Standard Operating Procedure for Mysis Sample Collection and Preservation

LG409

Revision 01, April 2015

TABLE OF CONTENTS

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 (between one hour after sunset and one hour before sunrise). Tows are taken from 3 m from the bottom in water shallower than 100 m and from 5 m from the bottom in water deeper than 100 m, using a 1 m diameter Mysis net described below. The net is lowered to the desired depth, left at depth for 60 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 remainder of 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 and ethanol.

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 US EPA GLNPO Standard Operating Procedures for Winter Operations (December 1994, or as amended).

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 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.
Weights, 10-20 lbs.
Winch with metering sheave and hydrographic line
Safety line for sample bucket
Garden hose
Red lights (fixtures and bulbs and extra bulbs) or headlamps with red light
Sieves (≤ 250µm Nitex mesh)
Soda water (club soda)
1-L plastic sample jar
Sample jar labels
Graduated cylinder with 50-100 mL capacity
Funnels for formalin Basins for sorting mysids Depth sensor (e.g., MK9 tag) Cable ties
1.5 mL screw-cap bullet tubes (up to 25 per station)
Holding tray for 1.5 mL bullet tubes (2-3 with at least 25 slots ea.) Squirt bottles; some for DI water and some for formalin
Plastic basin for formalin work
Forceps
Box of nitrile gloves
Eye protection
Funnel (non-formalin) for examining mysids

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).
95% Ethyl alcohol.

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 bullet tubes, pour 100 mL sucrose-buffered formalin solution (37% formaldehyde) into 1 L 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 A logbook or datasheet should record the following (See Appendix 1): Lake, Visit ID, Station ID, Day or Night, Sample Date, Personnel (surname), Station Depth, and Weather Conditions for each station. For each sample at a station, the datasheet should record the following: Sample ID, Sample Time, Starting Depth, Ending Depth, Wire Angle, Replicate Code, Number of bullet tubes of individual gravid females, and Remarks, esp. on weather and cloud cover. The sample jar labels should record Sample ID, Collection Date/Time, Start and End Tow Depths, and Replicate.

7.2 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.

7.2.1 If boat drifts substantially between initial station sounding and SeaBird profile, use judgment to determine a total depth intermediate between the two. Be sure to consider the typical depths of the region over which the boat has drifted. Note drift distance and depth difference under remarks in the field data sheet. Record how recorded depth was determined in field sheet.

7.3 Proper natural and artificial light conditions must be met for sampling to proceed. Mysis sampling must occur between 1 hour after sunset and 1 hour before sunrise. Deck lights must have been turned off and aft windows in wet lab covered prior to arrival at station. Aft lights in wet lab should be turned off 5 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.

7.4 Affix a depth sensor to the net to determine actual sampling depth. The MK9 tag is currently used.

7.5 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.

7.6 The deck hand should confirm station depth with the pilot house just prior to net deployment. 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.

7.7 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 60 seconds, retrieve at 0.5 m/s. Record the time and wire angle at the beginning of the retrieval.

7.9 Raise the net to about 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. The net is rinsed 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 it does not overflow when unclipped. Unclip the weight from the weight lead on the sample bucket, and the sample bucket from the cod end and immediately wash contents into a 1-L sample jar from the sample bucket. Use enough DI water to rinse down the sides of the sample jar so the mysids are not drying out on the sides.

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.

7.12 Bring into the wet lab and begin the preservation process (see section 8.0) while the next net is being deployed. Be sure to fill in label and record everything in the log book as each sample is taken. Also, be sure to record the sample date, time, and tow depth on the sample jar with permanent marker. This is helpful information for lab analysts as they observe patterns.

7.13 When finished with the Mysis tows, the deck lights may be turned on for the remainder of the station sample collections.

8.0 SAMPLE PRESERVATION

8.1 Add 20 mL soda water to each sample jar, and refrigerate the jar. This should be done as soon as possible after collection. Refrigerate the mysids 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 bullet tube. These are to be used for egg counts. Note how many mysids were removed and number of gravid females placed into tubes on field sheet. Set tubes 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 Immediately preserve mysids when washed back into tray. Use a pipet to fill bullet tubes containing gravid females with the 10% buffered formalin solution (3.7% formaldehyde). Under a fume hood, add 100 mL of stock buffered formalin solution (37% formaldehyde) to the 1 L sample jar of examined mysids. Insert bullet tubes into sample jar. Fill the 1-L sample jar with water. This results in a 10% sucrose-buffered formalin (3.7% formaldehyde) solution in the sample jar. Cap sample jar tightly. Wrap the cap and neck with parafilm to prevent leaks, and stow the sample storage bottle in a designated tote. The samples do not need to be kept cool.

9.0 FIELD QUALITY CONTROL

9.1 Cable Angle

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

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.

APPENDIX 1 – FIELD DATA SHEET FORMAT

GLNPO Mysis Field Sheets

Lake: _____ **Visit ID:** _____

Station ID	Day OR Night (D or N)	Sample Date (mm/dd/yyyy)	Personnel (surname)	Station Depth (m)

Weather Conditions:

Mysids

Sample ID	Sample Time (military)	Starting Depth (meters)	Ending Depth (meters)	Wire Angle (degrees)	Replicate Code	Number Bullet Tubes	Remarks

Entered into electronic file: _____/_____
(date/initials)