Standard Operating Procedure for Zooplankton Sample Collection and Preservation

LG402A

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1.0 SCOPE AND APPLICATION

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

2.0 SUMMARY OF METHOD

2.1 Two sampling tows are performed at each station. One tow is from 20 meters below the water surface to the surface using a 63-µm net. The other tow is from 100 meters below the surface to the surface using a 153-µm net. If the station depth is less than the specified depth, the tow is taken from two meters above the bottom to the surface. The tow net, with a screened sample bucket attached at the bottom, is lowered to the desired depth, and raised at 0.5 meters per second to collect zooplankton from the water column. After the net is lifted from the water, it is sprayed with a garden hose to wash the organisms down into the bucket. The sample is concentrated into the sample bucket and is transferred to a sample storage bottle. The organisms are narcotized with soda water and preserved with sucrose formalin solution.

3.0 SAFETY AND WASTE HANDLING

3.1 Refer to GLNPO Safety, Health & Environmental Compliance Manual (Version 9.3) 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 this method to comply with relevant chemical disposal and waste regulations as sited in *GLNPO Safety, Health & Environmental Compliance* Manual (Version 9.3). 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 wastes 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 Safety, Health & Environmental Compliance* Manual (Version 9.3) for more detailed descriptions of the potential risks associated with any chemicals used in this method. It is good laboratory practice to wear a lab coat, safety goggles, and gloves at all times.

3.4 During sampling, caution, common sense, and good judgement 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 Standard Operating Procedures for Winter Operations (available from GLNPO as Appendix N in the *QAPP US EPA Great Lakes National Program Office Open Lake Water Quality Sampling Surveys* (May 2017, revised 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

Plankton tow net, 63-µm pore size, 0.5-m diameter (D:L=1:3)
Plankton tow net, 153-µm pore size, 0.5-m diameter (D:L=1:3)
Tow net sample bucket with a 61-µm pore size metal screen
Tow net sample bucket with a 151-µm pore size metal screen
PVC framed hand sieve (53- µm)
Flowmeters - TSK (Tsurumi Seiki Co., Ltd.)
Weights, 10-20 lbs.
Net ring for flowmeter calibrations
Safety line for sample bucket
Garden hose and sprayer
Soda water (club soda)
500-mL plastic sample bottles
50 ml plastic or glass beaker with gradations
Graduated cylinder with 50 - 100 mL capacity
Hard-copy Zooplankton Sampling and Secchi Disk Data Form (printed on waterproof paper)
Gloves (neoprene)
Winch with metering sheave and wire

5.0 REAGENTS

Sucrose (crystalline), available as food-grade sugar
Formalin (37% solution of formaldehyde in water) in gallon jugs
Borax (powder), available in box as laundry additive
Rose Bengal biological stain (powder)

6.0 BUFFERED SUCROSE FORMALIN PREPARATION

6.1 Dissolve 227.4 grams of sucrose in 1 gallon of formalin (Haney and Hall, 1973)

6.2 Then dissolve 34.1 grams of borax in 1 gallon of sucrose formalin (Prepas 1978)

6.3 Store in original gallon jug, but revise label for additives (sugar and borax buffer) and date added.

7.0 SAMPLING PROCEDURE

7.1 At each site, the total water depth is obtained from the SeaBird profile.

7.2 The appropriate sample bucket (with matching mesh size, marked with blue tape for *63-µm*, orange tape for *153-µm)* is attached to the net (net ring is similarly marked with colored tape) and the net is attached to the winch cable by the three-point bridle. A nylon safety line clipped to the net frame is extended to the cod end of the net where it is attached to the sample bucket. A weight is clipped onto the bottom of the sample bucket.

7.3 The protective fleece cover is removed from the flowmeter, which has been mounted slightly off-center in the mouth of the net. The flowmeter ID number (four digits engraved on front of case) is recorded on the hard-copy Zooplankton Sampling and Secchi Disk Data Form (see Attachment 1). The cover is unscrewed and flipped up for reading, and then re-secured for deployment. The initial reading (three digits) is taken from the flowmeter using the upper two of the four dials (thousands and ten thousands value of lower two dials not necessary). The final singles digit is estimated by the reader from the dial position between markings for the top "tens" dial. Take care to read the dials properly, noting the direction of each dial (clockwise for "hundreds" and counter-clockwise for "tens"). Night readings are aided by a headlamp or small flashlight. THE FLOWMETER IS NOT MANUALLY ZEROED due to the fragility of the dials.

