Test Quality Assurance Plan
Standard Quality Assurance Project Plan
Shipboard Testing

Golden Bear Facility
Vallejo, California

5 November 2012
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BY: Nicholas Welschmeyer, PhD
Lead Investigator
Golden Bear Facility

CHECKED: Richard Muller
Quality Officer
Golden Bear Facility

APPROVED: William T. Davidson
Facility Director
Golden Bear Facility

APPROVED: Jim Burns
Dean of Sponsored Projects and Extended Learning
Golden Bear Facility

ACCEPTED: Verification Organization
Contents

Section 1  Project Summary ............................................. 1
  1.1  Project Management Contact Information ............................................. 2
  1.2  Golden Bear Facility Organization ............................................. 2

Section 2  Experimental Approach ............................................. 4
  2.1  Testing Nomenclature ............................................. 4
  2.2  Tank Cleaning and Inspection ............................................. 4
      Tank Cleaning ............................................. 4
      Final Inspection ............................................. 5
  2.3  Challenge Water Verification ............................................. 5
  2.4  BWTS Commissioning ............................................. 5
      Arrival Inspection ............................................. 6
      Installation ............................................. 6
      Service ............................................. 6
      Operational Check ............................................. 7
      BWTS Shakedown and Stress Testing ............................................. 7
      Lay-Up ............................................. 7
  2.5  Treatment System Operation ............................................. 7
      Standby ............................................. 8
      Ballasting Operation ............................................. 8
      Back Flushing Cycle ............................................. 8
      De-Ballasting Operation ............................................. 8
  2.6  Pumping and Piping Operation ............................................. 8
      Flushing and Draining Prior to Testing ............................................. 9
      Sequential Uptake or Combined Control Uptake Option ............................................. 9
      Treatment Uptake with Combined Control Uptake Option ............................................. 9
      Sequential Control Tank Uptake ............................................. 10
      Flushing and Draining Between Fill and Discharge ............................................. 10
      Treatment Tank Recirculation and Discharge ............................................. 11
      Control Tank Recirculation and Discharge ............................................. 12

Section 3  Sampling Procedures ............................................. 13
  3.1  Sampling Overview ............................................. 13
  3.2  Sampling Coordination ............................................. 14
  3.3  Water Quality Sampling ............................................. 15
3.4 Sample Handling and Custody Requirements .......................................................... 17

Section 4 Biological Efficacy Testing Protocols ......................................................... 18
4.1 Organisms ≥50 µm .................................................................................................. 19
4.2 Organisms 10 - 50 µm ......................................................................................... 20
4.3 Organisms <10 µm ............................................................................................... 22

Section 5 Whole Effluent Toxicity Testing Protocols .................................................. 23
5.1 Sample Collection ................................................................................................. 23
5.2 Testing and Reporting .......................................................................................... 23

Section 6 Data Collection, Reduction, and Validation .................................................. 24
6.1 Data Collection ....................................................................................................... 24
   Data Quality Indicators ......................................................................................... 24
   Engineering Data ................................................................................................. 25
   Water Quality ....................................................................................................... 26
   Biological Analysis .............................................................................................. 27
6.2 Data Reduction ....................................................................................................... 28
7.1 Data Validation ....................................................................................................... 29
   Test Cycle Automation Initiation, Tracking, and Error Logging ......................... 29
   Red-lining of Procedures ..................................................................................... 29
   Hand Log Tracking and Filing ............................................................................. 30
   Online Log Tracking and Filing ......................................................................... 30
   Verification Data Record ...................................................................................... 30

Section 8 Assessments ................................................................................................. 31
List of Figures

Figure 1 - Facility Operations............................................................................................................. 1
Figure 2 - Example Data Reduction Figure .......................................................................................... 28

List of Tables

Table 1 - Sampling Procedure Coordination ........................................................................................ 13
Table 2 - Water Quality Sample Parameters and Processing Details .................................................... 15
Table 3 - Sea-Bird TSG Continuous Monitoring Parameters ............................................................... 17
Table 4 - Valid Test Parameters, One Shipboard Test......................................................................... 18
Table 5 - Data Quality Indicators for Engineering Parameters .......................................................... 25
Table 6 - Data Quality Indicators for Continuous Monitoring Parameters ........................................ 26
Table 7 - Data Quality Indicators for Grab Sample Monitoring Parameters ........................................ 26
Table 8 - Data Quality Indicators for Biological Analyses .................................................................. 27
Table 9 - Example Data Reduction Table ............................................................................................ 28

Appendices

Appendix A: Project Training Requirements
Appendix B: GBF Chain-of-Custody
References

001 Environmental, Health, & Safety Plan, Golden Bear Facility, October 2010.


003 Physical Plant Description, Golden Bear Facility, October 2010.


005 Standard Operating Procedures, Golden Bear Facility, November 2012.


009 Vessel General Permit for Discharges Incidental to the Normal Operation of Vessels (VGP), US Environmental Protection Agency, 5 February 2009.


Section 1  Project Summary

The Golden Bear Facility (GBF) Standard Quality Assurance Project Plan (QAPP) establishes the quality assurance/quality control measures for executing the Standard Operating Procedures (SOPs), operating parameters and data collection requirements, and protocols for evaluating biological and water quality conditions. This QAPP is specific to the shipboard evaluation of ballast water treatment systems (BWTS) in accordance with the International Maritime Organization (IMO) Guidelines for Approval of Ballast Water Management Systems (G8) and the United States Coast Guard (USCG) Standards for Living Organisms in Ship’s Ballast Water Discharged in U.S. Waters, Final Rule (Final Rule).

The Standard QAPP for Shipboard Testing is one part of the Test Quality Assurance Plan (TQAP) that also consists of the following documents developed for each BWTS testing scheme:

- Project Plan (Plan);
- Standard QAPP for Land-based Testing (if applicable); and
- Standard Operating Procedures (SOPs).

The Project Plan is developed for each system and will specify any deviations from the SOPs.

![Facility Operations](image)

**Figure 1 - Facility Operations**

The procedures and methods provided in this QAPP reflect GBF’s desire to do the following:

- Achieve the objectives of the shipboard testing as stated in the Project Plan specific to each BWTS; and
- Utilize instrumentation and facilities at GBF that were assembled specifically for the purpose of viability testing, water quality analysis, and development of new treatment and analytical methodologies.
1.1 Project Management Contact Information

Contact information for the key personnel involved in the development and implementation of all GBF projects are as follows:

William T. Davidson, Facility Director/Chief Engineer  
Golden Bear Facility  
707.654.1304, cell 707.592.4267  
bdavidson@csum.edu  

Richard S. Muller, Facility Manager/Quality Officer  
Golden Bear Facility  
707.654.1258  
rmuller@csum.edu  

Veronica Boe, Facilities Program Administrator  
California Maritime Academy  
707.654.1156  
vboe@csum.edu  

Nicholas A. Welschmeyer, PhD, Lead Scientist  
Golden Bear Facility  
831.771.4439  
welschmeyer@mlml.calstate.edu  

ES&H Contacts—Moss Landing Marine Laboratories (MLML), 831.771.4400  
Jocelyn Douglas, MLML Health and Safety Officer, 831.771.4451, cell 831.750.9563  

Verification Organization  
(Assigned on a project specific basis)  

Ballast Water Treatment System Manufacturer Representative  
(Assigned on a project specific basis)

1.2 Golden Bear Facility Organization

The ability to conduct ballast water treatment technology verification tests requires careful coordination of pumping and piping systems, BWTS operation, sample collection, sample analysis, continuous water quality monitoring, water quality grab samples, and most importantly quality control.

Key GBF personnel will be given multiple responsibilities during testing. As such, responsibilities are described by GBF personnel below.

William T. Davidson, Facility Director/Chief Engineer

- Test Coordinator. Ensures that BWTS operation, pumping and piping operations, and sampling collection activities are coordinated and properly timed.
- Equipment Responsible Party. Responsible for all mechanical equipment involved with pumping and piping.
- Operator Supervisor. Reviews testing plan with Operator (see below for Operator responsibilities) such that the pumps and piping system are properly operated.
Rich Muller, Facility Manager / Quality Officer

- Quality Officer. Ensures that the procedures being utilized are current, and that all documentation is completed and logged into the automation system. Starts and stops the event. Checks function of the automation system during a test.
- Water Quality - Continuous Monitoring. Ensures that the continuous water quality system is calibrated, operating, and properly logging data. Provides feedback on water quality to the Scientist during decision making time before starting an uptake.
- Facility Manager. Ensures that all equipment and laboratory facilities are set-up for test day. Ensures that facilities are properly cared for, and secured following use.

Nicholas Welschmeyer, Lead Scientist

- Principal Investigator. Ensures that the procedures are conducted in accordance with scientific protocols. Responsible for validating test parameters, and conducting validity discussions with the Verification Organization.
- Sampling Team Lead. Ensures that sampling system is properly operated, and that required volumes are collected and processed.
- Biological Analysis. Ensures that biology analysis team conducts assessments in accordance with protocols. Reviews and assists when problems and questions arise. Reviews preliminary data, conferring with VO if and as needed.
- Water Quality – Grab Samples. Ensures that discrete, grab, samples are obtained, handled, and sent to the appropriate laboratories (internal or external). Receives and analyzes reports.

Staff with Single Task Assignments

- Nic Shields, David Coleman, Bill Schmid (Ballast System Operator). Ensures pump and valve line-up.
- John Coyle (Lead Operator). Acts as the Relief Coordinator and Relief Chief Engineer.
- Brian Maurer. Responsible for phytoplankton analysis and acts as GBF Laboratory Lead.

