

## STANDARD OPERATING PROCEDURE: Procedure for Algae/Small Protozoan Sample Collection

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### RECORD OF AMENDMENTS:

No.	Date	Type	No.	Date	Type
1	05/20/2011	Added references and text to "Introduction". Divided "Equipment List" into list of equipment and supplies. Added "Sample Handling Requirements". Updated "Procedure" throughout. Added references.	7		
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## **STANDARD OPERATING PROCEDURE**

### **Procedure for Algae/Small Protozoan Sample Collection**

#### **BACKGROUND**

The Great Ships Initiative (GSI) is a regional effort devoted to ending the problem of ship-mediated invasive species in the Great Lakes-St. Lawrence Seaway System and globally. In support of that goal, the GSI has established superlative freshwater ballast treatment evaluation capabilities at three scales—bench, land-based, and on board ship. Each scale is dedicated to addressing specific evaluation objectives. These include:

##### *GSI Bench-Scale Tests*

- Range finding for effective treatment dose against diverse freshwater taxa and water quality conditions;
- Generation of freshwater relevant chemical degradation curves; and
- Estimation of residual toxicity given diverse freshwater taxa and water quality conditions.

##### *GSI Land-Based Tests*

- Pre-certification testing, i.e., operational and biological performance (including residual toxicity) status-testing given scale-up and a range of challenge conditions; and
- Certification/verification testing, i.e., formal assessment of performance against international and other discharge standards.

##### *GSI Shipboard Tests*

- Confirmation of biological and operational treatment performance as expected in the ship environment;
- U.S. Coast Guard Shipboard Technology Evaluation Program (STEP) testing;
- Shipboard type approval testing;
- Ship discharge monitoring; and
- Methods development.

GSI awards its independent status-testing services to candidate systems only if technical and programmatic criteria are met. Decisions are based on third party technical assessments as well as GSI Advisory Committee programmatic input. Testing services are currently offered at no cost to the developer with the exception of transportation and system installation/removal costs. Instead, tests are supported by general project funds which derive from federal and state agency grants, Great Lakes port contributions, and in-kind contributions by local governments and universities.

GSI has no involvement, intellectual or financial, in the mechanics, design or market success of the actual treatment systems it tests. To ensure GSI remains completely independent and is uncompromised by any real or perceived individual or project bias, GSI subjects itself to rigorous quality management policies and procedures. In addition, GSI test activities are subject to rigorous QAQC procedures and documentation. This attention to quality management and QAQC assures the high quality and credible evaluation of both GSI and its findings.

## INTRODUCTION

This GSI Standard Operating Procedure (SOP) describes the methods used to collect samples of organisms at the GSI Land-Based Research, Development, Testing, and Evaluation (RDTE) Facility that meet the  $\geq 10$  and  $< 50 \mu\text{m}$  size classification of the International Maritime Organization's Convention (IMO, 2004) and the United States Environmental Protection Agency's Environmental Technology Verification Program's Generic Protocol (US EPA ETV; NSF International, 2010). In the Duluth-Superior Harbor of Lake Superior, these organism assemblages are primarily composed of algae, and smaller numbers of protozoans and rotifers. Continuous, time-integrated samples are collected during each intake and discharge operation into replicate 3.8 m<sup>3</sup> sample collection tubs, which fill from the bottom to provide a well-mixed sample. One-liter, whole-water samples are collected from each tub (as specified in the Test/Quality Assurance Plan (TQAP) or other planning documentation) immediately after the intake/discharge operation is complete to ensure to the greatest extent possible that a homogenous sample is collected. Sample collection times are recorded on pre-printed datasheets (Appendix 1 and 2).

## EQUIPMENT

- 3.8 m<sup>3</sup> sample collection tubs (up to six) with attached yardsticks, plankton net hooks and flexible drainspout hose with quick-connect fittings
- Removable drainspout nozzle with quick-connect fitting
- Laboratory notebook
- Preprinted sample collection datasheets
- Cooler (for storage and transport)

## SUPPLIES

- 1 L high-density polyethylene (HDPE) sample bottles
  - 5 L discard buckets
  - 4-20 L sample carboy for compositing (washed and re-used)
  - Protective eyeglasses/goggles and gloves (if a chemical treatment is to be tested)
  - Colored tape (various colors)
  - Preprinted sample bottle labels
- One clipboard, datasheet and pen for each sampling event

## SAMPLE HANDLING REQUIREMENTS

1. Collect samples in 1-L high-density polyethylene (HDPE) bottles. Protect samples from exposure to light by placing them immediately in a cooler.
2. Process and analyze samples immediately.
3. Preserve unanalyzed sample (*see GSI/SOP/LB/RA/SA/1 - Procedure for Algae/Small Protozoan Sample Analysis*).

