Standard Operating Procedure for Mysis Sample Collection and Preservation

LG409

Version 04, March 2021

TABLE OF CONTENTS

Standard Operating Procedure for Mysis Sample Collection and Preservation

1.0 SCOPE AND APPLICATION

1.1 This Standard Operating Procedure describes field sampling and preservation of mysid samples for the GLNPO open water Great Lakes surveys.

2.0 SUMMARY OF METHOD

2.1 Two replicate tows are performed at each station visited at night where the bottom depth is at least 30 meters. Retrieval should be initiated between one hour after sunset and 30 minutes before sunrise. Limit sampling to one tow if the second tow would start outside the sampling window. Tows are taken from 3 m off the bottom in water shallower than 100 m and from 5 m off the bottom in water deeper than 100 m, using a 1 m diameter *Mysis* net. The net is lowered to the desired depth, left at depth for 30 seconds, and raised at 0.5 m/s to collect mysids from the water column. After lifting the net from the water, it is sprayed with a hose to concentrate the animals in the sample bucket. The sample is transferred to a 1-L sample bottle, narcotized with soda water, and preserved with buffered sucrose formalin solution.

3.0 SAFETY AND WASTE HANDLING

3.1 Refer to GLNPO's Health, Safety, and Environmental Compliance Manual (May 1997, or as amended) and individual instrument procedural operations manuals for specific details on applicable 1) personal health and safety issues; 2) instrumental, chemical, and waste handling procedures; and 3) accident prevention. This applies to all EPA personnel, EPA contractors, or federal, state, or local government agencies, and persons who operate or are passengers onboard US EPA GLNPO vessels during all activities and surveys

3.2 It is the responsibility of the user of the method to comply with relevant chemical disposal and waste regulations as sited in GLNPO's Health, Safety and Environmental Compliance Manual (May 1997, or as amended). All applicable safety and waste handling rules are to be followed. All containers storing reagents, standards, controls, blanks, and wastes used in the laboratory must be properly identified through appropriate labeling and hazard definition. Good technique includes minimizing contaminated waste. Over-board discharges of chemical waste are forbidden.

3.3 Every chemical should be regarded as a potential health hazard and exposure to these compounds should be as low as reasonably achievable. Please refer to Appendix L in GLNPO's Health, Safety and Environmental Compliance Manual (May 1997, or as amended) for more detailed descriptions of the potential risks associated with formaldehyde.

3.4 During sampling, caution, common sense, and good judgment should dictate appropriate safety gear to be worn in any given situation on deck. Hard hats, gloves and steel-toed shoes must be worn in working conditions where there is a possibility of injury to the head, hands, or feet; however, if in doubt, please ask the Chemical Hygiene Officer.

3.5 Collecting samples in cold weather, especially around cold water bodies, carries the risk of hypothermia and frostbite. Sampling team members should wear adequate clothing for protection in cold weather. For specific information regarding sampling during cold conditions, please refer to the Winter Survey Plan, Appendix N of *QAPP US EPA Great Lakes National Program Office Open Lake Water Quality Sampling Surveys, May 2017, Updated March 2019*.

3.6 Collecting samples in extremely hot and humid weather carries the risk of dehydration and heat stroke. Sampling team members should carry an adequate supply of water or other liquids for protection against dehydration in hot weather.

3.7 Work vests must be worn while working on the fantail and Rosette deck.

3.8 Formaldehyde is a known carcinogen. During the preservation of samples, the formalin should be dispensed under a hood. A lab coat, gloves, and safety glasses or goggles should be worn.

4.0 EQUIPMENT AND SUPPLIES

4.1 Winch with metering sheave and hydrographic line
Mysid tow net, 500-µm pore size in upper 2/3, 250-µm pore size in lower 1/3, 1-m diameter (D:L = 1:2)
Tow net sample bucket with a 250-µm pore size Nitex mesh screen, 12 in. long
Safety line for sample bucket
Weights, 10-20 lbs.
Garden hose
Red lights (fixtures and bulbs and extra bulbs) or headlamps with red light
Hard-copy *Mysis* Sampling Data Form (printed on waterproof paper)
Sieves (≤ 250µm Nitex mesh)
Soda water (club soda)
Plastic sample jars, 1-L volume
Sample jar labels
Basins for sorting mysids (Pyrex casserole dishes work well)
Forceps for sorting and picking mysids
Screw-cap cryovials, 1.5 mL volume (up to 25 needed per station for gravid females)
Holding tray for 1.5 mL cryovials (2-3 with at least 25 slots ea.)
Wash bottles; some for DI water and some for formalin
Funnels; one non-formalin for examining mysids, one for formalin
Graduated cylinder with 50-100 mL capacity, for measuring formalin
Plastic basin for formalin work
Box of nitrile gloves (multiple sizes for different technicians)
Eye protection