The Test Coordinator (Coordinator) will provide logistics and timing assistance to the team. The Quality Officer (Quality) will report directly to the Facility Director (Director). Quality will be responsible for the data and information procedures outlined in Section 6 – Data Collection, Reduction and Validation. The Operator and the Scientist, while they will not report to Quality, will be responsible to provide Quality with the data control information. Refer to Appendix A for all key personnel training requirements.

A description of the facility and GBF personnel roles are further explained in the GBF Environmental, Health & Safety Plan, and Quality Management Plan.
Section 2 Experimental Approach

This section provides specific activities that GBF will conduct in preparation for BWTS testing and between BWTS test cycles.

2.1 Testing Nomenclature

GBF uses specific nomenclature to describe various activities. The following provides definitions and applies this nomenclature to each project:

- **Project.** A project is a collection of tests to verify a technology claim, or collection of experiments to prove a thesis. To verify that a BWTS meets the IMO and USCG Final Rule discharge standards, a project will consist of four shipboard test cycles performed in accordance with IMO G8 and USCG Final Rule.

- **Test.** A test is one replicate activity that compares the performance of a BWTS to a control. Each test will consist of a sequential or simultaneous (combined) treatment uptake through the BWTS and a control uptake with no treatment. The treatment and control ballast water uptake will have similar challenge water conditions as they will be ballasted in the same location. The uptake will be held separately in a treatment and control tank. The treated water will be discharged after a designated hold time and may be directed through the BWTS if treatment upon discharge is required. The control water will be discharged without treatment after a similar hold time.

- **Event.** An event is a discrete combination of activities, typically performed without stopping, resetting, or other breaks. It will typically take several events to complete a test.

- **Test Cycle.** A test cycle is one complete ballasting evolution including uptake to a Treatment Tank (3-154-1) and a Control Tank (3-154-2), a holding period, and discharge of the treated and control ballast water. A test cycle is a series of events that accomplishes one test.

2.2 Tank Cleaning and Inspection

Following guidance provided by the data sheets in SOP 9, Lead Operator (Operator) and Quality Officer (Quality) will ensure proper tank conditions are achieved prior to commencing tests. Test protocols require that the following tanks be opened, cleaned as required, inspected, and approved by the Operator before before shipboard testing commences:

- Treatment Tank (3-154-1); and
- Control Tank (3-154-2).

Tank cleaning will not be necessary in between subsequent shipboard tests. The Operator will properly vet and contract with a tank cleaning contractor for work onboard the vessel.

**Tank Cleaning**

All tanks that will be used for testing will be opened at manhole access covers (2 per tank). The covers will have safety rails installed, and mechanical portable ventilators will be installed at each tank’s weather deck vent terminus. All tanks will be ventilated continuously
throughout the entire tank cleaning process. After no less than 12 hours of ventilation, a certified marine chemist will test the tanks as “safe for men” and tank cleaning will commence. The tanks will continue to be checked by a certified and competent person for safe conditions at the beginning and end of each work day. All tank entry documentation will be maintained for inspection at the tank entry point.

Cleaners outfitted with appropriate personal protective equipment (PPE) will use high pressure washers to spray the control and treatment tanks with fresh water. An inspection by the Operator and contractor will determine how much, if any, mucking and disposal of silt or debris will be required. The silt will be mucked and loaded to appropriate containers staged on deck for later disposal per local requirements.

The Treatment Tank (3-154-1) will be pressure washed with a 200 ppm chlorine solution to disinfect all surfaces. After a contact time of not less than five minutes, all surfaces will be rinsed with fresh city water using the same high pressure washers. The wash down from the cleaning will be continuously pumped out of the ballast tank and transferred to municipal waste.

A final inspection by the Operator and contractor will confirm the removal of silt, debris, and wash water. In addition, random sampling of six (6) wet tank surface locations will be conducted in the Treatment Tank (3-154-1). A reading of less than 3 ppm free chlorine is considered passing.

**Final Inspection**

The Treatment Tank (3-154-1) will be pumped “dry” with no standing water and left ventilating for an additional 24 hours. Final inspection will be performed by the Operator, Quality, or assignee for acceptance. The tanks will be offered for inspection to the BWTS manufacturer representative and the Verification Organization (VO) prior to closing manhole access covers and ventilation removal.

### 2.3 Challenge Water Verification

Natural harbor or coastal water used during BWTS testing will be ballasted from various California and Pacific coastal and river areas during the T/S Golden Bear summer training cruise. All testing will be performed with ambient water, without adding or concentrating of any chemical or biological components.

Challenge water will be analyzed to verify the water quality and living organism concentrations meet IMO and USCG requirements according the appropriate salinity range as described in the Project Plan.

### 2.4 BWTS Commissioning

This section provides specific procedures required for commissioning the BWTS. A successful commissioning will integrate the BWTS with the GBF and confirm proper mechanical operation in accordance with the BWTS Technical Bulletin.

All commissioning efforts will be performed by the Operator. Commissioning checklists and data sheets included in SOP 4 will be provided to Quality on a daily basis.
**Arrival Inspection**

Upon BWTS arrival at the GBF staging area, an initial inspection will be performed to determine delivery condition and readiness for install. Utilizing the BWTS Commissioning Data Sheets, the Operator or assignee, the rigging and installation contractor, and Quality will inspect the system for the following:

- Overall condition and damage indication;
- Condition of the ISO container rack;
- Ballast water inlet and outlet connections (8-inch flange);
- Drain connection (4-inch flange);
- Electrical load requirements;
- Cable lengths and sizes for GBF-provided connectors;
- Fresh water and compressed air requirements; and
- Any additional requirements or conditions requiring specific care or address.

**Installation**

The Operator or assignee will utilize the BWTS Commissioning Data Sheets to ensure that the BWTS is properly installed and that all mechanical, electrical, and plumbing connections are secured.

Based on standard requirements for installing a 20-foot ISO container on the designated location onboard the T/S Golden Bear and any unique requirements identified at the arrival inspection, the Director will utilize a sub-contractor to conduct the following:

- Provide crane, rigging, and mechanical service to shift and secure the BWTS to the vessel; and
- Install all plumbing interfaces, including ballast in/out, drain, air, water, and any unique interfaces such as chemical injection or sampling lines.

Crane and rigging services will be planned and scheduled with GBF at least 24 hours prior to commissioning to allow enough time to plan alternate arrangements should they be needed. A suitable mobile crane and operator will be employed, along with a rigging crew to shift the BWTS from the CMA waterfront parking lot to the end of the pier near the stern of the vessel. The contracted rigging crew and crane operator will shift the container with the vessel’s crane into correct placement for securing to the container platform. The vessel’s Chief Mate will observe, inspect, and approve the securing of the container.

Electrical power interfaces will typically be installed by qualified GBF personnel under direction of vessel’s Chief Engineer, though an electrical contractor with facility oversight may be employed. Depending on the complexity of the BWTS user interface, the inter-connection to GBF’s Integrated Monitoring and Control System (IMACS) will be performed by qualified GBF personnel or contracted to the vessel’s automation contractor.

**Service**

Utilizing guidance from the BWTS Technical Bulletin and BWTS Commissioning Data Sheets, the Operator or assignee will ensure that the system is inspected and serviced by GBF personnel and/or an OEM technical representative under GBF supervision prior to placing the BWTS in operation. In general, this service would include inspection, installation, freedom of
mechanical linkages and pump shafts, operation of fitted valves and handwheels, and control interface condition. Any installed lubrication points shall be checked or lubricated per the BWTS Technical Bulletin.

**Operational Check**

Utilizing guidance from BWTS Technical Bulletin and the BWTS Commissioning Data Sheets, the Director or assignee will initiate operation of the BWTS. The BWTS will be placed into operation readiness by verifying proper installation of the following system elements and gradually bringing them on line:

- Pumps will be checked for proper rotation;
- Piping connections and covers will be checked under system pressure;
- Flow rates will be determined and confirmed; and
- Electrical power and control integration will be initiated and confirmed.

**BWTS Shakedown and Stress Tests**

Shakedown tests subject the BWTS to conditions within the BWTS specified limits. Stress tests subject the BWTS to slightly higher capacities than required for BE testing. The purpose of these tests is to verify that the BWTS will not have mechanical failures during BE tests. The shakedown and stress test procedures are provided in SOP 5 and are outlined below:

1. Sea-to-Sea ballasting through the BWTS for two (2) hours at approximately 200 m$^3$/hr (or 100% of capacity of the BWTS). If the BWTS requires a hold time prior to treated ballast water discharge, ballast water may be directed to a holding tank.
2. Start and stop cycles of the GBF ballast water pump and BWTS.
3. Overnight idle period.
4. Sea-to-Sea for four (4) hours at approximately 220 m$^3$/hr treatment (or 110% of capacity). If the BWTS requires a hold time prior to treated ballast water discharge, ballast water may be directed to a holding tank.
5. Operational tests according to SOP 16 to determine expected conditions during an uptake cycle.

**Lay-Up**

The BWTS will be laid-up after commissioning and shakedown tests, and between test cycles per the procedures provided in the BWTS Commissioning Data Sheets. The Director or assignee will oversee the performance of the lay-up procedures based on BWTS Technical Bulletin recommendations and guidance. In general, these procedures will consist of flushing the system with fresh water and opening power supply breakers to the BWTS, thus leaving in “wet” lay-up while installed on the vessel. If idle periods exceed one (1) month or if extended freezing periods are expected, the lay-up will also consist of draining the BWTS of all water.

