it may not be possible to record the bottom type until after the sediment collections are performed.
- 3. Record the presence and type of debris (if any), submerged aquatic vegetation (SAV) present, and/or macroalgae present in the area.
- 4. Make any general comments about the site that may be important during the data review portion of the assessment or any unusual characteristics about the site, including weather conditions.
- 5. Record directions to the launch site from an easily recognizable location (city or major road intersection).
- 6. Draw a simple sketch of the area.
  - Include the relative locations of the shoreline, launch point, X-site, Y-location, and, if different from the Y-location, sediment and fish collection locations. If sediment and fish were collected at different locations from each other, please indicate them separately (see **Section 5.4**). Include any other specific attributes of the site that may be important during data analysis.
  - A printed or copied section of a map with the pertinent information may be submitted in place of the scene sketch.
  - Upload this sketch/map to the NARS SharePoint site when you submit your data forms.
- 7. Record the names of the Field Crew Leader, fish taxonomist, and all crew members. The same name may be recorded twice if the Field Crew Leader is also the fish taxonomist.

## 5.2 SITE PHOTOGRAPH

Although not required, EPA encourages crews to take site photographs, especially if the site is associated with unusual natural or man-made features.

- Date-stamp any site photographs and include the site ID.
- Alternatively, start the photograph sequence with one image of an 8.5 × 11 inch piece of paper with the site ID, waterbody name, and date printed in large, thick letters.
- Keep a brief photograph log (site ID, number of photographs, time and date if not stamped by camera) and describe the subject of each photo *if it is not self-explanatory*.
- Field crews can upload these photos to the NARS SharePoint site.

## 5.3 SAMPLE COLLECTION

Even when the field crew makes every attempt to collect all samples, there will be some circumstances that will prevent all samples from being collected. When site conditions limit full completion of the standard sampling protocol, crews prioritize sample collection and follow a “checklist” for determining the order of sample completion:

1. Measure *in situ* water parameters and collect all water samples at all sites.

2. Collect benthic grab samples at all sites. Any size sediment grab is acceptable as long as it meets the definition of a “successful benthic grab” (see **Section 13.3**).  
*Note: Acceptable means:*
  - a) A sediment grab that meets the criteria for benthic samples; or
  - b) Enough sediment can be collected that will allow the crew to obtain the surficial sub-sample required for the sediment composite to send to the laboratory for abiotic indicator analysis (e.g., organics/metals, TOC, grain size, toxicity,  $\delta N^{15}$  isotopes in benthic organic matter).
3. Collect sediment composite material of sand-sized sediment grain or smaller (preferred size). If an acceptable sediment grab cannot be obtained at the Y-location or within a 37 m radius around the X-site, move to a secondary sediment collection area following the procedures in **Section 5.4.1** below. Flag and note the reason for limited/missing sediment samples. In the case of limited sediment, prioritize sample distribution in the following order of preference:
  - a) Toxicity [SEDX]
  - b) Organics/Metals [SEDO]
  - c) Total Organic Carbon [SEDC]
  - d) Silt/Clay (Grain Size) [SEDG]
  - e) Nitrogen Isotopes [D15N] at marine sites only*Indicate if any of the sediment samples were not successfully collected by marking the “no sample collected” box(es) in the App for each pertinent sample and supplying a reason for not collecting in the adjacent comment field.*
4. Collect fish for ecological contaminant [FTIS] analysis. For the ecological assessment, fish collections are targeted to areas within a 500 m radius of the X-site. After unsuccessful attempts within this area, crews may move outside of this radius and attempt to collect fish up to 1000 meters from the X-site (see **Section 5.4.2**). Unsuccessful deployment of fish collection gear or the absence of fish in the catch should not necessarily be used as a determining factor for rendering a site unsampleable.
5. Collect fish tissue plugs [FPLG].
6. Collect human health fish tissue sample [HTIS] at targeted Great Lakes sites. If suitable fish cannot be collected within 1000 meters of the X-site, crews may move out to a maximum of 1500 meters from the X-site in an effort to collect the human health fish tissue sample.

#### 5.4 SECONDARY SEDIMENT OR FISH COLLECTION ZONES

All water, benthos, sediment, and fish samples are expected to be collected at the same location (the Y-location), which is as close to the X-site as possible (within the 37 meter radius around the X-site). However, circumstances may require the field crew to relocate to a secondary location to collect an acceptable sediment grab and/or fish sample. If benthos, sediment, and/or fish are collected from a secondary or tertiary location, *in situ*

measurements and water collections do not need to be resampled. Guidelines for relocating to a secondary sample collection zone are covered in the sections below.

#### 5.4.1 SEDIMENT SAMPLES

1. If an acceptable sediment grab cannot be obtained at the Y-location where water samples were collected, move the vessel within the 37 m radius margin (of the X-site) and try to obtain the sediment sample. Use the site relocation method described previously (**Section 5.1.3**). On the **Sample Collection** form in the App, indicate the sediment collection zone by filling in the "within 37 m from X-site" bubble.
2. In cases where sediment sampling cannot be successfully conducted within 37 m of the X-site, grabs may be taken in a secondary sediment collection zone (e.g., > 37 m radius but within a 100 m radius (~0.05 nm) of the X-site) without re-collecting the water samples (**Figure 5.1**).  
Draw a second circle with a 100 m radius from the X-site on the site sketch or map. Place a mark on the map showing the relative location of the sediment collection zone and the approximate distance and direction from the X-site. Indicate in the comments section approximately how far and in what direction from the X-site the sediment was collected. On the **Sample Collection** form in the App, indicate the sediment collection location by filling in the "between 37-100 m from X-site" bubble. The data will be flagged for subsequent review.
3. Crews may use the same relocation procedures to move out to a maximum distance of 500 m from the X-site to locate suitable sediment sampling locations (after attempting to collect sediment from within the primary and secondary zones). Draw a 500 m radius circle on the site sketch or map indicating the sediment collection area and the approximate distance and direction from the X-site. Indicate in the comments section approximately how far and in what direction from the X-site the sediment was collected. On the **Sample Collection** form in the App, indicate the sediment collection zone by filling in the "between 100-500 m from X-site" bubble. The data will be flagged for subsequent review.
4. If a suitable location to collect sediment samples has not been found after a minimum of three collection attempts inside each of the acceptable relocation radii, sediment sampling is considered "complete" for the site. All appropriate explanations must be completed within the App, as well as pertinent "no sample collected" boxes.

**Note:** *The Field Crew Leader may choose to make additional sediment grab attempts.*

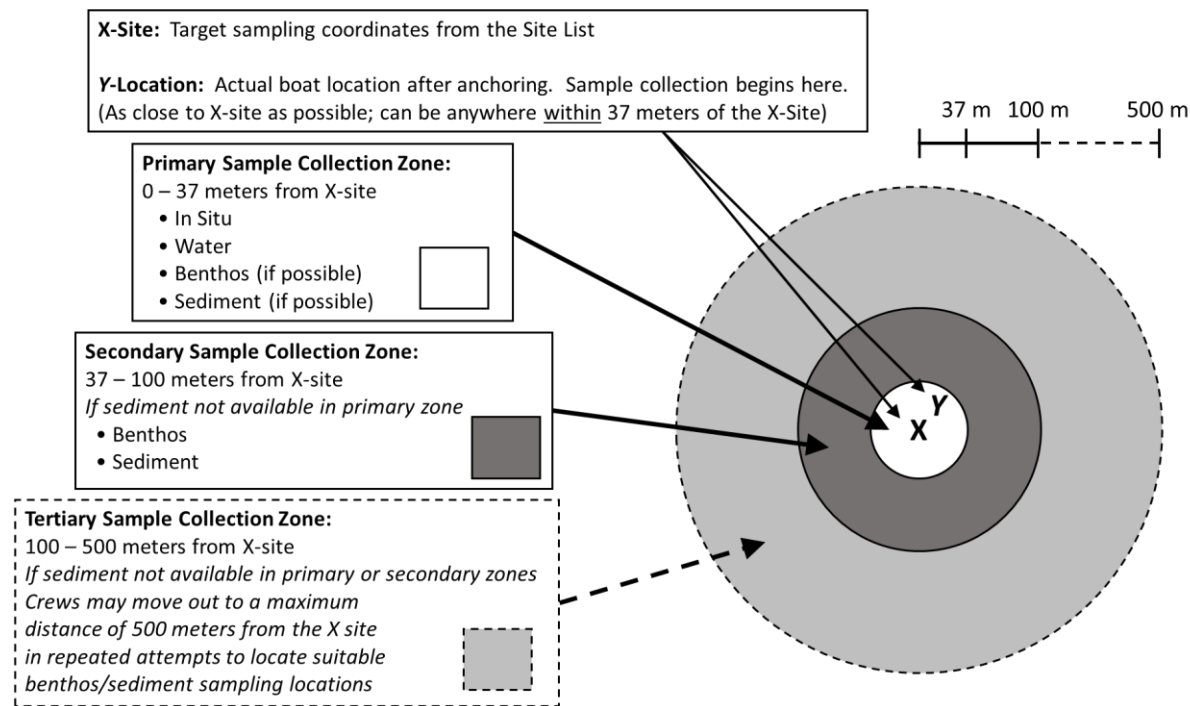


Figure 5.1 Primary, secondary and tertiary sample collection zones

#### 5.4.2 FISH SAMPLES

The primary fish collection zone at all sites is a radius 500 m from the X-site. Secondary fish tissue collection sites may be selected up to an additional 500 m beyond the original 500 m radius at all estuarine and Great Lakes sites (Figure 5.2).

Please observe the following guidelines when considering sampling locations for fish samples:

1. In order to move to a secondary fish tissue collection site, crews must be unsuccessful at obtaining target fish during a reasonable portion of the three hours allotted to fishing (at least 30 minutes and no more than two hours) within the original 500 m radius.
2. The crew must have attempted several sampling locations within the primary 500 m radius without success in order to move to the secondary fish collection zone.
3. When relocating crews should concentrate on signs of fish presence such as schools of bait fish just below the surface, predator activity or prey escape behavior on the surface of the water, overhead shading or favorable underwater habitat structure or bathymetric features within an additional 500 m from the X-site.
4. Record the coordinates of the site where fish were ultimately caught.
5. For the collection of the human health fish tissue sample ONLY, crews may move out to a maximum of 1500 meters from the X-site.



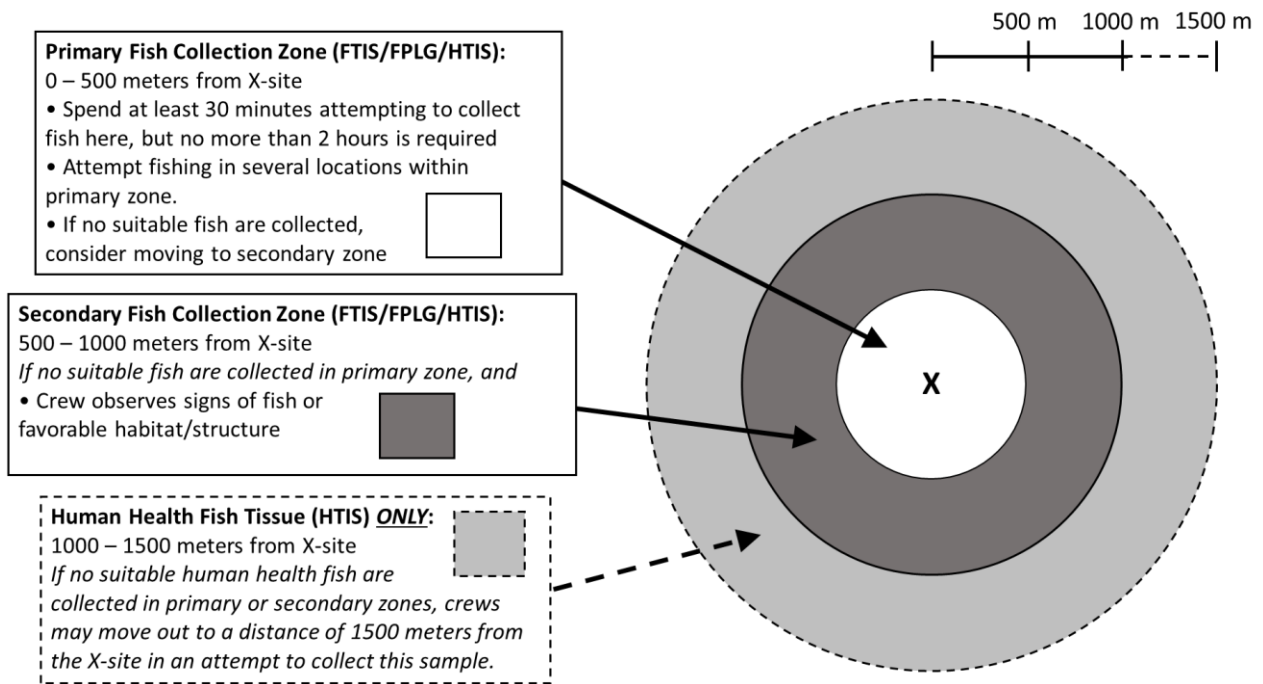


Figure 5.2 Primary and secondary fish collection zones

## 6 WATER QUALITY MEASUREMENTS

This section describes the procedures and methods for the field collection and analysis of the water quality indicators (*in situ* measurements, water column transparency, and light attenuation) from freshwater and marine coastal areas.

### 6.1 SUMMARY OF METHOD FOR IN SITU MEASUREMENTS OF WATER COLUMN TRANSPARENCY, DISSOLVED OXYGEN, pH, SALINITY, CONDUCTIVITY, TEMPERATURE, AND LIGHT ATTENUATION

Field crews obtain a hydrographic profile at each site (at the Y-location) by measuring DO, pH, salinity (marine sites) or conductivity (freshwater sites), and temperature using a multi-parameter water quality meter (or sonde). They also assess water column transparency using a Secchi disk and light attenuation using a PAR meter. The protocol requires measurements at the prescribed depths as the probe/sensor is both lowered and retrieved, starting just below the surface, progressing down to 0.5 m from the bottom, and returning to just below the surface.

#### 6.1.1 EQUIPMENT AND SUPPLIES

**Table 6.1** lists the equipment and supplies used to measure water column transparency, DO, pH, salinity/conductivity, temperature, and light attenuation. Crews record *in situ* measurements on the Hydrographic Profile form in the App.

*Table 6.1 Equipment & supplies: transparency, DO, pH, salinity/conductivity, temperature, & light attenuation*

<p><b>For taking measurements and calibrating the water quality meter</b></p>	<p>multi-parameter water quality meter with DO, pH, salinity/conductivity, and temperature probes.          extra batteries          de-ionized water (lab certified preferred, but not required)          calibration cups and standards          QCS (<i>used if internal meter checks are not possible</i>)          barometer to use for calibration          thermometer          Secchi disk (20 cm diameter, weighted) &amp; 100' line with clip (marked in 0.5 m intervals)          PAR meter (with LI-190 Quantum Sensor and LI-192 Underwater Quantum Sensor &amp; cables, independent datalogger)</p>
<p><b>For recording measurements</b></p>	<p>NCCA App Hydrographic Profile form</p>

### 6.2 SAMPLING PROCEDURE – WATER COLUMN TRANSPARENCY (SECCHI DEPTH)

A Secchi disk is a 20 cm black and white disk suspended from a non-stretch line that is marked in 0.5 m intervals. Field crews use a Secchi disk to measure water column to nearest 0.1 m transparency at every site (at the Y-location). The resulting measurement is called the Secchi disk transparency depth, or “Secchi depth” for short. Below are step-by-step procedures for measuring water column transparency.

**Note:** For valid Secchi depth readings, no sunglasses, hats, or any other devices that shade the eyes may be used by the person who is observing the disappearance and reappearance depths. The Secchi depth is assessed from the shady side of the boat and can only be measured during daylight hours. One crew member must make all three sets of Secchi measurements at a site, and it is desirable to have the same crew member complete Secchi depth readings throughout the entire field season whenever possible.

1. In the “Secchi Depth” section of the Hydrographic Profile Form in the App, record the time Secchi depth readings were started.
2. Slowly lower the Secchi disk until it is no longer visible. In the “DISAPPEARS” column, record the depth where the marking on the line meets the water level. Interpolate between the 0.5 m markings on the rope to the nearest 0.1 m.
  - If the disk hits the bottom before disappearing, water column transparency depth is greater than the water depth. Fill in the “Yes” circle in the App next to “Clear to Bottom?” and record the station depth as both the disappearance and reappearance depth in the “Reading 1” row in the App. No further measurements or recording are necessary in this case.
3. Slowly raise the Secchi disk until it just becomes visible and record the depth in the “REAPPEARS” column. Interpolate between the 0.5 m markings on the rope to the nearest 0.1 m.
4. Repeat steps 1-3 two more times, recording both disappearance and reappearance depths each time.
5. Use the comment space provided on the Hydrographic Profile Form in the App to comment on any measurements that the crew feels needs further explanation or when a measurement cannot be made.
6. Repeat the entire process if any one disappearance or reappearance measurement differs from the others by more than 0.5 m.

## 6.3 SAMPLING PROCEDURE – MULTI-PARAMETER SONDE

### 6.3.1 CALIBRATION

Crews calibrate the DO, pH, and salinity/conductivity meter functions of the multi-parameter water quality meter (or sonde) before collecting data at each site. If a crew is sampling multiple sites in a single day, a single calibration is sufficient for the day.

- Crews record the manufacturer and model number of the instruments in the Calibration/QA form in the App.
- Crews must calibrate their pH probe according to the manufacturer’s instructions and their own laboratory policies by using at least a 2-point calibration method. Crews will supply commercially purchased calibration standards (typically pH of 7 and 10 for 2-point calibration and pH of 4, 7, and 10 for 3-point calibration). Any pH standards used must reference NIST Standard Reference Material (SRM) certifications to be used in the calibration of the pH probe. This requirement applies for calibrations done both pre-sampling and post-sampling.
  - The calibration buffers must be accurate to 0.02 pH units or better.

- The calibration buffers should be replaced with fresh solutions every three to four days or sooner if the crew suspects it has become contaminated.
- Crews will also calibrate their conductivity/salinity probe according to the manufacturer's specifications and their own laboratory policies using a commercially supplied, traceable conductivity standard.
- Crews will re-check pH and conductivity/salinity calibration again after daily measurements are complete to document potential meter drift throughout the day.
- For instruments that are factory calibrated and checked (e.g., Sea-Bird Electronics meters, etc.), crews must ensure that factory-certified diagnostics have been completed according to manufacturer specifications (preferably conducted immediately prior to the sampling season) and provide documentation copies during assistance visits. Meters such as these do not require the daily calibration steps or the weekly diagnostic/QCS checks.
- Once each week, crews must verify that the meter is functioning properly by performing manufacturer recommended internal diagnostic checks. This is manufacturer and model specific, but typically involves accessing internal diagnostic readouts (e.g., pH millivolts, cell constants, and/or other diagnostic readings). Results of these checks must be recorded in a logbook or other documentation and saved for potential review.
- For those meters that do not have internal check capabilities, crews will check pH and conductivity against a commercially available QCS with properties similar to YSI 5580 confidence solution. The QCS is provided by the crew. Crews record the successful completion of the internal checks or the expected values and measured values of the QCS in the "Quality Control Check" section of the Calibration/QA form in the App.
- Crews using a commercially purchased pH QCS for the weekly quality checks should follow the guidelines below:
  - The pH QCS containers should be labeled with expected values and preparation dates.
  - The pH of the QCS should approximate the pH expected at sampling sites.
  - Crews should have centrally located bulk solutions to replenish allotments needed for quality checks every three to four days or sooner if the crew suspects it has become contaminated.
    - Bulk solutions should be replaced according to the manufacturer's specifications or at any time if crew suspects it has become contaminated.
- Crews use a commercially purchased primary conductivity/seawater standard to be used as the QCS for weekly quality checks of conductivity/salinity.
  - A secondary conductivity/seawater standard that is referenced against a certified standard may also be used.
    - If a secondary standard is used, then preparation and certification test procedures and results must be logged in a QA notebook and maintained by the state or contractor in-house QA personnel.
    - The standard should be representative of the conditions expected in the field (~0.5-35 ppt for marine waters).

- The conductivity/seawater calibration standard and QCS containers must be labeled with expected values and preparation dates.
- The standards should be replaced with fresh solutions every three to four days or sooner if the crew suspects they have become contaminated.
  - Bulk supplies of calibration standards and primary or secondary QCS may be maintained in a central location and used to replenish QA allotments.
  - Bulk solutions should be replaced according to manufacturer's specifications or if the crew suspects that they may have become contaminated.
- At least once per sampling season (usually in a laboratory before crews begin sampling), calibrate the temperature sensor against a National Institute of Standards and Technology (NIST)-traceable thermometer.
- If you observe any irregularities or calibration measurements that fall outside of the specified tolerance ranges use an alternate instrument if available and flag any affected data.

Specific information about calibrating each probe function is presented below.

#### 6.3.2 DISSOLVED OXYGEN METER

Calibrate the DO probe in the field against an atmospheric standard (i.e., ambient air saturated with water or water saturated with air) according to manufacturer's specifications and NCCA QA protocols prior to launching the boat. In addition, follow any of the manufacturer's recommendations for periodic comparisons with internal quality checks (cell constants, millivolt output, or other readings), or a DO chemical analysis procedure (e.g., Winkler titration) to check accuracy and linearity. Record results and report irregularities as described above.

#### 6.3.3 PH METER

Calibrate the pH meter in accordance with the manufacturer's instructions and with the field crew organization's existing Standard Operating Procedure (SOP).

After all *in situ* measurements have been completed for the sampling day, crews perform a post-measurement calibration check of the pH meter. Crews will record the Calibration Standard Value pH and the post-sampling measurement in the appropriate locations in the "Post-Measurement Calibration Check" section of the Calibration/QA form in the App. If significant drift (outside of manufacturer's specification) is detected, it may indicate that the meter is in need of service. Perform the required service or exchange devices as appropriate and if necessary, and flag any suspect measurements. Discontinue use of any meter that is not functioning properly.

Once a week, each crew must check their multi-parameter sonde using manufacturer recommended internal diagnostic checks (cell constants, millivolt output, or other readings) or against the QCS that they provide. In addition to recording the expected values and results, record the QCS date prepared in the appropriate sections of the

“Quality Control Check” section of the Calibration/QA form in the App. Report any calibration or QC irregularities as described above.

#### 6.3.4 SALINITY/CONDUCTIVITY METER

Prior to sampling each site, calibrate the salinity/conductivity meter in accordance with the manufacturer’s instructions. After the sampling day is complete, measure the salinity/conductivity of the calibration standard that was used earlier in the day to calibrate the instrument. Record the expected and post-measurement values as described above. Once a week, crews check the conductivity/salinity function using manufacturer recommended internal diagnostic checks (cell constants, millivolt output, or other readings) or against the QCS that they provide. Record results and report irregularities as described above.

#### 6.3.5 TEMPERATURE METER

When performing the once-a-season temperature sensor check, incorporate the entire temperature range encountered in the NCCA into the testing procedure and keep a record of test results on file. For use in this accuracy check, the temperature ranges below are from the NCCA 2010 dataset. On the Calibration/QA Form in the NCCA App, record two of the results (a high and a low temperature from the pertinent range) from the annual temperature check in the fields provided.

- Northeast:  $6.8\text{ °C} \leq T \leq 32.3\text{ °C}$
- Southeast:  $21.2\text{ °C} \leq T \leq 33.42\text{ °C}$
- Gulf Coast:  $22.4\text{ °C} \leq T \leq 36\text{ °C}$
- Great Lakes:  $3.54\text{ °C} \leq T \leq 30.9\text{ °C}$
- West Coast:  $9\text{ °C} \leq T \leq 24.1\text{ °C}$

See below methods for measuring DO, pH, salinity (marine sites) or conductivity (freshwater sites), and temperature.

### 6.4 SAMPLING PROCEDURE – DISSOLVED OXYGEN, pH, TEMPERATURE AND SALINITY/ CONDUCTIVITY

1. Measure the total water depth at the Y-location to the nearest 0.1 m and record on the Hydrographic Profile form in the App. If the sonde is attached to a data recorder, crews may submit the hydrographic profile data via an electronic file. If a crew chooses to use this option, ensure that all the data are saved correctly and check the “Submitted data via eFile” box on the form.
2. Lower the sonde into the water and record DO, pH, salinity/conductivity, and temperature measurements at the following depths: 0.1 m below the surface, 0.5 m below the surface, every 1 m from depths of 1.0 to 10.0 m, and if the site is greater than 10 m, every 5 m thereafter. Take the last set of measurements at 0.5 m from the bottom, making sure to not let the sonde touch the bottom. Record these results in the Hydrographic Profile form in the App.

- NOTE: if the station depth is less than 1 meter, take the measurements at 0.1 meters and mid-depth. Record the pertinent measurements at these two depths on both the upcast and the downcast.
3. Take a second reading at 0.5 m and then repeat the full sets of measurements at each of the same depth intervals as the probe is retrieved (upcast) in the Hydrographic Profile form in the App. Make sure to slide the ‘Upcast?’ toggle on the left side of the pertinent data rows in the App to indicate the measurements that were taken during the upcast (sliding the toggle to the right turns it green and indicates an upcast measurement). Two examples are provided below in **Table 6.2** that illustrate the depths at which measurements will be taken.
  4. Flag any measurements that the crew feels needs further comment or when a measurement cannot be made in the Hydrographic Profile form in the App. Note, measurements may be limited by the length of sonde cable and crews should record measurements as deep as the cable allows. However, at a minimum, crews should record to a depth of 30 m in these situations and comment about the cable limitation.
  5. After all *in situ* measurements have been completed for the sampling day, perform a “Post-Measurement Calibration Check” of the pH and conductivity probes. Record these values on the Calibration/QA form in the App.

Table 6.2 Example depth measurement intervals

<b>EXAMPLE 1:</b>	<b>EXAMPLE 2:</b>	<b>EXAMPLE 3:</b>
<i>Water Depth = 0.8 meters</i>	<i>Water Depth = 7.2 meters</i>	<i>Water Depth = 23.9 meters</i>
0.1 m	0.1 m	0.1 m
0.4 m	0.5 m	0.5 m
	1.0 m	1.0 m
	2.0 m	2.0 m
	3.0 m	3.0 m
	4.0 m	4.0 m
	5.0 m	5.0 m
	6.0 m	6.0 m
	6.7 m	7.0 m
		8.0 m
		9.0 m
		10.0 m
		15.0 m
		20.0 m
		23.4 m

## 6.5 PHOTOSYNTHETICALLY ACTIVE RADIATION (PAR) METER

Field crews measure photosynthetically active radiation using a PAR meter attached to a LI-COR® data logger. The PAR meter measures a vertical profile of light attenuation at each station. Measured light values are entered into a regression equation and used to determine the coefficient of attenuation in the water column. PAR sensors require no field calibration; however, they should be returned to the manufacturer at least every

two years for calibration. Crews measure PAR at the same depths as other water column indicators but should not be completed at the same time to prevent the multiparameter sonde from interfering with the PAR sensor. See procedures below for measuring light attenuation.

#### 6.5.1 SAMPLING PROCEDURE—LIGHT ATTENUATION (LI-1400 DATALOGGER)

1. Connect a deck sensor (LI-190 Quantum Sensor) to the BNC connector labelled I1 and an underwater sensor (LI-192 Underwater Quantum Sensor) to the BNC connector labelled I2 as described in **Section 4.2.1.2**. Enter the calibration factors (supplied by the manufacturer) for each probe if not already entered.
2. Place the deck sensor in an unshaded location on the boat to record the available ambient light.
3. Turn on the LI-1400 meter.
4. Press the View key.
5. Using the left or right keys, navigate to “NEW DATA” and press Enter.
6. Using the left or right keys, navigate until channel I1I is displayed; this shows the instantaneous reading for that channel.
7. Scrolling down will move the cursor to the second row of data.
8. Using the left or right keys, navigate until channel I2I is displayed; this shows the instantaneous reading for that channel allow viewing of both channels of instantaneous data at once.
9. Lower the underwater sensor, making sure that the sensor is facing up, on the SUNNY (or at least unshaded) side of the boat to a depth of 0.1 m (represents “surface”). Allow the readings to stabilize and press “Enter” to manually log the ambient (AMB) and underwater (UW) light readings in the datalogger. **NOTE: crews may choose to use alternate methods of recording the two sensor readings as long as both readings are recorded at the same instant. This may include using two people to view the two readings, taking a photograph of the screen, etc.**
10. Continue to lower the underwater sensor to each of the required depths (*same as other water quality measurements*):
  - a) 0.5 m
  - b) Every 1 m from 1.0 m to 10.0 m
  - c) Every 5 m thereafter for sites greater than 10 m
  - d) 0.5 m from the bottom
  - NOTE: if the station depth is less than 1 meter, take the measurements at 0.1 meters and mid-depth. Record the pertinent measurements at these two depths on both the upcast and the downcast.
11. Allow the readings to stabilize at each depth before pressing “Enter” or recording the values on the data form.
12. Repeat the procedure at the same depths, but in reverse order on the upcast.
13. Review the saved data by pressing Esc and using the right or left key to select “LOG DATA” and pressing Enter.
14. Select “View=ALL.” Press Enter.



15. Use the down key to scroll through stored data by date and time to find the data that were just logged. Press Enter to access logged data. Use the down key to view both of the sensor readings.
16. Record the values from both sensors ( $\mu\text{E}/\text{m}^2/\text{s}$ ), at the appropriate water depths of the underwater sensor, in the App. Record the deck sensor reading in the ambient (AMB) column, and the underwater sensor reading in the underwater (UW) column.
17. If the sensor hits bottom, allow two to three minutes for the disturbance to settle before taking the reading.
18. If the light measurements become negative before reaching the bottom measurement, terminate the profile at that depth and begin to take the upcast measurements.
19. If the underwater sensor begins reading negative values at startup, this likely indicates that the plug on the bottom of the underwater sensor is plugged in backwards. There is a yellow etched mark on the sensor bottom that should be aligned with the raised nub on the cable (see **Section 4.2.1**).

*Note: Pressing the On/Off key will only turn off the screen. To shut down the LI-1400 press the Fct key and use the right or left keys to navigate to "SHUTDOWN". Press Enter to shut down.*

## 7 TOTAL ALKALINITY [ALKT]

Total alkalinity (TA) is a characteristic of seawater that, in combination with other measurements, can be used to calculate total pH (i.e., coastal acidification) and the availability of carbonate ions used by marine organisms to produce structural materials such as corals and shells. TA is also used to calculate the fate of carbon that enters coastal waters in various forms and is useful as a direct indicator of seawater buffering capacity. TA is defined differently from the alkalinity measurements typically used in freshwater monitoring. In addition, the above seawater calculations are sensitive to tiny errors in TA determination, so monitoring programs aim for extreme care in the collection, handling, and analysis of TA samples.

### 7.1 SUMMARY OF METHOD

At marine sites only, two water samples will be taken from the Y-location using the EPA-provided hand-held peristaltic pump at 0.5 m below the water surface or mid-depth if station depth is less than 1.0 m. Store sample in cool, dark location (cooler) until ready to ship.

### 7.2 EQUIPMENT AND SUPPLIES

*Table 7.1 Equipment & supplies: total alkalinity sample collection*

<b>For collecting samples</b>	nitrile gloves Hand-operated peristaltic pump with flexible gas-impermeable tubing installed Threaded tubing adapters (3) Stainless steel 3/8 inch pipe to weight end of intake tube In-line disposable groundwater filter (0.45 µm) HDPE bottle (125 mL, white, rectangular) (2) Bucket, 5 gallon Electrical tape, plastic cooler with ice
<b>For recording measurements</b>	NCCA App Total alkalinity sample labels (2) fine-tipped indelible markers (for labels) clear tape strips

### 7.3 SAMPLING PROCEDURE

See below for step by step procedure for collecting total alkalinity. Collect at the Y-location and at marine sites only.