5.0 REAGENTS

5.1 Reagents can be ordered through chemical supply companies.

5.2 The reagents needed are as follows:
Sucrose (crystalline)
Formalin (37% solution of formaldehyde in water).

6.0 SUCROSE FORMALIN & 10% FORMALIN PREPARATION

6.1 For buffered sucrose formalin, dissolve 60 g sucrose in 1 L of formalin solution (37% formaldehyde) under a fume hood. Store in labeled plastic container.

6.2 For 10% formalin solution for cryovials, pour 100 mL sucrose-buffered formalin solution (37% formaldehyde) into a 1 L plastic bottle. Fill with water, to make a 10% buffered formalin solution (3.7% formaldehyde). Store in labeled plastic container.

7.0 SAMPLING PROCEDURE

7.1 The following should be recorded on a *Mysis* Sampling Data Form printed on waterproof paper (See Appendix E of the *QAPP US EPA Great Lakes National Program Office Open Lake Water Quality Sampling Surveys, May 2017, Updated March 2019*): Lake, Visit ID, Station ID, Sample Date, Personnel (initials, XXX), and Station Depth for each station. For each sample at a station, the datasheet should record the following: Sample ID, Sample Time, Sample Depth, Wire Angle, Replicate Code (RFS or FD1), Number of cryovials with individual gravid females, and Remarks, especially on weather and cloud cover. The preprinted sample jar labels record Sample ID, Lake, Station, and Survey.

7.2 Proper natural and artificial light conditions must be met for sampling to proceed. Deck lights must be turned off and aft windows in the wet lab should be covered by curtains prior to arrival at station. Aft lights in the wet lab should be turned off five minutes prior to arriving at the station. The deck should be lit for safety, but only red lights are permissible until *Mysis* tows are completed.

7.3 *Mysis* sample retrieval should be initiated at least 1 hour after sunset and at least 30 minutes before sunrise. Refer to LG402B Standard Operating Procedure for Secchi Depth Measurement Field Procedures for calculations of sunset and sunrise time (accessible at the R/V Lake Guardian SOP Repository available at login.glnpo.net). If time only allows for one tow within the prescribed time window, one tow should be collected, and this should be noted on the *Mysis* Sampling Data Form (field sheet). If it is uncertain whether the net retrieval would occur prior to 30 minutes before sunrise, either for a first or a second tow, it is better to collect a sample than not. The exact time net retrieval was initiated should be recorded on the field sheet. *Mysis* are still in the water column within 30 minutes of sunrise, at least in deeper lakes (see Figure 1); thus, collection of these “borderline” net tows is warranted.

At each site, the official total water depth is obtained from the SeaBird profile. However, the rosette cast (with the SeaBird profiler) is deployed after the *Mysis* tow, so the depth of the *Mysis* tow is determined by the depth sounder. If the boat drifts and bottom depth changes for the second tow, make a note of this in the field sheet and write down separate bottom depths for the two tows.