2.5 **Treatment System Operation**

Power will be supplied to the BWTS so the GBF automation system can remotely operate the BWTS control system and electrical components. The GBF automation system can place the BWTS in the following modes: Standby, Ballast, and De-ballast. The GBF automation system will be provided with alarm and fault status signals by the BWTS control system.
Standby
All operations start with the BWTS in Standby mode with all valves in the BWTS closed. The BWTS can enter Standby from the Ballasting or De-ballasting mode. The Operator will engage the Standby mode by selecting “Stop” on the automation system user interface. The BWTS will start its shutdown procedure, if applicable, and all BWTS valves will be closed by GBF personnel to isolate the BWTS.

Ballasting Operation
For a ballasting operation where treatment is required on uptake, the Operator and staff will prime the BWTS inlet piping and bleed air at the BWTS inlet. The Operator will then place the BWTS into Ballast mode by selecting the appropriate position on either the BWTS control system or GBF automation system user interface.

The BWTS will automatically undergo its startup procedure, treat the incoming challenge water, and allow treated water to pass through to the empty Treatment Tank (3-154-1).

Back Flushing Cycle
When filtration is part of the treatment process, the GBF ballast pumping and sampling operations will not be affected should the filtration need to initiate a back flush cycle during testing. Filter back flush will be directed overboard during treatment uptake or per the BWTS design if used during discharge.

Back flush initiation is typically triggered by differential pressure or timed cycle. In each cycle a percentage of inlet ballast water will be used to back flush the filter screen, and the effluent will be sent overboard as waste. Alternatively, back flush may be sent to the pump suction or holding tank when filtration is utilized during discharge. The pumping system is designed to deliver ballast water at a constant flow rate; however, the flow rate may be reduced during these back flush periods due to limits on pump speed and characteristics.

De-Ballasting Operation
For a de-ballasting operation where treatment is required upon discharge, the Operator and staff will prime the BWTS inlet piping and bleed air at the BWTS inlet. The Operator will place the BWTS into De-ballast mode by selecting the appropriate position on either the BWTS control system or GBF automation system user interface. The BWTS will automatically undergo its startup procedure in preparation for treatment.

If treatment is not required during ballast water discharge, water will bypass the BWTS and be directed through the sampling station to overboard.

2.6 Pumping and Piping Operation
The following sections outline the pumping and piping procedures during BWTS testing. Refer to the Piping System Line-up Diagrams in SOP 3 for detailed piping system instructions and logs. Refer to the Operation of BWTS and Facility Piping System Procedures in the SOP 16 for detailed BWTS instructions and logs.
### Flushing and Draining Prior to Testing

The GBF ballast piping system must be flushed with a mild bleach solution and then drained prior to an uptake event.

### Sequential Uptake or Combined Control Uptake Option

Shipboard testing uptake can take one of two forms, sequential or simultaneous “combined” uptake to treatment and control tanks. If sequential uptake is utilized, then treated ballast water uptake will be directed to the Treatment Tank (3-154-1) and untreated ballast water uptake will be directed to Control Tank (3-154-2) as two sequential uptake events. Alternatively, uptake may be combined so that uptake will simultaneously be directed to the Treatment Tank (3-154-1) and Control Tank (3-154-2) as one uptake event.

The uptake cycle procedure will be conducted as approved by the VO and detailed in the Project Plan.

### Treatment Uptake with Combined Control Uptake Option

Water from the sea chest will be pumped at a flow rate of 200 m$^3$/hr +/- 10% (or flow rate indicated in the Project Plan) through the BWTS and into the Treatment Tank (3-154-1) until the volume reaches a minimum of 200 m$^3$. If the Project Plan specifies a combined uptake option, the Control Tank (3-154-2) volume must also reach a minimum of 200 m$^3$. The steps for testing uptake with the combined control uptake option are outlined below.

1. Piping system will be arranged to take suction from the sea chest with the treatment pump and deliver water to the BWTS inlet.
2. The ballast treatment pump will be started, and air will be bled from the BWTS and ballast piping system.
3. The BWTS will be turned on and set to Ballast mode. Water will recirculate through the piping system and will be directed overboard until the BWTS is running.
4. Ballast water flow through the piping system will be maintained at the target flow rate by the ballast treatment pump.
5. Once the BWTS is ready and online it will automatically open its outlet valve, allowing treated ballast water or ballast water slip stream (for systems utilizing chemical dosing) to flush discharge piping. If a treated ballast water holding time is not required, the treated ballast water will be directed to the overboard. If a treated ballast water holding is required, then treated ballast water will recirculate via the ballast pump manifold.
   
   **Note:** If employing a combined control uptake option, then the lower pump recirculation path that allows untreated ballast water to bypass the BWTS will be maintained.
6. Water quality monitors will be placed online to provide continuous monitoring of the ballast water at the pump discharge.
7. The sampling station will be flushed with raw seawater while suction is taken from the sea chest. Flushed water will be discharged overboard or recirculated via the ballast pump manifold.
8. Once the sampling station is flushed and ready to begin collection, the following will occur simultaneously:
   - Treatment Tank (3-154-1) fill valves will be opened;
   - Discharge valve to overboard or recirculation valve will shut;
   *Note: If employing a combined control uptake option, then the BWTS will be bypassed at the ballast pump manifold and Control Tank (3-154-2) fill valves will be opened.

9. Sampling will begin by filling three sample tubs with uptake water, placing the sampling nets under the flowing uptake water and logging the start time into the automation system.

10. Uptake will be completed when the Treatment Tank (3-154-1) is confirmed at minimum volume of 200 m³. If employing a combined control uptake option, the Control Tank (3-154-2) will also be confirmed at a minimum volume of 200 m³. Upon uptake completion, the following will occur simultaneously:
   - The BWTS will shutdown and isolated;
   - The ballast treatment pump will stop;
   - The Treatment Tank (3-154-1) inlet piping will be double blocked and bled.
   *Note: If employing a combined control uptake option, then the Control Tank (3-154-2) inlet piping will also be double blocked and bled.

11. Sampling station will be adjusted so that the next sampling event will begin in the next set of tubs.

**Sequential Control Tank Uptake**

Water from the sea chest will be pumped at a flow rate of 200 m³/hr +/- 10% (or flow rate indicated in the Project Plan) through the BWTS and into the Treatment Tank (3-154-1) until the volume reaches a minimum of 200 m³. Following Treatment Tank 1 (3-154-1) uptake, the piping system will be realigned to begin filling the Control Tank (3-154-2) to a minimum volume of 200 m³. The steps for uptake into the Control Tank (3-154-2) are outlined below.

1. Once BWTS discharge is realigned to direct water to the Control Tank, the 01 Deck bypass valves will be opened
2. Flow rate will be managed using the ballast treatment pump and piping system.
3. Sampling will begin by filling three sample tubs with uptake water from the sea chest, placing the sampling nets under the flowing uptake water and logging the start time into the automation system.
4. Uptake will be completed when the Control Tank (3-154-2) is confirmed at a minimum volume of 200 m³. Upon completion of Control Tank uptake, the following will occur simultaneously:
   - The treatment pump will stop; and
   - All remaining valves left open will be secured.

**Flushing and Draining Between Fill and Discharge**

The piping system will be flushed with a mild bleach solution and drained between the uptake and discharge cycles to avoid contamination.
Treatment Tank Recirculation and Discharge

*Note:* The Treatment Tank (3-154-1) must be discharged BEFORE the Control Tank (3-154-2).

Following the required ballast water hold time indicated in the Project Plan, the Treatment Tank (3-154-1) will be discharged overboard as detailed in SOP 16. The ballast water discharge will be sampled in triplicate at the sampling station located on the Main Deck in accordance with the SOP 18. An overview of the recirculation and discharge procedures are provided below.

1. If treatment is required upon discharge, the piping system will be arranged to take suction from Treatment Tank 1 (3-154-1) and deliver ballast water to the BWTS inlet. The ballast water will recirculate directly to the pump suction until the BWTS is ready and running. If no treatment is required upon discharge, then the ballast water discharge will bypass the BWTS, be directed through the sampling station and overboard.
2. The ballast treatment pump will be started, and air will be bled from the piping system.
3. If required, the BWTS will be turned on and allow treated ballast water to recirculate. If the BWTS utilizes a filter that requires a back flush, any filter back flush will be directed per the BWTS design.
4. Ballast water flow through the piping system will be maintained at the target flow rate by the ballast treatment pump.
5. The direct pump recirculation route will be closed.
6. Water quality monitors will be placed online to provide continuous monitoring of the ballast water at the pump discharge.
7. The sampling station will be flushed with treated ballast water while the system continues to recirculate water from the Treatment Tank (3-154-1).
8. Once the sampling system is flushed and ready to begin collection, the following will occur simultaneously:
   - Discharge will be directed overboard and the treated water recirculation route will be closed; and
   - Sampling will begin by filling three sample tubs with treated ballast water discharge from Treatment Tank 1, placing the sampling nets under the flowing discharge water and logging the start time into the automation system.
9. Tank levels will be monitored as follows:
   - Target pumping rate will be maintained until tank water level reaches approximately 300 mm in height.
   - Pumping rate will be decreased to 50 m³/hr when the tank water level reaches 300 mm in height until the pump loses suction.
10. When the ballast treatment pump loses suction, it will be secured and the BWTS will shut down.
11. Piping to overboard will be closed immediately. This will ensure that the BWTS remains flooded during its shutdown cycle.
12. All remaining open valves will be secured.
13. Sampling station flow will naturally stop when the ballast treatment pump loses suction and is no longer pumping ballast water. The sampling station inlet valves will be shut and the sampling station secured.