Note: Alkalinity samples should be collected between the hydrographic profile measurements and when the water chemistry samples are collected.

### 7.3.1 SAMPLE COLLECTION

1. Fill out both total alkalinity sample labels with the Site ID, visit number, date, and salinity. The salinity value (0.5 m downcast) will auto-populate from the Hydrographic Profile form in the App. If the TA sample is not collected at the same time or location as the hydrographic profile and a new salinity value is determined, edit the salinity value in the Sample Collection form accordingly.
2. Put on nitrile gloves
3. Ensure that the sampling apparatus is set up with the intake tubing, pump, weight and threaded tubing adapters inserted into both ends of the intake tubing (**Figure 7.2**). The adapter at the inlet end of the tubing holds the weight in place while the adapter at the outlet side of the pump will receive the disposable filter.
4. Pre-assemble the filter assembly by attaching the short piece of tubing to the outlet side of a new filter (note the flow direction arrow on the side of the filter). This assembly will be attached to the outlet side of the pump after priming and flushing the system.
5. Fully submerge the inlet portion of the tube into a full bucket of site water and begin pumping to prime the system. Holding the pump and outlet tubing near the level of the bucket will help achieve prime more easily.
6. Once good flow is achieved through the tubing, fold and pinch the tube between the pump and the threaded outlet adapter and quickly remove the weighted inlet end from the bucket and lower the weighted end to desired sample collection depth (0.5 m below the surface or at mid-depth if station depth is less than 1.0 m). Flush the pre-filter tubing by hand cranking the pump for 30 seconds.
7. While pointing the inlet adapter upward, fold and pinch the tube between the pump and the threaded outlet adapter and attach the filter (with exit tubing) to the outlet adapter (do not allow water to enter the filter unless its exit opening is pointed upward). Continuing to point the filter outlet upward, crank the pump to fill the filter from the bottom up and expel the air. All pump cranking should be done slowly and carefully at a speed of approximately one to two revolutions per second until water is flowing and there are no observed bubbles in either filter or tubing. During this process, adjust the angle of the filter as needed to allow air bubbles to exit through the outlet tubing.
8. After all air is expelled, crank the pump for an additional 20 seconds to rinse filter and outlet tubing. It is no longer necessary to hold the filter upright once all air has been expelled.
9. Rinse sample container lids with a few ml of sample three times. The process of overflowing the sample bottle in Step 11 below will provide adequate rinsing of the container itself.
10. Put outlet tube in sample container so that it is all the way on the bottom. Fill sample container from the bottom in a controlled manner by turning the peristaltic pump slowly (approximately one to two revolutions per second).
11. Allow sample container to overflow with bubble-free water by at least three times the time needed to fill the container to the top (example: if it requires

- 10 seconds to fill the bottle, the overflow process should continue for 20 additional seconds for a total of 30 seconds).
12. While continuing to pump slowly, pinch the outlet tube before beginning to withdraw from the bottom so that, after withdrawal, the water surface is in the lower half of the threaded portion of the bottle neck (**Figure 7.1**). If the removal of the tube leaves the water surface too low in the bottle, start the overflow process again and try withdrawing the tube a small amount before pinching and resuming withdrawal.
  13. If any air bubbles appear in tubing while collecting the sample, restart the overflow timing in Step 11.
  14. Cap the bottle.
  15. Repeat the above steps for the second sample bottle.
  16. Tape both lids with electrical tape and place them in a cooler (on ice) and shut the lid. Do NOT freeze samples.
  17. Ensure the Sample ID is recorded on the Sample Collection form in the App.
  18. Once the samples are placed on ice, check the 'Chilled?' box on the form in the App.
  19. Both the time that the sample was collected and the salinity value are very important to the analysis of the TA sample:
    - a) In the Sample Collection form in the App, enter the time that the sample was collected as accurately as possible.
  20. Discard the filter after use, a new filter will be used at each site. Both pieces of tubing as well as the threaded adapters should be saved and reused.
  21. At the end of the sampling day, rinse the tubing, weight, and pump housing with DI water to avoid cross-contamination and corrosion.

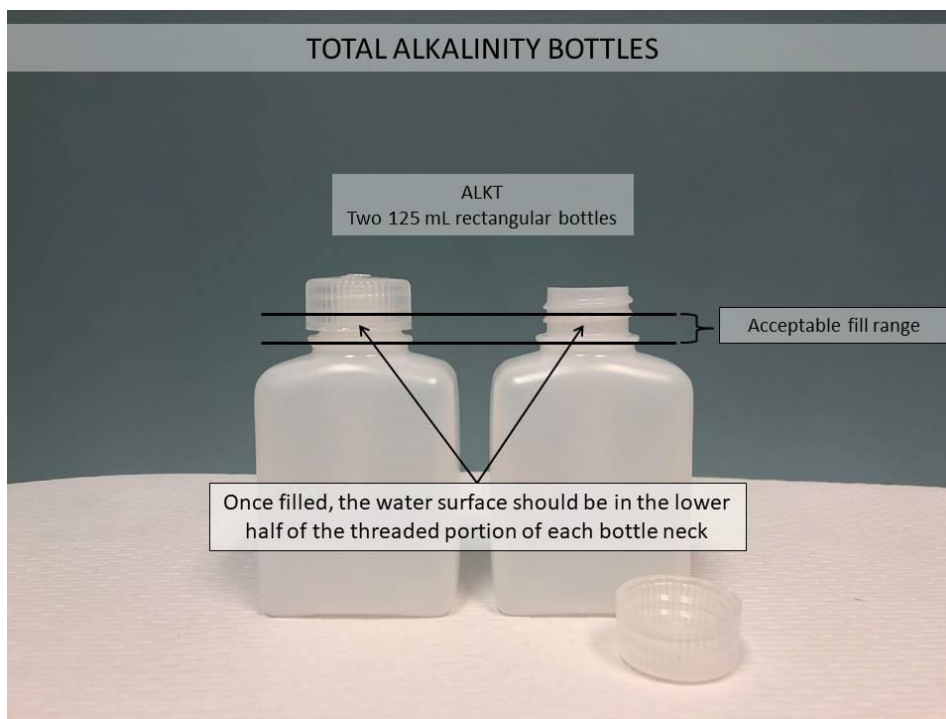


Figure 7.1 Target fill range for total alkalinity sample bottles

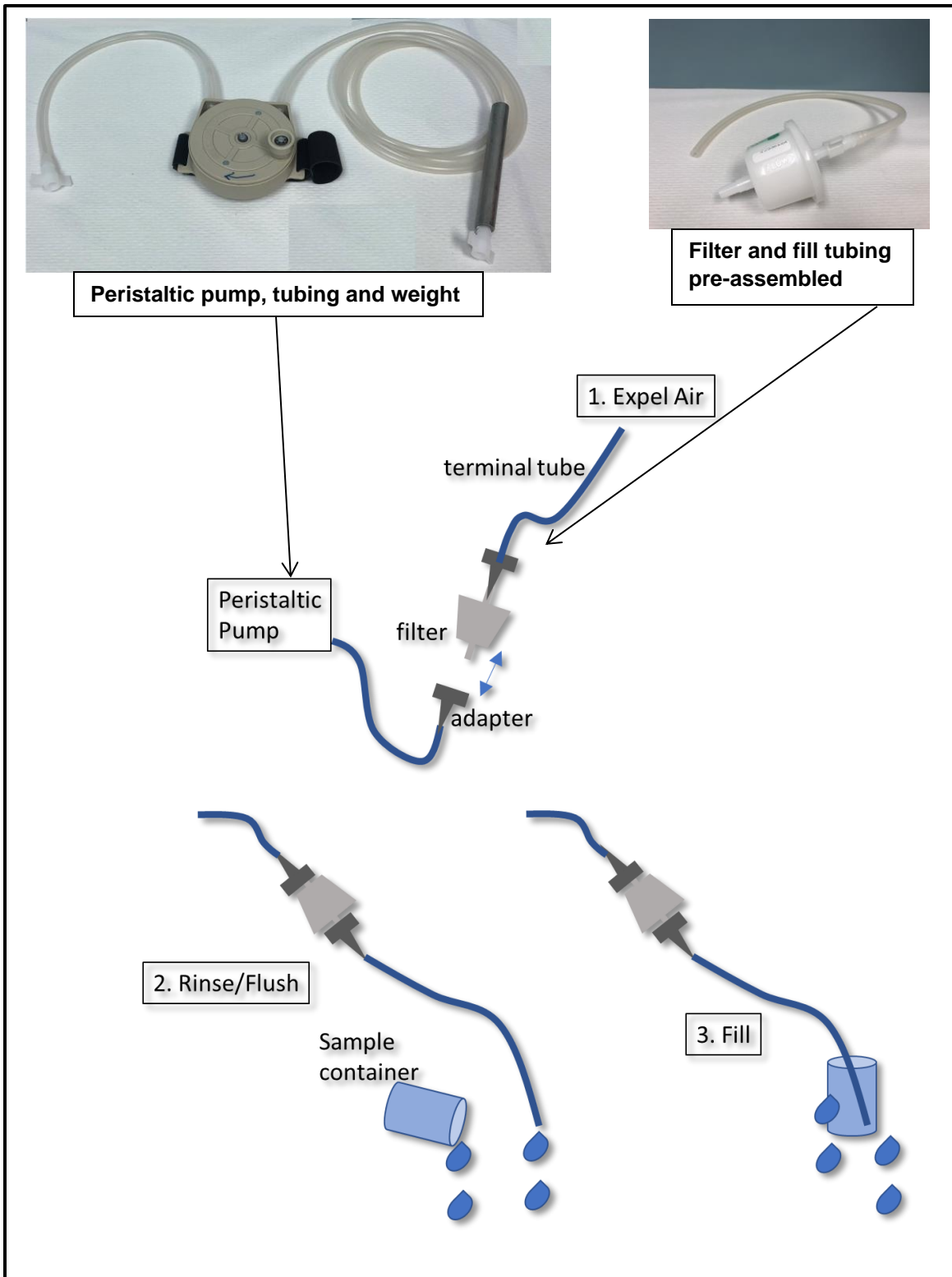


Figure 7.2 Total alkalinity filter detail  
Tubing from pump is flushed. Filter is connected to adapter, inverted and filled with sample to expel air. Additional water is run through to rinse tubing, filter, and bottle. Bottle filling procedure is then begun.

## 8 WATER CHEMISTRY [CHEM], CHLOROPHYLL-A [WCHL], AND NUTRIENTS [NUTS] SAMPLE COLLECTION AND PRESERVATION

This section describes the procedures and methods for the field collection and preservation of the water chemistry, chlorophyll-*a*, and dissolved nutrients samples from freshwater and marine coastal areas.

### 8.1 SUMMARY OF METHOD

The water chemistry samples will be analyzed for chlorophyll-*a* [WCHL], total nutrients including nitrogen and phosphorus [CHEM], and dissolved ammonia, nitrites, nitrates, and phosphorus [NUTS]. Collect the water samples at the Y-location, 0.5 meters below the surface (or mid-depth if station depth is less than 1.0 meter), with either a water pumping system or water sampling device such as a Niskin, Van Dorn, or Kemmerer bottle and transfer to a rinsed 250 mL amber HDPE bottle. Water for the chlorophyll-*a* sample will be collected and transferred to a separate reusable 2 L amber HDPE bottle. Store all samples in darkness on ice in a closed cooler. After you filter the chlorophyll-*a* sample, the filter must be kept frozen until ready to ship. A portion of the filtrate from the chlorophyll-*a* processing will be collected for the dissolved nutrient sample.

**Note:** Fecal indicator sample IS NOT collected with these samples.

### 8.2 EQUIPMENT AND SUPPLIES

Table 8.1 Equipment & supplies: water chemistry & chlorophyll-*a* sample collection

For collecting samples	water sampling device or water pumping system nitrile gloves HDPE bottle (250 mL, amber) [CHEM] HDPE bottle (2 L, amber) [WCHL] Electrical tape, plastic cooler with wet ice
For recording measurements	NCCA App water chemistry sample label fine-tipped indelible markers (for labels) clear tape strips

### 8.3 SAMPLING PROCEDURE

The following describes the sampling procedures for collecting water chemistry samples.

**Note:** Do not apply sunscreen or other chemical contaminants until after the sample is collected (or implement measures to reduce contamination by such chemicals if applied such as washing, wearing long gloves, etc.).

1. Collect the water chemistry samples at the Y-location, which is no more than 37 meters from the X-site (located via GPS).

2. Complete the CHEM sample label with Site ID, date collected, and visit number.
3. Attach the completed label to the 250 mL amber HDPE sample bottle and cover with clear plastic tape.
4. Put on nitrile gloves.
5. Using either a water sampling device or water pumping system, collect a water sample at 0.5 m below the surface (or mid-depth if station depth is less than 1.0 meter).
  - a. Rinse the sampling device and the sample containers three times with water from the site. To rinse a pumped sampling system follow your agency's SOP. If no SOP exists, flush long enough so that the amount of site water flushed is equal to at least three times the total volume of the sampling system (including tubing). Be sure to cap the bottles and rotate them so that the water contacts all the surfaces. Discard the water away from the sampling location if additional water is to be collected.
6. Fill the 250 mL amber HDPE bottle (for water chemistry) and the 2 L amber HDPE bottle (for chlorophyll-*a* and nutrients) with sample water.
7. Replace the lids and seal the lid of the 250 mL bottle tightly with electrical tape.
8. Place both samples in a cooler on ice at 4°C.
9. Record the collection data on the Sample Collection form in the NCCA App.
  - a) Enter the water chemistry sample ID on the Tracking Form in the NCCA App.
  - b) Once the sample is placed on ice, check the 'Chilled?' box in the App.
  - c) Note anything that could influence sample chemistry (heavy rain, potential contaminants, etc.) in the Comments section.
  - d) If the samples were not taken at the Y-location, enter the GPS coordinates of the sampling location and the reason for relocation in the comments field in the App.
10. Proceed to **Section 15.3** for instructions on processing chlorophyll-*a* and nutrients water sample to obtain a chlorophyll-*a* filter and the nutrients filtrate.

## 9 ALGAL TOXINS (CYLINDROSPERMOPSIN [MICX] AND MICROCYSTINS [MICZ])

Algae, including *Microcystis*, are microscopic organisms found naturally at low concentrations in water. Under optimal conditions (such as high light and calm weather, usually in summer), these organisms occasionally form a bloom, or dense aggregation of cells, that floats on the surface of the water forming a thick layer or “mat.” At higher concentrations, algal blooms are so dense that they resemble bright green paint that has been spilled in the water. These blooms potentially affect water quality as well as human health (some algae produce toxins) 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). Sometimes, this condition results in fish kills. The blooms can also be unsightly, often floating at the surface in a layer of decaying, odiferous, gelatinous scum.

Although the likelihood of people being affected by algal blooms is low, various health effects can occur following contact with or ingestion of algal toxins. People recreationally exposed (e.g., swimmers or personal watercraft operators) to algal blooms have also reported adverse effects. Health problems may occur in animals if they are chronically exposed to water with algal toxins present. Fish and bird mortalities have been reported in waterbodies with persistent algal blooms.

### 9.1 SUMMARY OF METHOD

Two water samples for algal toxin analysis are taken from the Y-location: one for both cylindrospermopsin and microcystin [MICX] and one for microcystin only [MICZ]. All field crews must collect water grab samples using the water chemistry sample collection device to fill two, 500 mL bottles. Collect these samples after the *in situ* measurements and water chemistry sample are collected. Store all samples on ice in a closed cooler.

### 9.2 EQUIPMENT AND SUPPLIES

Table 9.1 Equipment & supplies: algal toxins (cylindrospermopsin and microcystins)

<p><b>For collecting samples</b></p>	<p>nitrile gloves            water chemistry sample collection device            1 HDPE bottle (500 mL, white, round, wide mouth) [MICZ]            1 PETG bottle (500 mL, clear, square) [MICX]            Electrical tape, plastic            cooler with ice</p>
<p><b>For recording measurements</b></p>	<p>NCCA App            cylindrospermopsin sample label            microcystin sample label            fine-tipped indelible markers (for labels)            clear tape strips</p>



### 9.3 SAMPLING PROCEDURE

See below for step-by-step procedures for collecting both algal toxin samples. Collect both samples from the Y-location.

**Note:** *Make sure not to handle sunscreen or other chemical contaminants until after the sample is collected (or implement measures to reduce contamination by such chemicals if applied such as washing, wearing long gloves, etc.).*

#### 9.3.1 SAMPLE COLLECTION

1. Complete the MICZ and MICX sample labels with Site ID, date collected, and visit number.
2. At marine sites, also write the salinity (in ppt) on both of the labels.
3. Attach the completed labels to each of the 500 mL sample bottles and cover with clear plastic tape.
  - a) MICX = clear square 500 mL PETG bottle
  - b) MICZ = white round 500 mL HDPE bottle
4. Put on nitrile gloves.
5. Rinse the first 500 mL bottle three times with site water. Be sure to cap the bottle and rotate it so that the water contacts all the surfaces. Discard the water away from the sampling location if additional water is to be collected.
6. Fill the 500 mL bottle. Leave at least one inch of head space in the bottle to allow for expansion when frozen.
7. Replace the lid and seal tightly with electrical tape.
8. Repeat Steps 5-7 for the second 500 mL bottle.

#### 9.3.2 SAMPLE STORAGE

1. Place the 500 mL bottles in a cooler (on ice) and shut the lid.
2. Ensure the Sample IDs are recorded on the Sample Collection form in the App along with any pertinent sample information.
3. As soon as you return to your base site (hotel, lab, office, etc.), freeze sample bottles and keep frozen until shipping.
4. Once the samples are placed in the freezer, check the 'Frozen?' box on the form.

## 10 FECAL INDICATOR (ENTEROCOCCI, [ENTE])

Crews collect water samples to be tested for the presence of Enterococci. They filter water at the field site or a nearby location. The filters are sent to the lab for quantitative polymerase chain reaction (qPCR) analysis. **Two filters must be collected and frozen within six hours of collecting the water sample or the sample must be discarded and recollected.** Because of the time-sensitive nature of this technique, the position of the Enterococci water sample collection in the sampling sequence varies based upon whether and how fish will be collected at the site and how quickly the crew will be able to begin filtration.

In short, if the crew is using a passive fishing method or is able to filter the samples on the vessel, the Enterococci collection takes place immediately following the hydrographic profile. If the crew is using active fishing methods or will not be able to filter the sample until off the water, the collection of the Enterococci sample takes place at the end of the sampling day. This variation is based on balancing the need to protect the Enterococci sample from potential contamination with minimizing holding times once the sample is collected.

### 10.1 SUMMARY OF METHOD

Crews collect and preserve the fecal indicator sample at the Y-location using the method described in the Sampling Procedure (**Section 10.3**) below. In addition, crews observe the area around the X-site and record (on the Site Assessment form in the App) signs of disturbance that may contribute to the presence of fecal contamination to the waterbody.

### 10.2 EQUIPMENT AND SUPPLIES

*Table 10.1 Equipment & supplies: fecal indicator (Enterococci) sampling*

For collecting samples	nitrile gloves PETG bottle (250 mL, clear, square, pre-sterilized) sodium thiosulfate tablet wet ice cooler
For recording measurements	NCCA App

### 10.3 SAMPLING PROCEDURE

The following outlines the procedure for collecting the fecal indicator sample.

1. Put on nitrile gloves.
2. Using either a gloved hand (on smaller boats) or pole dipper (on larger vessels), lower the un-capped, inverted 250 mL sample bottle to a depth of 0.3 meters below the water surface (or mid-depth if station depth is less than 0.6 meters).
  - Avoid surface scum, vegetation, and substrates. Point the mouth of the container away from the boat. Right the bottle and raise it through the water column, allowing bottle to fill completely.

3. After removing the container from the water, discard a small portion of the sample to allow for proper mixing before filtering.
4. Add the sodium thiosulfate tablet, cap, and gently shake the bottle 25 times.
5. In the Sample Collection Form in the NCCA App, note the time and depth (typically 0.3 meters) of the Enterococci collection.
6. Immediately after collection, place the sample on wet ice.
7. Store the sample in a cooler on wet ice to chill (not freeze) for at least 15 minutes prior to beginning the filtration process. Do not hold samples longer than six hours before filtration and freezing.
8. The filtration procedure is contained in **Section 15.2**.

## 11 PHYTOPLANKTON [PHYT] (*GREAT LAKES ONLY*)

### 11.1 SUMMARY OF METHOD

At all Great Lakes sites, crews will collect a sample for phytoplankton analysis. Collect this sample from the Y-location at the same time and depth as the other water samples. Fill a 1 L white narrow-mouth HDPE bottle with water from the water sampling device or water pumping system. The phytoplankton sample must be preserved with Lugol's solution within two hours of collection. Store the samples in darkness inside a cooler with ice or in a refrigerator.

### 11.2 EQUIPMENT AND SUPPLIES

Table 11.1 Equipment & supplies: phytoplankton

For collecting and preserving samples	water sampling device or water pumping system nitrile gloves HDPE bottle (1 L, white, narrow mouth) wet ice cooler Lugol's solution Pipet (10 mL) Pipet Bulb Electrical tape, plastic
For recording measurements	NCCA App phytoplankton sample label fine-tipped indelible markers (for labels) clear tape strips

### 11.3 SAMPLING PROCEDURE

The text below describes the sampling and preservation procedures for phytoplankton samples. Collect the phytoplankton water sample at the Y-location along with the other water samples.

**Note:** Make sure not to apply sunscreen or other chemical contaminants until after the sample is collected (or implement measures to reduce contamination by such chemicals if applied such as washing, wearing long gloves, etc.).

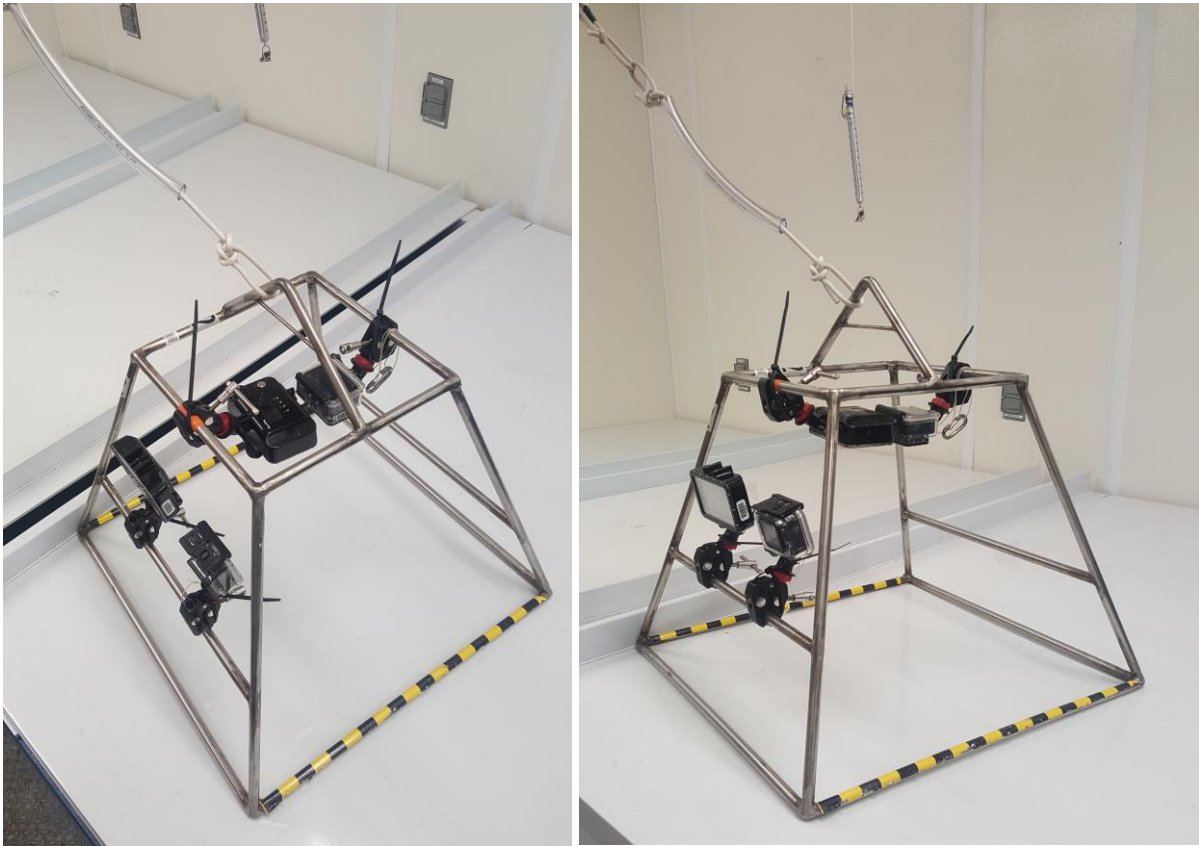
1. Complete the PHYT sample label with Site ID, date collected, and visit number.
2. Attach the completed label to the 1 L white narrow-mouth HDPE sample bottle and cover with clear plastic tape.
3. Put on nitrile gloves.
4. Using either a pre-rinsed pump system or a water sampling device, collect a water sample at 0.5 m below the surface (or mid-depth if station depth is less than 1.0 meter).
5. Rinse the sample bottle three times with site water. Be sure to cap the bottle and rotate it so that the water contacts all the surfaces. Discard the water away from the sampling location if additional water is to be collected.

6. Fill the sample bottle with sample water, leaving enough head space for 10 mL of Lugol's solution, and place in a cooler on ice at 4°C. Store the sample chilled and in darkness at all times.
7. The sample must be preserved by adding 10 mL of Lugol's solution to the bottle within two hours of collection.
8. After preservation, replace the lid and seal tightly with electrical tape.
9. Record the collection data on the Sample Collection form in the App. Include the depth of collection, time of collection, and time of preservation.
10. Ensure the sample ID is recorded on the Sample Collection form in the App.
11. After the sample is preserved, check the 'Preserved?' box on the form in the App.

## 12 UNDERWATER VIDEO [UVID] (GREAT LAKES ONLY)

### 12.1 SUMMARY OF METHOD

At Great Lakes sites only, crews will use underwater video cameras to capture at least 1 minute of benthic video at the Y-Location. Video will be used to document the benthic habitat composition and record the presence of invasive species like zebra and quagga mussels and round gobies, or other organisms. The underwater video carriage consists of a steel frame onto which two cameras and two lights are attached. One camera looks down (Camera A) and the other has an oblique view (Camera B). **Figure 12.1** shows the fully set up underwater camera assembly.



*Figure 12.1 Underwater video assembly. Includes cameras, lights, frame with scale markings on footings, and pre-attached leader.*

## 12.2 EQUIPMENT AND SUPPLIES

Table 12.1 Equipment & supplies: underwater video

Component	Description	Function
GoPro Hero 7 Black camera (2 – Units A and B)	Camera with waterproof housing, battery, and charging cord. Safety tether attached.	Record video
Lens covers (2)	Protective lens cover, removed for insertion into the dive housing. Replace when camera is taken out of housing.	Lens protection
Suptig LED underwater video lights (2 per rig)	Light with internal battery and charging cord.	Light
Video carriage	Stainless, welded, 18” tall; 21” wide at base. Leader with plastic tubing attached. Footings have black and yellow scale bar tape with each cube measuring 24 mm.	Frame for cameras for deployment from boat
Camera Clamps	2 pipe clamps with safety tethers	Attach camera to the video carriage
Light Clamps	2 GoPro clamps	Attach lights to the video carriage
Lowering line	100 ft rope, with carabiner and float attached.	Lowering video carriage
Media (Cards A and B)	Crews provided with at least 2 Micro SD cards for capturing, storing, and sending video to USEPA	Memory
USB Power Supply	USB power station	Recharging cameras and lights
Laptop/computer	Provided by crew	Transfer files and store data
<b>Supplies</b>	<b>Description/Function</b>	
Allen wrench	Used to tighten set screws on clamps	
Screwdriver	Used to tighten clamps	
Metal coin	Used to unscrew waterproof charging port on lights.	
Zip Ties	Used as back-up to secure clamps to carriage	
Lens cleaner cloth	Used to clean camera lens	
Carrying case	Protective case for storing and transporting cameras, lights and supplies	
Index cards and sharpie	Used to indicate site number in video at each site.	

## 12.3 UNDERWATER VIDEO CARRIAGE SET-UP

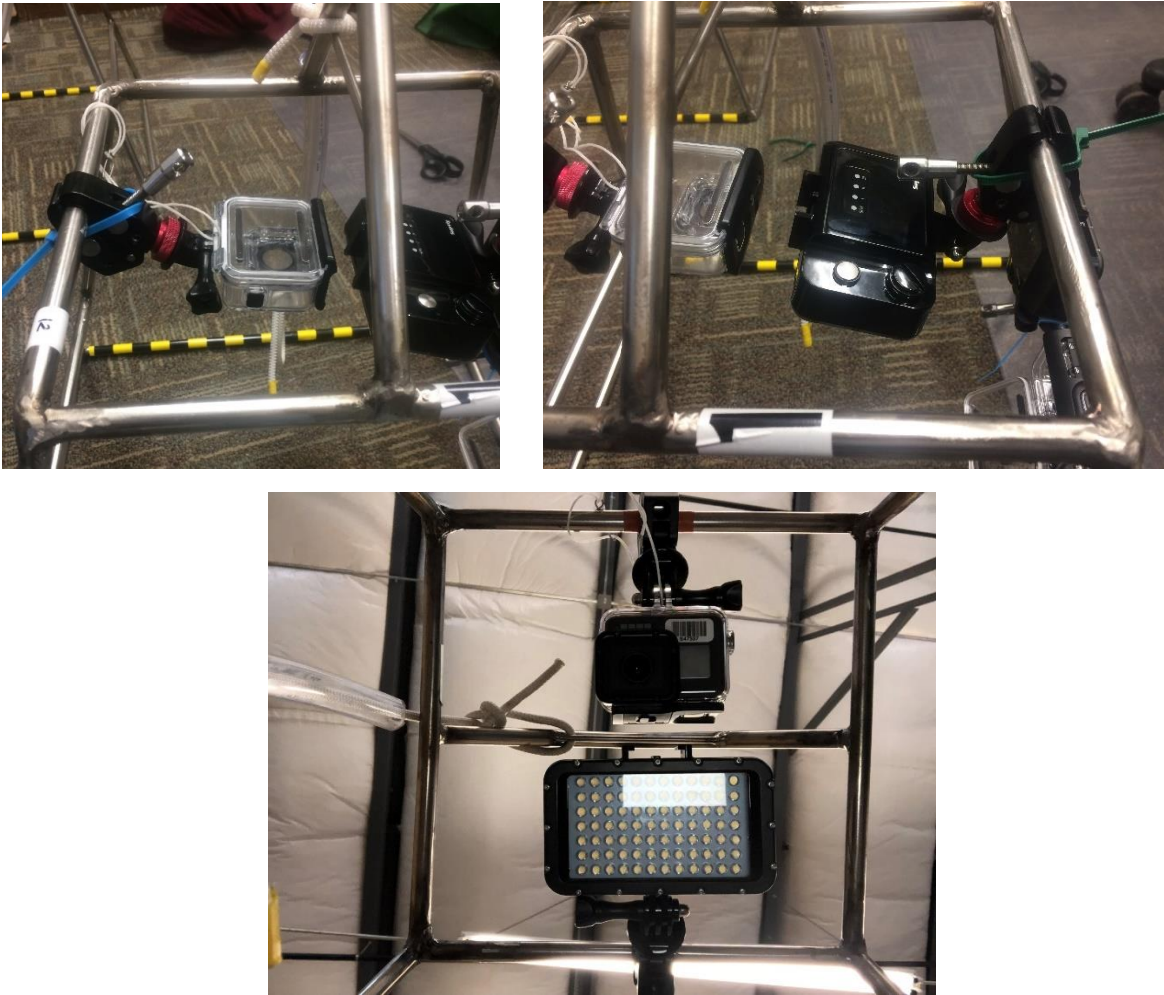
Underwater camera settings will be adjusted prior to shipment to field crews. Information here will allow field crews to verify camera setup and assemble the underwater camera system. Camera settings should not need to be changed. If settings have been altered between shipment and deployment, see **Table 12.2** to verify and restore settings. The video carriage system should be assembled for the field day and does not need to be disassembled between sites.