7.4 The winch operator deploys the net over the rail, with the net tender guiding the weight. Once extended, ensure that the net is not twisted, and if twisted, the act of unclipping the line attached to the net ring and spinning the net is effective in removing the twist. The winch operator then lowers the net so that the rim is at the surface of the water, at which point the net tender sets the cable sheave to zero.

7.5 The cable is deployed until the sheave reads 20 meters (63-µm net) or 100 meters (153-µm net). If the site depth is less than the specified sample depth, then the rim is only lowered to 2 meters above the bottom. Time and wire angle (off of the vertical plane) are recorded at this time. The net is then retrieved at 0.5 meters/second. The sheave reading is checked to be sure that it again reads zero as the net rim clears the water surface. If the cable is slipping or the sheave is not functioning properly, adjustments must be made to the cable metering system. Check to see whether the flowmeter is spinning as net exits the water, a sign that it is working properly.

7.6 The final flowmeter reading is then recorded on the hard-copy Zooplankton Sampling and Secchi Disk Data Form. If the difference of the two readings seems low, recheck. Evaluate whether clogging of the net could contribute to the low flow count. Replace the flowmeter if necessary.

7.7 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. Keep the collection bucket vertical during recovery to ensure that the sample bucket contents don't spill.

7.8 The upright sample collection bucket is swirled gently to concentrate the sample. For particularly dense samples, the bottom of the net can be used as a larger sieving area (by gently pouring the sample through the net mesh, then carefully rinsing the sample back into the bucket with a smaller volume of water). Check to ensure all material is washed off the net and the water level is below the top of the collection bucket before the bucket is detached. After removal from the net, the bucket is gently rinsed with deionized water from the outside, at least three times. The concentrated sample is gently poured into the sample bottle (with the mesh side of the sample collection bucket upward, so that organisms do not get stuck on the mesh during pouring). Be sure to leave headspace in the sample bottle to accommodate 40 mL of preservative. A 53-µm PVC framed hand sieve may be used if the sample is particularly dense and difficult to concentrate. Be sure to completely rinse all material into the sample bottle.

7.9 A final check is made to ensure that the sample ID on the hard-copy Zooplankton Sampling and Secchi Disk Data Form (see Attachment 1) is consistent with the sample ID on the bottle. Hand entered information including flowmeter ID, flowmeter readings, wire angle, site depth, bottom and top depth of tow, date, time (UTC military time), net mesh, weather information, and operator initials are checked. The flowmeter is rinsed with deionized water. The protective fleece cover is placed over the flow meter and the net is brought inside for storage.

8.0 SAMPLE PRESERVATION

8.1 The zooplankton samples should be refrigerated as soon as possible after collection. In the shipboard biology lab, 20 mL of soda water is measured with a small beaker and added to the samples to narcotize the organisms within 1 hour of sample collection.

8.2 The samples then stand for 30 minutes in a refrigerator.

8.3 Under a hood, 20 mL of sucrose formalin solution is added to the samples. A very small amount of Rose Bengal stain powder is added to each sample (using a pen cap tip), taking care with proper containment not to stain countertop in lab space.

8.4 The sample bottles are filled to the shoulder with deionized water and tightly capped, the cap and neck are wrapped with parafilm to prevent leaks, and the sample bottles are stowed in a designated tote in the aft lab at room temperature.

8.5 All the information recorded during the sampling process from the hard-copy Zooplankton Sampling and Secchi Disk Data Form (see Attachment 1) is entered into the appropriate spreadsheet worksheet and entered into the GLENDA shipboard database during the survey. The spreadsheet provides a continuous check that the initial flowmeter reading corresponds with the final flowmeter reading of the previous sampling site.

9.0 FIELD QUALITY CONTROL

9.1 Flowmeter Calibration

9.1.1 During each survey season, when calm weather permits, the flowmeter is calibrated. This should be done at the beginning of each cruise if possible. This is a good time to train new staff in flowmeter readings and to check consistency between readers for both watches. This is accomplished by lowering a designated calibration net ring (without the net cloth) with the two flowmeters to the 20 meters depth, raising it at 0.5 meters/second, and recording the initial and final flowmeter readings. This is repeated 20 times. Readings are recorded on the Zooplankton Net Flowmeter Calibration Form (see Attachment 2) and are eventually entered into the GLENDA database as calibration readings. Outliers can occur and should be removed, but no more than two outliers per 20 tows are permitted. If a flowmeter is erratic (high variability or low readings) it should be quickly replaced with a functional unit. The mean flowmeter value of these 20 readings is then used along with the reading during sampling (accounting for specific sample depth relative to 20 m) to calculate the net efficiency of samples in a spreadsheet.

