Guidelines for Collecting Ephemeral Data in the Arctic: PLANKTON

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Note: These guidelines are limited data collection aides that do not necessarily consider all possible scenarios under which samples may be collected. Use best professional judgment to modify these guidelines according to area-specific field conditions.

Guideline Objectives

The primary objective of this document is to provide guidelines on collecting plankton samples during the early stages of an oil spill in the Arctic to support Natural Resource Damage Assessment exposure and injury evaluations.

Sampling Objectives

Study exposure

- Assess the risk to plankton from exposures to oil constituents
- Quantify the composition, distribution, biomass, and densities of plankton (including ichthyoplankton, zooplankton, and phytoplankton) in background and oiled nearshore waters

Quality assurance/quality control

- Ensure the integrity the sample(s) throughout sampling, transport, and storage
- Ensure the reliability of biological characterizations

Collaboration

- Support other ongoing efforts including, but not limited to modeling of impacts to water-column resources (see Water, Shellfish Tissue, Fish guidelines), toxicity testing for injury assessment and assessing injury to higher trophic organisms that prey on plankton.

Before Field Sampling

- Assure that all personnel have required safety training and protective equipment for Arctic field work (not described in this guideline).
- Arctic weather conditions (e.g., wind direction and speed) are variable within a short timeframe. Be prepared for changing weather conditions, be aware of your surroundings, and take precautions to ensure the safety of the sampling team.

Study design

- It is important to have a defined sampling strategy prior to conducting fieldwork. Plankton are difficult to sample because of their inherent heterogeneity of distribution over space, depth, and time.
- The following terminology is used to define general to specific sampling geographies:
  - Area = general area of uniform characteristics, such as degree of oil exposure, physical setting, habitat types present, etc.
  - Location = a specific location that is representative of the area and contains the type of habitat to be sampled, such as an eelgrass bed or lagoon
  - Site = a specific point at which samples are collected or observations are made

- Plan ahead the number of locations and number of sites per location, taking into account level of effort, potential logistical limitations, weather conditions, and other issues that may compromise sample integrity.
- Review the guideline and resolve any area-specific issues. Area-specific modification of the guideline may be needed based on environmental conditions, geography, access to remote areas, and shipping capabilities.
- Contact the laboratories that will be receiving field samples for analysis and assure that they have the capacity to receive and analyze samples from the study. Follow relevant guidelines from the laboratory and consult with them about necessary modifications.
- The sampling strategy should have flexibility to be adjusted based on conditions in the field.
- Consult appropriate guidelines for the collection of other environmental media and biota concurrent with plankton sampling. Tarballs, sheens or other oil residues can be collected opportunistically for chemical analysis and fingerprinting.

Equipment

- Review the list of sampling equipment/containers, make adjustments as needed, and assure that all essential field materials are ready to be taken to the field.
- If not all sampling equipment is available, consult the alternative equipment guidelines or determine if other appropriate options are available.
- It may be necessary to coordinate with the laboratory that will receive the samples to assure that acceptable materials and conditions are used for sampling and sample storage and shipping.
- Do as much material preparation prior to field deployment, including: labeling sample jars using permanent markers or laboratory labels (e.g., peel and stick waterproof labels); etc.
- Make sure that all essential equipment is in working order and operational under Arctic field conditions, and that spare equipment and/materials are available.
- Store solvents carefully to prevent spillage. Follow regulations regarding the shipment and storage of chemicals.

Sampling Areas and Timing

- Follow a sampling plan/work plan if one is available.
- If a sampling plan is not available for ephemeral data collection immediately after a spill, data collection should focus on collecting samples from a range of unoiled, likely to be oiled, and already oiled areas.
- It is important to obtain reliable data that account for spatial and temporal variations of oil impacts.
- When sampling in remote areas with limited shipping capabilities, plan ahead to make sure that the integrity of samples is not compromised by ensuring that the processing laboratory receives the samples within their recommended holding time. Remember that it may take multiple days for shipments from remote areas to reach a laboratory facility. This last stage is the most important and requires due diligence until the samples are safely delivered.
- The number of locations and number of sites per location need to be considered accordingly, making sure that there is enough space in the coolers to accommodate all samples without sacrificing their integrity.
- Plan all sampling strategies within daylight hours, if possible. However, working from a vessel with deck lights that allow safe operations would permit nighttime sampling. This guideline may not apply during winter or much of the fall.
- The challenges of collecting samples in remote areas, particularly during winter, are great and require adequate planning and careful field implementation to attain the data quality required to meet the objectives of the sampling plan.