### 12.3.1 SETTING UP VIDEO CARRIAGE SYSTEM

Set up cameras on small boat video carriage as described in the steps below and as shown in **Figure 12.2** through **Figure 12.4**. The process of renaming files and backing the videos up to a computer will not require removal of the Micro SD cards from the cameras. If removal of a card is necessary, be sure not to interchange the cards so that each card only has either oblique or down-looking videos on it.

1. Make sure the camera settings are as shown in **Table 12.2**. Important note: unlike previous versions of the GoPro camera, the protective outer lens on the Hero 7 has been removed in order to fit in the dive housing. When handling the camera outside the housing for charging or downloading, use care to avoid scratching the lens, as it does not have a cover. If the camera needs to be stored outside the dive housing, put the protective lens cover back on the camera.
2. To remove camera from waterproof dive housing, push the button on top of black latch to the right, then lift front of latch up and release from the back cover. To place camera in waterproof dive housing, close the back cover and pull the black top latch over back cover. Push latch down to click into place. Ensure no debris is stuck in rubber O-ring such that the case would not be completely sealed.
3. Attach Camera A with Micro SD card A in the dive housing in the down-looking position as show in **Figure 12.2** and **Figure 12.3** using the clamp. Attach safety tether as shown. Use a screw driver or other tool to tighten the clamp. Add zip ties around clamps and carriage as back-up.
4. Attach the A light in the down-looking position opposite the camera as shown in **Figure 12.2** and **Figure 12.3** using the clamp. Tighten the clamps until the camera is held firmly. Add zip ties around clamps and carriage as back-up.
5. Attach Camera B with Micro SD card B in the oblique position as show in **Figure 12.3** and **Figure 12.4** using the clamp. To capture the foreground, the camera should be facing slightly downward rather than straight out from the frame. Attach safety tether as shown. Add zip ties around clamps and carriage as back-up.
6. Attach the B light in the oblique position next to camera B as shown in **Figure 12.3** and **Figure 12.4** using the clamp. Tighten the clamps until the camera is held firmly. Add zip ties around clamps and carriage as back-up.
7. Turn on the cameras and lights and check the aim. Down-looking camera should be pointing straight down with both of the taped video carriage arms visible in the frame for size referencing. Oblique camera should be tilted down approximately 20° from vertical to place the benthic horizon at the approximate midpoint of the image.
8. If a safe location is available for storage, cameras/lights can remain on the video carriage at the end of the day. If there is risk of theft or jostling, remove cameras and lights for storage. GoPro and dive housing can be removed by unscrewing GoPro clamp (with screwdriver if needed). When reassembling, make sure GoPro clamp is tight enough to prevent movement of the camera/light, but not overtightened, because the plastic parts can break with overtightening.





*Figure 12.2. GoPro camera A and light mounted on carriage.  
Note the orientation of clamps (screws for tightenting facing in), tether on camera, and cable ties on clamps.*



Figure 12.3. Approximate aiming angle of camera and lights for the oblique (B) and down-looking (A) cameras. Note the orientation of the tripod adapter (the fitting between the red locking thumbscrew and the camera or light) which is facing backward relative to the camera lens.

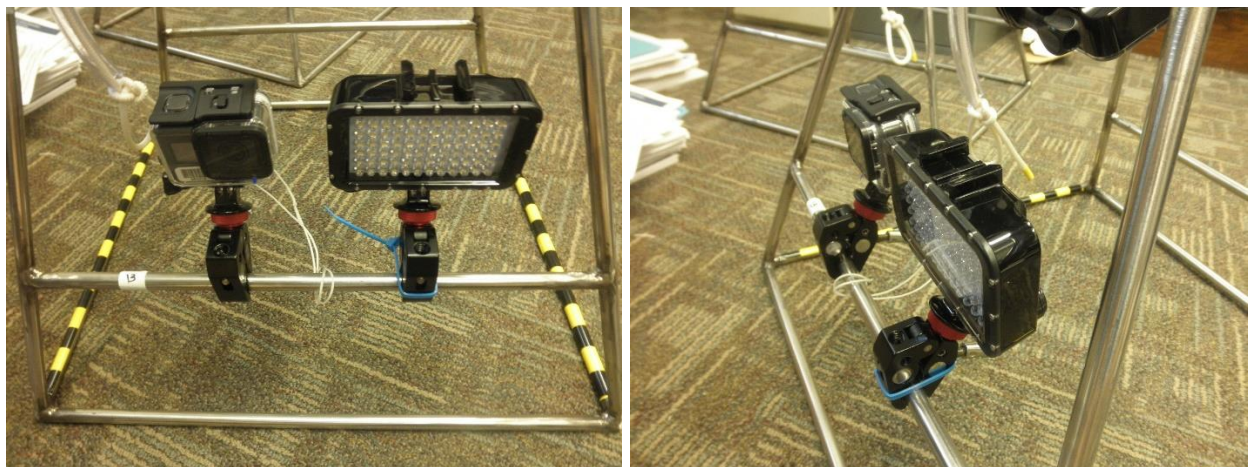


Figure 12.4. GoPro camera B and light mounted on carriage. Note the orientation of clamps (screws for tightening facing in), aiming of camera, tether on camera, and cable tie on light. To capture the foreground, the camera should be facing slightly downward rather than straight out from the frame.

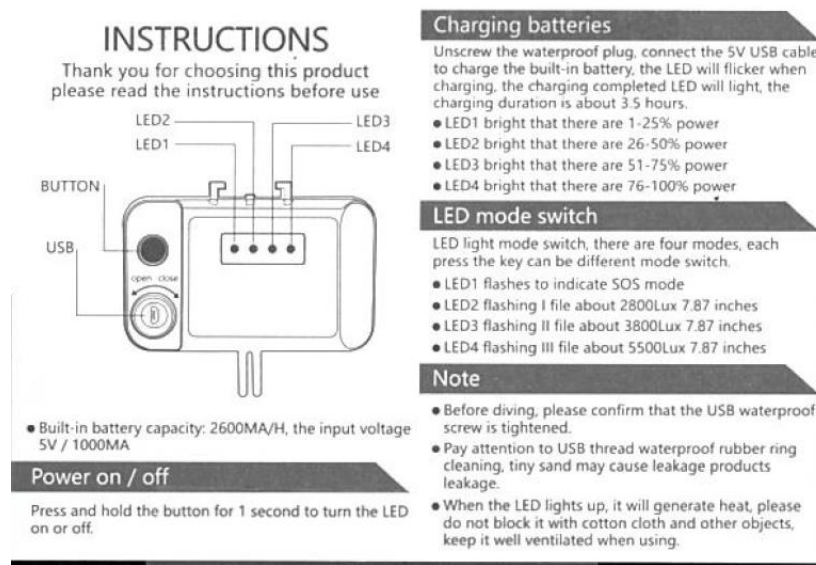


Figure 12.5. Manufacturer's instructions for lights.

### 12.3.2 OPERATING CAMERA AND LIGHTS

GoPro Camera settings should be pre-set when sent to field crews. Settings should match settings shown in **Table 12.2**. Directions for changing settings are also given in **Table 12.2**

To turn on the camera and/or to change setting, press the mode button on the right side of the camera. It is much easier to change settings with the camera out of the housing using touch screen, but be careful not to touch the lens, which does not have a lens cover in order to fit in waterproof housing.

- To enter settings mode, touch screen once and follow the directions in **Table 12.2**.
- To view saved video files, swipe bottom to top on back screen and select video to view.

Cameras should always be set to QuickCapture. When set to QuickCapture, pushing the record button on top of camera when the camera is off will turn the camera on and start recording. Pushing the same button again will stop recording and turn the camera off. This setting helps saves battery life.

The brightest light setting (mode 4) should be used for sites deeper than 3 m. A lower setting can be used for shallow sites to help preserve battery. Turn the light on by pressing and holding **ON** button. Press button again to toggle through light settings. **Table 12.2** also describes settings for lights. **Figure 12.5** includes manufacturer's instructions for lights.

Table 12.2 GoPro Hero 7 camera and light settings and directions.

GoPro Hero 7 Black		
Option	Setting	Directions
Mode	Video	Swipe screen left/right to switch among time lapse, video, and photo modes.
Resolution	1440	Touch Video Settings button on bottom of screen.
Frames per second	60	
Field of View	Wide	
Low light	Auto	
Stabilization	Off	
Protune	Off	
Short Clips	x	
Capture Mode	Video	Touch button in lower left of screen. Looks like video camera if set to video, looks like circular arrow if set to looping.
Voice Command/Voice Control (audio icon)	Off	Swipe down, icons are blue if on OR Swipe down, touch preferences, touch Voice Control.
Camera Beeps (music icon)	On	Swipe down, icons are blue if on
QuickCapture (bunny icon)	On	
Auto Screen Lock (lock icon)	Off	
Wireless Connections	Off	Swipe down, touch preferences, touch connections
Beep volume	High	Swipe down, touch preferences, touch General
Auto power off	Never	
LED	All on	
Video Compression	H.264 + HEVC	
Default Mode	Video	
Voice Control	Off	Swipe down, touch preferences, touch Voice Control
Landscape Lock	Off [important!]	Swipe down, touch preferences, touch Touch Display
Screensaver	1 min	
Brightness	80% or as needed	
GPS	On	Swipe down, touch preferences, touch Regional
Language	English	
Video Format	NTSC	
HDMI Output	Media	Swipe down, touch preferences, touch Input/Output
Audio input	N/A	
Suptig 84 LED light		
Light Level	I in <3m water to save battery; use IV in deep water.	Set to II at power on. Push on button to cycle through levels.

## 12.4 UNDERWATER VIDEO COLLECTION

At all Great Lakes sites, a minimum of 1 minute of video of the benthic habitat will be collected using an underwater video camera system. Video will be used to document the bottom composition and record the presence of invasive species like zebra and quagga mussels' round gobies, or other organisms.

### 12.4.1 SUMMARY OF METHOD

Follow directions in **Section 12.4.2** to deploy camera and collect underwater video. Before deploying, crews should always verify that all cameras and lights are fully

waterproof and have adequate battery/memory life, are on, and pointed correctly. After cameras and lights are turned on, the iPad displaying the NCCA App Verification Form or a notecard with the site and date is held in front of EACH camera lens (see **Figure 12.6**). When using the App screen, ensure date has been entered and is visible to the camera along with the blue header information. Make sure information is close enough to be read but not so close that information is cut off the screen. Video carriage is then lowered slowly to the bottom and slack is let out to prevent the carriage from dragging.

The screenshot shows the NCCA App interface for a verification form. The top status bar indicates the time is 8:38 AM on Tuesday, February 4th, with 98% battery. The app's header bar is blue and contains a 'Menu' button, the text 'NGL20\_IL-10001, Visit 1' and 'Version 2.6', and a green 'SAVE' button. Below this is a green banner with the text 'NCCA/NGLA 2020 VERIFICATION'. A white box with a red 'X' icon and the text 'This form has been thoroughly reviewed and is ready for submission' is displayed. The form itself has several input fields: 'Site name' with 'Lake Michigan', 'Date collected' with '06/12/2020' and a 'Today' button, 'Crew' with 'GL4' and a 'Select Date' button, 'Did you sample this site?' with 'YES' selected, 'Choose method used:' with 'Great Lakes' selected, 'Station Depth (m):' with '21.8', 'Arrival Time:' with '09:53' and a 'Now' button, and 'Depart Time:' with 'XX:XX' and a 'Now' button.

Figure 12.6 Example of verification form in the NCCA App showing header information prior to underwater camera deployment.

The target length of videos is at least one minute. In some cases (e.g., in swift currents or shipping lanes) it may be difficult to collect a full minute of video. In those cases, crews should collect whatever they can. Short videos are preferred over no videos, but if there is risk to crew or equipment, do not deploy the underwater video carriage. If the video carriage is dragging along bottom it should be retrieved immediately. In areas of swift current or suspected underwater obstructions (wood, marine debris), do not deploy the video carriage.

After video collection, the carriage is then retrieved, and cameras and lights are turned off. Video quality should be verified by reviewing on the GoPro camera after collecting at each site. Video section(s) of the Sample Collection form in the App should be filled out in the field, except file names.

Video is collected at the Y-Location. The camera can be deployed at approximately the same time as the in-situ measurements and water collection activities from the opposite side of the boat. Avoid heavy disturbance of the bottom with anchors or sediment samplers during the video. One person can operate and lower the cameras.

If a benthos grab is successful at the Y-Location, this is the only video that is collected. If the benthos grab is unsuccessful at the Y-Location, then the sediment collection protocol (**Section 13.3**) should be followed to move within 37 m, then 100 m, then 500 m of the X-site to obtain a successful benthos sample. When a successful benthos sample is obtained, a 2<sup>nd</sup> video should be collected at the same location as the benthic grab was collected. At the start of this video, scroll the benthic collection portion of the Sample Collection form on the App to just below the header and hold it in front of EACH lens (see **Figure 12.7**). Ensure that the distance from the X-site where the video is being collected is displayed (or print that information on the previously used notecard).

The screenshot shows the 'NCCA/NGLA 2020 SAMPLE COLLECTION' form. At the top, it says 'Benthic (BENT) (1L HDPE bottle)'. There are three radio buttons for 'Benthic Collection Location': 'Within 37m from X-site', 'Between 37-100m from X-site' (selected), and 'Between 100-500m from X-site'. Below that, 'Grab area (m2):' is a text box with '0.05'. 'Select grab type:' is a dropdown menu showing 'STANDARD\_PO...'. 'Number of grabs:' has radio buttons for '1' (selected) and '2'. A note says '\*\*\* Note: 2 grabs are required for samples less than 0.03m2 \*\*\*'. 'Sieve size:' has radio buttons for '0.5 mm' (selected) and '1.0 mm'. 'Depth (cm) (Should be >7 cm):' is a text box with '8.5'. 'No. of jars:' is a text box with '1'. 'Preserved?' has a checked checkbox. A 'SAVE' button is in the top right.

Figure 12.7 Example of sample collection form in the NCCA App showing header and benthic grab information prior to underwater camera deployment.

Fill out the information on the Sample Collection form in the App for the 2<sup>nd</sup> video. Additional space is included on the field form if multiple videos are collected for any other reason as well. At the end of each field day, video files will need to be transferred from GoPro cameras to a computer and backed up, at which point the rest of the field form (video filenames) can be completed. Directions for doing this are given in **Section 12.4.3**. Crews will also need to charge lights and camera batteries using charging cords and USB power supply.

#### 12.4.2 DEPLOYING GOPRO VIDEO CARRIAGE

The detailed steps for deploying GoPro Video carriage and collecting underwater video are shown below. The video carriage should be treated with the care and clean technique of a PAR sensor, Hydrolab sonde, or other delicate and expensive instrument. Store video carriage in a dedicated space where cameras will not be jostled between sites.

1. Before every deployment, check the following:
  - Both camera cases are fully closed
  - Both cameras have sufficient battery life
  - Both cameras have adequate memory space
  - Both cameras are both aimed correctly

- Both lights are on
  - Both lights and cameras are aimed correctly
  - Both charging ports on lights are closed securely
  - All clamps are tight
2. Attach the lowering line to the video carriage above the permanent leader with the carabiner provided. The purpose of the leader is to prevent the line from dropping inside the carriage and fouling the cameras or lights.
  3. Attach the other end of the lowering line to the boat and make sure there is always a float on the end of the lowering line in case it is dropped accidentally or must be jettisoned. If deploying in swift currents, do not tie off to the boat. Instead, have a second crew member hold the float end during deployment.
  4. Determine the approximate depth and clear 2x length of line to reach the bottom.
  5. Prepare the Site Verification screen on the App that displays the site ID and date (or notecard with site ID and date). For second videos and if the video is not collected at the Y-Location, display the benthic section of the Sample Collection form to show the site ID and if the video location is within 37 m or within 500 m of the X-site (or print on notecard).
  6. Turn on both lights:
    - a. Press on button once to turn on.
    - b. Three indicator lights show the brightness setting.
    - c. Set brightness to mode 4 for deployment, unless the site is very shallow. Toggle through brightness settings by pressing on button again. All four LEDs above the on button will be illuminated when set to brightest setting. If site is shallow, a lower setting can be used to save battery.
    - d. Verify light is on by waving hand in front of it.
  7. Start collecting video:
    - a. Turn on the GoPro cameras by pressing the red circle button (black square when inside waterproof housing) on top of the camera (Quick capture mode set to ON). You will hear three quick beeps indicating the camera is recording.
    - b. Hold the iPad screen or card with site information and date in front of EACH camera.
    - c. Double check the battery life and settings.
  8. Lower the camera slowly on the windward side of the boat until the camera hits the bottom. When bottom is reached, let out enough slack to prevent the boat from dragging the carriage.
  9. If there is risk of dragging, pull carriage up immediately and re-deploy if safe. In areas of swift current or suspected underwater obstructions (wood, marine debris), do not deploy the video carriage.
  10. Continue recording until you have captured at least 1 min of footage at the bottom.
  11. Retrieve carriage:
    - a. Pull up line. Pull up from bottom quickly to avoid dragging along the bottom. Avoid banging the rig against the side of the boat.
    - b. Stop video recordings by pressing the red circle button.
    - c. Turn off lights by pressing and holding on/off button until light goes off.

- d. Verify the light is turned off by waving hand in front of light.
12. Review video footage to ensure capture and quality:
  - a. Dry camera and remove camera from waterproof case, ensuring camera does not get wet.
  - b. Press on/mode button on right side of camera.
  - c. Swipe bottom to top to view files stored on camera.
  - d. The most recently collected video will play automatically. To see older videos, select the gallery icon in the upper left corner of the screen. The most recent video will be shown in the upper left. Touch the desired video to review, and touch play to watch.
  - e. Turn off camera by pressing **and holding** side on/mode button to turn off camera.
  - f. Return camera to waterproof case, snap shut, and re-collect if necessary.
13. The Sample Collection form in the App should be filled out in the field, except for the filenames for A (downlooking) and B (oblique) videos, which will be completed at the end of the day when files are transferred to a computer. Data should include:
  - a. GPS coordinates of video location
  - b. Distance from X-site (e.g., within 37 meters, 37-100 meters, or 100-500 meters)
  - c. Depth of video location
14. At the end of each field day, charge lights and camera batteries using charging cords and USB power supply.

#### 12.4.3 TRANSFERRING AND BACKING UP VIDEO FILES

Procedures for transferring video files via a USB cable to a computer and/or external hard drive are described below. Best practice is to always store videos in at least two locations in case of failure or damage to one.

1. Plug USB cable into Mini USB port on side of camera and laptop/computer. Take care not to scratch lens when camera is outside waterproof housing.
2. Turn on camera by pressing On/Mode button. Camera should show up as a drive on laptop. Note if camera “locks up” or stops responding, remove and replace the battery to reboot the camera.
3. Review each file for the site information at the beginning and change file name of the videos stored on the Micro SD card using the following convention:  
SITEID\_V1\_DATE\_X\_01, where:
  - SITEID -- the NCCA SiteID
  - V1 -- visit number, either V1 or V2
  - DATE -- the date collected in format YYYYMMDD
  - X -- either A for the downlooking camera or B for the oblique camera
  - 01 -- the number video collected (always 01 unless the camera is deployed more than once at a site)
    - Examples:
      - NGL20\_IL-10001\_V1\_20200612\_A\_01
      - NGL20\_IL-10001\_V1\_20200612\_B\_01



- NGL20\_IL-10001\_V1\_20200612\_A\_02
  - NGL20\_IL-10001\_V1\_20200612\_B\_02
4. Copy all files to the computer.
  5. Do not delete files from Micro SD card, as this is how files will be transferred to EPA. The file names on the computer and on SD card should be identical and both follow the naming convention above.
  6. If your Micro SD cards are getting close to full, request additional cards using the Supply Request Form.
  7. Complete field forms (**Figure 12.8**) by entering the filenames associated with the videos collected for each site.
  8. If no underwater video was taken at the site, fill in the “no sample collected” box and provide reason in the comment field.
  9. As the Micro SD cards fill, request an additional pair of cards and send both full Micro SD cards with videos to GLTED using a T7 FedEx label from your base kit. Be sure to request the replacement cards with enough lead time to receive and replace the cards before the cards are completely full to ensure videos can be taken at every site.
  10. In the NCCA App, access the Tracking Form for each site from which videos were collected and are being shipped. Mark each UVID sample (full set of videos from one site) as shipped and submit the tracking form.
  11. At the end of the field season, each crew will receive instructions on how to return all underwater camera equipment including all remaining Micro SD cards with videos saved to GLTED.
  12. Do not delete back-up videos on your computer for one year in case Micro SD cards are damaged or lost in transit.

12:49 PM Wed Mar 11 56%

Menu NGL20\_IL-10001, Visit 1 SAVE  
Version 2.8

### NCCA/NGLA 2020 SAMPLE COLLECTION

**Underwater: Digital Video Recording (UVID)** No Sample Collected  

Y-Location Video:

Latitude:  Longitude:  Depth (m):

Video taken:

Within 37m from X-site  Between 37-100m from X-site  Between 100-500m from X-site

A Camera Filename:  B Camera Filename:  

---

Additional Video: Use if more than one video is collected at the Y-Location or if the benthos sample is collected at a location other than the Y-Location.

Latitude:  Longitude:  Depth (m):

Video taken:

Within 37m from X-site  Between 37-100m from X-site  Between 100-500m from X-site

A Camera Filename:  B Camera Filename:  

---

Additional Video: Use if more than one video is collected at the Y-Location or if the benthos sample is collected at a location other than the Y-Location.

Latitude:  Longitude:  Depth (m):

Video taken:

Within 37m from X-site  Between 37-100m from X-site  Between 100-500m from X-site

A Camera Filename:  B Camera Filename:  

Figure 12.8 Example of sample collection form in the NCCA App, UVID information.

## 13 SEDIMENT COLLECTION

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Crews collect sediments for a variety of analyses. Field crews will sieve one or two sediment grabs and submit the resulting benthic infauna collection to the lab to be analyzed for species composition and abundance. Additional sediment grabs will be analyzed for chemical contaminants (organics/metals and TOC), grain size determination, acute whole sediment toxicity, and nitrogen isotopes in sediment. In order to provide the minimum volume of sediment for all analyses, crews may need to collect different numbers of grabs at different sites, based on sediment characteristics. While the biology (benthic assemblage) grab is being processed (sieved) by one crew member, other personnel collect the necessary grabs for chemistry, grain size, and toxicity tests. They composite the grabs, mix them and then split them into separate sample containers. Crews collect 2.5 L of sediment (1.5 liters at Great Lakes sites) to submit for chemistry (contaminants), toxicity, and grain size analyses. At marine sites only, an additional sample is collected for nitrogen isotopes.

### 13.1 SUMMARY OF METHOD

A 1/25 (0.04) m<sup>2</sup>, stainless steel, Young-modified Van Veen Grab (or similar) sampler is appropriate for collecting sediment samples for both biological and chemical analyses. The top of the sampler is either hinged or otherwise removable so the top layer of sediment can be easily removed for sediment contaminant, toxicity and the marine-only D15N sample collection. For crews sampling in the Great Lakes, a standard Ponar grab (box size 22.9 cm x 22.9 cm with depth of 9 cm) with removable top screens should be used for collecting sediments for benthic invertebrate analysis (USEPA 2001); other sediment grab devices may be used for sediment toxicity and contaminant samples at the crew's discretion. Record the dimensions and sample area of the grab used on the Sample Collection form in the App. The area of sediment the grab collects is important for data analysis. If the grab sampler size is less than 0.03 m<sup>2</sup>, take two grabs for the benthic macroinvertebrate collections and composite the sediment into the sieve.

## 13.2 EQUIPMENT AND SUPPLIES

Table 13.1 Equipment & supplies: sediment collection

<p><b>For collecting samples</b></p>	<p>Young-modified Van Veen (or Ponar) grab with grab stand          weights and pads for grab          nitrile gloves          plastic tub or bucket          0.5 mm stainless steel sieve          sieve box or bucket          electrical tape          forceps (fine-tipped)          funnel (wide-mouth)          Phosphate-free detergent such as Liquinox          Formalin (100% buffered) with stain          Graduated cylinder for measuring formalin          Rose Bengal Stain (for staining formalin solution)          Borax          ruler (cm)          wash bottle (for ambient water)          stainless steel mixing pot or bowl with lid          Spoon, stainless steel (15") or Teflon spoons/scoops/spatula          HDPE bottle(s) (1 L, wide-mouth) [BENT]          glass jar (2, 60 mL, amber) [SEDC, D15N]          glass jar (120 mL, amber) [SEDO]          plastic 6 mil bags (2, 1 quart) [SEDG]          Bucket w/screw top lid (0.6 gallon) for marine SEDX          Bucket w/snap top lid (1 quart) for Great Lakes SEDX          scrub brush          cooler with wet ice</p>
<p><b>For recording measurements</b></p>	<p>NCCA App          pencils (for inner labels)          fine-tipped indelible markers (for outer labels)          clear tape strips</p>

## 13.3 SAMPLING PROCEDURE

The following describes the sampling procedure to obtain sediment samples.

**Note:** *The sampler, spoons, and mixing bowl or bucket must be thoroughly rinsed with ambient water after sampling at each site to ensure no sediments remain. At the next station the sampler, spoons and mixing bowl or bucket must be washed with phosphate-free detergent such as Liquinox and rinsed with ambient water prior to use. This practice reduces the risk of the equipment carrying contaminants from site to site.*

*Do not apply sunscreen or other chemical contaminants until after the sample is collected (or implement measures to reduce contamination by such chemicals if applied such as washing, wearing long gloves, etc.). Be sure to use new clean nitrile gloves or wash gloves between stations if they are reused from one station to the next.*

1. Attach the sampler to the end of the winch cable with a shackle and tighten the pin.
2. Set the grab according to the manufacturer's instructions and disengage any safety device designed to lock the sampler open.
3. Lower the grab sampler through the water column such that travel through the last five meters is no faster than about 1 m/sec. This procedure minimizes the effects of bow wave disturbance to surficial sediments.
4. Allow a moment for the sampler to settle into the substrate and then allow slack on the cable. Letting the cable go slack serves to release the jaws of the sampler so they will close as the sampler is retrieved.
5. Retrieve the sampler and lower it into its cradle or a plastic tub on-board. Open the top and determine whether the sampling is successful or not.
  - A successful grab is one having relatively level, intact sediment over the entire area of the grab, and a sediment depth at the center of at least 7 cm for the benthic macroinvertebrate grab (see **Figure 13.1**).
  - Grabs containing no sediment, partially filled grabs, or grabs with shelly/rocky substrates or grossly slumped surfaces are unacceptable.
  - Grabs completely filled to the top, where the sediment is in direct contact with the hinged top, are also unacceptable.
  - It may take several attempts using different amounts of weight to obtain the first acceptable sample. More weight will result in a deeper bite of the grab. In very soft mud, pads may be needed to prevent the sampler from sinking into the mud. If pads are used, the rate of descent near the bottom should be slowed even further to reduce the bow wave.
6. If, after several attempts, only grabs less than 7 cm deep can be obtained, use the next successful grab regardless of the depth of sediment at the center of the grab.
  - Use the comments on the Sample Collection form in the App form to describe your efforts and be sure to accurately record the depth of the sediment captured by the grab.
  - Carefully drain overlying water from the grab. If the grab is used for benthic community analysis, the water must be drained into the container that will receive the sediment to ensure no organisms are lost.
  - Enter notes on the condition of the sample (smell, substrate, presence of organisms on the surface, etc.) in the Sediment Characteristics section of the Sample Collection form in the App.
7. If the grab sampler size is less than 0.03 m<sup>2</sup>, take two grabs for the benthic macroinvertebrate collections and composite the sediment into the sieve.
8. Process the grab sample for either benthic community analysis or chemistry/toxicity testing as described below.
9. Repeat steps 4-8 until all samples are successfully collected. To minimize the chance of sampling the exact same location twice, the boat engines can be turned periodically to change the drift of the boat, or additional anchor line can be let out.

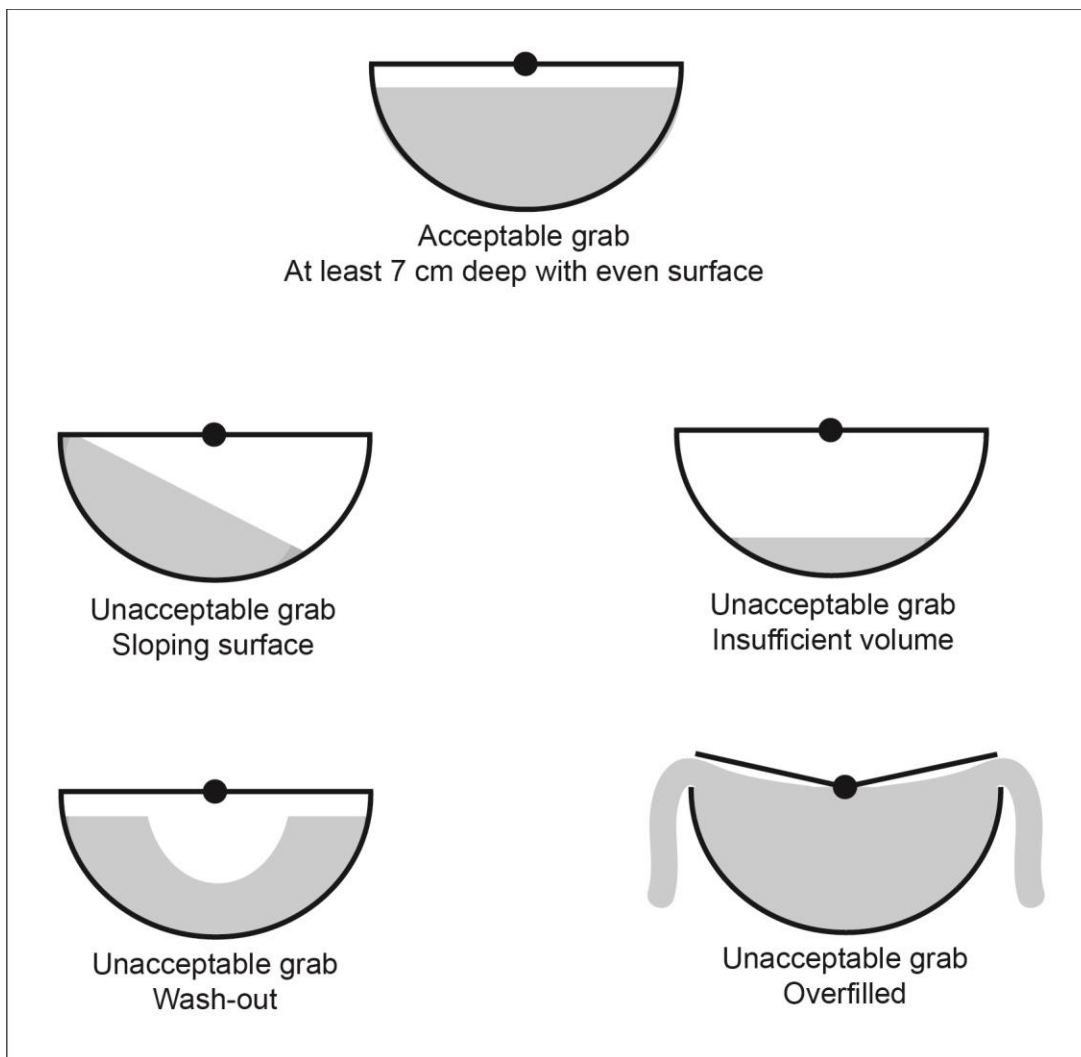


Figure 13.1 Illustration of acceptable & unacceptable grabs for benthic community analysis. An acceptable grab is at least 7 cm in depth, but not oozing out of the top of the grab and has a relatively level surface.