Control Tank Recirculation and Discharge

The Control Tank (3-154-2) will be discharged after the Treatment Tank (3-154-1). The discharge procedure is similar to those for the Treatment Tank (3-154-1). The primary differences include:

- If the BWTS was utilized during ballast water discharge, it will be secured during Control Tank (3-154-2) discharge and the 01 Deck bypass will be open.
- The required sample volumes for control discharge are less than treated ballast water discharge; therefore a different sampling station manifold will be used.
Section 3 Sampling Procedures

Sampling will be performed so that the Science team can conduct the biological analyses described in Section 4 - Biological Efficacy Testing Protocols. Table 1 below indicates the appropriate sampling procedure, sample replicates and sample volumes for each pumping system operation.

Table 1 - Sampling Procedure Coordination

<table>
<thead>
<tr>
<th>Pumping Operations</th>
<th>Sampling Procedures</th>
<th>Each Replicate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Rate * (m³/hr)</td>
<td>Name</td>
</tr>
<tr>
<td>Uptake</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treatment (Untreated)</td>
<td>200</td>
<td>3 x 1</td>
</tr>
<tr>
<td>Control (Untreated)</td>
<td>200</td>
<td>3 x 1</td>
</tr>
<tr>
<td>Discharge</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treatment (Treated)</td>
<td>200</td>
<td>3 x 3</td>
</tr>
<tr>
<td>Control (Untreated)</td>
<td>200</td>
<td>3 x 1</td>
</tr>
</tbody>
</table>

*aDuring tank stripping pumping rates slow down. Target flow rate may be adjusted as indicated in the Project Plan.

3.1 Sampling Overview

Ballast water sampling procedures vary per the ballasting operations below.

Ballast Water Uptake Untreated Water Samples (3 x 1.0 m³ and 3 x 22 L)

During ballast water uptake, untreated water will be pumped from the sea chest and directed to the Treatment Tank (3-154-1) and then the Control Tank (3-154-2). During each of sequential uptake events, three (3) continuous samples of at least 1.0 m³ will be taken upstream of the BWTS. If the combined control uptake option is utilized, untreated ballast will be directed to the Control tank and through the BWTS to Treatment Tank simultaneously. Only one uptake sample set will be required. Sampling will occur during the entire uptake and produce sets of three (3) triplicate samples for each uptake event totaling not less than 3.0 m³ for analysis of the ≥50 µm organism size class.

For each uptake sample, separate 22 L carboys will be continuously filled in triplicate with whole water (unsieved) ballast water from the same sample lines used to collect water for the ≥50 µm organism size class. This will produce three (3) triplicate samples of 22 L each for analysis of the 10 – 50 µm organism size class for each uptake event.

Ballast Water Discharge Treatment Tank Samples (3 x 3.0 m³ and 3 x 22 L)

During ballast water discharge from the Treatment Tank (3-154-1), integrated samples of treated ballast water in triplicate of at least 3.0 m³ each will be taken downstream the BWTS. This will provide a total of three (3) 3.0 m³ samples for a total volume of at least 9.0 m³. This method will assure sampling during the entire discharge event for analysis of the ≥50 µm organism size class.

For each discharge sample, separate 22 L carboys will be continuously filled in triplicate with whole water (unsieved) ballast water from the same sample lines used to collect water for the
≥50 µm organism size class. This will produce three (3) triplicate samples of 22 L each for analysis of the 10 - 50 µm organism size class.

**Ballast Water Discharge Control Samples (3 x 1.0 m³ and 3 x 22 L)**

During ballast water discharge from the Control Tank (3-154-2), integrated samples of untreated water will be taken in triplicate of at least 1.0 m³ each. This will provide a total of three (3) 1.0 m³ samples for a total volume of at least 3.0 m³. This method will assure sampling during the entire discharge event for analysis of the ≥50 µm organism size class.

For each discharge sample, separate 22 L carboys will be continuously filled in triplicate with whole water (unsieved) ballast water from the same sample lines used to collect water for the ≥50 µm organism size class. This will produce three (3) triplicate samples of 22 L each for analysis of the 10 – 50 µm organism size class.

Sampling pitots for each ballasting event are sized to maintain a consistent fluid velocity, despite different flow rates designated to event-specific sampling procedures.

### 3.2 Sampling Coordination

The Treatment Tank (3-154-1) and Control Tank (3-154-2) uptake may be combined or sequential; however, combined or sequential uptake to the Control Tank will depend on the Project Plan as approved by the VO.

The BWTS inlet piping will be primed before uptake to the Treatment Tank (3-154-1) by taking suction from the sea chest, directing water through the BWTS and then overboard (sea-to-sea). Alternatively, ballast water from the sea chest may be recirculated via the ballast pump manifold. As the BWTS is primed, the sampling team will set-up the sampling station. The Operator will verify suction is taken from the sea chest to fill each Treatment Tank with treated water.

After the Treatment Tank (3-154-1) reaches the required volume, the BWTS will be shutdown, and the Operator will redirect the piping system to fill the Control Tank (3-154-2). When the Control Tank reaches the desired volume, pumping will be stopped.

*Note:* When employing the combined control uptake option, both the Treatment Tank (3-154-1) and Control Tank (3-154-2) minimum volumes will be verified before the pump is stopped.

Ballast water discharge will proceed after ballast water is held for the time period indicated in the Project Plan. Treatment Tank 1 (3-154-1) discharge will be followed by the Control Tank (3-154-2) discharge.

If treatment will be required upon discharge, the BWTS inlet piping will be primed before Treatment Tank (3-154-1) discharge. Piping will be primed by directing treated water through the BWTS and recirculating through the pump suction. If no treatment is required upon discharge, then treated ballast water will bypass the BWTS during recirculation. The recirculation mode will enable the science team to set-up the sampling station.

The Operator will redirect the discharge to overboard when the science team is ready to take samples of the treated water in triplicate. The Control Tank (3-154-2) discharge will be
recirculated and bypass the BWTS. The untreated control water will be sampled and discharged to sea.

Communications between the Operator (opening and closing valves, operating the treatment system pump, and operating the BWTS) and the sampling team will be performed by radio, visual communications, and the automation system ballast order telegraph (BOT). Of these, the BOT is the primary and definitive means of communication.

The Scientist will supervise the science team as they prepare the sampling station by flushing all equipment, hoses, and other devices with raw seawater before uptake or discharge. During set-up, the sampling station will also balance flows into the tubs where the plankton nets are rigged. However, the sampling team will only send ballast water through the plankton nets when directed by the Operator. This is called “netting.” Only during an actual uptake or discharge are the samples netted in order to maximize the sample representativeness of the ballast tank contents.

### 3.3 Water Quality Sampling

Water quality grab samples will be taken at the time of ballast uptake to empirically define the chemical characteristics of water entering the control and treatment ballast tanks. These samples will be drawn directly from the sampling station using whole water (unsieved), collected in triplicate and with the time-integrated method as described above in Section 3.1 – Sampling Overview.

GBF monitors and records in-situ measurements from two Yellow Spring Instruments (YSI) sondes during testing. The sondes will be placed in the sample tubs for monitoring water quality throughout each uptake and discharge event. The data collected includes temperature, conductivity, salinity, dissolved oxygen, fluorescence, and turbidity. After the test event, the data is downloaded from the sonde to the online information system. Table 2 below provides information on each water quality sample method.

**Table 2 - Water Quality Sample Parameters and Processing Details**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Sample method/volume</th>
<th>Processing</th>
<th>Sample Storage</th>
<th>Analysis</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salinity</td>
<td>YSI 6600 handheld</td>
<td>Immediate</td>
<td>NA</td>
<td>Meter readings, handheld probe unit</td>
<td>Calibrated with Thermo-Fisher conductivity standards</td>
</tr>
<tr>
<td></td>
<td>probe</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Temperature</td>
<td>YSI 6600 handheld</td>
<td>Immediate</td>
<td>NA</td>
<td>Meter readings, handheld probe unit</td>
<td>Factory calibrated thermistor probe</td>
</tr>
<tr>
<td></td>
<td>probe</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dissolved Oxygen</td>
<td>YSI 6600 handheld</td>
<td>Immediate</td>
<td>NA</td>
<td>Meter readings, handheld probe unit</td>
<td>Calibrated with 100% air saturated water</td>
</tr>
<tr>
<td></td>
<td>probe</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pH</td>
<td>135 mL polypropylene bottle</td>
<td>Within 6 h of collection</td>
<td>Room °C</td>
<td>Beckman Model 70 pH meter</td>
<td>Two-point pH standardization</td>
</tr>
<tr>
<td>Parameter</td>
<td>Sample method/volume</td>
<td>Processing</td>
<td>Sample Storage</td>
<td>Analysis</td>
<td>Notes</td>
</tr>
<tr>
<td>-----------------------------------</td>
<td>----------------------</td>
<td>--------------------------------------------------------</td>
<td>--------------------------------------------------------------------------------</td>
<td>----------------------------------------------------</td>
<td>----------------------------------------------------------------------</td>
</tr>
<tr>
<td>Total Suspended Solids (TSS)</td>
<td>2 L polypropylene bottle</td>
<td>Volumetric filtration onto pre-weighed 0.5 µm membrane filter</td>
<td>Dried at 85 °C for 3 hours, stored in vacuum dessicator @ room °C, analyzed within 30 d</td>
<td>Gravimetric weight determination after drying to constant weight, ± 0.1 mg</td>
<td>Weighed on 5-digit balance, granite weighing table (MLML)</td>
</tr>
<tr>
<td>Transmittance</td>
<td>20 mL acid-washed glass vial</td>
<td>Water, unfiltered, directly into glass sample container for storage</td>
<td>Room °C</td>
<td>Light propagation analysis to determine beam transmittance.</td>
<td>Readings from 370 nm to 650 nm, at 10 cm and 25 cm.</td>
</tr>
<tr>
<td>Particulate Organic Carbon (POC)</td>
<td>2 L polypropylene bottle</td>
<td>Volumetric filtration onto pre-combusted GF/F filter</td>
<td>Dried at 65 °C for 48 h, stored in vacuum dessicator@ room °C, analyzed within 30 d</td>
<td>CHN combustion analysis</td>
<td>Combustion analysis on CEC 440 CHN Analyzer (MLML)</td>
</tr>
<tr>
<td>Dissolved Organic Carbon (DOC)</td>
<td>20 mL acid-washed glass vial</td>
<td>Water passed through GF/F filter, directly into glass sample container for storage</td>
<td>Frozen -20 °C, analyzed within 30 d</td>
<td>Catalytic oxidation</td>
<td>Contract analysis with McCampbell Analytical, Inc. (EPA approved), Pittsburg, CA</td>
</tr>
<tr>
<td>Chlorophyll a</td>
<td>1 L amber polypropylene bottle</td>
<td>Volumetrically harvested onto 25 mm GF/F filter, immediately extracted in 1.2 mL 90% acetone</td>
<td>90% acetone extracts stored @ -20 °C, analyzed within 30 d</td>
<td>Single-step fluorometric assay for chl a; C-8 HPLC for chlorophylls and carotenoids</td>
<td>Turner TD-700 filter fluorometer, calibrated with HPLC-purified authentic chl a standard (Welschmeyer 1990)</td>
</tr>
</tbody>
</table>