9.1.2 The flowmeter calibrations should be checked again at the middle of the cruise, if possible. Five to 10 readings are taken during the calibration check. When recording mid-survey flowmeter calibrations, circle "yes" in the Mid-survey Flowmeter Calibration section on the Zooplankton Net Flowmeter Calibration Form (see Attachment 2). If the average of these readings differs by more than 10% from the original calibration readings, and the differences cannot be explained by sampling conditions (i.e., rough seas or boat is drifting), then the meter needs to be serviced and re-calibrated by taking 20 additional readings.

9.1.3 If the flowmeters begin to give erratic readings (e.g. 50% efficiency) that do not correlate with changes in tow depth, line angle, or evidence of net clogging, the EPA Shift Supervisor and Field Team Lead should be consulted. The meters may need to be cleaned or replaced, particularly if not spinning upon leaving the water surface. Meters should be recalibrated after servicing. Keep an inventory of the flowmeters available on board including their performance history.

9.2 Cable angle

9.2.1 The cable line of the winch should be nearly vertical to obtain reproducible results. If the angle between the cable line and a vertical line drawn from the top of the cable line to the water surface exceeds 30° during retrieval, the sample is discarded, the net is washed, and the tow (steps 7.3 to 7.9) is repeated. If weather conditions continually produce drifting of the tow net such that the less than 30° requirement cannot be met, the EPA's Shift Supervisor must decide whether to seek haven until proper sampling can be performed.

9.3 Uninterrupted towing

9.3.1 If the tow is interrupted by stopping or changing the winch speed, the sample is discarded, the net is washed, and the tow repeated (steps 7.3. to 7.9).

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

9.5 The addition of the formalin preservative is performed within two hours of collection.

9.6 If the initial and final metering sheave readings do not correspond to within a meter, corrective action such as using a heavier weight, and/or lubricating the sheave is taken prior to rerunning the tow.

10.0 ZOOPLANKTON SAMPLING AND SECCHI DISK DATA FORM

10.1 The field technician should use the hard-copy Zooplankton Sampling and Secchi Disk Data Form to enter the relevant sampling data. The Zooplankton Sampling and Secchi Disk Data Form is used to record sampling data collected following two SOPs: LG402A Zooplankton Sample Collection and Preservation SOP (this SOP), and LG402B Secchi Depth Measurement Field Procedures SOP. A copy of this form can be found in Appendix E of the current *U.S. EPA GLNPO Open Lake Water Quality Sampling Surveys Quality Assurance Project Plan* (May 2017, revised March 2019) and is displayed in Attachment 1 of this SOP. The following table provides guidance on entering data in each field in the form. Fields that are preprinted on the form prior to the survey are indicated in the table and in Attachment 1, and do not need to be entered by the field technician. Fields that are not related to this SOP are grayed out in the table and Attachment 1.

Zooplankton Sampling and Secchi Disk Data Form	
Field Name	**Data Entry Instructions**
Survey ID	[preprinted]
Visit ID	[preprinted]
Station ID	[preprinted]
Sample Date	Enter the date the samples were collected in "mm/dd/yyyy" format
Personnel	Enter the initials of the personnel entering the data
Station Depth	Enter station depth (in meters)
Sample ID	[preprinted]
Sample Time	Enter the time the samples were collected in "(UTC, military)" time.
Depth Code	[preprinted]
QC ID Code	[preprinted]
Mesh Size	[preprinted]
Sample Depth	Enter the depth of the tow (in meters; generally 20 or 100)
Flowmeter Reading [Initial, Final, and Actual]	Enter the initial and final flowmeter readings, and subtract initial from final and put the result in the "Actual" column
Flow Meter #	Enter the flow meter ID number (####)
Net Angle	Enter the net angle off of vertical during net retrieval
Remarks	Enter any relevant comments on the zooplankton samples collected, as needed
Secchi Depth	[See LG402B Secchi Depth Measurement Field Procedures SOP]
Sample Time	[See LG402B Secchi Depth Measurement Field Procedures SOP]
Reader	[See LG402B Secchi Depth Measurement Field Procedures SOP]
Remarks	[See LG402B Secchi Depth Measurement Field Procedures SOP]