#### 13.4 PROCESSING PROCEDURE – BENTHIC MACROINVERTEBRATE [BENT] COMPOSITION AND ABUNDANCE

Grab samples obtained to assess the benthic macroinvertebrate community are processed as outlined below.

1. Record the deepest recorded salinity at sampling location and write on the BENT bottle label.
2. Measure the depth of the sediment at the middle of the sampler to the nearest ½ centimeter and record the value on the Sample Collection form in the App. The depth should be  $\geq 7$  cm if possible (see previous section).
  - Record the grab number in the app and any descriptive information about the grab, such as the presence or absence of a surface floc, color and smell of surface sediments, and visible fauna.

3. Dump the sediment into a clean basin (plastic tub or bucket) and then into a 0.5 mm mesh sieve. Place the sieve into a table (sieve box) containing water from the sampling station, a larger bucket, or place the sieve over the side of the boat.
  - Gently agitate the sieve to wash away sediments and leave organisms, detritus, sand particles, and pebbles larger than 0.5 mm. This method minimizes mechanical damage to fauna that is common when forceful jets of water are used to break up sediments.
  - A gentle flow of water over the sample is acceptable. Extreme care must be taken to assure that no sample is lost over the side of the sieve.
  - In the rare event that a federally listed benthic species is observed in the grab sample (e.g., listed freshwater mussels, black or white abalone, etc.), gently remove the individual from the sample and quickly return it to an area where it is unlikely to be sampled again to minimize stress. Make notes in the comments area in the NCCA App regarding any organisms removed from the sample, including best field identification and “ESA”.
4. Drain the water from the sieve and gently rinse the contents of the tray to one edge. Remove large non-living items such as rocks and sticks after inspecting them and ensuring that all benthic organisms are included in the collection.
  - Using either your fingers or a spoon, GENTLY scoop up the bulk of the sample and place it in the 1 L HDPE bottle (which should be placed in the sieve or a bucket in case some of the sample spills over).
5. Complete the BENT sample label with Site ID, date collected, visit number, and jar number.
6. Attach the completed label to the 1 L wide mouth sample bottle and cover with clear plastic tape.
7. Rinse the outside of the sample jar into the sieve, then, using a funnel, rinse the contents into the jar. The jar should be filled no more than one-half full.
  - If the quantity of sample exceeds 500 mL, place the remainder of the sample in a second container with a “2 of 2” label. For samples with a large amount of benthos, additional jars may be needed.
8. Use a pencil to fill out waterproof benthic infauna (BENT) label(s) with the pertinent sample information and place it inside the bottle(s). Be sure to include the sample ID and jar number.
9. Record sample collection location and the total number of jars on the Sample Collection form in the App.
10. Carefully inspect the sieve to ensure that all organisms are removed. Use fine forceps (if necessary) to transfer fauna from the sieve to the sample bottle. Once again, do not preserve organisms that have the potential to be a federally listed species.

11. A stained 100% percent buffered formalin solution is used to fix and preserve benthic samples. The solution should be mixed according to the directions in **Table 4.1**. 100 mL of the formalin should be added to each sample jar along with an additional teaspoon-full of borax to ensure saturation of the buffer. Rose Bengal stain is added to the stock formalin solution for use at all sites.
  - If rose bengal staining of sample is not evident, you may need to add more preservative.
  - Make sure that there is sufficient preservative to ensure everything gets preserved properly, then **fill the jar to the rim with seawater/lakewater to eliminate any air space**. This procedure eliminates the problem of organisms sticking to the cap because of sloshing during shipment.
  - Crews may choose to use a more dilute formalin solution in larger quantities as long as the end concentration of the preservative is at least six percent.
  - Once preserved, check the ‘Preserved?’ box on the Sample Collection form in the App.
12. After preservation, replace the bottle lid(s) and seal tightly with electrical tape. Gently rotate the bottle to mix the contents and place in the dark.
  - If the sample occupies more than one container, label all the sample bottles containing material from that grab together. All benthos jars from a single site will have the same sample ID number.
13. Prior to sieving the sample at the next site, use copious amounts of forceful water and a stiff brush to clean the sieve, thereby minimizing cross-contamination of samples. Be sure to rinse the brush between each sieve cleaning.

### **13.5 PROCESSING PROCEDURE – SEDIMENT COMPOSITION, CHEMISTRY, TOXICITY AND NITROGEN ISOTOPES**

In addition to grab samples collected for benthic community analysis, additional grabs are collected for chemical analyses (organics/metals and TOC), grain size determination, acute toxicity tests, and (at marine sites) nitrogen isotopes. The top two centimeters of these grabs are removed, homogenized, and split into these five sample types.

The samples are removed and processed in the order described below.

1. As each grab is retrieved, carefully examine it to determine acceptability. The grab is considered acceptable as long as the surface layer is intact. The grab need not be greater than 7 cm in depth for chemistry samples, but the other criteria outlined above apply (see **Section 13.3** and **Figure 13.1** above).
  - Carefully drain off, or siphon, any overlying water, and remove and discard large, non-living surface items such as rocks or pieces of wood. Remove any submerged aquatic vegetation (SAV) after recording its



presence in the sediment sample collection zone comment field on the Sample Collection form in the App.

**Note:** Great care must be taken to avoid contamination of this sample from atmospheric contaminants. **The boat engine should be turned off or the boat maneuvered to ensure the exhaust is downwind.** All containers, including the grab sampler, should be kept closed except when opening is necessary to remove or add samples.

2. A clean stainless steel or Teflon spoon that has been washed with phosphate-free detergent such as Liquinox and rinsed with ambient site water is used to remove sediments from grab samples for these analyses.
3. Remove the top 2 cm of sediment using the stainless steel or Teflon spoon. Sediment which is in direct contact with the sides of the sampler should be excluded as they may be contaminated from the device.
  - Place the sediment into a pre-cleaned (washed with phosphate-free detergent such as Liquinox and rinsed with ambient site water) stainless steel pot or bowl and place the pot in a cooler on wet ice (NOT dry ice). The sample must be stored at 4°C, and MUST NOT BE FROZEN.
4. Repeat obtaining sediment samples from the grab and compositing the sediment in the same stainless pot/bowl until a sufficient quantity of sediment has been collected for all samples (approximately 2.5L at marine sites and 1.5 liters at Great Lakes sites).
  - Stir sediment homogenate after every addition to the composite to ensure adequate mixing. Keep the container covered and in the cooler between grabs.
5. Record the location (zone) of the sediment collection on the Sample Collection form in the App. If sediment was collected from more than one zone, fill in the bubble of the zone where the majority of the sediment was collected and describe the proportions of sediment collected from each zone in the comments section for each sample.
6. Stir the sediment sample with a Teflon paddle or stainless steel spoon until it's thoroughly homogenized and takes on a uniform color and consistency. This will take between 2 and 10 minutes. Divide the composite into the sample types listed below. In the case of limited sediment, prioritize sample distribution in the order listed.
  - a) **ORGANICS and METALS [SEDO]:**
    - Complete the SEDO sample label with Site ID, date collected, and visit number.
    - Attach the completed label to the 120 mL (4 oz) glass sample jar and cover with clear plastic tape.
    - Using a clean stainless steel spoon, carefully place approximately 100 mL of sediment into the jar. CARE MUST BE TAKEN TO ENSURE THAT THE INSIDE OF THE JAR, CAP, AND THE SAMPLE IS NOT CONTAMINATED. Be sure that you leave ½ inch

headspace to avoid breakage due to possible sample expansion from freezing.

- Replace the lid and seal tightly with electrical tape, wrap the jar in the provided foam sleeve to protect it from breakage.
- Record any comments on the Sample Collection form in the App.
- Freeze the sample as soon as possible and keep frozen until shipped.
- Fill in the "frozen" bubble in the App to confirm that the sample has been frozen.

**b) SEDIMENT TOXICITY [SEDX]:**

- Complete the SEDX sample label with Site ID, date collected, and visit number.
- Attach the completed label to the 0.6 gallon plastic sample bucket (for marine samples) or 1 quart bucket (for Great Lakes samples) and cover with clear plastic tape.
- Using the stainless steel spoon, fill the bucket with the amount of sediment specified below:
  - For marine sites the preferred volume is 1800 mL (which will fill the 0.6 gallon bucket approximately 2/3 full) but if that is not possible the minimum volume required is 900 mL.
  - For Great Lakes sites the preferred volume is 900 mL (which will fill the bucket approximately ¾ inches from the rim of the 1 quart bucket) but if that is not possible the minimum required is 400 mL.
- Replace the lid and tighten so that the locking mechanism engages and holds the lid tightly closed.
- Record any comments on the Sample Collection form in the App.
- Place the sample on wet ice (NOT dry ice). The sample must be stored at 4°C, and **MUST NOT BE FROZEN**.
- Fill in the "chilled" bubble in the App to confirm that the sample has been chilled.

**c) TOTAL ORGANIC CARBON [SEDC]:**

- Complete the SEDC sample label with Site ID, date collected, and visit number.
- Attach the completed label to the 60 mL glass sample jar and cover with clear plastic tape.
- Using a clean stainless steel spoon, place approximately 50 mL of sediment into the jar. Be sure that you leave ½ inch headspace to avoid breakage due to possible sample expansion from freezing.
- Replace the lid and seal tightly with electrical tape, wrap the jar in the provided foam sleeve to protect it from breakage.

- Record any comments on the Sample Collection form in the App.
- Freeze the sample as soon as possible and keep frozen until shipped.
- Fill in the "frozen" bubble in the App to confirm that the sample has been frozen.

**d) SEDIMENT GRAIN SIZE [SEDG]:**

- Complete the SEDG sample label with Site ID, date collected, and visit number.
- Attach the completed label to the inner quart sized plastic sample bag and cover with clear plastic tape.
- Using a clean stainless steel spoon, place approximately 100 mL of sediment into the pre-labeled bag. Double bag the sample into a second quart sized plastic bag, ensuring that the tops of both bags are sealed tightly.
- Record any comments on the Sample Collection form in the App.
- Place the sample on wet ice (NOT dry ice). The sample must be stored at 4°C, and **MUST NOT BE FROZEN**.
- Fill in the "chilled" bubble in the App to confirm that the sample has been chilled.

**e) NITROGEN ISOTOPE (D15N) *marine sites only*:**

- Complete the D15N sample label with Site ID, date collected, and visit number.
- Attach the completed label to the 60 mL glass sample jar and cover with clear plastic tape.
- Using a clean stainless steel spoon, place approximately 50 mL of sediment into the jar. Be sure that you leave ½ inch headspace to avoid breakage due to possible sample expansion from freezing.
- Replace the lid and seal tightly with electrical tape, wrap the jar in the provided foam sleeve to protect it from breakage.
- Record any comments on the **Sample Collection** form in the App.
- Freeze the sample as soon as possible and keep frozen until shipped.
- Fill in the "frozen" bubble in the App to confirm that the sample has been frozen.

## 14 FISH TISSUE COLLECTION

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Crews collect fish at all NCCA sites. At revisit sites, ecological contamination of fish tissue or ecofish (FTIS) and fish tissue plugs (FPLG) collection should be attempted during visit 1. If a crew is unsuccessful collecting FTIS or FPLG during visit 1, then attempt to collect during visit 2. At Great Lakes revisit sites, crews that are unsuccessful at collecting the human health fish tissue (HTIS) sample during visit 1 are expected to attempt the collection of that sample during visit 2. Labs analyze whole body (also known as “ecological fish” or “ecofish”) tissue samples for concentrations of organic and inorganic contaminants. The results provide information about the ecological risks to wildlife associated with fish consumption. Refer to **Section 14.1** for detailed information regarding ecofish sample collection.

In addition to whole fish samples collected at all sites for ecological risk purposes, crews will also collect fish tissue plugs at all non-enhancement sites. These plugs can be taken from fish collected for the ecofish sample or crews can allow the fish to be released after the tissue plug sample is collected. The sample is analyzed for mercury concentrations and used to provide a measure of human health risk at all sites. Refer to **Section 14.2** for a detailed discussion of fish tissue plug collection.

Finally, crews at all 225 probabilistic nearshore Great Lakes sites (sites whose prefix begins with NGL20), all 38 Great Lakes island sites (sites whose prefix begins with ISA20), and all 12 Great Lakes park sites (sites whose prefix begins with NPA20) will collect a fish composite sample to analyze contaminants in fillet tissue for human health applications (HTIS). Refer to **Section 14.3** for detailed information regarding samples collected for human health fish tissue contaminant analysis. Note that human health fish tissue samples will **NOT** be collected at Lake Erie (LEA20) or Green Bay (GBA20) intensification sites.

When target fish are plentiful, crews in the Great Lakes will be able to submit specimens for both the ecofish and human health fish tissue collections. If specimens are less plentiful, crews may be able to split the sample between the two whole fish collection types and still meet the minimum criteria for each sample. For those instances, apply the fish distribution scheme described in Section 14.3.2.

At all sites and for all sample types, crews are never to collect species that are federally listed as threatened or endangered under the Endangered Species Act for tissue samples. If a federally listed species (e.g., fish, mammal, sea turtle, etc.) is encountered while fishing (netted, hooked, etc.), crew members are expected to immediately release the individual following identification in an area where it is unlikely to be captured again and cease sampling for five minutes to allow the individual to safely leave the area. Record the encounter with the listed species by selecting the ESA button in the NCCA App and record the species, number of individuals, and condition of the individuals. Prior to restarting fish collection, field crews should evaluate whether alternative fishing methods that are less likely to encounter listed species are available.

## 14.1 ECOLOGICAL CONTAMINATION FISH TISSUE COLLECTION [FTIS]

### 14.1.1 SUMMARY OF METHOD

Ecological fish tissue collection protocols require crews to collect at least five individuals of the target species, yielding a minimum of 300 g total mass from each site. These fish are to be collected within a 500 meter radius of the X-site (may expand to 1000 meters if needed - see below and **Figure 5.2**). Crews may collect these samples using any reasonable method (e.g., otter trawl, hook and line, gill net, seine, etc.) that is most efficient and the best use of available time on station.

For each attempted fish collection method, record equipment details, start and stop times, and fishing location(s) on the Eco Fish Collection form in the App. Also record sample ID, species retained, and specimen lengths on the Eco Fish Collection form in the App. Crews will also indicate the date of collection and the coordinates of the location where fish were ultimately caught.

Secondary fish tissue collection zones for ecofish and/or fish plugs may be selected up to an additional 500 m beyond the original 500 m radius at all estuarine and Great Lakes sites. Please observe the following guidelines:

1. In order to move to a secondary fish tissue collection zone, crews must be unsuccessful at obtaining target fish during a reasonable portion of the three hours allotted to fishing (at least 30 minutes and no more than two hours) within the original 500 m radius.
2. The crew must have attempted to collect fish at several sampling locations within the original 500 m radius without success.
3. When relocating crews should concentrate on signs of schools of bait fish just below the surface, predator activity or prey escape behavior on the surface of the water, overhead shading or favorable underwater habitat structure or bathymetric features within an additional 500 m from the X-site.
4. For collection of the human health fish tissue sample ONLY (if applicable), crews may move out to a maximum of 1500 meters from the X-site in an effort to collect this sample.
5. If fish are collected in more than one zone fill in the bubble of the zone where the majority of the fish were collected and describe the proportions of fish collected from each zone in the comments section for each sample in the App.

Crews working in each of the regional areas— Northeast, Southeast, Gulf, West Coast, and Great Lakes – collect different target fish species based on biogeographically specific lists. **Recommended Primary** and **Secondary** target species are given by region in the following tables:

- Northeast - **Table 14.2**
- Southeast - **Table 14.3**
- Gulf of Mexico - **Table 14.4**
- West - **Table 14.5**
- Great Lakes - **Table 14.6**

If a full composite sample is not collected after three hours of effort, crews may terminate the sampling, record the details of the sample, and submit as many fish as possible. If the target species are unavailable, the fisheries biologist selects an alternative available species (i.e., a species that is commonly present in the study area and in sufficient numbers to yield a composite) to obtain a fish composite sample. However, all attempts should be made to collect the targeted species if at all possible. Alternative fish species should be limited to bony fish. Cartilaginous fish and Moray eels (Family Muraenidae) should not be submitted for this indicator or for the fish plug sample. Regardless of the species that is ultimately collected, all fish in the composite **MUST** be of the same species and meet size requirements.

Crews are expected to know and be able to identify the federally listed species and state species of concern that have the potential to occur at a given sampling site. If a listed species is visually observed prior to initiating the sampling, allow the species to naturally depart the area without herding or harassing. If a listed species is encountered (stunned, netted, hooked, etc.), crews are expected to immediately release the fish following identification in an area where it is unlikely to be captured again and cease sampling for five minutes to allow the fish to safely leave the area.

Crews may spend additional time fishing (i.e., more than three hours) if desired. It is not recommended that crews purchase fish specimens dockside unless they can document that the purchased fish came from an area in close proximity to the X-site (i.e., within 1000 meters).

Crews identify specimens to species and measure the total length to the nearest millimeter. They record the taxonomic name (genus-species) and the length of each fish in the App. The preferred minimum length for a specimen for ecological risk purposes is 100 mm with a preferred length range of 100 - 400 mm. All individuals must be of similar size, such that the smallest individual in the composite is no less than 75% of the total length of the largest individual. Up to 20 individuals (a total of 300 g of whole body tissue is needed) should be collected and retained for analysis. If it is suspected that 20 individuals will yield less than 300 g total weight, additional specimens should be collected. The lengths of any additional fish should be recorded in the comment fields provided in the fish sample collection form in the App. At Great Lakes sites where crews will collect both ecological fish tissue and human health fish tissue samples, but they collect 10 or fewer fish, they must follow the fish distribution scheme described in **Section 14.3.2**.

### 14.1.2 EQUIPMENT AND SUPPLIES

Table 14.1 Equipment & supplies: eco fish tissue collection

<p><b>For collecting fish composite sample</b></p>	<p>scientific collection permit          Otter trawl (or other device to collect sufficient sample)          sampling vessel (including boat, motor, trailer, oars, gas, and all required safety equipment) Coast Guard-approved personal flotation devices          Global Positioning System (GPS) unit          nitrile gloves          livewell and/or buckets          measuring board (millimeter scale)          scale (in grams)</p>
<p><b>For storing and preserving fish composite sample</b></p>	<p>zip-top bag(s) (plastic, 2 gallon)          Plastic bag (large, composite)          zip-top bag(s) (sandwich size) – for labels          cooler          plastic cable tie          dry ice or wet ice (for temporary transport)          side cutter (cleaned with phosphate-free detergent such as Liquinox between sites)</p>
<p><b>For documenting the fish composite sample</b></p>	<p>NCCA App          fish tissue sample labels          fine-tipped indelible markers (for labels)          Tyvek label tag with grommet          clear tape strips</p>

The procedures for collecting and processing ecological fish composite samples are presented below. If fish plugs are to be collected from specimens in the ecofish collection, complete the steps in **Section 14.2** before packaging the ecofish collection.

**Note:** Do not handle any food, drink, sunscreen, or insect repellent until after the composite sample has been collected, measured and bagged (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. Select at least five fish, with a minimum total weight of 300 grams, to include in the eco fish composite. If needed, 20 or more fish may be composited to reach the minimum weight of 300 grams. The selected fish must meet the following criteria:
  - All fish are of the same species.
  - The preferred specimen length is between 100 and 400 mm; if after sufficient fishing only smaller or larger fish of the target species are available, those will be accepted.

- 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.
- All fish for one site visit are collected as close to the same time as possible, but no more than one week apart.

*Note: Individual fish may have to be frozen until all fish to be included in the composite are available for delivery to the designated laboratory.*

4. Identify the fish to species and record the scientific name on the Eco Fish Collection form in the App.  
*Note: Accurate taxonomic identification is essential in assuring and defining the composited organisms submitted for analysis. Individuals from different species may not be composited in a single sample. Submit only one species per site.*
5. Measure each individual fish 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.
6. Record collection method and equipment details, start and stop times, and fishing location(s) on the Eco Fish Collection form in the App. Record sample ID, species name, and specimen lengths on the Eco Fish Collection form in the App. Make sure the sample ID recorded on the collection form match those on the sample labels.
7. While wearing clean nitrile gloves, remove each fish retained for analysis from the clean holding container(s). If needed, dispatch larger fish using the most humane method available.
8. Place all fish from the composite in a two-gallon zip-top bag. Take care to prevent fish spines from piercing the bag. If spines are likely to puncture the bag, break off or clip the spines with a side-cutter or other appropriate tool (cleaned with phosphate-free detergent such as Liquinox and rinsed with ambient site water before use at each site) and place the spine in the bag with the fish. Use additional bags if all the fish collected for a composite will not fit in a single two-gallon bag.
9. Weigh the composite bag(s) to determine if enough fish have been collected to reach a minimum weight of 300 grams.
10. Prepare interior and exterior FTIS sample labels for the two-gallon bag(s), ensuring that the label information matches the information recorded on the Eco Fish Collection form in the App. **Be sure to record scientific name and minimum and maximum lengths on the labels.**
  - Place the interior label inside a small (sandwich-size) zip-top bag and place the bag inside the two-gallon bag with the fish composite.
  - Affix the exterior label to the two-gallon bag and cover with clear plastic tape. If additional two-gallon bags are used, fill out extra labels with the same sample ID and information for each bag and label accordingly (i.e., bag 2 of 2).
11. Double-bag all specimens in the composite by placing all two-gallon bag(s) from the site inside a large plastic bag.



12. Prepare a sample label for the outer bag, ensuring that the label information matches the information recorded on the Eco Fish Collection form in the App. **Be sure to record scientific name and minimum and maximum lengths on the sample label.**
13. Affix the sample label to a Tyvek tag and cover with clear plastic tape. Thread a cable tie through the grommet in the Tyvek tag and seal the outer bag with the cable tie.

#### 14.1.3 SAMPLE STORAGE AND SHIPPING PREPARATION

1. After the sample is packaged, immediately place it on dry ice for shipment.
  - Check the "frozen" box on the Eco Fish Collection form in the App to confirm that the sample has been frozen.
  - Packaged samples may be placed on wet ice in coolers if they will be transported to a laboratory or other interim facility to be frozen before shipment.
  - Samples may be stored on wet ice for a maximum of 24 hours.
  - Freeze the samples within 24 hours of collection at  $\leq -20^{\circ}\text{C}$  and store the frozen samples until shipment within two weeks of sample collection. Crews may ship the frozen fish sample along with the other frozen samples from the site using a cooler with a dry ice insert or they may ship the ecofish separately. Frozen samples should be packed on at least 20 pounds of layered dry ice and shipped to the batched sample lab via priority overnight delivery service.

Table 14.2 Northeast region primary and secondary marine target species - whole body fish tissue collection (Ecofish)

NORTHEAST REGION PRIMARY ECOFISH TARGET SPECIES			
FAMILY	SCIENTIFIC NAME	COMMON NAME	FISH PLUG LIST*
Ictaluridae	<i>Ameiurus catus</i>	White catfish	Primary
	<i>Ictalurus punctatus</i>	Channel catfish	Primary
Moronidae	<i>Morone americana</i>	White perch	Primary
Paralichthyidae	<i>Paralichthys dentatus</i>	Summer flounder	Primary
Pleuronectidae	<i>Pseudopleuronectes americanus</i>	Winter flounder	Primary
Sciaenidae	<i>Cynoscion regalis</i>	Gray weakfish	Primary
	<i>Sciaenops ocellatus</i>	Red drum	Primary
Sparidae	<i>Stenotomus chrysops</i>	Scup	Primary
NORTHEAST REGION SECONDARY ECOFISH TARGET SPECIES			
FAMILY	SCIENTIFIC NAME	COMMON NAME	FISH PLUG LIST*
Achiridae	<i>Trinectes maculatus</i>	Hogchoaker	
Anguillidae	<i>Anguilla rostrata</i>	American eel	Secondary
Atherinopsidae	<i>Menidia menidia</i>	Atlantic silverside	
Batrachoididae	<i>Opsanus tau</i>	Oyster toadfish	
Ephippidae	<i>Chaetodipterus faber</i>	Atlantic spadefish	
Moronidae	<i>Morone saxatilis</i>	Rock fish (or striped bass)	Secondary
Mugilidae	<i>Mugil cephalus</i>	Black mullet	
Pomatomidae	<i>Pomatomus saltatrix</i>	Bluefish	Secondary
Sciaenidae	<i>Bairdiella chrysoura</i>	Silver perch	
	<i>Menticirrhus saxatilis</i>	Northern kingfish	
Serranidae	<i>Centropristis striata</i>	Black sea bass	
Triglidae	<i>Prionotus carolinus</i>	Northern searobin	
	<i>Prionotus evolans</i>	Striped searobin	

\* Indicates whether species also occurs in the primary or secondary fish plug list (see Table 14.8).

Table 14.3 Southeast region primary and secondary marine target species - whole body fish tissue collection (Ecofish)

SOUTHEAST REGION PRIMARY ECOFISH TARGET SPECIES			
FAMILY	SCIENTIFIC NAME	COMMON NAME	FISH PLUG LIST*
Ariidae	<i>Ariopsis felis</i>	Hardhead sea catfish	Primary
	<i>Bagre marinus</i>	Gafftopsail sea catfish	Primary
Paralichthyidae	<i>Paralichthys albigutta</i>	Gulf flounder	Primary
	<i>Paralichthys dentatus</i>	Summer flounder	Primary
	<i>Paralichthys lethostigma</i>	Southern flounder	Primary
Sciaenidae	<i>Cynoscion arenarius</i>	Sand weakfish (or seatrout)	Primary
	<i>Cynoscion nebulosus</i>	Speckled trout	Primary
	<i>Cynoscion regalis</i>	Gray weakfish	Primary
	<i>Leiostomus xanthurus</i>	Spot croaker	Primary
Sparidae	<i>Lagodon rhomboides</i>	Pinfish	
SOUTHEAST REGION SECONDARY ECOFISH TARGET SPECIES			
FAMILY	SCIENTIFIC NAME	COMMON NAME	FISH PLUG LIST*
Cichlidae	<i>Tilapia mariae</i>	Spotted tilapia	
Haemulidae	<i>Haemulon aurolineatum</i>	Tomtate	
Sciaenidae	<i>Bairdiella chrysoura</i>	Silver perch	
	<i>Menticirrhus americanus</i>	Southern kingfish	
Serranidae	<i>Centropristis striata</i>	Black sea bass	

\* Indicates whether species also occurs in the primary or secondary fish plug list (see Table 14.8).

Table 14.4 Gulf region primary and secondary marine target species - whole body fish tissue collection (Ecofish)

GULF REGION PRIMARY ECOFISH TARGET SPECIES			
FAMILY	SCIENTIFIC NAME	COMMON NAME	FISH PLUG LIST*
Ariidae	<i>Ariopsis felis</i>	Hardhead sea catfish	Primary
	<i>Bagre marinus</i>	Gafftopsail sea catfish	Primary
Paralichthyidae	<i>Paralichthys albigutta</i>	Gulf flounder	Primary
	<i>Paralichthys dentatus</i>	Summer flounder	Primary
	<i>Paralichthys lethostigma</i>	Southern flounder	Primary
Sciaenidae	<i>Cynoscion arenarius</i>	Sand weakfish (or seatrout)	Primary
	<i>Cynoscion nebulosus</i>	Speckled trout	Primary
	<i>Cynoscion regalis</i>	Gray weakfish	Primary
	<i>Leiostomus xanthurus</i>	Spot croaker	Primary
	<i>Micropogonias undulatus</i>	Atlantic croaker	Primary
	<i>Sciaenops ocellatus</i>	Red drum	Primary
Sparidae	<i>Lagodon rhomboides</i>	Pinfish	
GULF REGION SECONDARY ECOFISH TARGET SPECIES			
FAMILY	SCIENTIFIC NAME	COMMON NAME	FISH PLUG LIST*
Carangidae	<i>Caranx hippos</i>	Crevalle jack	
	<i>Chloroscombrus chrysurus</i>	Atlantic bumper	
Diodontidae	<i>Chilomycterus schoepfii</i>	Burrfish	
Gerreidae	<i>Eucinostomus gula</i>	Silver jenny	
Haemulidae	<i>Orthopristis chrysoptera</i>	Pigfish	
Ictaluridae	<i>Ictalurus furcatus</i>	Blue catfish	
Lepisosteidae	<i>Lepisosteus oculatus</i>	Spotted gar	
Lutjanidae	<i>Lutjanus griseus</i>	Gray snapper	
Sciaenidae	<i>Pogonias cromis</i>	Black drum	
Serranidae	<i>Diplectrum formosum</i>	Sand perch	
Triglidae	<i>Prionotus scitulus</i>	Leopard searobin	

\* Indicates whether species also occurs in the primary or secondary fish plug list (see Table 14.8).

Table 14.5 Western region primary and secondary marine target species - whole body fish tissue collection (Ecofish)

WESTERN REGION PRIMARY ECOFISH TARGET SPECIES			
FAMILY	SCIENTIFIC NAME	COMMON NAME	FISH PLUG LIST*
Atherinopsidae	<i>Atherinops affinis</i>	Topsmelt silverside	
Cottidae	<i>Leptocottus armatus</i>	Pacific staghorn sculpin	Primary
	<i>Oligocottus rimensis</i>	Saddleback sculpin	
Cynoglossidae	<i>Symphurus atricaudus</i>	California tonguefish	
Embiotocidae	<i>Cymatogaster aggregata</i>	Shiner perch	Primary
	<i>Embiotoca lateralis</i>	Striped seaperch	Primary
Gasterosteidae	<i>Gasterosteus aculeatus</i>	Three-spined stickleback	
Paralichthyidae	<i>Citharichthys sordidus</i>	Pacific sanddab	Primary
	<i>Citharichthys stigmaeus</i>	Speckled sanddab	Primary
	<i>Paralichthys californicus</i>	California flounder	Primary
Pleuronectidae	<i>Isopsetta isolepis</i>	Butter sole	
	<i>Parophrys vetulus</i>	English sole	Primary
	<i>Platichthys stellatus</i>	Starry flounder	Primary
	<i>Psettichthys melanostictus</i>	Pacific sand sole	
Sciaenidae	<i>Genyonemus lineatus</i>	White croaker	Primary
Serranidae	<i>Paralabrax nebulifer</i>	Barred sand bass	Primary
	<i>Paralabrax maculatofasciatus</i>	Spotted sand bass	Primary
WESTERN REGION SECONDARY ECOFISH TARGET SPECIES			
FAMILY	SCIENTIFIC NAME	COMMON NAME	FISH PLUG LIST*
Batrachoididae	<i>Porichthys notatus</i>	Plainfin midshipman	
	<i>Porichthys myriaster</i>	Specklefin midshipman	
Embiotocidae	<i>Amphistichus argenteus</i>	Barred surfperch	Secondary
Paralichthyidae	<i>Xystreurus liolepis</i>	Fantail sole	
Pleuronectidae	<i>Hypsopsetta guttulata</i>	Diamond turbot	Secondary
	<i>Microstomus pacificus</i>	Dover sole	Secondary
	<i>Lepidopsetta bilineata</i>	Rock sole	
	<i>Lyopsetta exilis</i>	Slender sole	
Sciaenidae	<i>Umbrina roncador</i>	Yellowfin croaker	

\* Indicates whether species also occurs in the primary or secondary fish plug list (see Table 14.8).