GBF may also operate an inline Sea-bird SBE 21 thermosalonograph (TSG) for redundant monitoring of ballast water during testing. The TSG will measure the conductivity (i.e. salinity), temperature, dissolved oxygen, transmissivity (Wet Labs Transmissometer) and chlorophyll florescence (Turner Cyclops) and display real time data on the IMACS. The data is post processed according to Sea-bird’s recommendations and transferred to DVD after each test cycle.

Sea-bird TSG continuous monitoring parameters are indicated in Table 3 below.
Table 3 - Sea-Bird TSG Continuous Monitoring Parameters

<table>
<thead>
<tr>
<th>Sensor</th>
<th>Maker</th>
<th>Type</th>
<th>Calibration Procedure</th>
<th>Reporting Units</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature</td>
<td>Sea-Bird Electronics</td>
<td>SBE 21</td>
<td>Nominally 1/year</td>
<td>°C</td>
<td>-5 to +35</td>
</tr>
<tr>
<td>Conductivity</td>
<td>Sea-Bird Electronics</td>
<td>SBE 21</td>
<td>Nominally 1/year</td>
<td>S/m</td>
<td>0 to 7</td>
</tr>
<tr>
<td>Transmissometer</td>
<td>Wet Labs</td>
<td>C-Star</td>
<td>Nominally 1/year</td>
<td>% transmittance</td>
<td>0-100</td>
</tr>
<tr>
<td>(25 cm)</td>
<td></td>
<td></td>
<td></td>
<td>beam attenuation</td>
<td></td>
</tr>
<tr>
<td>Dissolved Oxygen</td>
<td>Sea-Bird Electronics</td>
<td>SBE 43</td>
<td>Nominally 2/year</td>
<td>mg/L</td>
<td>120% of saturation</td>
</tr>
<tr>
<td>Fluorometer</td>
<td>Turner Designs</td>
<td>Cyclops-7</td>
<td>In-house, before each</td>
<td>µg/L</td>
<td>0.1 to 50</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>experimental run</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

3.4 Sample Handling and Custody Requirements

All biological efficacy (BE) and water quality samples will be taken by the GBF science team. All samples will be clearly labeled with the unique test event number and an associated identifier. The unique test event number and associated identifier will match all continuous monitoring data and reports generated by the GBF automation and online information system.

A chain-of-custody will be completed for all samples that are not immediately analyzed at the GBF laboratory and are transferred to either a subcontracted laboratory or Moss Landing Marine Laboratory (MLML). Refer to Appendix B for an example GBF chain-of-custody.

In the case samples are transferred to a subcontracted laboratory, the analysis laboratory chain-of-custody will be completed and will remain with the samples at all times.
Section 4 Biological Efficacy Testing Protocols

Biological efficacy (BE) testing procedures for determination of BWTS performance are detailed in this section of the QAPP. BE test methods are evolving continuously. There are few routine, time-tested methods for plankton viability determination at this time, such as standardized methods published by USEPA, ASTM, or otherwise. Therefore, methods must be devised to meet the quantitative challenges of regulatory performance standards. Detailed biological assays are included in SOP 20 to 29.

Test validation parameters are provided in the Project Plan specific to the BWTS and are duplicated in Table 4 below for convenience. Please refer to the Project Plan for a detailed explanation of these parameters, actions to perform if one or more parameters are not met, and interpretation of levels and durations.

Table 4 - Valid Test Parameters, One Shipboard Test

<table>
<thead>
<tr>
<th></th>
<th>Uptake Cycles</th>
<th>Discharge Cycles</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Criteria</td>
<td>Treatment</td>
</tr>
<tr>
<td>Treatment Line and Tank</td>
<td></td>
<td>Treatment</td>
</tr>
<tr>
<td>Average^a (m³/hr)</td>
<td>200 ± 10%</td>
<td>200 ± 10%</td>
</tr>
<tr>
<td>Total Volume (m³)</td>
<td>≥ 200</td>
<td></td>
</tr>
<tr>
<td>Control Line and Tank</td>
<td></td>
<td>Treatment</td>
</tr>
<tr>
<td>Average^a (m³/hr)</td>
<td>200 ± 10%</td>
<td>200 ± 10%</td>
</tr>
<tr>
<td>Total volume (m³)</td>
<td>≥ 200</td>
<td></td>
</tr>
<tr>
<td>Combined Sample Volume (m³)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Uptake and Control Discharge</td>
<td>≥ 3</td>
<td></td>
</tr>
<tr>
<td>Treatment Discharge</td>
<td>≥ 9</td>
<td></td>
</tr>
<tr>
<td>Ballast Hold Duration (hours)</td>
<td>48+</td>
<td></td>
</tr>
<tr>
<td>Water Quality</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Salinity (PSU)</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>Temperature (°C)</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>DOC (mg/L)</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>POC (mg/L)</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>TSS (mg/L)</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>Uptake Living Populations</td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥ 50 µm (organisms/m³)</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>10 - 50 µm (organisms/mL)</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>Control Living Populations</td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥ 50 µm (organisms/m³)</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>10 - 50 µm (organisms/mL)</td>
<td>10</td>
<td></td>
</tr>
</tbody>
</table>

^a During tank stripping pumping rates slow down. Target flow rate may be adjusted as indicated in the Project Plan.

The BE tests will provide numeric counts of living organisms defined either by size category or specific pathogen. Methods for living organism counts to be used by GBF science team are detailed below, according to size and pathogen classification. Refer to Table 8 for a summary of the critical measurements of organism viability made in shipboard tests, along with notes on methods and processing techniques.
4.1 Organisms ≥50 µm

Organisms ≥50 µm will be collected at each of two replicate sampling stations mounted on the Main Deck of T/S Golden Bear. Each sampling station provides metered flow into the mouth of the sampling tubs and/or zooplankton nets (three simultaneous replicates). Sample water from the appropriate source such as treatment uptake, control uptake, treatment discharge, or control discharge will be directed to each tub. Organisms ≥50 µm in minimum physical dimension will be collected with Nitex mesh zooplankton nets having a nominal pore size of 50 µm, measured on the diagonal. Counts of live and dead organisms ≥50 µm will be determined using the ‘poke and probe’ method under 30x (nominal) dissecting microscope observation.

In the case of uptake samples, whole water (unseived) is retained in the tubs and filtered just prior to analysis. Discharge samples are directed through zooplankton nets mounted in each tub. In all cases, sample water is diverted at uniformly metered flow rates to yield continuous samples integrated over the each ballasting operation. The plankton nets are positioned within large polyethylene containers that retain filtrate water to a user-controlled height, thus bathing the plankton net in surrounding water to reduce damage to live captured organisms. Three replicate 1.0 m$^3$ samples will be collected during uptake sampling. Three replicate 3.0 m$^3$ samples will be collected for treatment discharge sampling. Three replicate 1.0 m$^3$ samples will be collected for control discharge sampling. The larger collection volume for treatment discharge provides statistical confidence in samples where the total number of live organisms is expected to be low.

Rates of approximately 20-25 L/min per replicate will provide adequate flow to collect samples of at least 1.0 m$^3$ of organisms in ≥50 µm size class during the uptake events. The sample flow rate will be increased by a factor of 3x for treatment discharge sampling; output from the calibrated flow meters will be logged continuously by the GBF monitoring system.

Each replicate sampling station will be fit with additional spare nets: three (3) replicate nets to collect the bulk of the sample, and three (3) spares that can be quickly positioned in-line in case clogging becomes a problem. GBF’s experience indicates that most sample volumes of 1.0 m$^3$ will be easily handled by a single net.

Concentrated organisms will be immediately transported to the GBF laboratory to determine the live counts by microscopy. The samples will be maintained at ambient water temperature in a darkened, insulated container that is plumbed with flowing surface seawater from the ship. Cod end contents of the sample nets will be adjusted quantitatively to 400 mL with filtered seawater (0.7 µm GF/F filtrate) and counted immediately. Sample aliquots of 10 mL will be placed in a serpentine counting tray and observed under a stereo microscope; nominally, at approximately 30x magnification for determination of live/dead counts.