Area selection

- Sampling locations should be representative of areas that have been or may be oiled by the spill and unoiled reference areas.
- Use trajectory models, conceptual models, overflight information, SCAT data, or other tools to determine what areas have been oiled and which ones are likely to be oiled.
- Samples should also be collected from locations known or suspected to be affected by other natural or anthropogenic sources of contamination (e.g., oil seeps, coal, peat, mining, combustion engines), as these will be important to differentiate background sources and levels of contamination.
- It may be necessary to prioritize sampling locations. In this case, highest priority samples are to be collected from nearshore water adjacent to sensitive habitats, biologically productive, or highly relevant for human use. Collecting pre-oiling nearshore water adjacent to sensitive/productive areas that are likely to be oiled by the spill in the near future is also a priority. Sampling at unoiled "control" areas and sampling other sources of contamination should be prioritized based on the ephemerality of the data and relative importance to developing a NRDA case.
- Plankton samples should be collected pre-oiling, if possible, as soon as practical after oiling, and periodically thereafter. Sampling frequency should be defined in the study design.
- Use a computer or conceptual model of the extent of water-column contamination or an appropriate power analysis to determine the number and location of samples:
  - Sample along exposure gradients, starting in the cleanest zone, at regular intervals proportional to the exposure area
  - Minimum guidelines under normal conditions are at least three samples per waterbody location of relatively uniform oceanographic and oiling exposure
  - If logistical limitations are a concern, sample collection MUST be prioritized by selecting a minimum of 1 reference/pre-oiled location and 2 heavily oiled locations
- When appropriate take duplicate samples from the same site and following the same steps as the preceding sample. This is not the same as collecting three replicates from each site/depth. A minimum of one duplicate sample should be collected at every third sampling site, or following specifications of the work plan. As a general rule of thumb, duplicates are 10% of all samples, but consideration should be given to sample storage capacity. Do not split samples unless these types of samples are specified in the work plan.
- When present, plankton samples from nearshore lagoons with connectivity or potential connectivity to the marine environment should be obtained.
- Preferably, plankton samples should be collected from boats, though collection by wading in shallow water is also possible.

Collaboration

- Plankton samples from nearshore areas can be collected in conjunction with water, snow, or ice sampling.
- Close collaboration and coordination with other ongoing ephemeral sampling efforts is important.

Field Sampling Methods

Sampling Equipment/Containers

Note: The amount of equipment required depends on the sampling plan, desired sample volumes, and logistics. Analytical laboratories may provide required sampling and sample storage and transport materials – contact the receiving lab before preparing to collect samples in the field.

- Coolers – for sample storage and transport
- Ice packs/Collapsible jugs − for storage temperature regulation (if ambient temperature exceeds 4°C)
- Thermometer or temperature logger (1 per cooler)

- Conical nets: 10-µm Nitex® mesh or similar net (for larger phytoplankton), tow-net (mesh sizes range from 64 µm to 505 µm; e.g., bongo net) ideally equipped with flowmeters – for collection of plankton samples.
- Zooplankton bucket
- Niskin or similar sampler
- Shadowed Image Particle Profiling and Evaluation Recorder (SIPPER) (optional) – Though expensive and requiring significant logistics for shipping and deployment, use of this equipment avoids the issue of contamination of plankton nets and provides in-situ information
- Pencils, waterproof pens, waterproof labels, markers
- Ice corer for sampling under ice
- Disposable nitrile gloves (preferred), insulated nitrile-coated gloves (less ideal)
- Insulated shoulder-length rubberized gloves preferred for water sampling under extreme cold conditions
- 1 L glass jars, amber glass preferred – for plankton and water sampling
- 100 mL plastic centrifuge tubes – for samples collected for purposes other than chemical analysis
- Lugol and 10% buffered formalin (in seawater) (preferred), or in 95% ethanol (less ideal) – for sample preservation
- Packaging materials for glass jars (e.g., bubble wrap, sorbent pads, tape) – may be provided by the analytic laboratory
- Field Sample Forms (template in Appendix A)
- Chain of Custody forms (see Chain of Custody guideline)
- Evidence tape (see Chain of Custody guidelines)
- Field notebook (waterproof paper), other guidelines as needed (Water, Fish, Subtidal and Intertidal Sediment guidelines)
- GPS, camera (with spare batteries), and photo scales
- Packaging materials for glass jars (e.g., bubble wrap, sorbent pads, tape) – may be provided by the analytic laboratory
- Suitable disposal bags for oiled PPE and disposable sampling materials