Table 14.6 Great Lakes primary and secondary target species - whole body fish tissue collection (Ecofish)

GREAT LAKES PRIMARY ECOFISH TARGET SPECIES			
FAMILY	SCIENTIFIC NAME	COMMON NAME	FISH PLUG LIST*
Catostomidae	<i>Moxostoma macrolepidotum</i>	Shorthead redhorse	Primary
Centrarchidae	<i>Ambloplites rupestris</i>	Rock bass	Primary
	<i>Lepomis gibbosus</i>	Pumpkinseed	Primary
	<i>Lepomis macrochirus</i>	Bluegill	Primary
	<i>Micropterus dolomieu</i>	Smallmouth bass	Primary
	<i>Pomoxis annularis</i>	White crappie	
	<i>Pomoxis nigromaculatus</i>	Black crappie	
Cottidae	<i>Cottus bairdii</i>	Mottled sculpin	
	<i>Cottus cognatus</i>	Slimy sculpin	
Cyprinidae	<i>Couesius plumbeus</i>	Lake chub	
	<i>Cyprinus carpio</i>	Common carp	Primary
	<i>Pimephales notatus</i>	Bluntnose minnow	
Esocidae	<i>Esox lucius</i>	Northern pike	Primary
	<i>Esox masquinongy</i>	Muskellunge	Primary
Gadidae	<i>Lota lota</i>	Burbot	Primary
Gasterosteidae	<i>Gasterosteus aculeatus</i>	Three-spined stickleback	
Gobiidae	<i>Neogobius melanostomus</i>	Round goby	
	<i>Proterorhinus marmoratus</i>	Tubenose goby	
Ictaluridae	<i>Ameiurus nebulosus</i>	Brown bullhead	Primary
	<i>Ictalurus punctatus</i>	Channel catfish	Primary
	<i>Noturus flavus</i>	Stonecat	
Moronidae	<i>Morone americana</i>	White perch	Primary
	<i>Morone chrysops</i>	White bass	Primary
Osmeridae	<i>Osmerus mordax</i>	American/ rainbow smelt	
Percidae	<i>Gymnocephalus cernuus</i>	Ruffe	
	<i>Perca flavescens</i>	Yellow perch	Primary
	<i>Percina caprodes</i>	Logperch	
	<i>Sander canadensis</i>	Sauger	
	<i>Sander vitreus</i>	Walleye	Primary
Percopsidae	<i>Percopsis omiscomaycus</i>	Trout-perch	
Salmonidae	<i>Coregonus artedi</i>	Cisco/ lake herring	
	<i>Coregonus clupeaformis</i>	Lake whitefish	Primary
	<i>Oncorhynchus qorbuscha</i>	Pink salmon	
	<i>Oncorhynchus kisutch</i>	Coho salmon	Primary
	<i>Oncorhynchus mykiss</i>	Rainbow trout	Primary
	<i>Oncorhynchus tshawytscha</i>	Chinook salmon	Primary
Sciaenidae	<i>Salvelinus namaycush</i>	Lake trout	Primary
	<i>Aplodinotus grunniens</i>	Freshwater drum	Primary
GREAT LAKES SECONDARY ECOFISH TARGET SPECIES			
FAMILY	SCIENTIFIC NAME	COMMON NAME	FISH PLUG LIST*
Catostomidae	<i>Catostomus catostomus</i>	Longnose sucker	
	<i>Catostomus commersonii</i>	White sucker	Secondary
	<i>Moxostoma anisurum</i>	Silver redhorse	
Centrarchidae	<i>Micropterus salmoides</i>	Largemouth bass	
Clupeidae	<i>Alosa pseudoharengus</i>	Alewife	
	<i>Dorosoma cepedianum</i>	American gizzard shad	
Cyprinidae	<i>Cyprinella spiloptera</i>	Spotfin shiner	
	<i>Luxilus cornutus</i>	Common shiner	
	<i>Notropis stramineus</i>	Sand shiner	
Esocidae	<i>Esox niqer</i>	Chain pickerel	
Fundulidae	<i>Fundulus diaphanus</i>	Banded killifish	
	<i>Fundulus majalis</i>	Striped killifish	
Ictaluridae	<i>Ameiurus melas</i>	Black bullhead	
Salmonidae	<i>Prosopium cylindraceum</i>	Round whitefish	
	<i>Salmo trutta</i>	Brown trout	Secondary
	<i>Salvelinus fontinalis</i>	Brook trout	
	<i>Salvelinus fontinalis x namaycush</i>	Splake	

\* Indicates whether species also occurs in the primary or secondary fish plug list (see Table 14.9).

## 14.2 FISH TISSUE PLUG [FPLG]

### 14.2.1 SUMMARY OF METHOD

Because many fish spend their entire life in a particular water body, they can be important indicators of water quality, especially for toxic pollutants (e.g., pesticides and trace elements). Toxic pollutants, which may be present in the water column or sediments at concentrations below our analytical detection limits, can be found in fish tissue above detection limits due to bioaccumulation.

Typical fish tissue collection methods require the fish to be sacrificed, whether it be a whole fish or a skin-on fillet tissue sample. This can be problematic when there is a need to collect large trophy-sized fish for contaminant analysis or when a large sample size is necessary for statistical analysis. The following method collects fish tissue plugs instead of a skin-on fillet. One fish tissue plug for mercury analysis will be collected from each of two fish of at least 190 mm of the same species (one plug per fish) from the target list (below) at every site. These fish are collected during the ecological fish tissue collection effort (**Section 14.1**). In order of preference, fish tissue plugs should be collected from 1) an ecological fish specimen that will be sent to the lab (when size and species requirements overlap), or 2) (if all required HTIS and FTIS specimens have been collected) a live fish that will be released after the plug has been collected. When possible, select larger individuals from which to collect the fish plugs. **Do not remove fish plugs from specimens that are part of the human health fish composite sample collection.** A tissue plug sample is collected by inserting a biopsy punch into a de-scaled area of dorsal muscle section of a fish. After the plug has been collected, ecofish specimens are frozen according to the protocol in **Section 14.1**; if a plug is collected from a live fish, antibiotic salve is placed over the wound and the fish is released.

### 14.2.2 EQUIPMENT AND SUPPLIES

**Table 14.7** lists the equipment and supplies necessary for field crews to collect fish tissue plug samples. Record the fish tissue plug sampling data in the Fish Tissue Plug Samples section of the Eco Fish Collection form in the App.

*Table 14.7 Equipment & supplies: fish tissue plugs*

<p><b>For fish tissue plug samples</b></p>	<p>antibiotic salve            cooler with dry ice            cooler with wet ice            dip net            biopsy punch (sterile, disposable)            fish collection gear (trawl, nets, livewell, etc.)            disposable forceps (sterile)            glass scintillation vial (20 mL)            nitrile gloves            measuring board            aspirator bulb            scale (in grams)            scalpel (disposable, sterile)</p>
<p><b>For recording measurements</b></p>	<p>NCCA App            fish tissue plug sample label            fine-tipped indelible markers (for labels)            clear tape strips</p>

### 14.2.3 SAMPLING PROCEDURE

The fish tissue plug indicator samples will be collected using the same gear and procedures used to collect the ecological and/or human health fish tissue samples, and collection occurs within the same area as other fish collections. Samples should be taken from the species listed in the target list (primary and secondary species) found in **Table 14.8** and **Table 14.9**. When ecofish specimens meet the size (190 mm) and species requirements for fish plug samples, the plugs should be taken from the ecofish prior to placing on ice. If ecofish specimens do not meet the size and species requirement for fish plugs, fish plugs should be taken from live fish and the fish are released with antibiotic salve on the wound, as in step 14 below. If the recommended primary and secondary species are unavailable, the fisheries biologist will select an alternative species (i.e., a species that is commonly consumed by people in or around the study area, with specimens that have a minimum length of 190 mm) to obtain a sample from the species that are available. Alternative fish species should be limited to bony fish. Cartilaginous fish and Moray eels (Family Muraenidae) should not be submitted for this indicator or for the ecofish sample. The alternative genus and species must be written in to the NCCA App. In no instance should fish plugs be removed from specimens submitted for the human health fish tissue sample.

In order of preference, crews should try to submit species from 1) the Primary Target List; 2) the Secondary Target List; and 3) any other commonly consumed, available fish. It is recognized that there are species not on these lists that may be culturally or regionally important food sources, essential to subsistence fishers or increasingly popular among food trends. For these reasons, the guidance for selecting species for fish plug samples is purposefully inclusive.

Please note: There are no invertebrate organisms on this list. Crab, shrimp, mollusks, lobsters, etc., will not be used in assessment of mercury content in fish plugs. If invertebrate species are submitted for FPLG samples, those data will be reported as MISSING for the associated sites.

The procedures for collecting and processing fish plug samples are presented below.

1. Spread out a cooler liner bag on a flat surface for your workspace.
2. Prepare the FPLG sample label with Site ID, date collected, and visit number.
3. Attach the completed label to the 20 milliliter scintillation vial and cover with clear tape.
4. Put on clean nitrile gloves before handling the fish.  
*Note: Do not handle any food, drink, sunscreen, or insect repellent until after the plug samples have been collected (or implement measures to reduce contamination by such chemicals if applied such as washing, wearing long gloves, etc.).*
5. Rinse potential target species/individuals in ambient water to remove any foreign material from the external surface and place in clean holding containers (e.g., livewells, buckets). Return non-target fishes or small specimens to the water.
6. Retain two individuals of the same target species from each site. The fish should be:
  - large enough to collect a fish plug yielding ~ 0.5 grams (wet weight) of tissue,
  - on the recommended primary or secondary target list (if not available select an alternative species present),
  - both the same species,
  - both satisfy legal requirements of harvestable size (or weight) for the sampled water body, or at least be of consumable size and
  - of similar size, so that the smaller individual is no less than 75% of the total length of the larger individual,
  - at least 190 mm in length.*NOTE: Whenever possible, larger specimens should be selected over smaller specimens.*
7. Remove one fish retained for analysis from the clean holding container(s) (e.g., livewell) using clean nitrile gloves.
8. Measure the fish to determine total body length. Measure total length of the specimen in millimeters 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). The minimum acceptable length for a fish used for any fish plug sample is 190 mm.
9. Weigh the fish in grams using the fish weigh scale.
10. Note any anomalies (e.g., lesions, cuts, sores, tumors, fin erosion) observed on the fish.



11. Record sample ID, species, and specimen length and weight in the Fish Tissue Plug Samples section of the Eco Fish Collection form in the App.
12. On a meaty portion of the left side, dorsal area of the fish between the dorsal fin and the lateral line, clear a small area of scales with a sterile disposable scalpel.
13. Wearing clean nitrile gloves, insert the 8 mm biopsy punch into the dorsal muscle of the fish through the scale-free area. The punch is inserted with a slight twisting motion cutting the skin and muscle tissue. Once full depth of the punch is achieved, a slight bending or tilting of the punch is needed to break off the end of the sample. Remove biopsy punch taking care to ensure sample remains in the punch.  
*Note: The full depth of the punch should be filled with muscle tissue, which should result in collecting a minimum of 0.25 to 0.35 grams of fish tissue for mercury analysis.*
14. If the fish is to be released, apply a generous amount of antibiotic salve to the plug area and gently return the fish to the water. If the fish is part of the ecofish collection, return the fish to the ecofish holding area without the application of antibiotic.
15. Using an aspirator bulb placed on the end of the biopsy punch, give a quick squeeze, blowing the tissue sample into the 20 mL scintillation vial.
16. Place the vial with sample immediately on dry ice for temporary storage.
17. Repeat steps 2-15 for the second fish, to collect a second fish plug sample. Place the second plug in the same scintillation vial as the first. The two plugs should provide at least 0.5 grams of tissue. NOTE: If two qualifying fish cannot be caught, both plugs may be taken from the same fish.
18. Replace the lid and seal tightly with electrical tape, insert the vial into the "bubble bag" to protect it from breakage, and then place it into the zip-top bag. Place the sample in a cooler with dry ice
19. Dispose of gloves, scalpel, and biopsy punch.

#### 14.2.4 SAMPLE STORAGE

1. Keep the samples frozen on dry ice or in a freezer at  $\leq -20^{\circ}\text{C}$  until shipment.
2. Frozen samples will subsequently be packed on dry ice and shipped to the batched sample laboratory via priority overnight delivery service within one week of collection. Please see **Appendix C: Shipping and Tracking Guidelines** for next steps.

Table 14.8 Primary and secondary marine target species for fish plug collection

PRIMARY MARINE FISH PLUG TARGET SPECIES		
FAMILY	SCIENTIFIC NAME	COMMON NAME
Ariidae	<i>Ariopsis felis</i>	Hardhead sea catfish
	<i>Bagre marinus</i>	Gafftopsail sea catfish
Cottidae	<i>Leptocottus armatus</i>	Pacific staghorn sculpin
Embiotocidae	<i>Cymatogaster aggregata</i>	Shiner perch
	<i>Embiotoca lateralis</i>	Striped seaperch
Ictaluridae	<i>Ameiurus catus</i>	White catfish
	<i>Ictalurus punctatus</i>	Channel catfish
Moronidae	<i>Morone americana</i>	White perch
Paralichthyidae	<i>Citharichthys sordidus</i>	Pacific sanddab
	<i>Citharichthys stigmaeus</i>	Speckled sanddab
	<i>Paralichthys albigutta</i>	Gulf flounder
	<i>Paralichthys californicus</i>	California flounder
	<i>Paralichthys dentatus</i>	Summer flounder
	<i>Paralichthys lethostigma</i>	Southern flounder
	<i>Parophrys vetulus</i>	English sole
Pleuronectidae	<i>Platichthys stellatus</i>	Starry flounder
	<i>Pseudopleuronectes americanus</i>	Winter flounder
Sciaenidae	<i>Cynoscion arenarius</i>	Sand weakfish (or seatrout)
	<i>Cynoscion nebulosus</i>	Speckled trout
	<i>Cynoscion regalis</i>	Gray weakfish
	<i>Genyonemus lineatus</i>	White croaker
	<i>Leiostomus xanthurus</i>	Spot croaker
	<i>Micropogonias undulatus</i>	Atlantic croaker
	<i>Sciaenops ocellatus</i>	Red drum
Serranidae	<i>Paralabrax maculatofasciatus</i>	Spotted sand bass
	<i>Paralabrax nebulifer</i>	Barred sand bass
Sparidae	<i>Stenotomus chrysops</i>	Scup
SECONDARY MARINE FISH PLUG TARGET SPECIES		
FAMILY	SCIENTIFIC NAME	COMMON NAME
Anguillidae	<i>Anguilla rostrata</i>	American eel
Embiotocidae	<i>Amphistichus argenteus</i>	Barred surfperch
	<i>Amphistichus rhodoterus</i>	Redtail surfperch
	<i>Embiotoca jacksoni</i>	Black perch
	<i>Hyperprosopon argenteum</i>	Walleye surfperch
Moronidae	<i>Morone saxatilis</i>	Rock fish (or striped bass)
Paralichthyidae	<i>Hippoglossina oblonga</i>	Fourspot flounder
Pleuronectidae	<i>Hippoglossoides platessoides</i>	American dab
	<i>Hypsopsetta guttulata</i>	Diamond turbot
	<i>Limanda ferruginea</i>	Yellowtail flounder
	<i>Microstomus pacificus</i>	Dover sole
Pomatomidae	<i>Pomatomus saltatrix</i>	Blue fish
Sciaenidae	<i>Menticirrhus undulatus</i>	California whiting
Scorpaenidae	<i>Scorpaena guttata</i>	California scorpionfish
	<i>Sebastes caurinus</i>	Copper rockfish
	<i>Sebastes entomelas</i>	Widow rockfish
	<i>Sebastes flavidus</i>	Yellowtail rockfish
	<i>Sebastes melanops</i>	Black rockfish
	<i>Sebastes mystinus</i>	Blue rockfish
	<i>Sebastes paucispinis</i>	Bocaccio
Serranidae	<i>Paralabrax clathratus</i>	Kelp bass

Table 14.9 Primary and secondary Great Lakes target species for fish plug collection

PRIMARY GREAT LAKES FISH PLUG TARGET SPECIES		
FAMILY	SCIENTIFIC NAME	COMMON NAME
Catostomidae	<i>Moxostoma macrolepidotum</i>	Shorthead redhorse
Centrarchidae	<i>Ambloplites rupestris</i>	Rock bass
	<i>Lepomis gibbosus</i>	Pumpkinseed
	<i>Lepomis macrochirus</i>	Bluegill
	<i>Micropterus dolomieu</i>	Smallmouth bass
Cyprinidae	<i>Cyprinus carpio</i>	Common carp
Esocidae	<i>Esox lucius</i>	Northern pike
	<i>Esox masquinongy</i>	Muskellunge
Ictaluridae	<i>Ameiurus nebulosus</i>	Brown bullhead
	<i>Ictalurus punctatus</i>	Channel catfish
Gadidae	<i>Lota lota</i>	Burbot
Moronidae	<i>Morone americana</i>	White perch
	<i>Morone chrysops</i>	White bass
Percidae	<i>Perca flavescens</i>	Yellow perch
	<i>Sander vitreus</i>	Walleye
Salmonidae	<i>Coregonus clupeaformis</i>	Lake whitefish
	<i>Oncorhynchus kisutch</i>	Coho salmon
	<i>Oncorhynchus mykiss</i>	Rainbow trout
	<i>Oncorhynchus tshawytscha</i>	Chinook salmon
	<i>Salvelinus namaycush</i>	Lake trout
Sciaenidae	<i>Aplodinotus grunniens</i>	Freshwater drum
SECONDARY GREAT LAKES FISH PLUG TARGET SPECIES		
FAMILY	SCIENTIFIC NAME	COMMON NAME
Catostomidae	<i>Catostomus commersonii</i>	White sucker
Ictaluridae	<i>Ictalurus furcatus</i>	Blue catfish
Salmonidae	<i>Salmo trutta</i>	Brown trout

### 14.3 HUMAN HEALTH FISH TISSUE COLLECTION [HTIS] (*GREAT LAKES NEARSHORE AND LAKE MICHIGAN ENHANCEMENT SITES ONLY*)

#### 14.3.1 SUMMARY OF METHOD

Field crews collect human health fish composite samples at all 225 of the Great Lakes nearshore sites (i.e., sites whose prefix begins with NGL20), all 38 Great Lakes island sites (sites whose prefix begins with ISA20), and all 12 Great Lakes National Park sites (sites whose prefix begins with NPA20). This will result in human health fish tissue being targeted at 45 sites per lake, plus the 38 island sites and 12 park sites in Lake Michigan. If a Great Lake site has been designated as a human health fish tissue site and is dropped, a replacement site is identified following procedures described in Section 2.3.2 and human health fish tissue should be collected at the replacement site. At revisit sites in the Great Lakes, crews that are unsuccessful at collecting the human health fish tissue sample (HTIS) during visit 1 are expected to attempt the collection of that sample during visit 2. Note that human health fish tissue samples will **NOT** be collected at Lake Erie (LEA20) and Green Bay (GBA20) enhancement sites.

Labs analyze fillet tissue for mercury, polychlorinated biphenyls (PCBs), per- and polyfluoroalkyl substances (PFAS), and fatty acids.

This section contains the sampling procedures and target species for human health fish composite collection. Note that the human health fish species table (**Table 14.11**) includes 25 primary target species and 18 secondary fish species. Field crews must attempt to collect a primary target species wherever possible. If primary target species are not available at a particular site, then the field crew collects a composite of one of the secondary fish species. In the event that a crew is unable to collect fish which are on the human health species list, then the field crew should contact the Great Lakes Human Health Fish Tissue Manager or Great Lakes Fish Tissue Trainer.

As with the ecological fish tissue samples, crews collect human health fish tissue samples using any reasonable method that represents the most efficient or best use of the available time on station (e.g., hook and line, gill net, or otter trawl). However, in contrast to the allowable procedures for ecological fish tissue samples, **crews may not purchase fish for human health fish tissue collection**. Record sample collection information on the Human Health Fish Collection form in the App.

For each attempted fish collection method, record equipment details, start and stop times, and fishing location(s) on the Human Health Fish Collection form in the App. Record sample ID, species retained, and specimen lengths on the Human Health Fish Collection form in the App.

Identify and measure the specimens collected for each composite sample. Record the scientific name (genus and species) and total length for each specimen on the Human Health Fish Collection form in the App. Human health fish composites 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 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 human health fish 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 submitted as part of the human health fish composite sample should remain intact and be submitted as whole specimens. **Crews should not take fish plugs from human health fish tissue specimens.**

#### 14.3.2 FISH TISSUE DISTRIBUTION SCHEME

Ideally, at Great Lakes sites where crews will collect both human health fish tissue samples (HTIS) and ecological fish tissue samples (FTIS), they will successfully collect 10 or more fish of the same species that are each  $\geq 190$  mm in length. That would allow them to retain 5 fish for the HTIS sample and 5 (or more) for the FTIS sample. However, if 10 fish are not available at a site, field teams will apply a fish distribution or “fish-splitting” scheme. It is important to understand that the 5 HTIS fish must be the same species and the 5 FTIS fish must be the same species, but the HTIS sample and the FTIS sample from a site may be different species. (Note that the following fish distribution scheme would only apply when the same species of fish is collected and available for both human health and ecological samples).

If only a single fish is collected at a site, it should be retained as the Ecological (FTIS) sample.

If sampling yields two fish of the same species, one will be the Human Health (HTIS) sample and one will be the FTIS sample. If an odd number of fish of the same species are collected at a site, the “extra” fish should be included in the HTIS sample. For example, if sampling yields three fish of the same species, two of them will be saved as the HTIS sample and one fish will be retained as the FTIS sample (See **Figure 14.1**). Obviously, in cases where an even number of fish (of the same species) are collected from a site, the number of specimens will be split evenly between the HTIS sample and the FTIS sample.

## Fish Tissue Sample Fish Distribution Scheme




















	Human Health (HTIS)	Ecological (FTIS)
<i>Reminder: Apply this scheme only when all fish are the same species</i>		
<b>If... 1 fish collected</b>		
<b>2 fish collected</b>		
<b>3 fish collected</b>		
<b>4 fish collected</b>		
<b>5 fish collected</b>		
<b>6 fish collected</b>		
<b>7 fish collected</b>		
<b>8 fish collected</b>		
<b>9 fish collected</b>		
<b>10 fish collected</b>		

Figure 14.1 Fish Tissue Distribution Scheme to be used at all Great Lake Sites with the prefix NGL20, ISA20, or NPA20.

### 14.3.3 EQUIPMENT AND SUPPLIES

Table 14.10 lists the equipment and supplies necessary for field crews to collect human health fish composite samples. Additional human health fish collection supplies can be ordered through the Supply 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 human health fish composites. Detailed procedures for collecting and processing fish composite samples are presented below.

Table 14.10 Equipment & supplies: human health fish tissue collection

<b>For collecting fish composite sample</b>	scientific collection permit gill net, otter trawl, hook and line (or other device to collect sufficient sample) 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)
<b>For storing and preserving fish composite sample</b>	aluminum foil (solvent rinsed) polyethylene tubing (food-grade) large plastic (composite) bags coolers plastic cable ties dry ice (for preservation) or wet ice (for temporary transport)
<b>For documenting the fish composite sample</b>	NCCA App human health fish tissue sample labels fine-tipped indelible markers (for labels) Tyvek label tag with grommet clear tape strips

#### 14.3.4 SAMPLING PROCEDURE

**Note:** Do not handle any food, drink, sunscreen, or insect repellent 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 14.11)  
**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 Human Health Fish Collection form in the App:
  - 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). If needed, dispatch each fish using the most humane method available.
7. Wrap each whole fish 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 Human Health Fish Collection form in the App. **Be sure to record the fish genus and species 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. Prepare a Sample Identification Label for the outer bag, ensuring that the label information matches the information recorded on the Human Health Fish Collection form in the App. **Be sure to record fish genus and species 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.

#### 14.3.5 SAMPLE STORAGE AND SHIPPING PREPARATION

1. After the fish sample is packaged, keep the sample chilled using either of the following options (**option “a” preferred**):
  - a) (**preferred option**) immediately place the fish sample in a cooler of dry ice until it can be properly frozen (at  $\leq -20^{\circ}\text{C}$  in a laboratory or other interim facility) or shipped to Microbac Laboratories (Baltimore, MD);
    - If fish samples are held on dry ice in the field, the field crew should replenish the supply of dry ice at least daily until the samples can be properly frozen or shipped.
    - Keep all specimens designated for a particular fish composite sample in the same cooler for transport.
  - b) (**alternate option for temporary holding or transport**) immediately place the fish sample in a cooler with wet ice (for temporary holding only).
    - 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 designated for a particular fish composite sample in the same cooler for transport.
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 human health fish samples. Remember to record the tracking number on the Tracking Form in the App before submitting it to NARS IM.

- b) Freeze the fish samples within 24 hours of collection at  $\leq -20^{\circ}\text{C}$  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 14.11 Primary and secondary Great Lakes target species for human health fish tissue collection

PRIMARY HUMAN HEALTH FISH TISSUE TARGET SPECIES		
FAMILY	SCIENTIFIC NAME	COMMON NAME
Centrarchidae	<i>Ambloplites rupestris</i>	Rock bass
	<i>Micropterus dolomieu</i>	Smallmouth bass
	<i>Micropterus salmoides</i>	Largemouth bass
	<i>Pomoxis annularis</i>	White crappie
	<i>Pomoxis nigromaculatus</i>	Black crappie
Cyprinidae	<i>Cyprinus carpio</i>	Common carp
Esocidae	<i>Esox lucius</i>	Northern pike
	<i>Esox masquinongy</i>	Muskellunge
	<i>Esox niger</i>	Chain pickerel
Ictaluridae	<i>Ictalurus punctatus</i>	Channel catfish
Gadidae	<i>Lota lota</i>	Burbot
Moronidae	<i>Morone americana</i>	White perch
	<i>Morone chrysops</i>	White bass
Percidae	<i>Perca flavescens</i>	Yellow perch
	<i>Sander canadensis</i>	Sauger
	<i>Sander vitreus</i>	Walleye
Salmonidae	<i>Coregonus clupeaformis</i>	Lake whitefish
	<i>Oncorhynchus gorbuscha</i>	Pink salmon
	<i>Oncorhynchus kisutch</i>	Coho salmon
	<i>Oncorhynchus tshawytscha</i>	Chinook salmon
	<i>Oncorhynchus mykiss</i>	Rainbow trout
	<i>Salmo salar</i>	Atlantic salmon
	<i>Salmo trutta</i>	Brown trout
Salvelinidae	<i>Salvelinus namaycush</i>	Lake trout
Sciaenidae	<i>Aplodinotus grunniens</i>	Freshwater drum
SECONDARY HUMAN HEALTH FISH TISSUE TARGET SPECIES		
FAMILY	SCIENTIFIC NAME	COMMON NAME
Catostomidae	<i>Carpoides cyprinus</i>	Quillback
	<i>Catostomus catostomus</i>	Longnose sucker
	<i>Catostomus commersonii</i>	White sucker
	<i>Hypentelium nigricans</i>	Northern hogsucker
	<i>Ictiobus cyprinellus</i>	Bigmouth buffalo
	<i>Ictiobus niger</i>	Black buffalo

Centrarchidae	<i>Lepomis cyanellus</i>	Green Sunfish
	<i>Lepomis gibbosus</i>	Pumpkinseed
	<i>Lepomis gulosus</i>	Warmouth
	<i>Lepomis macrochirus</i>	Bluegill
	<i>Lepomis megalotis</i>	Longear Sunfish
Ictaluridae	<i>Ameiurus melas</i>	Black bullhead
	<i>Ameiurus natalis</i>	Yellow bullhead
	<i>Ameiurus nebulosus</i>	Brown bullhead
Salmonidae	<i>Coregonus artedi</i>	Cisco/ lake herring
	<i>Coregonus hoyi</i>	Bloater
	<i>Prosopium cylindraceum</i>	Round whitefish
	<i>Salvelinus fontinalis</i>	Brook trout

## 15 FINAL SITE ACTIVITIES

After sampling, crews complete a visual site assessment and, upon return to the launching location, the field crew must perform a post-measurement calibration check of the multi-parameter sonde, review all data forms and labels, inspect samples, complete tracking forms, ship or store samples, submit tracking forms, submit data forms, clean sampling equipment, and inventory supplies. Activities described in this section are summarized in Figure 15.1.

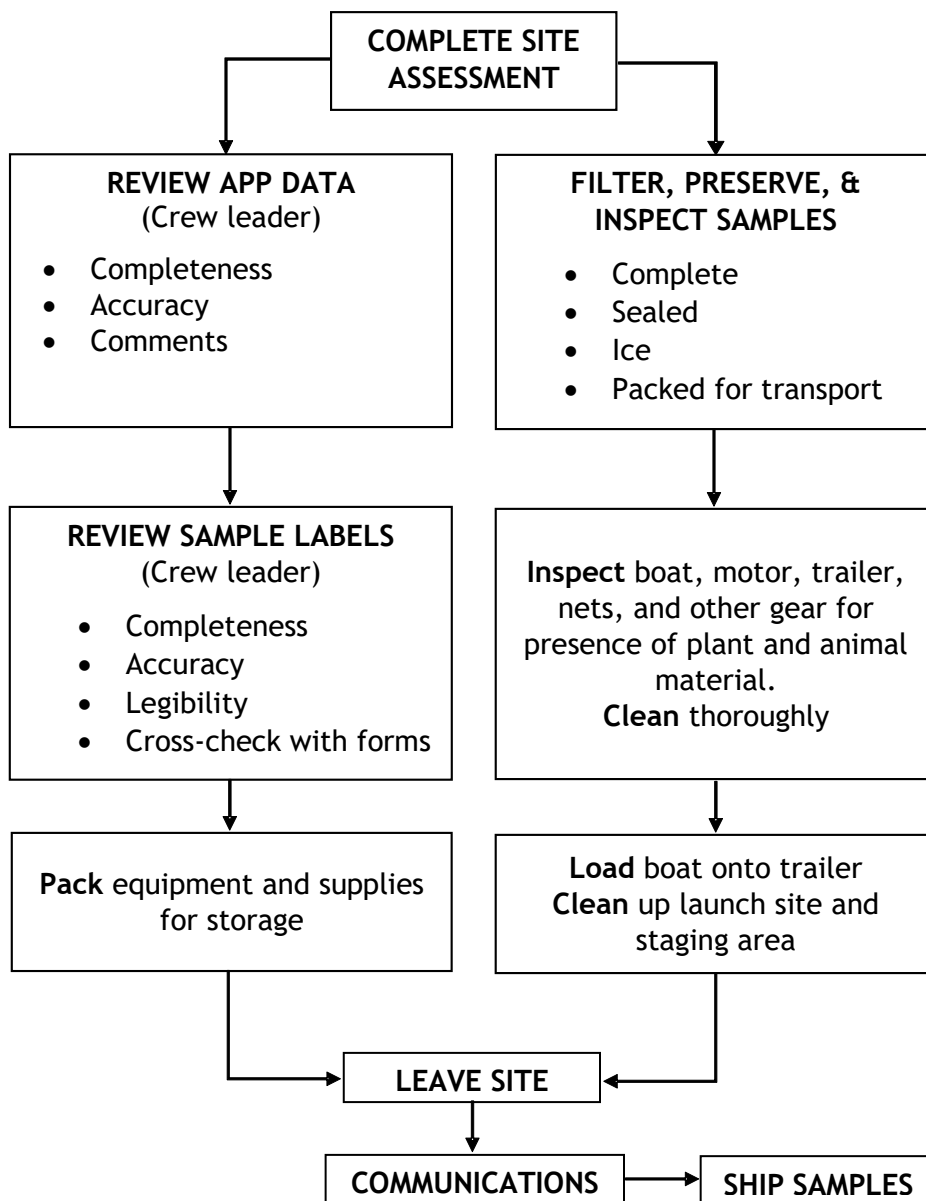


Figure 15.1 Final site activities summary

## 15.1 GENERAL SITE ASSESSMENT

After sampling, complete the Site Assessment form in the App. Record all observations from the site that were noted during the course of the visit. The Site Assessment form is by no means comprehensive, and crews are encouraged to record any additional pertinent observations in the General Assessment form in the App.