## PROCEDURE

### Preparation

1. Clean and label sample bottles, composite sample carboys and discard buckets with the relevant sample identification nomenclature according to *GSI/SOP/LB/G/O/4 – Procedure for Cleaning Sampling Equipment at the GSI Land-Based RDTE Facility* and *GSI/SOP/G/RA/SC/3 – Procedure for Labeling Samples Collected at the GSI Land-Based RDTE Facility*.
2. All sample collection bottles must be properly labeled with the word “Phytoplankton” to identify the sample type and the following information: Date of Sample Collection, Sample ID Code, and Sample Collection Location.
3. Prepare all data recording sheets (use Appendix 1 for intake samples and Appendix 2 for discharge samples) and attach to clipboards. Completed datasheets must be stored in a ballast treatment system-specific three ring binder that has been assigned an identification number by the GSI QAQC Staff.

### Sample Collection

1. Drain sample water from each sample collection tub, as specified in the TQAP or other planning documentation; by opening the drainspout valve (see *GSI/SOP/LB/RA/SC/1 - Procedure for Collecting Biological Samples via In-Line Sample Ports*). To remove potentially unmixed water from the drainspout piping, fill a 5 L bucket with the sample water and discard the contents, or use for rinse water.
2. Direct the sample water flow into a 1 L HDPE sample bottle until just overflowing, cap it and place it in a cooler. Record any discarded water volumes on the datasheet (Appendix 1 for intake samples and Appendix 2 for discharge samples).

### Sample Compositing

*Note: The sample compositing procedure is specific to the ballast water treatment system being tested; therefore, exact details regarding sample compositing will be outlined in the TQAP or other planning documentation.*

1. Prepare composite sample collection containers (carboys) to be used by rinsing with water (distilled or “city”) beforehand, draining so that only trace amounts of water remain, and closing any spigots.
2. Combine all relevant samples (if forming a composite) immediately following collection by pouring the contents of all appropriate (control or treatment), 1 L sample bottles into a labeled carboy. After filling, close and invert the carboy gently approximately six times to mix the composite sample. Open the spigot and allow it to drain onto the ground for

three seconds, and then collect a 1 L subsample of the composite sample using a 1 L sample bottle. Seal the final sample bottle and discard the remaining water in the carboy.

3. In many cases, treated samples are collected from three sample collection tubs in individual 1 L bottles. These 1 L samples are composited into the clean, 4 L (or larger) carboy. The carboy is inverted six times to mix and 1 L of mixed sample is immediately poured into a 1-L HDPE sample bottle, comprising the composited sample for further analysis.
4. Transfer the 1 L sample to the on-site laboratory for immediate analysis (*see GSI/SOP/LB/RA/SA/1 - Procedure for Algae/Small Protozoan Sample Analysis*). Analyze samples within 1.5 hours of sample collection.

### **Following Completion of Sampling and Analysis**

1. Wash all bottles and carboys used during sampling between sampling events according to the procedure outlined in *GSI/SOP/LB/G/O/4 – Procedure for Cleaning Sampling Equipment at the GSI Land-Based RDTE Facility*.
2. Document any deviations from this SOP, issues of significance, and remedial actions taken during each sampling event in a treatment-system specific laboratory notebook or datasheet.
3. Provide a copy of each treatment-specific laboratory notebook and sample collection datasheets to the GSI QAQC Staff, either by scanning the records and posting the subsequent PDF file on the GSI internal SharePoint website or by creating an exact hard copy of the records.

### **QUALITY ASSURANCE/QUALITY CONTROL (QA/QC)**

1. Conduct all quality assurance/quality control procedures according to *GSI/QAQC/QAPP/LB/1 - Quality Assurance Project Plan for Great Ships Initiative (GSI) Land-Based Tests (2011)*.
2. Follow all procedures outlined in this SOP. Any SOP amendments must be approved by the GSI Principal Investigator and Director. Deviations must also be communicated to the GSI Senior QAQC Officer and recorded on *GSI/FORM/G/1 - GSI Standard Operating Procedure Deviation Form*.

### **DATA STORAGE AND ARCHIVING**

1. Store and archive data according to *GSI/QAQC/QAPP/LB/1 - Quality Assurance Project Plan for Great Ships Initiative (GSI) Land-Based Tests (2011)*.
2. Archive all hard- and electronic-copies of data and records generated for a period of at least seven years.

## REFERENCES AND RELATED DOCUMENTS

Eaton AD, Clesceri LS, Rice EW & Greenberg AE, Eds. (2005). Standard Methods for the Examination of Water & Wastewater.