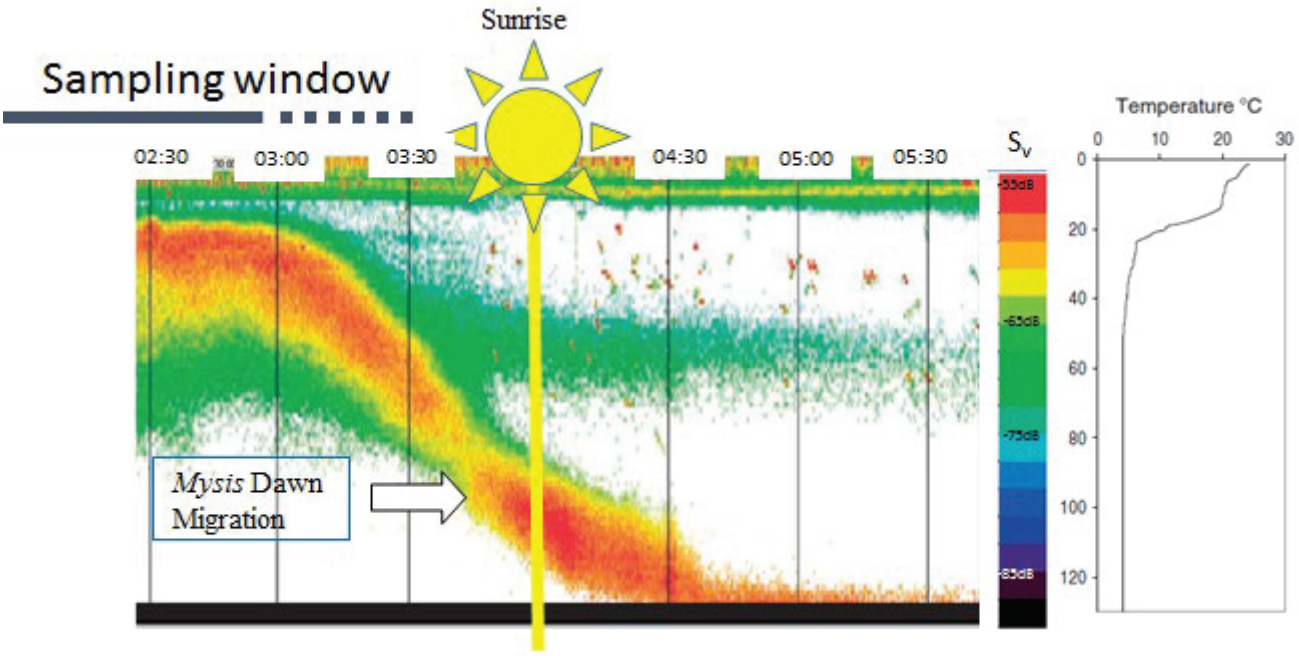

Figure 1. Acoustic trace of downward migration of *Mysis diluviana* in Lake Ontario on July 31, 1995 from 2:25 to 5:40 AM local time at a 130 m deep station. Vertical lines represent 30 minutes, sunrise at 4:00 given as a heavy line, and sampling window is up to 30 minutes prior to sunrise. From Rudstam (2009). Color represents mysid biomass. Temperature profile is also given.

7.4 The 250-µm sample bucket is attached to the net and the net is attached to the winch cable. A rope bridle is clipped to the net frame and extended to the cod end of the net where it is attached to the sample bucket. A weight is added to the lower end of the rope and the bridle is adjusted so that the frame of the net (not the mesh netting) supports the weight.

7.5 The deck hand should confirm station depth with the pilot house just prior to net deployment. It is best if the crew also checks the rosette depth for the last time the station was visited; if the last rosette depth is much shallower, consider towing the net to a shallower depth. If the depth is less than 100 m, send the net to bottom depth minus 3 m. If the depth is equal to or greater than 100 m, send the net to bottom depth minus 5 m. Request permission from pilot to tow Mysis net from this depth.

7.6 Mysid samples do not need to be collected in Western and Central basin of Lake Erie or at stations with bottom depths shallower than 30 m.

7.7 The winch operator deploys the net so that the rim is at the surface of the water and then sets the cable sheave to zero. Lower the net slowly to the specified depth.

7.8 After letting the net sit at depth for 30 seconds, retrieve it at 0.5 m/s. Record the tow time as the time at the beginning of the net retrieval on the field sheet. Also record the wire angle at the beginning of the net retrieval on the field sheet.

7.9 Raise the net to about the chest level of the collector. If the net has inverted or acquired any mud upon retrieval, another cast will have to be completed, after rinsing out the net and sample bucket and re-confirming station depth with the pilot house. Mud on the outside of the cod-end or on the anchor, but not inside the net and cod-end does not necessitate re-casting the net. Be sure to note any mud on casts in the 'Remarks' section of the field sheet, whether or not the cast was kept. Upon retrieval, rinse down gently from the outside with ambient temperature lake water to wash all of the organisms off the net cloth and into the sample bucket. Once the net has been rinsed, pull the weight inboard by the weight

leads, being careful not to tip the sample bucket. Lower the Mysis net until the frame rests a few inches below the top of the bulwarks.

7.10 Spray the remainder of the net. You may need to gently swirl the sample bucket or gently tap its mesh screen to concentrate the sample so that it does not overflow when unclipped. Unclip the weight from the weight lead on the sample bucket, unclip the sample bucket from the net, and immediately wash the contents into a 1-L sample jar. After several rinses, look inside sample bucket with a headlamp to ensure all individual mysids are transferred to the sample jar. Use enough DI water to rinse down the sides of the sample jar so the mysids do not get stuck on the sides and dry out.

7.11 Attach the next sample bucket to the net and the weight to the weight lead. Repeat the procedure for the second (replicate) *Mysis* tow if the retrieval of the second tow can be started 30 minutes before sunrise.