### 15.1.1 SHORELINE ACTIVITIES AND DISTURBANCES

Rank shoreline activities and disturbances at the site. Consider only the shoreline that is ecologically significant to, adjacent to, and visible from the X-site. Do not consider the shoreline that is not in the same estuary, waterbody and/or embayment as the X-site. If the shore cannot be seen from the X-site (due to weather conditions or distance), note in the comments section the reason that the shoreline assessment was not possible. If an activity or disturbance is present, fill in the appropriate bubble: “L” for low, “M” for medium or “H” for high indicating the level of each.

*Note: If an activity or disturbance is not observed, do not fill in any bubble. Also be sure to fill in the ‘super bubble’ at the top the activities and disturbances section of the App to verify that blank fields indicate absence of the specific type of activity or disturbance.*

### 15.1.2 SITE CHARACTERISTICS

Record the general characteristics of the site. When assessing site characteristics, look at a 200 m radius around the X-site. Rank the site on a scale of 1 to 5, with 1 indicating “pristine” or “appealing” and 5 indicating “highly disturbed” or “unappealing.” As with other aspects of the general visual assessment, all crew members contribute to the final ranking. Observations of site characteristics will be understandably subjective, but provide valuable information on crew impressions of the overall character of the site. The NCCA analysts use crew observations to help explain data and results. For example, the assessment of visible trash in water (aquatic trash) will provide data for the U.S. EPA’s Trash Free Waters Program. If any items listed are visible in the water from the X-site, fill in a bubble estimating the amount each type of trash. If none are visible, leave the bubbles empty. If possible, list “Other plastic items”, types of “Fishing gear” and “Other” items not accounted for above. Additional information on aquatic trash may be written in the General Assessment area at the crew’s discretion. Document the dominant land use around the X-site. If dominant land use is “forest,” estimate the age class. Document the weather conditions on the day of sampling, as well as any extreme weather conditions just prior to sampling.

*Note: If there is no land within 200 meters of the X-site, leave the dominant land use section blank.*

### 15.1.3 GENERAL ASSESSMENT

Record any additional information and observations in this narrative section. Include observations on biotic integrity, presence of SAV, presence and abundance of endangered and/or exotic species, local anecdotal information, or any other pertinent information about the site or its adjacent areas. Record any observations that may be useful for future data interpretation.

## 15.2 PROCESSING THE FECAL INDICATOR

### 15.2.1 SUMMARY OF METHOD

At each site, crews collect and filter water samples for fecal indicator analyses. Upon receipt of the filters, the lab uses qPCR analysis to quantify Enterococci bacteria trapped on the filter.

### 15.2.2 EQUIPMENT AND SUPPLIES

**Table 15.1** provides the equipment and supplies needed for field crews to filter the fecal indicator sample. The filtering apparatus for this indicator **MUST** be sterile (i.e., a new unused filter funnel with pre-loaded filter is used for each filtration). Because some implements (forceps, centrifuge tube, etc.) will be reused for subsequent filtering of the chlorophyll-*a* sample at the same site, Enterococci must be filtered **before** filtering chlorophyll-*a* samples.

*Table 15.1 Equipment & supplies: Enterococci processing*

<p><b>For processing samples</b></p>	<p>nitrile gloves            sterile screw-cap graduated 50 mL centrifuge tube (for measuring sample)            Filtration flask (side arm, 500 mL)            rubber stopper (#8 white, with 10 mm hole) and small filter funnel adapter            2 filtration units (white base, sterile 100 mL units, includes pre-loaded filter for ENTE) + 1 extra for revisit sites            vacuum pump (electric or hand)            sterile phosphate buffer solution            2 sterile disposable forceps            2 sterile microcentrifuge tubes containing sterile glass beads (chilled on dry ice during pre-sampling activities) + 1 extra for blank filter (at revisit sites)            bubble bag (3 microcentrifuge tubes at revisit sites; 2 at all other sites)            dry ice            cooler</p>
<p><b>For recording measurements</b></p>	<p>NCCA App            fine-tipped indelible markers (for labels)            fecal indicator sample labels (2 or 3 vial labels and 1 bag label)            clear tape strips</p>

### 15.2.3 PROCESSING PROCEDURE - FECAL INDICATOR FILTER BLANK

At revisit sites (sites that will be visited twice in the index period for QA purposes), not only do crews filter the Enterococci samples, but they also prepare a filter blank to be sent to the lab for analysis during both Visit 1 and Visit 2. A filter blank is prepared **prior** to filtering the Enterococci sample. See below for filter blank field processing procedure.

1. Put on nitrile gloves.
2. Set up the sample filtration apparatus on a flat surface and attach the vacuum pump (**Figure 15.2**). Set out:
  - a. 50 mL sterile centrifuge tube,
  - b. One bottle of chilled phosphate buffer solution (PBS), and
  - c. Two sterile forceps.

3. Attach the filter funnel with pre-loaded sterile filter to the filtering flask with reusable rubber stopper and adapter.
4. Measure 20 mL of the chilled PBS with the sterile graduated centrifuge tube and pour into the filter funnel.
5. Replace the cover on the filter funnel and use the vacuum pump to generate a vacuum of no more than seven inches of Hg (or ~3.4 psig). Keep pumping until all liquid is in filtrate collection flask.
6. Remove the filter funnel from the base without disturbing the 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).
7. Insert the filter into the chilled microcentrifuge tube (with beads) open end first (pointed end up). Replace and tighten the screw cap.
8. Record the filter blank information on the Sample Collection Form in the App.
9. Prepare a sample label [Filter: Blank] by recording the volume of PBS filtered.
10. Affix the sample label to the microcentrifuge tube. Do **NOT** place tape on either the label or the cap of the microcentrifuge tube.
11. Insert the tube into the bubble envelope. Place the bubble envelope on dry ice while waiting to process the remaining filters.
12. Proceed to **Section 15.2.4** for processing the water sample collected for Enterococci.

#### 15.2.4 PROCESSING PROCEDURE - FECAL INDICATOR SAMPLE

The filtering apparatus must be sterile when filtering the fecal indicator sample. A separate, sterile, filter funnel pre-loaded with a filter will be provided for each sample collected and processed. Crews must filter and freeze the fecal indicator sample **within six hours of collection**. See below for field processing procedures.

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 the sample filtration apparatus on a flat surface and attach the vacuum pump (**Figure 15.2**). Set out:
  - a. 50 mL sterile centrifuge tube,
  - b. one bottle of chilled PBS, and
  - c. two sterile forceps.
3. Attach the filter funnel with pre-loaded sterile filter onto the filtering flask with reusable rubber stopper and adapter.
4. Gently shake the sample bottle 25 times to mix well.
5. Using the 50 mL sterile graduated centrifuge tube, measure 25 mL of the mixed water sample and pour into the filter funnel.
6. Replace the cover on the filter funnel. Use the vacuum pump to generate a vacuum of no more than seven inches of Hg (or ~3.4 psig). Keep pumping until all liquid is in the filtrate collection flask.
7. If the first 25 mL volume passes readily through the filter, add another 25 mL and continue filtration. If the filter clogs before completely filtering the first or second

- 25 mL volume, discard the filter and, using a new sterile filter funnel with pre-loaded filter, repeat the filtration using a lesser volume.
8. Pour approx. 10 mL of the chilled PBS into the same graduated centrifuge tube used for measuring the water sample. Cap the tube and shake five times. Remove the cap and pour the rinse into the filter funnel to rinse the filter.
  9. Filter the rinsate and repeat with another 10 mL of chilled PBS.
  10. Remove the filter funnel from the base without disturbing the 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).
  11. Insert the filter into the chilled microcentrifuge tube (with beads)—open end first (pointed end up). Replace and tighten the screw cap.
  12. Record the volume of water sample filtered through the filter (minimum is 25 mL, target is 50 mL) and the volume of PBS used to rinse each filter on the Sample Collection form in the App. Record the filtration start time (beginning of first filter) and finish time (end of second filter) for the sample.
  13. Prepare a corresponding sample label (Filter:1 or Filter:2), ensuring that the volume filtered on the label matches the information recorded on the Sample Collection form in the App.
  14. Affix the sample label to the microcentrifuge tube. Do **NOT** place tape on either the label or the cap of the microcentrifuge tube.
  15. Insert the tube into the bubble envelope. Place the bubble envelope on dry ice while processing the second filter.
  16. Repeat steps 1 to 15 for the second filter, using a new sterile filter funnel with pre-loaded filter. It is important that the same sample volume be filtered through each filter.
  17. Prepare an exterior label for the bubble envelope [ENTEROCOCCI (ENTE) - BAG], ensuring that the label information (site ID, date, visit #, volume filtered, sample ID) matches the information recorded on the Sample Collection form in the App. Affix the exterior label on the outside of the bubble envelope and cover with clear plastic tape.
  18. Place the bubble envelope in the zip-top bag and then on dry ice for preservation during transport and shipping.

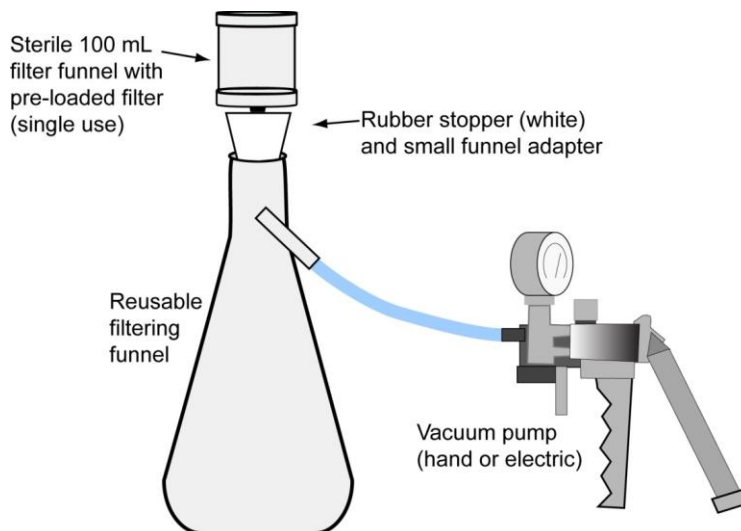


Figure 15.2 Filtering set-up for Enterococci filtering

## 15.3 PROCESSING THE CHLOROPHYLL-A & DISSOLVED NUTRIENTS INDICATORS

### 15.3.1 SUMMARY OF METHOD

At each site, crews collect and filter water samples for chlorophyll-*a* and dissolved nutrient analyses. The chlorophyll-*a* sample is submitted to the lab as residue on a Whatman GF/F filter. Upon receipt of the filters, the lab extracts the pigment from the filter and quantifies it using flourometry. A portion of the filtrate produced from collecting the chlorophyll-*a* sample is submitted to the laboratory and processed for dissolved nutrients. In order to avoid cross-contamination, a new filter funnel will be used at each site. This filter funnel is provided in each site kit.



### 15.3.2 EQUIPMENT AND SUPPLIES

Table 15.2 Equipment & supplies: chlorophyll-*a* & dissolved nutrients processing

<p><b>For filtering chlorophyll-<i>a</i> sample</b></p>	<p>Filters - Whatman 47 mm glass fiber GF/F 0.7 <math>\mu</math> - Box          Nutrients filtering chamber          Silicone grease          Filtration unit (blue base filter funnel, 250 mL unit)          rubber stopper (#8 blue, with 15 mm hole) and large filter funnel adapter          vacuum pump (electric or hand)          DI water          nitrile gloves          forceps          graduated cylinder (250 mL)</p>
<p><b>For recording measurements</b></p>	<p>NCCA App          chlorophyll-<i>a</i> &amp; dissolved nutrients sample labels          fine-tipped indelible markers (for labels)          clear tape strips</p>
<p><b>For sample collection and preservation</b></p>	<p>Centrifuge tube (sterile, screw-top, 50-mL) in leak-proof bag          aluminum foil square          HDPE bottle (250 mL, white)          cooler with dry ice          electrical tape</p>

### 15.3.3 PROCESSING PROCEDURE

Below presents the field procedures for processing chlorophyll-*a* and dissolved nutrient samples. The steps below describe using the nutrients filtering chamber supplied in the base kit. Crews have the option of using a side-arm filtering flask or other filtrate collection device in place of the nutrients chamber. If a flask or other device is used, it is important to NOT use the same flask/device as is used for the filtering of Enterococci. Doing so will lead to potential contamination of the nutrients sample with phosphate buffer used to rinse the Enterococci filter. If a flask or other filtrate collection device is used to collect the filtered nutrients sample (as opposed to collecting the sample directly into the nutrients bottle with a chamber), the collection device must be rinsed three times with filtered sample water before allowing any sample to enter the bottle.

**Note:** Crews must make every attempt to process chlorophyll-*a* samples in subdued light, out of direct sunlight.

1. Complete the NUTS sample label with Site ID, date collected, and visit number.
2. Attach the completed label to the 250 mL clear HDPE sample bottle and cover with clear plastic tape.
3. Set up the nutrients filtering chamber on a flat surface, insert the sample bottle into the chamber, and attach the vacuum pump (**Figure 15.3**).
4. Put on nitrile gloves.
5. Crews will use a 250 mL filter funnel (with blue bottom), rubber stopper, and adapter that are specifically designated for chlorophyll filtering (i.e., not the same ones used for the Enterococci filtering). A new filter funnel will be provided in each site kit and should not be reused. The stopper and adapter are to be cleaned with DI water between sampling events. Prior to filtration of the sample, rinse the filter funnel adapter and graduated cylinders three times with DI water. After assembling the

filtering apparatus and attaching the filter funnel to the nutrients chamber with the correct stopper and adapter, remove the cup portion of the filter funnel from the blue base. Remove the pre-loaded filter (which has a faint grid pattern on it) but leave the white support pad in place.

6. Use clean forceps to place a Whatman GF/F 47 mm 0.7 micron filter on the support pad with the gridded/pressed side of the filter facing down, making sure both the support pad and filter are centered on the base.
7. Reattach the funnel portion of the filter funnel to the base by pressing it straight down firmly until it snaps into place. This will firmly hold the filter in place.
8. Remove the 2 L amber chlorophyll-*a* collection bottle from cooler and shake to mix the sample. Using the graduated cylinder, measure and pour 250 mL of water into the filter holder, replace the cover, and use the vacuum pump to draw a small portion of the sample through the filter. Do not exceed seven inches of Hg of vacuum ~3.4 psig or a filtration duration of more than five minutes for a single sample volume, to avoid cell damage or loss of contents during filtering.
  - a. If the lid of the filtration chamber does not seal adequately, apply a small amount of silicone grease to the gasket on the underside of the lid.
  - b. Applying downward pressure to the lid during initial application of vacuum will also help the lid seal.
9. Use the first 10-20 mL of filtrate to rinse the 250 mL sample bottle and discard the rinsate. Be sure to cap the bottle and rotate it so that the filtered water contacts all the surfaces. Replace the bottle and chamber cap and continue filtering. Repeat the rinse of the sample bottle with an additional two rinses of filtered site water and discard the rinsate.
10. If the filter clogs before 250 mL of site water will pass through the filter, discard the filter and water remaining in the filter funnel, rinse the filter funnel with DI water, install a new filter, and repeat the procedures using 100 mL of site water.
11. Observe the filter for readily visible color. If there is visible color, proceed to the next step; if not, filter additional aliquots until color is visible on the filter or until a maximum of 2,000 mL have been filtered.
12. After collecting 250 mL of filtered site water in the dissolved nutrients sample bottle, remove the 250 mL HDPE bottle. Replace the lid and seal tightly with electrical tape. Submit this filtrate for dissolved nutrient analyses.
13. Move the filter funnel and adapter to a side-arm filter flask to complete the filtering process. Additional filtrate will be discarded.
14. Record the dissolved nutrients sample information on the Sample Collection form in the App. Place the sample on wet ice.
15. After achieving a readily visible stain on the filter and collecting the filtrate for dissolved nutrient analyses, record the actual sample volume filtered in the Chlorophyll-*a* section on the Sample Collection form in the App and on the sample label.
16. Attach the completed label to the 50 mL centrifuge tube and cover with clear plastic tape.
17. Rinse the graduated cylinder and upper portion of the filter funnel 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.

18. 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. Place the folded filter into the 50 mL screw-top centrifuge tube used previously for measuring the Enterococci sample and replace the cap.
19. Tighten the cap as tightly as possible. The cap will seal tightly after an additional  $\frac{1}{4}$  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 electrical tape.
20. Wrap the 50 mL tube in a foil square and place in the provided zip-top plastic bag.
21. Close the plastic bag and place it on dry ice.
  - i. *NOTE: if the chlorophyll filtering process did not yield at least 250 mL of filtered site water, install a new GF/F filter and continue filtering site water until 250 mL of filtrate has been collected for the dissolved nutrients sample. Be sure to collect the filtrate prior to any rinsing of the filter funnel with DI water as directed in Step 17.*

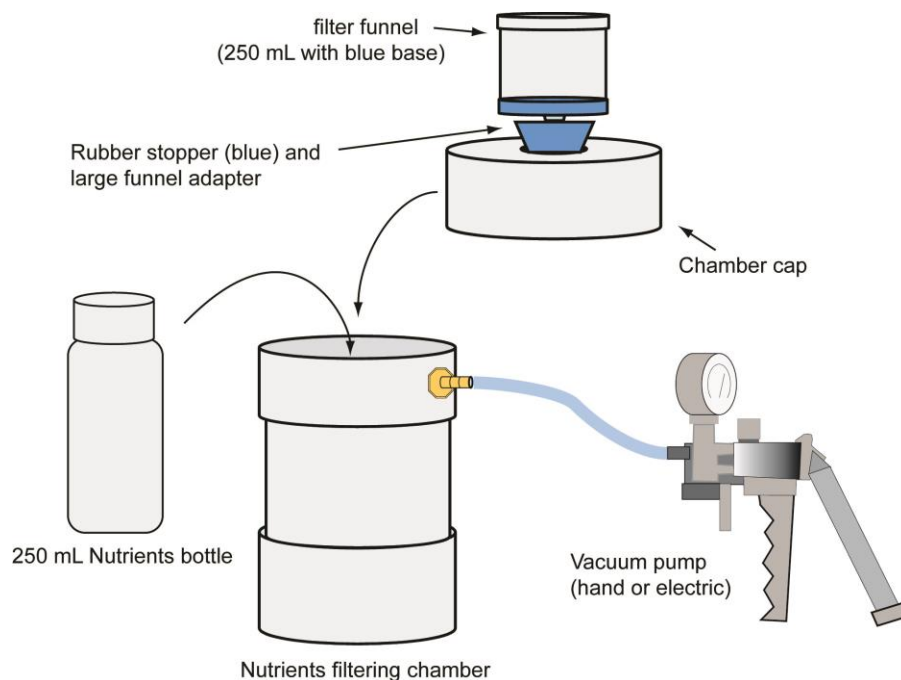


Figure 15.3 Filtering set-up for chlorophyll-a and nutrients filtering

#### 15.4 POST-MEASUREMENT CALIBRATION CHECK OF MULTI-PARAMETER SONDE

After all *in situ* measurements have been completed for the sampling day, the crew must perform a post-measurement calibration check of the multi-parameter sonde. To do this, measure the pH and conductivity of one of each of the respective calibration standards that were used earlier in the day to calibrate the instrument. Record these values in the Post-Measurement Calibration Check section of the Calibration/QA form in the App. If

significant drift is detected as defined by the manufacturer, the meter may need service and data collected since the last successful calibration and post-measurement calibration check should be flagged. Discontinue use of any meter that is not functioning properly.

### 15.5 FIELD DATA & TRACKING FORM REVIEW

The Field Crew Leader is ultimately responsible for reviewing the App submission and/or all data forms for completeness, legibility, accuracy, and consistency. The following are some checks to perform on the data forms:

- Ensure that all required data forms for the site have been fully completed.
- Confirm that the Site ID, visit number, and date of visit are correct.
- Ensure that the water chemistry sample ID has been entered on the Tracking Form in the App.
- Verify the accuracy and clarity of all recorded information.
- Ensure that any pertinent data explanations are entered into the respective comments sections.
- Ensure that comments are clear, with no “shorthand” or abbreviations.
- Make sure that any targeted sample that was not collected has a comment recorded as to why the sample was not collected.
- Ensure that shipping/airbill tracking numbers have been recorded in the Tracking Form in the App prior to shipping samples.

If information is missing from the forms, the Field Crew Leader must complete the missing sections. When utilizing the NCCA App, the Field Crew Leader must ensure that the data is submitted. The receipt of a submission is a confirmation that the data has been reviewed by the Field Crew Leader.

### 15.6 SAMPLE PACKAGING AND LABEL REVIEW

All samples must be appropriately preserved and packaged for transport. The following are some checks to perform on the labels:

- All samples are collected. If *obtainable* samples are missing, the crew must reschedule a site visit or return to the site that same day to complete collection of the missing samples.
- All samples are labeled.
- All labels are complete, legible, accurate, and consistent.
- Although the labels are preprinted with the sample IDs, review the labels and forms in the App to ensure consistent sample ID information was utilized.
- Each label is covered with clear plastic tape (except those on the ENTE sample vials).
- Inspect the integrity of each sample container; be sure there are no leaks. Make sure that all sample containers are properly sealed.
- Verify that all sample containers are properly preserved for storage or immediate shipment.

If information is missing from the labels, the Field Crew Leader must complete the missing sections. The Field Crew Leader must also verify the integrity of all samples. The Field Crew Leader must reconcile any disagreements between sample IDs on the data forms in the NCCA App and labels before tracking forms are transmitted to NARS IM and samples are packaged and sent to the labs.

## 15.7 SAMPLE SHIPMENT & TRACKING FORM SUBMITTAL

Each shipping group has been assigned a “T” number to help crews identify the correct section of the Tracking Form in the App to use when sending samples. This “T” number is located above each of the sample groups in the Tracking Form in the App. Crews will also find reference to the same “T” numbers on the individual samples labels, on the packing slips that crews will include in the coolers, and on the top of the pre-printed FedEx return labels provided in the site kits.

Crews submit tracking information via the NCCA App and include packing slips in the coolers when they send samples to the labs. Refer to **Appendix C: Shipping and Tracking Guidelines** for additional details on preparing samples for shipping.

### 15.7.1 TIME-SENSITIVE SAMPLES

The field crew must ship or deliver time-sensitive samples (i.e., water chemistry (CHEM), chlorophyll-*a* (WCHL), and dissolved nutrients (NUTS)) to the appropriate analytical laboratory (WRS Corvallis or approved state lab) so that the samples will arrive within 48 hours of collection. Therefore, crews must send them via Priority Overnight shipping, preferably the same day as collection, but no later than the following day. **Reminder: FedEx does not deliver shipments on Sunday or start shipments on Saturday or Sunday, so you must ensure samples are shipped by Friday afternoon to allow for a Saturday delivery. Be sure to verify the last EXPRESS drop off time at the FedEx facility you plan to use.**

The Field Crew Leader or his/her designee will complete the T-1 section of the Tracking Form in the App for the samples being shipped. Shipping details which include the destination lab, date shipped, FedEx airbill number, sender, and sender’s phone number will be entered for the group of samples. Once details are saved in the App, a date will appear in the shipped column of the Tracking Form.

The Field Crew Leader or his/her designee will submit the Tracking Form via the NCCA App along with any and all data forms. This initial submission serves as both the notification of a sampling event and the tracking of the designated samples. 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).

The field crew will place the samples and the appropriate packing slip (in a waterproof bag or plastic sleeve) in the cooler provided with the site kit. The field crew will attach the appropriate pre-addressed FedEx airbill from the site kit marked for the WRS 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 to ensure its actual pick up. **If there are samples listed on the packing that are not included in the cooler, line out the sample IDs for the samples not included.**

### 15.7.2 OTHER SAMPLES

Samples that are less time sensitive will be shipped in batches, according to the chart in **Appendix C: Shipping and Tracking Guidelines**. See **Section 16: Post-Sampling Activities** for further guidance.

## 15.8 EQUIPMENT CLEANUP & CHECK

After each sampling event, crews will need to clean all sampling gear using the guidance provided in **Table 15.3**. These steps are for general cleaning and do not include any additional steps necessary to decontaminate equipment for the known or suspected presence of nuisance species.

*Table 15.3 General cleaning of sampling gear after each site*

Equipment Type	Cleaning Method
Water collection and filtering equipment	Rinse 3 times with DI water (no detergent)
Sediment collection/processing equipment	Wash with a phosphate-free detergent such as Liquinox, rinse with DI water
Sieve box/bucket	Use copious amounts of forceful water and a stiff brush to clean the sieve. Be sure to rinse the brush between each sieve cleaning.
Fish collection/processing gear	Clean with 1% bleach solution, rinse with tap water and/or DI water

Field crews must take appropriate precautions to avoid transfer of national and regional invasive species of concern. Nuisance species of concern in the U.S. include zebra mussels (*Dreissena polymorpha*), mitten crabs (*Eriocheir sinensis*) and Eurasian ruffe (*Gymnocephalus ceinuus*). In the Great Lakes, Viral Hemorrhagic Septicemia (VHS) is an invasive and deadly fish virus that is threatening Great Lakes fish. VHS was identified as the cause of large fish kills in Lakes Huron, St. Clair, Erie, Ontario and the St. Lawrence River in 2005 and 2006. To reduce the risk of transferring nuisance species and pathogens, all equipment and gear must be cleaned and disinfected prior to traveling over land from one field site to another. For specific techniques to disinfect boats and gear in the Great Lakes, please see **Section 15.8.3**.

Online resources regarding invasive species:

- Aquatic Nuisance Species Task Force (<http://www.anstaskforce.gov>)
- U.S. Geological Survey Nonindigenous Aquatic Species website (<http://nas.er.usgs.gov>)
- *Protect Your Waters* website, co-sponsored by the U.S. Fish and Wildlife Service (<http://www.protectyourwaters.net/hitchhikers>)
- Sea Grant Program (<http://www.sgnis.org>)

- USDA Animal and Plant Health Inspection Service (<http://aphis.usda.gov>)

#### 15.8.1 BOAT & TRAILER CLEANUP

While your organizations likely have protocols in place to account for these precautions, the following are some procedures and checks to perform on your equipment:

1. Load the boat on the trailer.
2. Drain all bilge water from the boat.
3. Inspect the boat, motor, and trailer for evidence of weeds and other macrophytes.
4. Clean the boat, motor, and trailer as completely as possible before leaving the launch site.
  - Follow any state or other requirements associated with nuisance species, pathogens and/or viruses.

#### 15.8.2 POST SAMPLING EQUIPMENT CARE

1. Inspect sampling gear (seines, dip nets, sieves, foul weather gear, boots, etc.) for evidence of mud, snails, plant fragments, algae, animal remains, or debris. Rinse and remove using brushes or other tools. Use one of the procedures below to disinfect gear if necessary. Let dry.
2. Pack all equipment and supplies in the vehicle and trailer for transport.
3. Keep equipment and supplies organized so they can be inventoried using the equipment and supply checklists (**Appendix A: Equipment and Supplies Lists**).
4. 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.

#### 15.8.3 ADDITIONAL DECONTAMINATION INFORMATION

Additional precautions to prevent transfer of Whirling Disease spores, New Zealand mudsnails, and amphibian chytrid fungus are important for Great Lakes sites. Before visiting the site, research the site and determine if it is in an area where one of these organisms are known to exist. Contact the local or State fishery biologist to confirm the presence or absence of these organisms.

If the site is listed as “positive” for any of the organisms, or no information is available, *avoid using felt-soled wading boots*. After sampling, disinfect **all** fish and benthos sampling gear and all other equipment that came into contact with water or sediments (i.e., waders, boots, etc.) by one of the following procedures:

##### Option A:

1. Soak gear in a 10% household bleach solution for at least 10 minutes, or wipe or spray on a 50% household bleach solution and let stand for five minutes.
2. Rinse with tap water (do not use sea or lake water) and remove remaining debris.

3. Place gear in a freezer overnight, soak in a 50% solution of Formula 409® antibacterial cleaner for at least 10 minutes or soak gear in 120°F (49°C) water for at least 1 minute.
4. Dry gear in direct sunlight (at least 84 °F) for at least four hours.

Option B:

1. Soak gear in a solution of Sparquat® (4-6 oz. per gallon of water) for at least 10 minutes (Sparquat is especially effective at inactivating whirling disease spores).
2. Place gear in a freezer overnight or soak in 120°F (49°C) water for at least one minute.
3. Dry gear in direct sunlight (at least 84 °F) for at least four hours.

Clean and dry other equipment prior to storage.

- Rinse coolers with clean water to remove any dirt or debris on the outside and inside.
- Make sure water quality meter probes are rinsed with deionized water and stored moist.
- Rinse all equipment used to collect and filter water samples three times with deionized water. Place sampling equipment in a clean location for use at the next site.
- Check nets for holes and repair or locate replacements.
- Inventory equipment and supply needs and relay orders through the fillable PDF Supply Request form.
- Remove GPS and multi-parameter sonde, and set up for pre-departure checks and calibration. Examine the oxygen membranes for cracks, wrinkles, or bubbles. Replace if necessary, allowing sufficient time for equilibration.
- Recharge/replace batteries as necessary.
- Replenish fuel and oil.
- If a commercial car wash facility is available, thoroughly clean vehicle and boat (hot water pressurized rinse—no soap).

**Note:** Handle and dispose of disinfectant solutions properly, and take care to avoid damage to lawns or other property.



## 16 POST-SAMPLING ACTIVITIES

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### 16.1 SAMPLE SHIPPING

Samples that are less time sensitive will be shipped in batches, according to the chart in **Appendix C: Shipping and Tracking Guidelines**. The Field Crew Leader or his/her designee will complete the pertinent section(s) (e.g., T-2, T-3, T-4, and/or T-5) of the Tracking Form in the App for the samples being shipped. Shipping details which include the destination lab, date shipped, FedEx airbill number, sender, and sender's phone number will be entered for the group of samples. Once details are saved in the App, a date will appear in the shipped column of the Tracking Form.

The Field Crew Leader or his/her designee will submit the Tracking Form via the NCCA App. 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).

The Field Crew Leader will place the samples and the correct batch packing slip (in a waterproof bag or plastic sleeve) in a requested batch shipment cooler. **If there are samples listed on the packing that are not included in the cooler, line out the sample IDs for the samples not included.** The Field Crew Leader will attach the appropriate pre-addressed FedEx airbill from the site kit 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 its actual pick up. Once the package is in the possession of FedEx, the IM Team and FLC will track the package to its destination and take steps necessary to ensure its timely delivery.

### 16.2 DATA SUBMITTAL

For crews utilizing the mobile App, after the Field Crew Leader has reviewed form content at the end of your sampling day, click the SUBMIT menu button and choose the form(s) 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](mailto:NARSFieldData@epa.gov). Copy yourself, 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.

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 automatically take the place of previous data. It is not necessary to re-submit data forms that were unchanged however.