11.0 ZOOPLANKTON NET FLOWMETER CALIBRATION FORM

11.1 The field technician should use the hard-copy Zooplankton Net Flowmeter Calibration Form to enter the relevant sampling data for flowmeter calibration (See Section 9.1 above). A copy of this form can be found in Appendix E of the current *U.S. EPA GLNPO Open Lake Water Quality Sampling Surveys Quality Assurance Project Plan* (May 2017, revised March 2019) and is displayed in Attachment 2 of this SOP. The following table provides guidance on entering data in each field in the form. Fields that are preprinted on the form prior to the survey are indicated in the table and in Attachment 2, and do not need to be entered by the field technician.

Zooplankton Net Flowmeter Calibration Form	
Field Name	**Data Entry Instructions**
Survey ID	[preprinted]
Station ID	[preprinted]
Date	Enter the date the samples were collected in "mm/dd/yyyy" format
Time	Enter the time the samples were collected in "(UTC, military)" time
Mid-survey Calibration	Circle the relevant answer: [yes or no]
Flowmeter ID	Enter the flowmeter ID number (####)
Mesh Size	[preprinted]
Winch Operator	Enter the initials of the winch operator
Meter Reader	Enter the initials of the meter reader
Tow number	[preprinted]; sequential tow number for each of the 20 flowmeter recordings (initial and final) as described in Section 9.1.1
Depth	Enter the depth (in meters) associated with each flowmeter reading/tow number
Revolutions	Enter initial and final flowmeter readings associated with each tow number
Line Angle	Enter the angle of the line off of vertical associated with each flowmeter reading/tow number
Comments	Enter any relevant comments on the flowmeter calibration, as needed

12.0 REFERENCES

12.1 Haney, J.F., and D.J. Hall. 1973. Sugar-coated *Daphnia*: A preservation technique for Cladocera. Limnol. Oceanogr. 18: 331-333.

12.2 Prepas, E. 1978. Sugar frosted *Daphnia*: An improved fixation technique for Cladocera. Limnol. Oceanogr. 23: 557-559.

Attachment 8:
Zooplankton Sampling and Secchi Disk Data Form

ER2011 | Zooplankton and Secchi Disc Data | Spring 2020 GLNPO's WQS

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

Sample ID	Sample Time (UTC, military)	Depth Code	QC ID Code	Mesh Size (µm)	Sample Depth (meters)	Flowmeter Reading			Flow Meter #	Net Angle, degrees	Remarks
						Initial	Final	Actual			
[preprinted]		[preprinted]	[preprinted]								

Secchi Depth (meters)	Sample Time (UTC, military)	Reader (Initials) XXX	Remarks

If Secchi disk measurement was not collected, please list "T" for time or "W" for weather. If the time is between one hour before sunset and one hour after sunrise, then the sampler should not collect a Secchi disk measurement.

Method: LG 402

Entered into electronic file _____ (Initials)

Attachment 9:
Zooplankton Net Flowmeter Calibration Form

GLNPO's WQS

Zooplankton Net Flowmeter Calibration

Survey ID	Station ID	Date (mm/dd/yyyy)	Time (Shiptime, military)	Mid-survey Calibration* (Circle answer)
[preprinted]	[preprinted]			Yes or No

Flowmeter ID (number)	Mesh Size (µm)	Winch Operator (initials) XXX	Meter Reader (initials) XXX
	153-µm [preprinted]		

Tow number	Depth	Revolutions	Line Angle
1			
2			
3			
4			
5			
6			
7			
8			
9			
10			
11			
12			
13			
14			
15			
16			
17			
18			
19			
20			

Flowmeter ID (number)	Mesh Size (µm)	Winch Operator (initials) XXX	Meter Reader (initials) XXX
	63-µm [preprinted]		

Tow number	Depth	Revolutions	Line Angle
1			
2			
3			
4			
5			
6			
7			
8			
9			
10			
11			
12			
13			
14			
15			
16			
17			
18			
19			
20			

Comments:

*The flowmeter calibrations should be checked again at the middle of the cruise, if possible. Five to ten readings are taken during the calibration check. When recording mid-survey flowmeter calibrations, circle "yes" in the "Mid-survey Flowmeter Calibration" section above.
NOTE: Refer to Attachment A of the WQS QAPP, LG400, or LG401 for more information on integrated samples.

03/26/2004