All efforts will be made to enumerate live organisms quickly, since the primary metric in ballast treatment performance tests is the determination of live organism counts. Live organisms will be counted first, followed by enumeration of dead organisms in the same sample aliquot. Organisms will be designated as ‘live’ if they are fully intact and actively moving, exhibited an escape response when probed with a fine needle, or showed any internal/external movement. Organisms will be recorded as ‘dead’ if no activity or movement of any kind was observed, or if organisms were not intact. This viability determination is commonly referred as the ‘poke and probe’ technique. Totals and fractional portions of live
and dead organisms will be tabulated, and all assays will be manipulated volumetrically (if required) so that organism concentrations (number/volume) can be reported. Two separate archive samples will be preserved in 4% buffered formalin to allow for the inspection of general taxonomic diversity under less pressing time constraints and kept for at least six (6) months.

4.2 Organisms 10 - 50 µm

It is widely recognized that the absolute numerical determination of viable unicellular protists in the 10 - 50 µm size class is ambiguous (Dobroski 2009). This follows from the fact that visible signs in distinguishing live from dead protists are usually not evident (except in the case of motile representatives). For this reason, several corroborative methods will be used onboard ship to evaluate the diverse assemblage of microbiota occurring in the 10 - 50 µm size class. The methods will include techniques that specifically yield estimates of living cell concentrations. Whole water (unsieved) for use in preparing the 10 - 50 µm size class samples will be collected in 22 L carboys, as described in Section 3 - Sampling Procedures.

The GBF science team will use a size fractionation technique to isolate organisms in the 10 - 50 µm size class for all assays listed below. This is easily accomplished by concentrating the smaller than 50 µm filtrate (passed through a 50 µm sieve) on to custom made Nitex filters of 10 µm pore size on the diagonal (Nitex product 03/7-2) and re-suspending the retained particles for direct use in the assays below. GBF has been able to concentrate samples 10x to 100x greater than ambient concentrations utilizing this method; this significantly increases the analytical sensitivity and precision of techniques used to measure low concentrations of living organisms expected in treatment samples. Two separate archive samples will be preserved in 1% glutaraldehyde, to allow for the inspection of general taxonomic diversity under less pressing time constraints, and kept for at least six (6) months.

Flow Cytometric Analysis of Live Cells Utilizing Fluorescein Diacetate (FDA) Vital Stain – Corroborative Assay

GBF will maintain a Becton-Dickinson FACScan flow cytometer onboard ship throughout the two-three month experimental period to be used for quantitative analysis of living cell concentrations utilizing FDA vital stain protocol (Geary et al 1997; Hayakawa et al. 2008). FDA is a colorless reagent that freely passes through cell membranes and, when acted upon by living cellular esterase activity, is converted to the brilliant green fluorescent product, fluorescein, which readily marks living cells for flow cytometric detection. GBF will focus primarily on the detection of larger phytoplankton cells, as opposed to colorless heterotrophs, since phytoplankton provide natural red chlorophyll fluorescence; this yields a robust, two-color discrimination (red/green) for quantitative cytometry. The cytometric technique depends on the detection of obvious cell populations using optical scattering and fluorescence signals. GBF will rely on natural ‘red’ chlorophyll fluorescence to determine natural phytoplankton population targets, as this provides the optimal optical discrimination to identify the ‘green’ fluorescent live cells after the addition of FDA. Inert fluorescent bead standards of 10 µm and 50 µm will be used to roughly establish the cytometric region of analysis (based on forward scatter), thus allowing GBF to gate out the more numerous, small cells (< 10 µm) that are sure to be present in all samples. Visual observation via microscopy will also be utilized to insure gate is set to capture those organisms that are close to but exceed 10 µm in minimum dimension.
**Visual Epifluorescence Detection of Living 10 - 50 µm Cells Utilizing FDA and CMFDA Tracers - Corroborative Assay**

Visual enumeration of live cells in the 10 - 50 µm size class will be made using similar protocol to that described for flow cytometry. In this case, two fluorescent markers, FDA (fluorescein diacetate) and CMFDA (chloromethylfluorescein diacetate), will be applied simultaneously to maximize visual fluorescent signals and to minimize color fade during the counting procedure. FDA and CMFDA will be added to a final concentration of 5 µM and 2.5 µM, respectively, and incubated for 10 minutes before mounting in a covered 1 mL counting chamber for epifluorescence enumeration utilizing blue excitation and green emission.

**Chlorophyll-based Most Probable Number (MPN) Determination of Living Cell Concentration - Required Assay**

Photoautotrophic growth will be measured from long term incubations (14d) by using whole-cell chlorophyll fluorescence analysis as a sensitive indicator of cellular growth. MPN culture arrays of serially-diluted sample water will be prepared with F/2 seawater media (adjusted for ballast water salinity) in clear micro-tubes (0.5 mL volume) that can be read directly in a Spex Fluoromax 2 spectrofluorometer onboard ship. The MPN array will be constructed to yield optimized detection of 10 living organisms per mL (Woomer et al., 1990). Nominally the MPN culture arrays will include 30 tubes per sample (5 replicates x 6 dilution levels). MPN cultures will be maintained in an illuminated incubator at 5°C above natural temperature (to promote rapid growth) and monitored every other day for fluorometric indication of chlorophyll growth, defined as a two-fold increase in chlorophyll fluorescence relative to values scored at time zero. Triplicate MPN arrays will be set up for each sample, thus dictating the use of small growth tubes to conserve space within the incubator for all samples acquired from the three separate ballasting test cycles described here. One member of the GBF science team will remain onboard the T/S Golden Bear after the last ballast test cycle to complete the daily fluorometric analysis of MPN arrays for a minimum 14 day incubation period for each sample.

**C-14 Primary Production Experiments**

The radiotracer C-14 technique will be applied to uptake, control and treatment samples to yield physiological measurements of photosynthesis (carbon fixation rates), primarily in support of treatment technologies requiring metabolic confirmation of efficacy (e.g., UV treatment). Tests will be initiated and terminated at Moss Landing Marine Laboratory (MLML) in a walk-in cold room nominally held at 13°C. Samples will be prepared in triplicate, acid-washed polycarbonate bottles (125 mL), inoculated with C-14 (2 µCi) and incubated for nominally 24 hours under continuous, constant illumination provided by high intensity LED lamps; bottles will be rotated continuously on motorized plankton wheels to ensure uniform irradiance exposure and to prevent settling of cells. C-14 processing will follow that of Welschmeyer et al. (1993). Whole water sample aliquots will be harvested onto GF/F filters (0.7 µm) to estimate photosynthetic rates (µgC L⁻¹ d⁻¹). Total dissolved inorganic carbonate (DIC) will be estimated from salinity (µgC /L as DIC = 810xPSU) for determination of DIC specific activity (dpm/gC). Chlorophyll specific photosynthetic rates will be computed from Chl measurements made on the same water samples.
Thus, the following three methods for enumerating living organisms in the 10 - 50 µm size class will be used:

- Viability staining with FDA, flow cytometric detection;
- Viability staining with FDA and CMFDA; and
- MPN culture by chlorophyll detection.

It is hoped that the use of independent and corroborative methods will add confidence in the efficacy testing for the problematic 10 - 50 µm organism size class.

### 4.3 Organisms <10 µm

The GBF science team will use five assays to detect living organisms in the <10 µm organism size class. The first assay is the bulk assay for total cultivable bacteria and the remaining four assays are directed to specific microbial pathogens, *Escherichia coli*, *Enterococci sp.*, *Vibrio cholerae* serotype 01, and *V. cholerae* serotype 0139.

**Bulk Heterotrophic Bacteria Plate Counts**

A traditional sterile plating technique will be used to enumerate colony forming units (CFU) of the bulk bacterial community passing through a 10 µm sieve. Volumetric (100uL) sample aliquots (in triplicate) will be spread on sterile marine agar plates (Difco, 100 mm dia.) and incubated overnight (maximum of 24 hours) under dark conditions and at room temperature. Plates will be photographed in a digital image analyzer (Bio Rad Fluor S-Max) and enumerated using colony-counting software provided with the instrument. Data will be tabulated as CFU/mL.

**Microbial Pathogens**

Test kits based on quantitative colony forming unit (CFU) measurements specific to *E. coli* and *Enterococci* will be used onboard ship using sterile protocol. *E. coli* and *Enterococci* will be assayed using the Colilert® and Enterolert® test kits (Idexx, Inc.), which are based on MPN methodology and species-specific chromogenic reactions. Both assays will be prepared in triplicate using heat sealed dilution trays (Quanti-Tray® Idexx, Inc.) incubated at 35°C for 24 hours. *V. cholera* will be assayed using test kits for the 01 and 0139 serotypes, Cholera Smart® II and Bengal Smart® II, respectively (New Horizons Diagnostics, Inc.). The kits were originally produced for the purpose of obtaining quick (20 min) *V. cholera* presence/absence tests in fecal stool samples; this method is rapid, but relatively insensitive. MLML has worked with New Horizons (Larry Loomis, CEO) to show that tolerance limits for *V. cholera* of less than 1 CFU/100 mL can be achieved with a 48 hour, 35°C incubation using Cholera Smart kits as packaged by the manufacturer (determined from quantitative dilutions of actively growing cultures of *V. cholerae* 01 and 0139 serotypes). The GBF science team will use the prolonged incubation for *V. cholera* to achieve positive/negative scores at less than 1 CFU/100mL. All of the microbial assays above will be completed in triplicate, and all waste solutions will be bleach-sterilized before disposal.
Section 5 Whole Effluent Toxicity Testing Protocols

Samples may be collected for the purpose of whole effluent toxicity (WET) testing during a treatment discharge in accordance with US EPA Vessel General Permit as a requirement of the USCG Final Rule. A local and experienced toxicity lab, Pacific EcoRisk (PER), has been contracted to perform toxicity sampling and analysis. Samples for WET testing will be collected concurrently with BE sampling. No BE analysis will be performed on the WET test samples.