### 16.3 DATA AND TRACKING REMINDERS

It is very important to submit the data and tracking forms **immediately after every sampling event**. Prompt submissions allow the FLC to closely track sampling progress. More importantly, it enables NARS IM to track samples that were collected at each site versus those that were not, and to immediately track the shipment of the time-sensitive samples after each sampling event.

The field crews must promptly report any field sampling problems to the FLC and report sample tracking or data reporting problems to NARS IM. They will follow up with the EPA NCCA 2020 Lead throughout the sampling period.

The EPA Logistics Coordinator serves as the central point of contact for information exchange among field crews, the management and QA staff, the NARS IM staff, and the public. The EPA Logistics Coordinator and Contractor FLC contact information can be found on **Table 1.1** of this manual.

### 16.4 SITE EVALUATION SPREADSHEET SUBMITTAL

Throughout the field season or at the end of the field season, EPA HQ needs field crews to submit their updated Site Evaluation Spreadsheets. These are critical to determining site weights used in data analysis. Please submit these forms to the FLC and EPA Logistics Coordinator within two weeks of completion of your last site.

## 17 FIELD QUALITY CONTROL

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The NCCA program requires that all cooperators and field crews follow strict QA and QC guidelines. Standardized training and data forms set the foundation to help ensure that data quality standards for field sampling are met. In addition, repeat sampling and field evaluation and assistance visits address specific aspects of the data quality standards for the NCCA.

### 17.1 STANDARDIZED TRAINING

All Field Crew Leaders must attend a formal three day NCCA training prior to participating in field sampling for the NCCA and all field crew members are encouraged to attend. The training, which is divided into classroom and hands-on field sessions, is designed to reduce sampling variability, and subsequently ensure data comparability from crew to crew and site to site. Standardized training allows the EPA to collect field crew input that will help to identify potential sampling pitfalls and troubleshoot solutions. The entire three day training session is required to qualify a crew for sampling activities.

### 17.2 STANDARDIZED FIELD DATA COLLECTION APP

All field crews collect and record data using an app. The app serves several purposes. First, it ensures that crews measure and record the same parameters. Second, it promotes efficient data entry and minimizes the opportunities for data transcription errors. Finally, the app facilitates field data quality control reviews when data are received at NARS IM.

Paper field forms and the NARS App have been developed for data collection and contain the same data.

### 17.3 REPEAT SAMPLING

The NCCA collects temporal repeat samples in order to estimate site measurement and index period variance. Repeat sampling provides data that can be used to evaluate the potential for the NCCA design to estimate status and detect trends in the target site population.

During the field season, crews will revisit approximately 7% of the target sites as designated in the EPA site list with “RVT2” in the panel code. In order to ensure that sampling procedures are as comparable as possible from the first visit to the second visit, the same field crew who initially sampled the site also conducts the revisit. During site revisits, crews collect the full set of samples and *in situ* measurement parameters (except eco fish and fish plug samples, which are targeted only on the first visit). At Great Lakes revisit sites, crews that are unsuccessful at collecting the human health fish composite sample (HTIS) during visit 1 are expected to attempt the collection of that sample during visit 2. When sampling sites are identified as revisit sites, crews collect Enterococci filter blanks during both the initial visit and the revisit. The crews must always collect the filter blanks before the sample is filtered. See **Section 15.2.3** for the procedure for collecting filter blanks.

The NCCA identifies sites targeted for repeat visits in the state's site draw. The number of repeat visit sites varies from state to state, depending on the number of base sites drawn within the state. If a site selected for repeat sampling is dropped, then the alternate site assigned to replace it becomes the revisit site. The time elapsed between the initial and repeat site visits should be as long as possible within the index period, but not shorter than two weeks.

#### **17.4 FIELD EVALUATION AND ASSISTANCE VISITS**

A rigorous program of field and laboratory evaluation and assistance visits supports the quality assurance and control for the NARS. The following sections focus only on the field evaluation and assistance visits.

By coupling assistance visits conducted early in the data collection process with uniform training, sampling variability associated with specific implementation or interpretation of the protocols will be significantly reduced. Field evaluation and assistance visits provide an opportunity to ensure that crews follow field procedures and meet minimum quality control requirements. In addition, assistance visits allow for uniform evaluation of the standard NCCA data collection methods. When widespread problems or confusion surround a given method, the information from assistance visits contributes to refining the method for sites that are yet to be sampled and in future field manuals.

The field evaluators observe and review the information listed on the Field Evaluation and Assistance Visit Checklist. An assistance visit has been scheduled to evaluate each unique crew collecting and contributing data under this program. If unforeseen events prevent the EPA from evaluating every crew, the NCCA Quality Assurance Coordinator (QAC) will rely on the data review and validation process to identify unacceptable data that will not be included in the final database. If inconsistencies cannot be resolved, the QAC may contact the Field Crew Leader for clarification.

#### 17.4.1 SPECIFICATIONS FOR QC ASSURANCE

Field evaluation and assistance personnel are trained in the specific data collection methods detailed in this FOM. A plan and checklist for field evaluation and assistance detail the methods and procedures that will be evaluated. The plan and checklist are included as Attachment D in the QAPP and will be posted on the SharePoint site for crews to access. **Table 17.1** summarizes the plan, the checklist, and corrective action procedures.

*Table 17.1 General information noted during field evaluation*

<b>Field Evaluation Plan</b>	<ul style="list-style-type: none"> <li>• Regional Coordinators or another assigned trained individual arrange the field assistance visit with each field crew, ideally within the first two weeks of sampling.</li> <li>• The Evaluator observes the performance of a crew through one complete set of sampling activities.</li> <li>• If the crew misses or incorrectly performs a procedure, the Evaluator notes it on the checklist and immediately points it out so the mistake can be corrected on the spot.</li> <li>• The Evaluator reviews the results of the evaluation with the field crew before leaving the site, noting positive practices as well as problems.</li> </ul>
<b>Field Evaluation and Assistance Visit Checklist</b>	<ul style="list-style-type: none"> <li>• The Evaluator observes all pre-sampling activities and verifies that equipment is properly calibrated and in good working order, and that NCCA protocols are followed.</li> <li>• The Evaluator 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.</li> <li>• The Evaluator confirms that the field crew has followed NCCA protocols for locating the site.</li> <li>• The Evaluator observes the complete set of sampling activities, confirming that all protocols are followed.</li> <li>• The Evaluator will record responses or concerns, if any, on the Field Evaluation and Assistance Visit Checklist.</li> </ul>
<b>Corrective Action Procedures</b>	<ul style="list-style-type: none"> <li>• If the 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 and minimize adverse effects on data quality.</li> <li>• If the Evaluator finds major deficiencies in the field crew operations, the Evaluator must contact the NCCA QA Coordinator immediately (e.g., within 24-48 hours) so that additional correction actions can be taken.</li> </ul>

The EPA anticipates that evaluation and assistance visits will be conducted with each field crew early in the sampling and data collection process, and that corrective actions will be conducted in real time. The role of the Evaluator is to provide additional training and guidance so that the procedures are being performed in a manner consistent with the Field Operations Manual, all data are recorded correctly, and paperwork is properly completed at the site. If the field crew misses or incorrectly performs a procedure, the Evaluator will note the error on the checklist, immediately point it out and direct the crew to correct it on the spot.

#### 17.4.2 REPORTING

Upon completion of the sampling operations, the Evaluator will review the results of the evaluation with the Field Crew before leaving the site (if practicable). The evaluator will note positive practices and problems (termed weaknesses if they *might* affect data quality or deficiencies if they would adversely affect data quality). The Evaluator ensures that all

crew members understand the findings and can perform the procedures properly in the future. The Evaluator will record field crew responses or concerns, if any, on the Field Evaluation and Assistance Visit Checklist. After the Evaluator completes the Field Evaluation and Assistance Visit Checklist, including a brief summary of findings, all field crew members must read and sign off on the evaluation.

If after directing the crew to correct problems, 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. If the Evaluator finds major deficiencies in the field crew operations (e.g., major misinterpretation of protocols, equipment or performance problems that will adversely affect data quality), they must be reported to the following QA official:

- Brian Hasty, EPA Field Logistics Coordinator

The Field Logistics Coordinator will contact the NCCA QA Lead and the NCCA Project Lead to determine the appropriate course of action. Data records from sampling sites previously visited by this field crew will be checked to determine whether any sites must be resampled.

## 18 LITERATURE CITED

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Aquatic Nuisance Species Task Force (<http://www.anstaskforce.gov>)

U.S. Geological Survey Nonindigenous Aquatic Species website (<http://nas.er.usgs.gov>)

*Protect Your Waters* website, co-sponsored by the U.S. Fish and Wildlife Service  
(<http://www.protectyourwaters.net/hitchhikers>)  
Sea Grant Program (<http://www.sgnis.org>)  
USDA Animal and Plant Health Inspection Service (<http://aphis.usda.gov>)

The Code of Federal Regulations (49 CFR Section 173.150)

National Coastal Condition Assessment 2015: Quality Assurance Project Plan (EPA-841-R-14-005)  
National Coastal Condition Assessment 2020: Site Evaluation Guidelines (EPA-841-R-14-006)  
National Coastal Condition Assessment 2020: Field Operations Manual (EPA-841-R-14-007)  
National Coastal Condition Assessment 2020: Laboratory Operations Manual (EPA-841-R-14-008)



## APPENDIX A: EQUIPMENT AND SUPPLIES LISTS

### BASE KIT

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

**Note:** *Sodium thiosulfate tablets, filters, 1 Liter HDPE bottles, aluminum foil squares, and disposable nitrile gloves will be provided in the base kit; you may order more throughout the field season if needed.*

Kit Type	Item	Quantity	Protocol(s)
Regular	Aluminum foil squares - pack of 25	2	Chlorophyll A
Regular	Antibiotic Salve	1	Fish plug
Regular	Aspirator bulb	1	Fish Plug
Regular	Centrifuge tube stand	1	Chlorophyll A
Regular	Centrifuge tubes (screw-top, 50-mL) (extras)	10	Enterococci, Chlorophyll A
Regular	Clear tape strips - packs of 25	6	General
Regular	Electrical tape, plastic - roll	4	General
Regular	FedEx airbills (non-chilled batch, frozen batch, data)	10	Shipping
Regular	FedEx Dangerous Goods label (Class 9, for dry ice shipments)	10	Shipping
Regular	Filters - Whatman 47 mm glass fiber GF/F 0.7 $\mu$ - Box	1	Chlorophyll A
Regular	Filtration flask (side arm, 500 mL)	1	Chlorophyll A, Dissolved Nutrients
Regular	Filtration unit (Sterile blue base 250 mL with funnel, cap, and filter holder) - spares	5	Chlorophyll A, Dissolved Nutrients
Regular	Filtration unit (white base, sterile, 100 mL units, includes pre-loaded filter for ENTE) - spares	5	Enterococci
Regular	Filtration unit adapter (large)	3	Chlorophyll A
Regular	Filtration unit adapter (small)	3	Enterococci
Regular	Fish weigh scale, case, and spare batteries†	1	Fish plug
Regular	Forceps (fine-tipped, watchmakers types)	1	Benthics
Regular	Forceps (sterile, disposable) - spares	5	Enterococci, Chlorophyll A
Regular	Funnel (wide-mouth)	1	Benthics
Regular	Gloves (nitrile) - box	1	General
Regular	Graduated cylinder (100 mL)	1	Benthics
Regular	Graduated cylinder (250 mL)	1	Chlorophyll A
Regular	HDPE bottle (1 L, white, wide-mouth) (extras)	6	Benthics
Regular	HDPE bottle (2 L, amber)†	1	Chlorophyll A
Regular	Lowering line (100') with clips (marked in 0.5 m intervals)†	1	Depth Secchi
GL Only	Lugol's Solution	1	Phytoplankton
Regular	Microcentrifuge tubes containing glass beads (extras or for filter blanks)	5	Enterococci

Kit Type	Item	Quantity	Protocol(s)
Regular	Nutrients filtering chamber†	1	Dissolved Nutrients
Regular	Packing tape (extra rolls)	2	Shipping
Regular	Packing tape (on holder)	1	Shipping
Regular	PAR Meter with frame, sensors and LI-1400 dataloger	1	Water Profile
Marine Only	Peristaltic pump with flexible gas-impermeable tubing installed	1	Total Alkalinity
GL Only	Pipet (10 mL)	2	Phytoplankton
GL Only	Pipet Bulb	1	Phytoplankton
Regular	Plastic cable ties - spares	10	Eco Fish Tissue
Regular	Rubber bands (spares)	20	Sediment collection
Regular	Rubber stopper (#8 blue, with 15 mm hole)	2	Chlorophyll A, Dissolved Nutrients
Regular	Rubber stopper (#8 white, with 10 mm hole)	2	Enterococci
Regular	Rubbermaid Roughneck tote (3 gallon)	1	General
Regular	Secchi disk (20 cm diameter, weighted)†	1	Water Profile
Regular	Shipping supplies organizer	1	Shipping
Regular	Sieve bucket (500 µm)†	1	Benthics
Regular	Silicone grease	1	General
Regular	Sodium thiosulfate tablets (in vial)	1	Enterococci
Regular	Spoon, stainless steel (15")	1	Sediment collection
Regular	Tyvek tag with grommet - spares	10	Eco Fish Tissue
GL Only	Underwater video camera kit (includes frame, 2 cameras, 2 lights, lowering line with float, tools, Micro SD cards, etc.)	1	Underwater Video
Regular	Vacuum hand pump and clear plastic tubing†	1	Enterococci, Chlorophyll A, Dissolved Nutrients
Regular	Wash bottle (1 L Nalgene), One for ambient water, One for DI	2	Sediment collection, Chlorophyll A, Dissolved Nutrients
Marine Only	Weight, stainless steel pipe, for TA intake tube	1	Total Alkalinity
Regular	Zip-top bags (2 gallon for ecofish) - extras	10	Eco Fish Tissue
Regular	Zip-top bags (sandwich size) - for ecofish labels - extras	10	Eco Fish Tissue

† Item supplied if needed

## SITE KITS

A **site kit** will be provided to the field crews for each sampling site. Site kits are specific to marine sites and Great Lakes sites. 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. Each site kit will include a label packet (specific to marine or Great Lakes sites) and will also include necessary coolers and shipping supplies for all samples collected. Prior to sampling, inspect each site kit to ensure all supplies are included. Some items may not be used at all sites and should be held until the end of the field season and shipped back.

### MARINE SITE KIT

Item	Quantity per Site Kit	Protocol(s)
Bucket w/screw top lid (0.6 gallon) for marine SEDX	1	Sediment Toxicity (Marine)
Centrifuge tube (sterile, screw-top, 50-mL) in leak-proof bag	1	Enterococci, Chlorophyll A
Cooler Liner (batch size, GLEC)	1	Shipping
Cooler liner (medium size, WRS)	1	Shipping
FedEx air bills (pre-addressed) plus handle tags, zip ties, etc.	1	Shipping
Filtration unit (blue base, 250 mL with funnel, cap, and filter holder)	1	Chl-A, Dissolved Nutrients
Filtration unit (white base, 100 mL, with pre-loaded ENTE filter)	2	Enterococci
Fish Tissue Plug Kit (includes vial, scalpel, punch, forceps, gloves, bubble bag, and zip-top bag)	1	Fish Tissue Plugs
Forceps (sterile, disposable)	2	Enterococci, Chlorophyll A
Glass jar (120 mL, amber)	1	Sediment Organics/Metals
Glass jar (60 mL, amber)	1	Sediment TOC
Glass jar (60 mL, amber)	1	D15N
HDPE bottle (1 L, white, wide-mouth)	1	Benthics
HDPE bottle (125 mL, white, rectangular)	2	Total Alkalinity
HDPE bottle (250 mL, amber)	1	Water Chemistry
HDPE bottle (250 mL, white, round)	1	Dissolved Nutrients
HDPE bottle (500 mL, white, round, wide mouth)	1	Algal Toxin
In-line disposable groundwater filter (0.45 µm)	1	Total Alkalinity
Label and Packing slip packet	1	General
Microcentrifuge tubes with glass beads (in bubble and zip-top bag)	2	Enterococci
PETG bottle (250 mL, clear, square, pre-sterilized)	1	Enterococci
PETG bottle (500 mL, clear, square)	1	Microcystin
Plastic 6 mil bags (1 qt)	2	Sediment grain size
Plastic bag (large, composite)	1	Eco Fish Tissue
Plastic cable tie	1	Eco Fish Tissue
Sterile phosphate buffered solution (PBS)	1	Enterococci
Tyvek Tags w/Grommet	1	Eco Fish Tissue
Zip-top bags (plastic, 2 gallon)	2	Eco Fish Tissue
Zip-top bags (sandwich size) – for labels	2	Eco Fish Tissue

## GREAT LAKES SITE KIT

Item	Quantity per Site Kit	Protocol(s)
Bucket w/snap top lid (1 quart) for Great Lakes SEDX	1	Sediment Toxicity (GL)
Centrifuge tube (sterile, screw-top, 50-mL) in leak-proof bag	1	Enterococci, Chlorophyll A
Cooler Liner (batch size, GLEC)	1	Shipping
Cooler liner (medium size, WRS)	1	Shipping
FedEx air bills (pre-addressed) plus handle tags, zip ties, etc.	1	Shipping
Filtration unit (blue base, 250 mL with funnel, cap, and filter holder)	1	Chl-A, Dissolved Nutrients
Filtration unit (white base, 100 mL, with pre-loaded ENTE filter)	2	Enterococci
Fish Tissue Plug Kit (includes vial, scalpel, punch, forceps, gloves, bubble bag, and zip-top bag)	1	Fish Tissue Plugs
Forceps (sterile, disposable)	2	Enterococci, Chlorophyll A
Glass jar (120 mL, amber)	1	Sediment Organics/Metals
Glass jar (60 mL, amber)	1	Sediment TOC
Glass jar (60 mL, amber)	1	D15N
HDPE bottle (1 L, white, narrow mouth)	1	Phytoplankton
HDPE bottle (1 L, white, wide-mouth)	1	Benthics
HDPE bottle (250 mL, amber)	1	Water Chemistry
HDPE bottle (250 mL, white, round)	1	Dissolved Nutrients
HDPE bottle (500 mL, white, round, wide mouth)	1	Algal Toxin
Label and Packing slip packet	1	General
Microcentrifuge tubes with glass beads (in bubble and zip-top bag)	2	Enterococci
PETG bottle (250 mL, clear, square, pre-sterilized)	1	Enterococci
PETG bottle (500 mL, clear, square)	1	Microcystin
Plastic 6 mil bags (1 qt)	2	Sediment grain size
Plastic bag (large, composite)	1	Eco Fish Tissue
Plastic cable tie	1	Eco Fish Tissue
Sterile phosphate buffered solution (PBS)	1	Enterococci
Tyvek Tags w/Grommet	1	Eco Fish Tissue
Zip-top bags (plastic, 2 gallon)	2	Eco Fish Tissue
Zip-top bags (sandwich size) – for labels	2	Eco Fish Tissue

## HUMAN HEALTH FISH TISSUE SAMPLING KIT

A human health fish tissue (HTIS) kit will be provided to the field crews for selected sampling sites (separately from site kits). This kit will include materials for sampling HTIS at one Great Lakes nearshore site. 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 human health fish tissue kit to ensure all supplies are included. These kits include:

	Item	Quantity	Protocol
<b>HH FISH TISSUE KIT</b>	Aluminum foil	5	Packaging
	Cooler (blue)	1	Storage & Shipping
	Dry ice (Class 9) shipping label	1	Shipping
	FedEx airbill (pre-addressed)	1	Shipping
	Nitrile gloves	5 pairs	Packaging
	Plastic bags (large, composite)	1	Packaging
	Plastic cable ties	12	Packaging
	Polyethylene tubing (heavy-duty, food grade)	1 roll	Packaging
	Tyvek tags with grommets	1	Packaging

## CREW SUPPLIED EQUIPMENT

	Item	Quantity	Protocol
<b>GENERAL EQUIPMENT</b>	Active/passive fish sampling device (e.g., trawl, seine, hook & line, etc.)		Fish Collection
	Phosphate-free detergent such as Liquinox		Sediment Collection
	Barometer (for calibration)		Water Profile
	Batteries (AA)		GPS, Water Profile
	Bleach (1-10% solution)		Decontamination
	Borax		Sediment Collection
	Buckets (large)		Sediment Collection
	Calibration cups & standards		Profile
	Cell phone, 2-way radios, walkie talkies		General
	Clipboard(s)	1-2	General
	De-ionized water (lab certified preferred, not required)		Water Profile
	Digital camera (with extra memory card & batteries)		General
	Dip net	1	Fish Collection
	Dry ice	~50 lbs/site	Shipping
	Fine-tipped, indelible markers		General
	Formalin (100% buffered) with stain		Sediment Collection
	Fuses (10 amp)		Underwater Video
	GPS unit (with manual & reference card, extra battery pack);		General
	Knife		General
	Livewell/buckets with aerator		Fish Collection
	Maps & access instructions		General
Measuring board (mm scale)	1	Fish Collection	

	Item	Quantity	Protocol
	Multi-parameter probe water quality meter (with pH, DO, temperature, and conductivity/salinity probes – e.g., Hydrolab, YSI, etc.)	1	Water Profile
	NCCA 2020 Fact Sheets (available on NARS SharePoint)	10	General, Outreach
	PAR meter (with LI-190 Quantum Sensor and LI-192 Underwater Quantum Sensor & cables, independent datalogger)	1	Water Profile
	Pencils (#2)	5	General
	Plastic tub or bucket	1	Sediment Collection
	QCS – quality check solution	If needed	Water Profile
	Rose Bengal stain	1 bottle	Sediment Collection
	Ruler (in cm)	1	General
	Sampling permits/permission letters		General
	Side cutter	1	Ecofish, Human health fish collection
	Scissors	1	General
	Scrub brush	1	Sediment Collection
	Sieve box/frame (if necessary)	1	Sediment Collection
	Spare parts	Various	Multi-probe
	Stainless steel or Teflon spoons (large & small), spatulas, & scoops		Mixing and dispensing sediment
	Stainless steel mixing pot or bowl with lid	1	Sediment Collection
	Stop watch	1	Underwater Video
	Thermometer	1	Water Profile
	Water sampling device (e.g., Niskin) or pump system	1	Chlorophyll A Dissolved Nutrients Phytoplankton Water Chemistry Microcystin
	Weights & pads for grabs		Sediment Collection
	Wet ice	~50 lbs/site, additional for shipping	Shipping
	Young-modified Van Veen grab sampler (0.04 m <sup>2</sup> ) OR standard OR Petite Ponar sampler with grab stand, plastic tub, drop line, pinch pin	1	Sediment Collection
<b>BOAT EQUIPMENT</b>	Anchor (with 75 m line or sufficient to anchor in 50 m depth)		
	Boat horn		
	Bow/Stern lights		
	Emergency tool kit		
	Extra boat plug		
	Fire extinguisher		
	First aid kit		
	Float (to attach to anchor)		
	Gas Can		
	Hand bilge pump		

	Item	Quantity	Protocol
	Motor		
	PFDs (1/person)		
	Pingers		
	Sonar unit		
	Spare prop		
	Spare prop shear pin		
	Type IV PFD (throwable life saving device)		

## APPENDIX B: SAMPLE LABELS & PACKING SLIPS

### SAMPLE LABELS (MARINE)

**\*\*MARINE\*\***

<p><b>WATER CHEMISTRY (CHEM)</b> Site ID: _____ Date: ____/____/2020 Visit #: <input type="radio"/>1 <input type="radio"/>2 <b>999000</b></p> <p><b>T1</b></p>	<p><b>WATER COLUMN CHLOROPHYLL (WCHL)</b> Site ID: _____ Date: ____/____/2020 Visit #: <input type="radio"/>1 <input type="radio"/>2 Volume Filtered: _____mL <b>999001</b></p> <p><b>T1</b></p>
<p><b>NUTRIENTS (NUTS)</b> Site ID: _____ Date: ____/____/2020 Visit #: <input type="radio"/>1 <input type="radio"/>2 <b>999002</b></p> <p><b>T1</b></p>	<p><b>ALGAL TOXIN (MICX)</b> (PETG Bottle (clear, square bottle)) Site ID: _____ Date: ____/____/2020 Visit #: <input type="radio"/>1 <input type="radio"/>2 Salinity: _____(‰) <b>999003</b></p> <p><b>T3</b></p>
<p><b>ALGAL TOXIN (MICZ)</b> (HDPE Bottle (round Nalgene bottle)) Site ID: _____ Date: ____/____/2020 Visit #: <input type="radio"/>1 <input type="radio"/>2 Salinity: _____(‰) <b>999004</b></p> <p><b>T3</b></p>	<p><b>BENTHIC INFAUNA (BENT)</b> Site ID: _____ Date: ____/____/2020 Visit #: <input type="radio"/>1 <input type="radio"/>2 Salinity: _____(‰) Jar 1 of ____ <b>999005</b></p> <p><b>T4</b></p>
<p><b>SEDIMENT TOC (SEDC)</b> Site ID: _____ Date: ____/____/2020 Visit #: <input type="radio"/>1 <input type="radio"/>2 <b>999006</b></p> <p><b>T3</b></p>	<p><b>SEDIMENT GRAIN SIZE (SEDG)</b> Site ID: _____ Date: ____/____/2020 Visit #: <input type="radio"/>1 <input type="radio"/>2 <b>999007</b></p> <p><b>T2</b></p>
<p><b>SEDIMENT ORGANICS/METAL (SEDO)</b> Site ID: _____ Date: ____/____/2020 Visit #: <input type="radio"/>1 <input type="radio"/>2 <b>999008</b></p> <p><b>T3</b></p>	<p><b>SEDIMENT TOXICITY (SEDX)</b> Site ID: _____ Date: ____/____/2020 Visit #: <input type="radio"/>1 <input type="radio"/>2 <b>999009</b></p> <p><b>T2</b></p>
<p><b>SEDIMENT NITROGEN (D15N)</b> Site ID: _____ Date: ____/____/2020 Visit #: <input type="radio"/>1 <input type="radio"/>2 <b>999010</b></p> <p><b>T3</b></p>	<p><b>TOTAL ALKALINITY (ALKT)</b> Site ID: _____ Date: ____/____/2020 Visit #: <input type="radio"/>1 <input type="radio"/>2 Salinity: _____(‰) Jar 1 of 2 <b>999011</b></p> <p><b>T2</b></p>
<p><b>TOTAL ALKALINITY (ALKT)</b> Site ID: _____ Date: ____/____/2020 Visit #: <input type="radio"/>1 <input type="radio"/>2 Salinity: _____(‰) Jar 2 of 2 <b>999011</b></p> <p><b>T2</b></p>	<p><b>Sample Type:</b> _____ Site ID: _____ Date: ____/____/2020 Visit #: <input type="radio"/>1 <input type="radio"/>2 Sample ID: _____</p>



**FISH TISSUE PLUG (FPLG)**  
Site ID: \_\_\_\_\_  
Date: \_\_\_\_/\_\_\_\_/2020 Visit #: 1 2  
**999012**

**T3**

**ECO FISH TISSUE - INNER BAG \_\_\_\_ OF \_\_\_\_**  
Site ID: \_\_\_\_\_  
Date: \_\_\_\_/\_\_\_\_/2020 Visit #: 1 2  
Genus Species: \_\_\_\_\_  
Length (mm) Min.: \_\_\_\_\_ Max.: \_\_\_\_\_  
**999013**

**ECO FISH TISSUE - INNER BAG \_\_\_\_ OF \_\_\_\_**  
Site ID: \_\_\_\_\_  
Date: \_\_\_\_/\_\_\_\_/2020 Visit #: 1 2  
Genus Species: \_\_\_\_\_  
Length (mm) Min.: \_\_\_\_\_ Max.: \_\_\_\_\_  
**999013**

**T5**

**ECO FISH TISSUE - OUTER BAG**  
Site ID: \_\_\_\_\_  
Date: \_\_\_\_/\_\_\_\_/2020 Visit #: 1 2  
Genus Species: \_\_\_\_\_  
Length (mm) Min.: \_\_\_\_\_ Max.: \_\_\_\_\_  
**999013**

**ENTEROCOCCI (ENTE) - BAG**  
Site ID: \_\_\_\_\_  
Date: \_\_\_\_/\_\_\_\_/2020 Visit #: 1 2  
Vol. Filt: 1 \_\_\_\_ mL 2 \_\_\_\_ mL  
**999014**

Filter : 1  
Vol. Filt: \_\_\_\_ mL  
999014

Filter : 2  
Vol. Filt: \_\_\_\_ mL  
999014

Filter : Blank  
Vol. Filt: \_\_\_\_ mL  
999014

**Benthic Infauna (BENT)**  
Site ID: \_\_\_\_\_  
Date: \_\_\_\_/\_\_\_\_/2020 Visit #: 1 2  
Collector(s): \_\_\_\_\_  
Jar \_\_\_\_ of \_\_\_\_  
**SAMPLE ID: \_\_\_\_\_**

**BENTHOS – EXTRA JAR**  
Site ID: \_\_\_\_\_  
Date: \_\_\_\_/\_\_\_\_/2020 Visit #: 1 2  
Jar \_\_\_\_ of \_\_\_\_  
**SAMPLE ID: \_\_\_\_\_**

**ECO Fish Tissue (FTIS)**  
Site ID: \_\_\_\_\_  
Date: \_\_\_\_/\_\_\_\_/2020 Visit #: 1 2  
Length (mm) Min.: \_\_\_\_ Max.: \_\_\_\_  
Bag \_\_\_\_ of \_\_\_\_  
**SAMPLE ID: \_\_\_\_\_**

**BENTHOS – EXTRA JAR**  
Site ID: \_\_\_\_\_  
Date: \_\_\_\_/\_\_\_\_/2020 Visit #: 1 2  
Jar \_\_\_\_ of \_\_\_\_  
**SAMPLE ID: \_\_\_\_\_**

### SAMPLE LABELS (GREAT LAKES)

**GREAT LAKES**	
<p><b>WATER CHEMISTRY (CHEM)</b> Site ID: _____ Date: ____/____/2020 Visit #: <input type="radio"/>1 <input type="radio"/>2 <b>999020</b></p> <p><b>T1</b></p>	<p><b>WATER COLUMN CHLOROPHYLL (WCHL)</b> Site ID: _____ Date: ____/____/2020 Visit #: <input type="radio"/>1 <input type="radio"/>2 Volume Filtered: _____mL <b>999021</b></p> <p><b>T1</b></p>
<p><b>NUTRIENTS (NUTS)</b> Site ID: _____ Date: ____/____/2020 Visit #: <input type="radio"/>1 <input type="radio"/>2 <b>999022</b></p> <p><b>T1</b></p>	<p><b>ALGAL TOXIN (MICX)</b> (PETG Bottle (clear, square bottle)) Site ID: _____ Date: ____/____/2020 Visit #: <input type="radio"/>1 <input type="radio"/>2 <b>999023</b></p> <p><b>T3</b></p>
<p><b>ALGAL TOXIN (MICZ)</b> (HDPE Bottle (round Nalgene bottle)) Site ID: _____ Date: ____/____/2020 Visit #: <input type="radio"/>1 <input type="radio"/>2 <b>999024</b></p> <p><b>T3</b></p>	<p><b>BENTHIC INFAUNA (BENT)</b> Site ID: _____ Date: ____/____/2020 Visit #: <input type="radio"/>1 <input type="radio"/>2 Jar 1 of ____ <b>999025</b></p> <p><b>T4</b></p>
<p><b>SEDIMENT TOC (SEDC)</b> Site ID: _____ Date: ____/____/2020 Visit #: <input type="radio"/>1 <input type="radio"/>2 <b>999026</b></p> <p><b>T3</b></p>	<p><b>SEDIMENT GRAIN SIZE (SEDG)</b> Site ID: _____ Date: ____/____/2020 Visit #: <input type="radio"/>1 <input type="radio"/>2 <b>999027</b></p> <p><b>T2</b></p>
<p><b>SEDIMENT ORGANICS/METAL (SEDO)</b> Site ID: _____ Date: ____/____/2020 Visit #: <input type="radio"/>1 <input type="radio"/>2 <b>999028</b></p> <p><b>T3</b></p>	<p><b>SEDIMENT TOXICITY (SEDX)</b> Site ID: _____ Date: ____/____/2020 Visit #: <input type="radio"/>1 <input type="radio"/>2 <b>999029</b></p> <p><b>T2</b></p>
<p><b>PHYTOPLANKTON (PHYT)</b> Site ID: _____ Date: ____/____/2020 Visit #: <input type="radio"/>1 <input type="radio"/>2 <b>999035</b></p> <p><b>T2</b></p>	<p>Site ID: _____ Date: ____/____/2020 Visit #: <input type="radio"/>1 <input type="radio"/>2 <b>Sample ID: _____</b></p>
<p>Site ID: _____ Date: ____/____/2020 Visit #: <input type="radio"/>1 <input type="radio"/>2 <b>Sample ID: _____</b></p>	<p>Site ID: _____ Date: ____/____/2020 Visit #: <input type="radio"/>1 <input type="radio"/>2 <b>Sample ID: _____</b></p>