Optional (if single-use sampling equipment are not available):

- Sufficient quantities of pre-cleaned or disposable single-use equipment are preferable. If equipment will be reused in the field, decontamination is necessary and will require the following materials:
  - Reusable sampling equipment
  - Solvents for cleaning sampling equipment – acetone, methanol, or hexane (Capillary GC Pesticide Residue Grade or equivalent) – consider shipping/airline regulations for solvents
  - Teflon solvent squirt bottles
  - Laboratory-grade, certified-clean distilled water (preferred), store-bought distilled water (less ideal); laboratory-grade detergent
  - Approved, sealed container for collecting solvent rinsate for disposal

Quality Assurance/Control

- Rinsate blanks should be collected if there is a risk of cross contamination from reuse of sampling equipment. After cleaning the equipment in accordance with the procedures described in this method, rinse the clean equipment with solvent or cleaning solution and collect the rinsate in a sample jar. Note on the field sample form where and how rinsate blanks were collected.

Good Sampling Practices and Decontamination

- Good field practices and the development of a consistent sampling routine will help provide for the integrity of the samples and their validity in environmental assessments.

- Check for major rips or holes in the mesh, especially in the lower 1/3 of the net. If holes are detected, repair them or replace the net.
- Make sure that there are no bubbles in the flowmeters. Check to insure that the flowmeter rotor spins freely and does not wobble, i.e., the shaft is not bent.
- Disposable nitrile gloves should be worn when sampling and changed between each sample collected or as necessary to prevent cross contamination.
- Disposable nitrile gloves can be worn over low-profile insulated gloves (e.g., neoprene gloves) in cold conditions and should be changed between samples to prevent cross contamination if they become contaminated or damaged. If nitrile gloves are not available or will not fit over insulated gloves in cold conditions, insulated nitrile-coated gloves may be an alternative, but extra precautions will have to be taken to prevent sample contamination; gloves will need to be cleaned with soap and clean water between samples and should not come in contact with the sample or with the surfaces of glassware or tools that will be in direct contact with the sample. Similar precautions should be taken when using insulated shoulder-length rubberized gloves.
- To reduce the need for field decontamination, use pre-cleaned and/or disposable equipment and tools.
- If disposable sampling equipment are not available, reusable sampling equipment MUST be decontaminated between samples collected for chemical analysis. To decontaminate sampling nets if they become visibly oiled and any other sampling equipment prior to each use:
  - Wash nets/equipment/sampling utensil with laboratory-grade detergent and clean with a triple clean-water rinse. Cleaning with laboratory-grade water is preferred, though store-bought distilled water is a less ideal alternative and, as a last resort, “background” water from an up-current clean area can be used.
- Take precautions to avoid cross-contamination of the site from oil on boots and other gear.
- Potential sources of contamination while sampling from vessels (exhaust fumes, oily surfaces) are a concern. Work up-wind of any exhausts, and designate clean areas for handling samples. Segregate dirty/clean areas. Layout clean surfaces to work on and replace frequently.
- Contamination by surface slicks is of great concern. Document presence of slicks, weather, wave conditions, etc. which might suggest mixing of surface oil during sampling.