*GSI/FORM/G/1 - GSI Standard Operating Procedure Deviation Form*

*GSI/QAQC/QMP/1 – Great Ships Initiative Quality Management Plan (2010).*

*GSI/QAQC/QAPP/LB/1 - Quality Assurance Project Plan for Great Ships Initiative (GSI) Land-Based Tests (2011).*

*GSI/SOP/G/RA/SC/2 – Procedure for Labeling Samples Collected at the GSI Land-Based RDTE Facility.*

*GSI/SOP/LB/G/O/1 – Procedure for Operating the GSI Land-Based RDTE Facility.*

*GSI/SOP/LB/RA/SA/1 – Procedure for Algae/Small Protozoan Sample Analysis.*

*GSI/SOP/LB/RA/SC/1 – Procedure for Collecting Biological Samples via In-Line Sample Ports.*

*GSI/SOP/LB/RA/SC/3 – Procedure for Algae/Small Protozoan Sample Collection.*

Great Ships Initiative website: [www.greatshipsinitiative.org](http://www.greatshipsinitiative.org).

Great Ships Initiative Standard Operating Protocols: <http://www.nemw.org/GSI/protocols.htm>.

IMO (2004). International Convention for the Control and Management of Ships Ballast Water and Sediments. As adopted by consensus at a Diplomatic Conference at IMO, London, England, February 13 2004.

NSF International (2010). Generic Protocol for the Verification of Ballast Water Treatment Technology. Version 5.1. EPA/600/R-10/146. Produced for the USEPA, Environmental Technology Verification Program in Conjunction with U.S. Coast Guard, Environmental Standards Division and U.S. Naval Research Laboratory, Center for Corrosion Science and Engineering. NSF International, Ann Arbor, Michigan.

## **APPENDIX 1**

### **GSI Intake Sample Collection Datasheet (GSI/FORM/LB/C/1)**

Ballast Treatment System Test Sample Collection Form (GSI/FORM/LB/C/1)							
Sample Collection Tub Intake (F) Samples				System Name: _____			
Date				Test #			
Duration	Days			Hours			
Collection Team							
Sample Collection Scenario - Fill Control and Treatment Tanks at same time							
Note which tanks are being filled	C1 T1			C2 T2			
Collect water in up to 6 tubs							Harbor ZP
							Pre-Trial
Sample Point and Port	SP3A	SP3B	SP3C	SP2A	SP2B	SP2C	
Tub #	4	5	6	1	2	3	
					12456	3	
Time start line flush			Time valves operated manually			Time end fill	
Time Tubs start fill							
Total amount of water in tank (US Gal)	Treatment			Control			
	SP3A	SP3B	SP3C	SP2A	SP2B	SP2C	
Height of water placed in Sampling Tub							
	4 (PT)	5 (PT)	6 (T)	1	2	3	
Total amount of water placed in Sampling Tub (US Gal)							
Collect phytoplankton, microbial, chemistry and zooplankton samples as directed by workplan; record sonde data on Sonde Datasheet							
Time phytoplankton (PP) collected							
Phyto discard/sample volume (l)							
Time microbes (MA) collected							
Microbial sample volume (l)							
Time zooplankton (ZP) collected							
Weather:	Time:				Sky:		
	Air Temp:				Wind:	@	kts.
	Water Temp:				Water Level:	in.	Above LWD
Notes:							

## **APPENDIX 2**

### **GSI Discharge Sample Collection Datasheet (GSI/FORM/LB/C/2)**

<b>Ballast Treatment System Test Sample Collection Form (GSI/FORM/LB/C/2)</b>								
<b>Sample Collection Tub Discharge (D) Samples</b>				<b>System Name:</b> _____				
Date				Test #				
Collection Team								
Sample Collection Scenario - Drain <b>Treatment</b> and then <b>Control</b> Tank								
Note which tanks are being used	C1 T1			C2 T2				
	Collect <b>Treatment (T)</b> water in up to 3 tubs				City Water	Collect <b>Control (C)</b> water in up to 3 tubs		
Sample Point and Port	SP10C	SP10B	SP10A	SP10B	SP10C	SP10B	SP10A	
TUB #	4	5	6	5	1	2	3	
Time pump started								
Time Tubs start filling								
Time pump stopped								
Total volume discharged from Tank (US Gal)	<b>Treatment</b>				<b>Control</b>			
Height of water placed in Sampling Tub	SP10C	SP10B	SP10A	SP10B	SP10C	SP10B	SP10A	
	4 (T)	5 (T)	6 (T)	5 (W)	1	2	3	
Total volume of water placed in Sampling Tub (US Gal)								
Collect phytoplankton, microbial, chemistry and zooplankton samples as directed by workplan; record sonde data on Sonde Darasheet								
	4	5	6	5	1	2	3	
Time phytoplankton (PP) collected								
Phyto discard/sample volume (l)								
Time microbes (MA) collected								
Microbial sample volume (l)								
Time zooplankton (ZP) collected								
Time 2nd cod end collected								
Notes:								