5.1 Sample Collection

A representative from PER will collect the WET test samples from a sample port downstream of the ballast water sampling station. For treatment discharge, a total volume of 150 L will be collected for WET testing during each toxicity sampling event. Water will be collected near the beginning, middle, and end of the treatment discharge. 50 L will be collected approximately 5 minutes after the discharge begins, 50 L will be collected approximately 20-30 minutes into the discharge, and 50 L will be collected approximately 50 minutes into the discharge. All sample water will then be mixed (at the PER laboratory under controlled conditions) to make a composite sample that is representative of the entire treatment tank discharge.

If testing control discharge, a total volume of 75 L will be collected for toxicity testing during each toxicity sampling event. Water will be collected near the beginning, middle, and end of the control tank discharge. 25 L will be collected approximately 5 minutes after the discharge begins, 25 L will be collected approximately 20-30 minutes into the discharge, and 25 L will be collected approximately 50 minutes into the discharge. All sample water will be mixed at the PER laboratory under controlled conditions to make a composite sample that is representative of the entire control tank discharge.

5.2 Testing and Reporting

WET testing will be performed in accordance with BWTS technology requirements and as approved by the VO. Both acute and chronic toxicity testing will be performed on the discharge samples. Three taxonomic groups will be evaluated: algae, crustaceans, and fish.

A report containing the toxicity test results will be prepared by PER and provided directly to GBF.

The Toxicity Study Design and Summary of Toxicity Test Conditions tables prepared by PER will be included in the GBF Final Report Appendices when toxicity testing is required.
Section 6  Data Collection, Reduction, and Validation

This section outlines the quality control procedures for collections and verification of project data and information.

6.1  Data Collection

GBF collects and records information about the pumping system, piping system, continuous monitoring equipment, and BWTS status. The information is gathered by the GBF automation and online information system. The GBF automation system is comprised of a computer network which monitors and securely records a wide array of field sensors such as valve position indicators, sample flow meters, and water quality instruments. User interfaces are located throughout the facility to provide visual indicators of the piping system, BWTS, and sampling station status to the GBF team. Further, the automation system facilitates the generation of reports based on the stored data. For a detailed description of the automation system, refer to the GBF Physical Plant Description.

The online information system is a computer network for handling and storing test documentation. Personnel may access the online information to view standard and test specific procedures, view secure collected data, and generate reports. User forms allow online entry of data directly into the information system. Hand logs may also be utilized during testing and will be scanned into the online information system for GBF records.

The BWTS will be expected to have its own data collection system in accordance with IMO G8 and USCG Final Rule. All data collected by the BWTS will be downloaded and transferred to the GBF online information system at the completion of each test cycle.

Data Quality Indicators

Statistical analyses will be carried out on data obtained for all performance measurements to assess data quality. Six data quality indicators (DQIs) will be used to interpret the degree of acceptability of the data and will be reported in a table comparing DQI criteria to recorded results. The protocol provided in this section will be used to assess the acceptable limits and criteria of the following DQIs:

- Representativeness;
- Accuracy;
- Precision;
- Bias;
- Comparability; and
- Completeness.

At the conclusion of each test event, the data will be processed and compared to the assigned DQI criteria. These are not pass/fail criteria, but rather an indicator of the quality of the collected data. An explanation is required for criteria that are not met. In subsequent test events, the procedure may be modified to improve data quality, or the criteria may be lowered.
Engineering Data

Engineering data will be collected and stored by the automation system for post processing. In general, the data should correlate with other monitored parameters during each test event.

For example, a pumping rate of 250 m$^3$/hr should result in a tank level corresponding to 250 m$^3$ of ballast water after one hour. An additional example includes agreement between the various sensors in the facility. If the temperature monitor at the pump discharge reads 20°C, and the temperature monitor at the treatment system discharge reads 10°C, then this does not follow the expected trend and will be investigated.

Instrumentation will be calibrated by a combination of factory set points and practical tests. For example, the sample tubs have graduated marks against which the total volume recorded by the sample flow meters can be compared. At the conclusion of each test event, the engineering data will be processed and compared to the assigned DQI criteria in Table 5 below.

**Table 5 - Data Quality Indicators for Engineering Parameters**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Indicator</th>
<th>Metric</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ballast Water Flow - Rosemount 8711 Flow Meter</td>
<td>Representativeness</td>
<td>90%</td>
<td>Flow meters are continuously online and recording. A temporary system fault is possible.</td>
</tr>
<tr>
<td></td>
<td>Accuracy</td>
<td>± 3%</td>
<td>Instrument performance is ± 0.3% of flow as per factory calibration.</td>
</tr>
<tr>
<td></td>
<td>Precision</td>
<td>0.1</td>
<td>Cubic meters per hour. Display on unit.</td>
</tr>
<tr>
<td></td>
<td>Bias</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Comparability</td>
<td>10%</td>
<td>All ballast water passes at least two of the three flow meters allowing a comparison between them.</td>
</tr>
<tr>
<td></td>
<td>Completeness</td>
<td>90%</td>
<td>Data is automatically recorded in the automation system.</td>
</tr>
<tr>
<td>Ballast Water Volume - TMS LevelCom Tank Level Indicator</td>
<td>Representativeness</td>
<td>90%</td>
<td>Indicator measures the water column in the forward portion of the tank. If ship is trimmed aft, it will miss a small portion of this water when the tank level is very low.</td>
</tr>
<tr>
<td></td>
<td>Accuracy</td>
<td>± 5%</td>
<td>Some variation is expected due to the shape of the tanks changing at various heights.</td>
</tr>
<tr>
<td></td>
<td>Precision</td>
<td>0.1</td>
<td>Meters. This is a function of the system analog signal to the automation system.</td>
</tr>
<tr>
<td></td>
<td>Bias</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Comparability</td>
<td>10%</td>
<td>Variation when compared to flow meter and time calculation while tank is being filled/emptied.</td>
</tr>
<tr>
<td></td>
<td>Completeness</td>
<td>90%</td>
<td>Data is automatically recorded in the automation system.</td>
</tr>
<tr>
<td>Sample Water Flow - Seametrics SPX</td>
<td>Representativeness</td>
<td>90%</td>
<td>Flow meters are continuously online and recording. A temporary system fault is possible.</td>
</tr>
<tr>
<td></td>
<td>Accuracy</td>
<td>± 5%</td>
<td>Instrument performance is +/- 1% of full scale.</td>
</tr>
<tr>
<td></td>
<td>Precision</td>
<td>0.3</td>
<td>Gallons per minute on display unit.</td>
</tr>
<tr>
<td></td>
<td>Bias</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Comparability</td>
<td>10%</td>
<td>Variation between the three sample tubs that take simultaneous samples.</td>
</tr>
<tr>
<td></td>
<td>Completeness</td>
<td>90%</td>
<td>Data is automatically recorded in the automation system.</td>
</tr>
</tbody>
</table>
Water Quality

GBF has integrated continuous monitoring equipment that will provide continuous water quality measurements in real time for viewing and verification. In-situ instrumentation and laboratory analysis will also be utilized.

Continuous and grab sample data will be evaluated using the DQI criteria in Table 6 and Table 7, respectively.

Table 6 - Data Quality Indicators for Continuous Monitoring Parameters

<table>
<thead>
<tr>
<th>Sensor</th>
<th>Maker</th>
<th>Type</th>
<th>Calibration Procedure</th>
<th>Reporting Units</th>
<th>Range</th>
<th>Accuracy</th>
<th>Precision</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature</td>
<td>Sea-Bird Electronics</td>
<td>SBE 21</td>
<td>Nominally 1/year</td>
<td>°C</td>
<td>-5 to +35</td>
<td>0.01</td>
<td>0.001</td>
</tr>
<tr>
<td>Conductivity</td>
<td>Sea-Bird Electronics</td>
<td>SBE 21</td>
<td>Nominally 1/year</td>
<td>S/m</td>
<td>0 to 7</td>
<td>0.001</td>
<td>0.0001</td>
</tr>
<tr>
<td>Transmissometer (25 cm)</td>
<td>Wet Labs</td>
<td>C-Star</td>
<td>Nominally 1/year</td>
<td>% transmittance or beam attenuation</td>
<td>0-100</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Dissolved Oxygen</td>
<td>Sea-Bird Electronics</td>
<td>SBE 43</td>
<td>Nominally 2/year</td>
<td>mg/L</td>
<td>120% of saturation</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Fluorometer</td>
<td>Turner Designs</td>
<td>Cyclops -7</td>
<td>In-house, before each experimental run</td>
<td>µg/L</td>
<td>0.1 to 50</td>
<td>±5%</td>
<td>0.01</td>
</tr>
</tbody>
</table>

*When compared to wet chemistry.