Sample Collection Methods

- Use field data forms included in the work plan, if one is available. Otherwise, use forms in Appendix A. Coordinate data form development/modification with the data management group.
- Because GPS units will be used to record locations and times, make sure that all units are using the same coordinate system, datum, reporting units, and correct time. Follow the recommended GPS datum of the study plan, if one is available. Alternatively, set the default to WGS84.
- Record GPS coordinates for each sample site.
- Photograph the sampling site prior to sample collection to document the site conditions, as well as the sample collected. Make sure each photograph or series can be later associated with the corresponding sampling locations (e.g., through use of GPS Photo link software or by keeping a detailed photo log with waypoints and/or lat/long). Do not delete or alter any photographs. The numbering sequence of photographs uploaded from your camera must not have any gaps (see Field Photography guideline).
- Avoid sampling under sheens and oil slicks, but if unavoidable, clear surface oil prior to plankton sampling. If possible, place sorbent boom upstream of the sampling site to temporarily divert oil. Alternatively, use sorbents to remove oil inside the net prior to collection of plankton.
- If possible consult a plankton expert when developing the study design to determine what net size is appropriate for the study. Modifications of this guideline may be needed depending on the types of plankton that are being targeted and/or the nets that are available for plankton sampling. Note that species composition, plankton size, life stages, etc., captured will vary depending on the net mesh size used for sampling. For example:
  - Mesh size as small as 64 µm can be used to sample phytoplankton and micro-zooplankton

  - Mesh size up to 3 mm can be used to capture the largest zooplankton
  - Standard mesh size used in the Arctic are typically 150 µm and 505 µm for small and large taxa, respectively
- Vertical, double-oblique and horizontal deployment speed needs to be considered and recorded for each type of net used in plankton tows.
- To sample phytoplankton qualitatively (presence/absence, taxonomic surveys) (less ideal):
  - Lower a 10-µm Nitex® mesh or similar net to a given depth, allow it to settle for 30 seconds and pull it slowly to the surface
  - Slowly pull the neck back to the surface. If bow waves or obvious disturbances of the water column or water surface are observed discard sample and start again
  - After retrieving the net from the water, place the mouth of the net into a 1 L sample-collecting bottle and drain the sample. Collect samples in triplicate
- To sample phytoplankton quantitatively:
  - Open the, Niskin or similar sampler (e.g., 4 L) by raising the end seals and set the trigger mechanism
  - Lower the sampler to the desired depth, send the messenger down to close the seals, and retrieve the sampler to the surface
  - Carefully, remove the sampling bottle, gently shake it and transfer contents into a 1 L sample bottle leaving a 3-5 cm headspace
  - Preserve samples with 3 mL of Lugol's solution or 0.05-1% by volume, followed by preservation with 20 mL of an acidified formalin solution (2% by volume). The second preservation step is important for preserving the color of algae. If space in coolers is limited and/or there are concerns about transport of preservatives, omit the second preservation step
    - Lugol's solution can be prepared as follows: 100 g I, 200 g KI, 200 mL glacial acetic acid, and 2000 mL of distilled water. This solution MUST be stored in a dark bottle because of its sensitivity to light
    - Acidified formalin solution can be prepared as follows: equal volumes of formaldehyde (37%) and glacial acetic acid
    - Both of these solutions can be prepared in advance and used up to 2 months of preparation. Label each bottle including the name of the preservative and the date of preparation
    - Cap the bottle and place it a cooler, minimizing exposure to light
- To sample zooplankton quantitatively:
  - Select the appropriate zooplankton mesh size tow-net (64 µm to 505 µm) equipped with a flowmeter. A flowmeter is important because it allows an estimation of the volume of water that passes through the net. This information, in combination with counts of organisms caught in the net, zooplankton concentration per volume of seawater can be inferred
  - Soak the body of the tow-net in water for 2 minutes and rinse the net to dislodge any attached material
  - Attach the zooplankton bucket/bongo with plug in place, and fill a Nalgene squirt bottle with seawater that has been filtered through the net mesh
  - For vertical sampling, which targets smaller zooplankton, lower the net to the appropriate depth in a vertical position, allow the net to settle for 10 seconds, and raise it vertically at average speed of 1 knot (0.5 m/s) to minimize avoidance of the net by fast-swimming zooplankton. This sampling should performed from a stationary or anchored ship, or from a fixed platform
  - For double-oblique sampling, which targets larger and more mobile zooplankton, lower the net wide from the surface to the desire depth, continue sampling at the desire depth for 30 seconds, and retrieve the net back to the surface. This sampling should performed with the ship moving at an average speed of 2 knots (~1 m/sec)
  - For horizontal sampling, useful when characterizing zooplankton at different depths, lower the net to the target depth, pull the net at that depth for approximately 5 min at average speed of 1