**FISH TISSUE PLUG (FPLG)**  
Site ID: \_\_\_\_\_  
Date: \_\_\_\_/\_\_\_\_/2020 Visit #: 1 2  
**999012**

**T3**

**ECO FISH TISSUE - INNER BAG \_\_\_\_ OF \_\_\_\_**  
Site ID: \_\_\_\_\_  
Date: \_\_\_\_/\_\_\_\_/2020 Visit #: 1 2  
Genus Species: \_\_\_\_\_  
Length (mm) Min.: \_\_\_\_\_ Max.: \_\_\_\_\_  
**999013**

**ECO FISH TISSUE - INNER BAG \_\_\_\_ OF \_\_\_\_**  
Site ID: \_\_\_\_\_  
Date: \_\_\_\_/\_\_\_\_/2020 Visit #: 1 2  
Genus Species: \_\_\_\_\_  
Length (mm) Min.: \_\_\_\_\_ Max.: \_\_\_\_\_  
**999013**

**T5**

**ECO FISH TISSUE - OUTER BAG**  
Site ID: \_\_\_\_\_  
Date: \_\_\_\_/\_\_\_\_/2020 Visit #: 1 2  
Genus Species: \_\_\_\_\_  
Length (mm) Min.: \_\_\_\_\_ Max.: \_\_\_\_\_  
**999013**

**ENTEROCOCCI (ENTE) - BAG**  
Site ID: \_\_\_\_\_  
Date: \_\_\_\_/\_\_\_\_/2020 Visit #: 1 2  
Vol. Filt: 1 \_\_\_\_\_ mL 2 \_\_\_\_\_ mL  
**999014**

Filter : 1  
Vol. Filt: \_\_\_\_\_ mL  
999014

Filter : 2  
Vol. Filt: \_\_\_\_\_ mL  
999014

Filter : Blank  
Vol. Filt: \_\_\_\_\_ mL  
999014

**Benthic Infauna (BENT)**  
Site ID: \_\_\_\_\_  
Date: \_\_\_\_/\_\_\_\_/2020 Visit #: 1 2  
Collector(s): \_\_\_\_\_  
Jar \_\_\_\_ of \_\_\_\_  
**SAMPLE ID: \_\_\_\_\_**

**ECO Fish Tissue (FTIS)**  
Site ID: \_\_\_\_\_  
Date: \_\_\_\_/\_\_\_\_/2020 Visit #: 1 2  
Length (mm) Min.: \_\_\_\_\_ Max.: \_\_\_\_\_  
Bag \_\_\_\_ of \_\_\_\_  
**SAMPLE ID: \_\_\_\_\_**

**BENTHOS – EXTRA JAR**  
Site ID: \_\_\_\_\_  
Date: \_\_\_\_/\_\_\_\_/2020 Visit #: 1 2  
Jar \_\_\_\_ of \_\_\_\_  
**SAMPLE ID: \_\_\_\_\_**

**BENTHOS – EXTRA JAR**  
Site ID: \_\_\_\_\_  
Date: \_\_\_\_/\_\_\_\_/2020 Visit #: 1 2  
Jar \_\_\_\_ of \_\_\_\_  
**SAMPLE ID: \_\_\_\_\_**

**HH FISH TISSUE WHOLE (HTIS)**

Site ID: \_\_\_\_\_  
Date: \_\_\_\_/\_\_\_\_/2020 Visit #: 1 2  
Genus Species: \_\_\_\_\_  
Length: \_\_\_\_\_ mm

**999016.01**

**HH FISH TISSUE WHOLE (HTIS)**

Site ID: \_\_\_\_\_  
Date: \_\_\_\_/\_\_\_\_/2020 Visit #: 1 2  
Genus Species: \_\_\_\_\_  
Length: \_\_\_\_\_ mm

**999016.02**

**HH FISH TISSUE WHOLE (HTIS)**

Site ID: \_\_\_\_\_  
Date: \_\_\_\_/\_\_\_\_/2020 Visit #: 1 2  
Genus Species: \_\_\_\_\_  
Length: \_\_\_\_\_ mm

**999016.03**

**HH FISH TISSUE WHOLE (HTIS)**

Site ID: \_\_\_\_\_  
Date: \_\_\_\_/\_\_\_\_/2020 Visit #: 1 2  
Genus Species: \_\_\_\_\_  
Length: \_\_\_\_\_ mm

**999016.04**

**HH FISH TISSUE WHOLE (HTIS)**

Site ID: \_\_\_\_\_  
Date: \_\_\_\_/\_\_\_\_/2020 Visit #: 1 2  
Genus Species: \_\_\_\_\_  
Length: \_\_\_\_\_ mm

**999016.05**

**HH FISH TISSUE WHOLE (HTIS) - BAG**

Site ID: \_\_\_\_\_  
Date: \_\_\_\_/\_\_\_\_/2020 Visit #: 1 2  
Genus Species: \_\_\_\_\_  
Length range: \_\_\_\_ mm to \_\_\_\_ mm

**T6**

**999016**

## APPENDIX C: SHIPPING AND TRACKING GUIDELINES

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### TRACKING FORMS IN THE APP

Each shipping group has been assigned a “T” number to help crews identify the correct section of the Tracking Form in the App to use when sending samples. This “T” number is located above each of the sample groups in the Tracking Form in the App. Crews will also find reference to the same “T” numbers on the individual samples labels, on the packing slips that crews will include in the coolers, and on the top of the pre-printed FedEx return labels provided in the site kits. Crews submit tracking information via the NCCA App and include packing slips in the coolers when they send samples to the labs. 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.

#### 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., Sample Collection, Eco Fish Collection, and/or Human Health Fish Collection).
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, airbill 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](mailto:NARSFieldData@epa.gov). Copy yourself, 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.

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:

#### T1 – DAILY WATER CHEMISTRY SAMPLES

- Complete the T1 section of the tracking form for the samples that are shipped immediately after each sampling event
  - water chemistry (CHEM)
  - chlorophyll A (WCHL)
  - dissolved nutrients (NUTS).
- Send the tracking form and all data forms from the site to the IM Team via the NCCA App. This serves as the “status report” for that sampling event.
- 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 from two sites may be shipped together in a single cooler if they were collected on the same day.
- Samples need to be shipped on as much fresh wet ice as will fit in the cooler liner.
- Water chemistry samples should be shipped within 24 hours of collection.

#### T2 – CHILLED BATCHED SAMPLES (MARINE = DAILY, GREAT LAKES = WEEKLY)

- Use this section of the App tracking form for shipping batches of chilled samples:
  - Sediment toxicity (SEDX)
  - Sediment grain size (SEDG)
  - Total alkalinity (ALKT at marine sites only)
  - Phytoplankton (PHYT) at Great Lakes sites only
- At marine sites, ship 1 day’s worth of samples (up to 3 sites) together in a single cooler.
- At Great Lakes sites, ship up to 7 site’s worth of samples 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.
- Samples need to be shipped on as much fresh wet ice as will fit in the cooler liner.
- At marine sites, chilled batched samples should be shipped the same day as sampling or the next day.
- At Great Lakes sites, chilled batched samples should be shipped at least every week

### T3 – FROZEN BATCHED SAMPLES

- Use this form for shipping batches of frozen samples:
  - Algal Toxins - Microcystins and Cylindrospermopsin (MICX)
  - Algal Toxins - Microcystins (MICZ)
  - Enterococci (ENTE)
  - Fish tissue plugs (FPLG)
  - Sediment TOC (SEDC)
  - Sediment Organics/Metals (SEDO)
  - Nitrogen isotope (D15N) *marine sites only*
  - Ecofish samples (FTIS) (may be shipped separately as a standalone T5 shipment)
- 2-3 site's worth of samples may be shipped together in a single cooler, depending on whether the ecofish sample is included and the size of the fish comprising that sample.
- 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 with approximately 20 pounds of dry ice in a cooler with a two-piece dry ice liner.
- Frozen batched samples should be shipped at least every week.

### T4 – NON-CHILLED: BATCHED SAMPLES

- Use this section of the App tracking form for shipping batches of non-chilled samples:
  - Benthic Macroinvertebrates (BENT)
- Up to 12 site's worth of samples may be shipped together in a single cooler, depending on whether more than one bottle of sample was collected at a site.
- 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.

- Samples need to be shipped with absorbent material and no ice. Place all samples and absorbent material inside the cooler liner.
- Non-chilled batched samples should be shipped every 2-3 weeks.

**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.*

#### T5 – ECO FISH TISSUE

- Use this section of the App tracking form for shipping batches of frozen eco fish (FTIS) samples.
- Eco Fish samples may be sent in the same cooler as the other frozen batched samples (T3) listed above or may be sent separately.
- 2-4 site's worth of samples may be shipped together in a single cooler, depending on whether eco fish are included and the size of the eco fish sample.
- 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.
- Samples need to be shipped with approximately 20 pounds of dry ice in a cooler with a two-piece dry ice liner.
- Eco Fish samples should be shipped at least every 2 weeks.

#### T6 – HUMAN HEALTH WHOLE FISH TISSUE COMPOSITE SAMPLE – NGL20, ISA20, AND NPA20 SITES ONLY

- Use this section of the App tracking form for shipping frozen human health fish tissue samples (HTIS).
- Only one human health fish composite sample may be shipped in a single cooler.
- Ship the sample to the lab in the same 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 2 weeks of collection.



### T7 – UNDERWATER VIDEO UVID FORM [GREAT LAKES ONLY]

- Use this section of the App tracking form for shipping the EPA-provided Micro SD Cards containing all underwater video recorded during the season.
- Before shipping, make backups of the video files for your records and as a backup in the event the forms are lost during shipping.

### SHIPPING GUIDELINES

Samples will be shipped according to the chart in **Appendix C: Shipping and Tracking Guidelines**. The Field Crew Leader will complete the appropriate section(s) of the Tracking Form in the App for the samples being shipped and will submit tracking via the App. The field crew will place the samples and the packing slip (in a waterproof bag or plastic sleeve) in a shipment cooler. 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. The field crew will attach the appropriate pre-addressed airbill from the site kit 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 its actual pick up. Once the package is in the possession of FedEx, the IM Team and FLC will track the package to its destination and take steps necessary to ensure its timely delivery. Prior to shipping, there are a few other guidelines to be aware of:

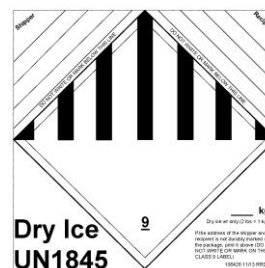
Preservation	Holding Time	Shipping
<ul style="list-style-type: none"><li>• See chart for specific preservation information for each sample</li></ul>	<ul style="list-style-type: none"><li>• Note the holding time window for each sample</li><li>• Ensure that samples will be shipped in time for the lab to be able to process them within the allowable holding time frame</li></ul>	<ul style="list-style-type: none"><li>• Samples may be shipped on wet ice, dry ice, or with no ice</li><li>• Secure the cooler with strapping tape</li><li>• See dry ice shipping protocols</li></ul>

## Wet Ice

- Ensure that the ice is fresh immediately prior to shipment;
- Line the cooler with a large plastic liner bag.
- Place samples and ice inside the cooler liner and seal the liner with the provided cable tie.
- Secure the cooler lid with packing tape.

## Dry Ice

- **Note:** *Not all FedEx locations will accept shipments containing dry ice. Dry ice shipments can be shipped from “FedEx staffed” locations. 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/Wal-Mart/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.*
- Attach the provided FedEx airbill:
  - Ensure that the label indicates the amount of dry ice in the package.
- 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 same 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.
- Secure the cooler lid with packing tape. Do not completely seal the entire edge of the cooler such that 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.



## No Ice

- Line the cooler with a large plastic liner bag.
- Surround the jars with crumpled newspaper or other absorbent material
- If the cooler is not full, add material to keep all bottles upright to prevent leakage.

## Water Chemistry [CHEM]

- Ship within 24 hours
- Ship 250 mL brown HDPE bottle
- Confirm label completed & taped
- Seal with plastic electrical tape
- Place in cooler liner
- Ship on wet ice

## Chlorophyll-*a* [WCHL]

- Ship with CHEM and NUTS samples
- Ship foil wrapped centrifuge tube
- Confirm label completed & taped
- Seal with plastic electrical tape
- Place in provided leak-proof zip-top bag
- Place in cooler liner
- Ship on wet ice

## Dissolved Nutrients [NUTS]

- Ship within 24 hours
- Ship 250 mL white HDPE bottle
- Confirm label completed & taped
- Seal with plastic electrical tape
- Place in cooler liner
- Ship on wet ice

### Sediment Grain Size [SEDG]

- Ship daily at marine sites and within 1 week at GL sites
- Ship in plastic bag (quart size, double bagged)
- Confirm label completed & taped
- Place in lined cooler with other chilled batched samples
- Ship on wet ice

### Sediment Toxicity [SEDX]

- Ship daily at marine sites and within 1 week at GL sites
- Ship in bucket (0.6 gal for estuarine, 1 quart for Great Lakes)
- Confirm label completed & taped
- Tighten the lid securely and ensure it will not loosen in shipping
- Place in lined cooler with other chilled batched samples.
- Ship on wet ice

### Total Alkalinity [ALKT] (Marine only)

- Ship within 24 hours
- Ship two 125 mL white rectangular HDPE bottles
- Confirm label completed & taped
- Seal with plastic electrical tape
- Place in cooler liner
- Ship on wet ice

### Phytoplankton [PHYT] (GL only)

- Ship within 1 week
- Ship in HDPE bottle (1 L, white, narrow mouth)
- Confirm preserved with 10 ml Lugol's solution
- Confirm label completed & taped
- Seal with plastic electrical tape
- Place in cooler lined cooler with other chilled batched samples
- Ship on wet ice

### Algal Toxins [MICX] and [MICZ]

- Ship at least every week
- Freeze after collection
- Ship in HDPE bottle (500 mL, white, wide-mouth)
- Confirm labels completed & taped
- Place in cooler lined with dry ice insert along with other frozen batched samples
- Pack cooler with 20 lbs of dry ice

### Enterococci [ENTE]

- Ship at least every week
- Ship in frozen, microcentrifuge tubes
- Confirm labels completed
- Place each tube in small bubble bag with label on outside
- Place bag in zip-top bag
- Place in cooler lined with dry ice insert along with other frozen batched samples
- Pack cooler with 20 lbs of dry ice

### Fish Plugs [FPLG]

- Ship at least every week
- Freeze after collection
- Ship in glass scintillation vial
- Confirm label completed & taped
- Place vial in small bubble bag and then in zip-top bag
- Wrap packing material around bag to prevent breakage
- Place in cooler lined with dry ice insert along with other frozen batched samples
- Pack cooler with 20 lbs of dry ice

### Sediment TOC [SEDC]

- Ship at least every week
- Ship in frozen, glass jar (60 mL) (leave headspace)
- Confirm label completed & taped
- Seal with plastic electrical tape
- Place jar in foam sleeve
- Place in cooler lined with dry ice insert along with other frozen batched samples
- Pack cooler with 20 pounds of dry ice. Pack with fill material such as newspaper if necessary to ensure no shifting

Sediment  
Organics/Metals  
[SEDO]

- Ship at least every week
- Ship in frozen, glass jar (120 mL) (leave headspace)
- Confirm label completed & taped
- Seal with plastic electrical tape
- Place jar in foam sleeve
- Place in cooler lined with dry ice insert along with other frozen batched samples
- Pack cooler with 20 pounds of dry ice. Pack with fill material such as newspaper if necessary to ensure no shifting

Nitrogen Isotopes  
[D15N]

- Ship at least every week
- Ship in frozen, glass jar (60 mL) (leave headspace)
- Confirm label completed & taped
- Seal with plastic electrical tape
- Place jar in foam sleeve
- Place in cooler lined with dry ice insert along with other frozen batched samples
- Pack cooler with 20 pounds of dry ice. Pack with fill material such as newspaper if necessary to ensure no shifting

Benthic  
Macroinvertebrates  
[BENT]

- Ship every 2-3 weeks
- Preserve benthos samples immediately upon collection
- Ship in HDPE bottle (1 L, white, wide mouth)
- Confirm label completed & taped
- Seal with plastic electrical tape
- Surround the jars with crumpled newspaper, vermiculite or other absorbent material
- Place in cooler liner
- Ship with NO ice

Ecological Whole Fish  
Tissue  
[FTIS]

- Ship at least every 2 weeks
- Freeze after collection, as soon as possible (-20 cooler)
- Ship in bags
- Confirm label completed & taped
- Place in cooler lined with dry ice insert along with other frozen batched samples
- Pack cooler with 20 lbs of dry ice

## Human Health Whole Fish Tissue

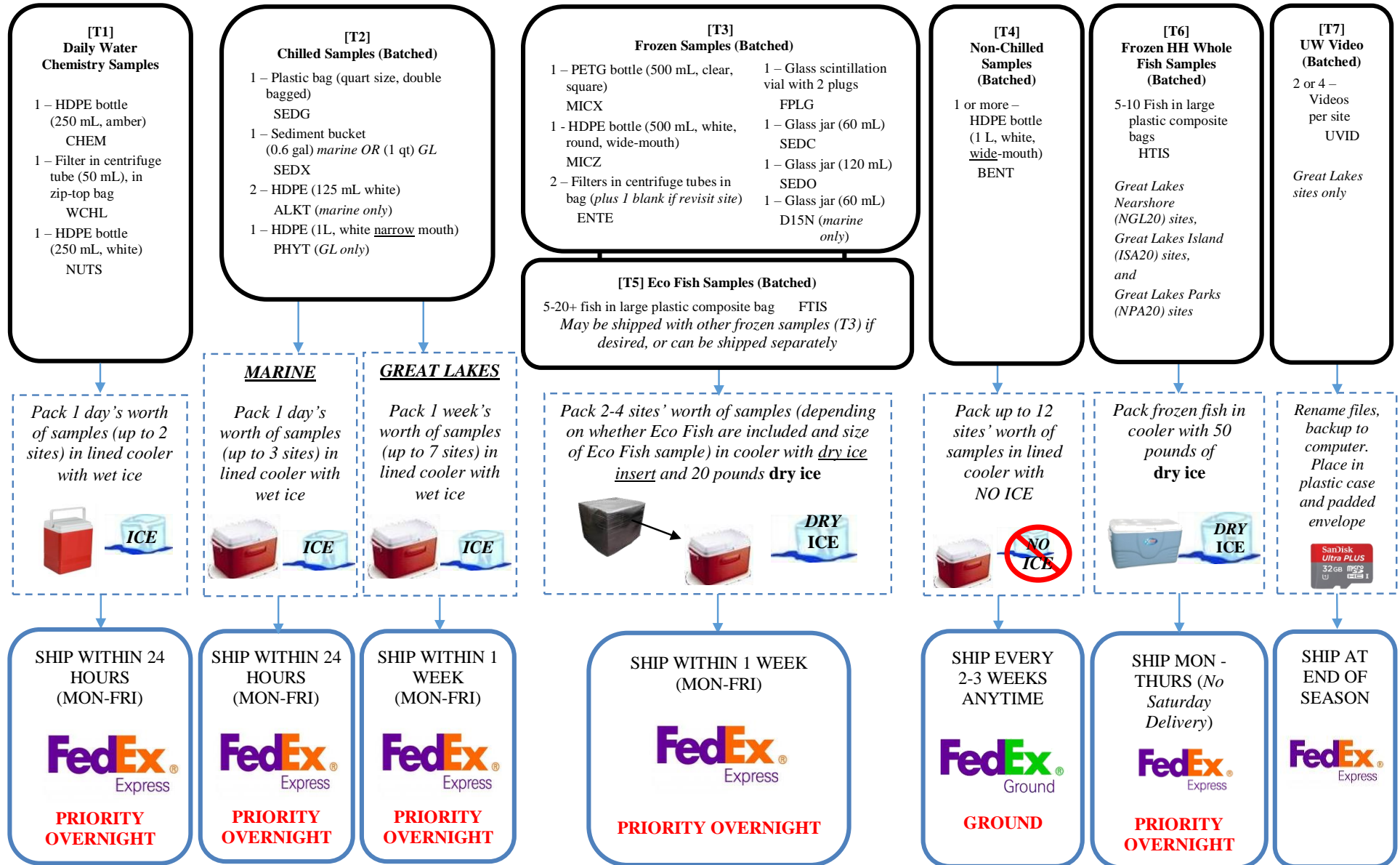
[HTIS]

(NGL20, ISA20, and  
NPA20 sites only)

- Ship at least every 2 weeks
- Freeze after collection, as soon as possible (-20 °C cooler)
- Ship in bags
- Confirm label completed & taped
- Pack cooler with 50 lbs of dry ice

## Underwater Video [UVID] (GL only)

- Ship at end of season or as Micro SD cards get full
- Back up files to computer hard drive
- Be sure files are named appropriately
- Package EPA-provided Micro SD cards in plastic cases
- Ship in FedEx envelope





SHIPPING GROUP	SAMPLE	SAMPLE TARGET VOLUME	CONTAINER	PRESERVATIVE	PACKAGING FOR SHIPMENT	HOLDING TIME
<b>DAILY WATER CHEMISTRY SAMPLES (T1)</b>	Water Chemistry (CHEM)	250 mL	HDPE bottle (250 mL, amber)	Wet ice	Line cooler with heavy plastic bag cooler liner  Ship in cooler with wet ice	24 hours (ship same day as sampling or next day) to <b>WRS lab - Corvallis or approved State lab</b>
	Chlorophyll- <i>a</i> (WCHL)	Collection: 2L	HDPE bottle (2 L, amber)	<i>Wet ice prior to filtering</i>		
		Processing: readily visible stain on filter, max 2000 mL	Filter in 50 mL centrifuge tube (foil wrapped)	Dry ice in field		
	Dissolved Nutrients (NUTS)	250 mL of filtrate from chl- <i>a</i> filtering	HDPE bottle (250 mL, white)	Wet ice in field		
<b>CHILLED BATCHED SAMPLES (T2)</b>	Sediment Grain Size (SEDG)	100 mL	Plastic bag (double bagged, quart size)	Wet ice in field	Line cooler with heavy plastic bag cooler liner  Ship in cooler with wet ice	<b>MARINE:</b> 24 hours (ship same day as sampling or next day) to <b>GLEC lab – Traverse City</b>  <b>GREAT LAKES:</b> Batch; ship at least every week to <b>GLEC lab – Traverse City</b>
	Sediment Toxicity (SEDX)	MARINE: target = 1800 mL minimum = 900 mL	MARINE = screw top bucket (0.6 gal)	Wet ice in field		
		GREAT LAKES: target = 900 mL, minimum = 400 mL	GREAT LAKES = snap lid bucket (1 qt)			
	Total Alkalinity (ALKT) <i>Marine only</i>	125 mL in each of 2 bottles	HDPE bottles (2) (125 mL, white)	Wet ice in field		
Phytoplankton (PHYT) <i>Great Lakes only</i>	1 L	HDPE bottle (1 L, white, narrow-mouth)	Add 10 mL Lugol's solution Wet ice in field Hold chilled in the dark			
<b>FROZEN BATCHED SAMPLES (T3)</b>	Algal Toxins (MICX)	500 mL (leave headspace)	PETG bottle (500 mL, clear, square)	Wet ice in field, Freeze as soon as possible	Ship in cooler with <u>2-piece dry ice insert</u> and 20 pounds of DRY ICE        <b>Note:</b> Frozen Batched (T3) and Ecofish samples (T5) may be shipped together or separately	Batch, ship within 1 week to <b>GLEC lab – Traverse City</b>
	Algal Toxins (MICZ)	500 mL (leave headspace)	HDPE bottle (500 mL, white, wide-mouth, round)	Wet ice in field, Freeze as soon as possible		
	Enterococci (ENTE)	Collection: 250 mL	HDPE bottle (250 mL, pre-sterilized, clear)	<i>Wet ice prior to filtering (at least 15 minutes)</i>		
		Processing: Two 50 mL filtrations	2 filters in microcentrifuge tubes	Dry ice in field; hold in freezer; MUST be filtered & frozen within 6 hours of collection;		
		Filter blanks (revisit sites only): One 20 mL filtration of PBS	1 filter in microcentrifuge tube	Hold in freezer or on dry ice		
	Fish Tissue Plugs (FPLG)	2 plugs	glass scintillation vial (20 mL)	Dry ice in field; Hold in freezer or on dry ice		
	Sediment Total Organic Carbon (SEDC)	50 mL (leave headspace)	glass jar (60 mL, amber)	Dry ice in field		
	Sediment Organics/Metals (SEDO)	100 mL (leave headspace)	glass jar (120 mL, amber)	Dry ice in field		
Nitrogen Isotopes (D15N) <i>Marine only</i>	50 mL (leave headspace)	glass jar (60 mL, amber)	Dry ice in field			
<b>ECOFISH SAMPLES (T5)</b>	Whole Fish Tissue Sample (FTIS)	5-20+ fish (300 g whole body tissue)	2 gallon self-sealing bags Large outer bag	Dry ice in field; Hold in freezer		
<b>NON-CHILLED BATCHED SAMPLES (T4)</b>	Benthic Macroinvertebrates (BENT)	All organisms in grab(s)	HDPE bottle(s) (1 L, white, wide-mouth)	Stained formalin solution	Ship in cooler lined with plastic bag cooler liner	Batch, ship at least every 2-3 weeks to <b>GLEC lab – Traverse City</b>
<b>HUMAN HEALTH WHOLE FISH* (T6)</b>	Human Health Whole Fish Tissue Sample (HTIS)* <i>Great Lakes only</i>	5-10 whole fish (500 g of fillet weight) minimum fish length of 190 mm	Wrapped individually in solvent rinsed foil Sealed in poly tubing Large outer plastic bag	Dry ice in field; Hold in freezer	Ship in provided HTIS cooler with 50 pounds of DRY ICE	Batch, ship weekly (except on Fridays, Saturdays, or the day before Federal holidays) to <b>HTIS lab</b>
<b>UW VIDEO (T7)</b>	Underwater Video (UVID) <i>Great Lakes only</i>	1 minute videos of benthic habitat on both A and B cameras	2 Micro SD Cards (one per camera)	Rename files on Micro SD Cards and back up files to computer	Place Micro SD Card in plastic case, Ship in padded envelope	Ship when cards are nearly full and/or at end of season to <b>EPA Duluth lab</b>

\* HUMAN HEALTH FISH COMPOSITE SAMPLE IS COLLECTED AT ALL 225 PROBABILISTIC NEARSHORE GREAT LAKES SITES (PREFIX = NGL20), ALL 38 GREAT LAKES ISLAND SITES (PREFIX = ISA20), AND ALL 12 GREAT LAKES PARK SITES (PREFIX = NPA20).

## APPENDIX D: MICROPLASTICS COLLECTION AND PROCESSING

At specifically targeted sites in the northeast, crews will collect a sample of sediment for the analysis of microplastics. Microplastics have been found in every aquatic environmental matrix on the globe from coastal waters to arctic ice, and deep-sea trench sediments. These reports demonstrate that the likelihood of exposure to microplastics by aquatic organisms is ever-increasing. There are many routes of contamination for microplastics to enter the aquatic environment (e.g., surface run-off, wastewater treatment plants, and poor trash management). Once in aquatic systems, due to bio-fouling and aggregation, sediments are the likely ultimate sink for most microplastics. Sediments also serve as the habitat for many organisms at the base of aquatic food chains and therefore act as a vector for many anthropogenic contaminants, including possibly microplastics, to enter food webs. Quantification of microplastics in sediments is one of the first steps to understand the fate and effects of microplastics in aquatic food webs including those that may ultimately affect humans. By better understanding how much microplastics are present in marine coastal sediments, we improve our ability to predict potential microplastics exposure to humans.

This methodology describes the procedure for sampling sediments for microplastic analysis. The sampling procedure is very similar to conventional sediment sampling for other anthropogenic contaminants (e.g., metals, PCBs, etc.). However, special care must be taken to avoid accidental contamination of the sediment sample by the collectors' clothing or sources of plastics associated with the sampling vessel (e.g., fraying polymer lines). To address this source of contamination, while the sediment sample is being collected, an "air blank" will be collected to allow quantification of any microplastics entering the sample via airborne-sources.

*Equipment & supplies: microplastics collection and processing*

<b>For collecting samples</b>	nitrile gloves sediment sampling collection device 2 (500mL) glass jars 1 metal spatula or stainless steel teaspoon Electrical tape, plastic
<b>For recording measurements</b>	microplastics sample label fine-tipped indelible markers (for labels) clear tape strips

1. Rinse all collection equipment with sea water in between sites to remove sediment debris, followed by DI water to avoid cross contamination.
2. Collect sediment using appropriate collection device (the same device used for the collection of sediment for chemical analysis will suffice).
3. Minimize exposure to synthetic fibers:

- wear cotton laboratory coats or cotton t shirts. All crew members should wear the same color, but **not** white, blue or black. Note the color worn on the sample label.
  - Note the color of the nitrile gloves worn on the sample label.
  - Minimize exposure to fraying ropes or other plastic items onboard. Do not use synthetic wipes such as Kimwipes for equipment cleaning.
4. After retrieving the grab sampler, ensure the grab is acceptable (e.g., level sediment layer, not overfilled, etc. - see **Figure 13.1**).
  5. Open one of the glass jars before transferring sediment to the sample jar to collect an air blank. Position this empty air blank jar open to the atmosphere, close to the sediment sample whenever it's exposed to air.
  6. Using a metal spatula or stainless steel teaspoon to transfer sediment directly from the grab sampler to the 500 mL glass sample jar, collect the top 5 cm of the grab.
    - The target collection volume is 400mL of sample, which will likely require multiple grabs, depending on the size of your grab sampler and the depth of sediment in each collection.
    - Leave approximately 1 inch of headspace in the sample jar.
  7. Close both the sample and air blank jars and secure the lids with electrical tape.
  8. Fill out sample labels for the both the sample and air blank jars and cover with clear tape.
  9. If plastic debris is present on the boat (i.e., - shedding equipment or rope), take a small sample of plastic and retain it in aluminum foil for potential analysis. This will help reduce uncertainty in the air blank contamination.
    - Label this sample with an extra label from the label packet with the same sample ID as the air blank sample.
  10. Ship the sample and air blank to the lab with samples of benthic macroinvertebrates, or whenever is convenient. No chilling or freezing is necessary
    - Be sure to keep each sample jar upright to help prevent leakage.
    - Pad all glass jars with packing material to avoid breakage.