8.0 SAMPLE PRESERVATION

8.1 Add 20 mL of soda water to each sample jar and refrigerate the jar. This should be done as soon as possible after collection. Refrigerate the sample for at least 30 minutes.

8.2 Once narcotized, carefully wash the sample jar into a sorting tray and scan for gravid females. Remove up to 25 gravid females per station (total from both replicate tows) using forceps, placing each in a cryovial. These are to be used for brood counts. Note the number of gravid females removed and placed into cryovials on the field sheet. Set cryovials aside in holding tray. Carefully wash the remaining mysids and other zooplankton from the sorting tray back into sample jar through a funnel.

8.3 Under a fume hood, use a disposable plastic pipette to fill each cryovial containing a gravid female with the 10% buffered formalin solution (3.7% formaldehyde, prepared ahead, see section 6.2), and add these filled cryovials into the sample jar. Then add stock buffered formalin solution (37% formaldehyde) to achieve a concentration of 10% buffered formalin solution in the 1-L sample jar (add 100mL stock formalin solution and up to 900 mL water in the 1-L jar of examined mysids). Cap sample jar tightly. Wrap the cap and neck with parafilm to prevent leaks and stow the sample storage bottle in a designated container. The samples do not need to be kept cool after preservation.

9.0 FIELD QUALITY CONTROL

9.1 Wire Angle

9.1.1 The wire line of the winch should be nearly vertical to obtain reproducible results. An effort should be made for the wire to be as vertical as possible. Record the actual wire angle at beginning of retrieval and note any apparent drifting of the net during retrieval.

9.2 Uninterrupted Towing

9.2.1 If the tow is interrupted by stopping or changing the winch speed, the sample is discarded, the net is washed, and the tow is repeated (steps 7.4 to 7.10).

9.3 The addition of the club soda is performed within an hour of collection.

9.4 The addition of formalin preservative is performed within 2 hours of collection.

10.0 *MYSIS* SAMPLING DATA FORM

The field technician should use the hard copy *Mysis* Sampling Data Form to enter the relevant sampling data. A copy of this form can be found in Appendix E of the WQS QAPP and is displayed in Attachment 1 of this SOP. The following table provides guidance on entering data in each field in the

form. See LG101 Electronic Field Information Recording for an explanation of Survey ID, Visit ID, Station ID and Sample ID.

Mysis Sampling Data Form	
Field Name	**Data Entry Instructions**
Survey ID	[preprinted; example "MI1921"]
Visit ID	[preprinted; example "M017G19"]
Station ID	[preprinted; example "MI 17"]
Sample Date	Enter the date on which the sample was collected in "mm/dd/yyyy" format
Station Depth	Enter the station depth provided by the pilot house from the depth sounder (not from the Rosette deployment; in meters); recheck and record for each tow
Personnel, Initials	Enter full initials of personnel entering data on the form
Sample ID	[preprinted; example "19GM40S22"]
Sample Time	Enter the time at which the mysis sample retrieval was initiated in "UTC, military" format
Depth Code	[preprinted; "Mysis"]
QC ID Code	[preprinted; "RFS", "FD1"]
Sample Depths – Starting	Enter the starting sample tow depth (e.g., near the bottom depth; in meters)
Wire Angle	Enter the actual angle off of vertical at beginning of retrieval (in degrees) for the wire line of the winch.
Number of Cryovials	Enter the number of cryovials used for individual gravid females
Remarks	Indicate special circumstances for the tow (e.g., mud on the net, net was inverted, drifting of the net, cast was discarded and why, tow not taken and why, weather conditions, light meter reading)

11.0 REFERENCES

Rudstam, L.G., 2009. Other zooplankton, in: Likens, G.E. (Ed.), Encyclopedia of inland waters. Elsevier, Oxford, UK, pp. 667-677

Attachment 1: *Mysis* Sampling Data Form

LG409 Mysid Field Sheet | Mysis Sampling Data | Summer 2019 GLNPO's WQS

Survey ID	Visit ID	Station ID	Sample Date (mm/dd/yyyy)	Station Depth (m)	Personnel, Initials
[preprinted]	[preprinted]	[preprinted]			

Sample ID	Sample Time (UTC, military)	Depth Code	QC ID Code	Sample Depth (m)	Wire Angle (degrees)	Number Cryovials	Remarks
[preprinted]		[preprinted]	[preprinted]				

Method: LG 409

Entered into electronic file _____ (Initials)