knot (0.5 m/s), and quickly retrieve the net back to the surface. This type of sampling is also useful for surface sampling in shallow waters. In these cases, place the net on the water surface and once it is half way submerged, start pulling the net at an upward angle
  - At the surface, rinse down the outer sides of the net 3 times with seawater avoiding the net opening, and do not let the net drop below the surface
  - Separate the bucket from the net, place the lower end of the bucket into a sample bottle. Remove the plug and drain the bucket contents
  - Rinse the bucket contents into the sample tube with the squeeze bottle previously filled with filtered net water
  - Preserve zooplankton samples in 10% buffered formalin (in seawater) (preferred), or in 95% ethanol (less ideal)
  - Do not freeze preserved samples
- Rinse the net and bucket with clean seawater between sites.
- When sampling through ice:
  - Clear loose ice and snow away from the sampling location and drill through the ice
  - Clean the drill-hole area from potential sources of contamination, and allow several minutes for the water to flow freely under the ice before taking a sample
  - Carefully lower the net and follow the rest of the sampling procedure and preservation as described above
  - To sample ice plankton, collect a 7.5-10.5 cm diameter core and cut the bottom 2-4 cm of ice. Melt the ice in surface water filtered through 0.2 µm polycarbonate membranes, recording the volume of water used
  - Preserve samples for phytoplankton analysis with Lugol's solution followed by an acidified formalin solution (see Ice guidelines)
  - Preserve samples for zooplankton analysis in 10% buffered formalin (in seawater) (preferred), or in 95% ethanol (less ideal)
- To sample plankton quantitatively *in-situ* (only if resources and personnel are available): The SIPPER (Shadowed Image Particle Profiling and Evaluation Recorder) can be used to collect high-resolution information on the distribution of zooplankton, phytoplankton, larval fish, and detritus within a 100 cm^2 sampling area as it moves through the water. The SIPPER is also equipped with a CTD (conductivity, temperature, depth) scan, oxygen sensor, fluorometer, transmissometer and CDOM (colored dissolved organic matter) fluorometer. When using this sampling strategy:
  - Tow the SIPPER horizontally and vertically through the water at speeds between 1-4 knots. SIPPER tows require at least an hour per 100 m depth
  - Conduct multiple tows at day and night in control/least oiled and contaminated waters
  - Sampling locations, depths, number of transects, and aerial extent, will be adaptively selected. Ideally, distinct areas of relative uniform exposure would be sampled once (1 SIPPPER vertical profile) every 100 m
  - SIPPER operation will require expertise and training, and will require on board ship space
- Plankton samples from nearshore areas should be collected in conjunction with water sampling for basic environmental parameters (i.e., temperature, salinity). If practical, deploy a CTD to collect oceanographic parameters at every sampling site.

Sample Labeling and Record Keeping

- Verify that all samples are properly labeled, and that field sample forms are properly filled out.
- Follow chain of custody procedures for securing samples and complete chain of custody forms (See Chain of Custody guidelines).
- Complete the Chain of Custody form, noting where each plankton sample was collected, sampling equipment used, time/date of collection, size and container type, and sampler name.

- Make special notation on the Chain of Custody form about any problems or observations during sampling.
- Maintain strict chain of custody during sample storage and transportation.
- Record the sample # on both the label and lid. Record the following on the field sample form:
  - Sample collection site (NRDA sample grid ID and GPS coordinates)
  - Sample matrix (plankton)
  - Sample #, date/time
  - Sampling method (bottle, net), sample collection depth, distance from shoreline
  - Sample #; date/time; station location; GPS coordinates, water depth
  - Characteristics of suspended material in the water sample: texture, color, biota, vegetation, debris, odor, etc.
- All sample numbers must be unique. Use the sample number convention provided by data management if available. Otherwise, the sample number should consist of a sample team ID and sequential numbers. For example AKA-0001, AKA-0002, etc.
- If sample volume is split between two jars, both jars should receive the same sample ID and be recorded on a single line of the Chain of Custody form.
- Documenting oil exposure is best accomplished with photography, video, and good field notes and sketches using standard shoreline assessment methods.
- Keep a detailed photo log so that each photograph can be labeled.
- Note any deviations from the recommended guidelines in the field book.

Preservation/Holding Times

- Follow chain of custody procedures for sample storage and shipping.
- Ship highly oiled samples separate from lightly or unoiled samples to reduce risk of cross-contamination.
- Immediately following collection, place all preserved plankton samples in a cooler. DO NOT FREEZE samples preserved in formalin. Refrigeration temperature shall be recorded upon sample storage and monitored and recorded periodically to ensure proper refrigeration.
- In below-freezing temperatures, collapsible jugs of warm water can be used in the cooler between samples to prevent them from freezing.
- Preserve samples immediately after collection, and discard samples not preserved within one hour of collection.
- Do NOT use freshwater when sampling and preserving plankton samples.
- Do NOT keep plankton tows that contain sediments.
- Tape lids on sample bottles so that they do not accidentally come off.
- Use packing material, such as bubble wrap or sorbent pads, around glass jars to prevent breakage during transport and shipping.
- Ship samples directly to the laboratory as soon as practical, overnight (preferred), with completed Chain of Custody forms. If necessary, samples can be stored under specified conditions and with complete chain of custody until they can be shipped. Assure that samples are packaged to protect them from breakage, shipping containers are sealed and use ice packs or dry ice to maintain storage temperatures during shipment to the lab.
- NEVER discard any samples even if these have exceeded their recommended holding times or storage temperatures.

Key References

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Appendix A
Supporting Documentation- Field Data Form Examples

Unique field data forms may be included in the work plan if one has been developed, otherwise, use the attached form.

- Print the form on weather-resistant paper (if available). Make more than enough copies of the form before going into the field.
- Fill out forms with waterproof pen or permanent marker. Do not use pencil or biro (erasable) ink.
- Make any additional notes that do not fit on the form in a field notebook and indicate the presence of associated additional notes on the field data form.
- Fill in blanks with "N/A" if data are not applicable or not available. Avoid leaving blank values on data forms.
- Do not erase or black out erroneous entries on the field data forms. Errors should be corrected by crossing out the entry with a single line and signing and dating the strike-through.
- Electronic versions of field data forms are available. Coordinate data entry with NRDA data management personnel.

Attached form:
- Plankton Sample Collection Form

NRDA Sample Collection Form - PLANKTON

Lead Sampler's Name/Phone		**Sampler Team Code**		**Vessel Name**	
Lead Sampler's Affiliation		**Resource Group**		**Wind Speed**	
NRDA Contact/Phone		**Resource Group Leader**		**Wind Direction**	
Incident Name		**Habitat (e.g., lagoon)**			
General Location Description		**Sample date (mm/dd/yyyy)**			

Location Code	Sample Number	Sampling Gear / Size	Tow Start Time	Tow Start Latitude	Tow Start Longitude	Flow Meter Start	Tow End Time	Tow End Latitude	Tow End Longitude	Flow Meter End	Tow Depth or Max Depth	Tow Length	Sample Container	Sample preservation	Instrument Data	Sample Notes
NRDA Sample Grid ID	*TEAM ID - Sequential Numbers*	*Net type / Mesh Size (uM)*	*(24-hr clock, local time)*	*Latitude in DD XX.XXXXXX*	*Longitude in DD - YY'Y.YYYYYY*		*(24-hr clock, local time)*	*Latitude in DD XX.XXXXXX*	*Longitude in DD - YY'Y.YYYYYY*		*Depth (m)*	*Distance (m)*	*Jar, tube, etc.*	*Preservative (Formalin, Ethanol, etc.) or NA*	*Instrument Used (CTD, LISST, etc.) / Instrument Metadada File Name*	

Survey Notes - (flow meter serial number, weather, wildlife observed, photos, etc.)

Relinquished by:				**Received by:**			
Date	**Time**	**Signature -Field Sampler**	**Print Name- Field Sampler**	**Date**	**Time**	**Signature - Sample Runner/ Command Post**	**Print Name - Sample Runner/ Command Post**