Table 7 - Data Quality Indicators for Grab Sample Monitoring Parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Maker</th>
<th>Type</th>
<th>Calibration Procedure</th>
<th>Reporting Units</th>
<th>Range</th>
<th>Accuracy</th>
<th>Precision</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature</td>
<td>YSI</td>
<td>6560</td>
<td>Nominally 1/year</td>
<td>Degrees C</td>
<td>-5 to +50</td>
<td>0.15</td>
<td>0.1</td>
</tr>
<tr>
<td>Conductivity</td>
<td>YSI</td>
<td>6560</td>
<td>Nominally 1/year</td>
<td>S/m</td>
<td>0 to 100</td>
<td>0.5%</td>
<td>0.1</td>
</tr>
<tr>
<td>Dissolved Oxygen</td>
<td>YSI</td>
<td>6150</td>
<td>Nominally 1/year</td>
<td>mg/L</td>
<td>0 to 50</td>
<td>0 to 20: ±0.1 or 1%, whichever is greater</td>
<td>0.01</td>
</tr>
<tr>
<td>Chlorophyll α</td>
<td>YSI</td>
<td>6025</td>
<td>In-house per exper. run</td>
<td>µg/L</td>
<td>0 to 400</td>
<td>0.1 µg/L Chl</td>
<td>~0.1</td>
</tr>
<tr>
<td>Turbidity</td>
<td>YSI</td>
<td>6136</td>
<td>In-house per exper. Run</td>
<td>NTU</td>
<td>0 to 1000</td>
<td>±0.1</td>
<td>0.1 NTU</td>
</tr>
<tr>
<td>pH</td>
<td>Beckman</td>
<td>70</td>
<td>In-house per exper. Run</td>
<td>pH units</td>
<td>0 to 14</td>
<td>±0.1</td>
<td>±0.03</td>
</tr>
<tr>
<td>Total Suspended Solids (TSS)</td>
<td>Proweight</td>
<td>Filter</td>
<td>Calibrated 1/year, checked per exper. run</td>
<td>mg/L</td>
<td>5 to 100</td>
<td>±2</td>
<td>2.60%</td>
</tr>
<tr>
<td>Particulate Organic Carbon (POC)</td>
<td>GF/F</td>
<td>Filter</td>
<td>In-house per exper. Run</td>
<td>mg/L</td>
<td>0.1 to 100</td>
<td>±0.15</td>
<td>7.50%</td>
</tr>
<tr>
<td>Dissolved Organic Carbon (DOC)</td>
<td>GF/F</td>
<td>Filter</td>
<td>Per exper. run, at McCampbell Analytical, Inc.</td>
<td>mg/L</td>
<td>0.5 to 100</td>
<td>±0.1</td>
<td>20%</td>
</tr>
</tbody>
</table>
Biological Analysis

Biological DQIs are determined empirically by the GBF science team based on actual sample measurements made in the GBF laboratory. By design, GBF has developed procedures that generate a high level of replication.

Biological analysis data will be evaluated using the DQIs in Table 8 below.

**Table 8 - Data Quality Indicators for Biological Analyses**

<table>
<thead>
<tr>
<th>Sample Description</th>
<th>Analytical method</th>
<th>Data Quality Indicators (DQI) (^a)</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Organisms ≥50 µm (#/m³)</td>
<td>Visual determination of live status (30x magnification)</td>
<td>Expected CV(^b) &lt;31%</td>
<td>DQI based on ‘uptake’ and ‘control’ samples which provide highest live organism densities</td>
</tr>
<tr>
<td>2a. Organisms 10–50 µm Method A MPN (MPN/mL)</td>
<td>MPN determination of cultivable phytoplankton; fluorometric determination of growth</td>
<td>Expected CV &lt;60%</td>
<td>DQI based on ‘uptake’ and ‘control’ samples which provide highest live organism densities</td>
</tr>
<tr>
<td>2b. Organisms 10–50 µm Method B Flow cytometry (#/mL)</td>
<td>Flow cytometric determination of live cells tagged w/ fluorescein diacetate (FDA)</td>
<td>Expected CV &lt;42%</td>
<td>DQI based on ‘uptake’ and ‘control’ samples which provide highest live organism densities</td>
</tr>
<tr>
<td>2c. Organisms 10–50 µm Method C Epifluorescence microscopy (#/mL)</td>
<td>Visual epifluorescence determination of live cells tagged w/ FDC and CMFDA</td>
<td>Expected CV &lt;25%</td>
<td>DQI based on ‘uptake’ and ‘control’ samples which provide highest live organism densities (experience limited to one complete ballast sequence)</td>
</tr>
<tr>
<td>3a. Organisms &lt; 10 µm Bulk bacterial plate counts (CFU/mL)</td>
<td>Total colony forming units (CFU) on agar substrate</td>
<td>Expected CV &lt;90%</td>
<td>DQI based on ‘uptake’ and ‘control’ samples which provide highest live organism densities</td>
</tr>
<tr>
<td>3b. Pathogens E. coli (MPN/100 mL)</td>
<td>MPN determination IDEXX proprietary, enzyme-based MPN kit (Colilert™)</td>
<td>Expected CV &lt;64%</td>
<td>DQI based on ‘uptake’ and ‘control’ samples which provide highest live organism densities</td>
</tr>
<tr>
<td>3c. Pathogens Enterococci (MPN/100 mL)</td>
<td>MPN determination IDEXX proprietary, enzyme-based MPN kit (Enterolert™)</td>
<td>Expected CV &lt;42%</td>
<td>DQI based on ‘uptake’ and ‘control’ samples which provide highest live organism densities</td>
</tr>
<tr>
<td>3d. Pathogens <em>Vibrio cholerae</em> serotype 01 (detection limit &lt;1CFU/mL)</td>
<td>Antibody detection; New Horizons Diagnostics, Cholera Smart™ II</td>
<td>N.A.</td>
<td>No observation of <em>V. cholera</em> (Type 01) &gt;1 CFU/100 mL has been found in any sample analyzed at GBF (n=36)</td>
</tr>
<tr>
<td>3e. Pathogens <em>Vibrio cholerae</em> serotype 0139 (detection limit &lt;1CFU/mL)</td>
<td>Antibody detection; New Horizons Diagnostics, Bengal Smart™ II</td>
<td>N.A.</td>
<td>No observation of <em>V. cholera</em> (Type 0139) &gt;1 CFU/100 mL has been found in any sample analyzed at GBF (n=36)</td>
</tr>
</tbody>
</table>

\(^a\) DQI is estimated as twice the empirically measured coefficient of variation (CV) for sample replicates analyzed by GBF science staff for the given assay.

\(^b\) CV = standard deviation/mean*100, where the sample standard deviation is \(\sqrt{\sum(x - \bar{x})^2/(n - 1)}\)
6.2 Data Reduction

All testing data will be collected, analyzed, and reported in a uniform format. Specifically, all data from time-integrated sample collections such as net samples and microbe/chemistry ‘grab’ samples will be collected in triplicate corresponding to the three individual sampling ports configured at the sampling station. Additionally, each replicate sampling container, including each net and each 22 L ‘grab’ carboy, will be sampled in triplicate for sample aliquots corresponding to the assays described in SOP 20 to 36. In effect, each ballasting event will yield $3 \times 3 = 9$ analyses of the parameters indicated above in Section 6.1 – Data Collection.

Data means and standard deviations will be calculated and reported as figures and tables. Example formats for data reporting are provided in Figure 2 and Table 9 below.

*Note:* The example figure is a graphical representation of the same tabular data.

![Busan: Live Zooplankton >50 µm (Analyst 1)](image)

**Figure 2 - Example Data Reduction Figure**

**Table 9 - Example Data Reduction Table**

<table>
<thead>
<tr>
<th>Location</th>
<th>Sample</th>
<th>Live Zoop (#/m³)</th>
<th>Dead Zoop. (#/m³)</th>
<th>% Live</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Busan</td>
<td>1U-A</td>
<td>45425</td>
<td>8282</td>
<td>23523</td>
<td>3351</td>
</tr>
<tr>
<td></td>
<td>1U-B</td>
<td>36907</td>
<td>9328</td>
<td>17643</td>
<td>3219</td>
</tr>
<tr>
<td></td>
<td>1U-C</td>
<td>55564</td>
<td>6965</td>
<td>18048</td>
<td>3337</td>
</tr>
<tr>
<td>Busan</td>
<td>1C-A</td>
<td>27004</td>
<td>3096</td>
<td>16956</td>
<td>5042</td>
</tr>
<tr>
<td>(Control)</td>
<td>1C-B</td>
<td>19684</td>
<td>4219</td>
<td>12845</td>
<td>2128</td>
</tr>
<tr>
<td></td>
<td>1C-C</td>
<td>21750</td>
<td>2110</td>
<td>11136</td>
<td>1437</td>
</tr>
<tr>
<td>Busan</td>
<td>1T-A</td>
<td>0</td>
<td>0</td>
<td>4857</td>
<td>1052</td>
</tr>
<tr>
<td>(Treatment)</td>
<td>1T-B</td>
<td>0</td>
<td>0</td>
<td>3879</td>
<td>433</td>
</tr>
<tr>
<td></td>
<td>1T-C</td>
<td>0</td>
<td>0</td>
<td>6649</td>
<td>823</td>
</tr>
</tbody>
</table>
7.1 Data Validation

Each project related task will be tracked either through the automation system, online information system, or hand logs which will be scanned into the online information system.

Quality will consult with the Director, Scientist, and Operator to confirm the correct version of the GBF standard and project specific documents. Quality will confirm the correct versions are available through the online information system by utilizing data sheets provided in SOP 7 - Quality Control Checklist for Cycle (Shipboard).

Prior to the start of a test cycle, Quality will determine which aspects of the automation system will be used for data monitoring and if the GBF team will need to maintain hand logs. To make this assessment, Quality will open, view, practice, and determine the availability of each of the data collecting tools in the automation system. Upon this determination, Quality will update the data sheets to record which data collection sets will be recorded on hand logs or online. Quality will notify all team members of the correct document versions and record method.

Test Cycle Automation Initiation, Tracking, and Error Logging

Prior to the start of a test cycle, Quality will work with the Operator and Scientist to produce an automation and online system status report. The report will include calibration records for field instrumentation; a system check of all monitored parameters; and a system check of all screens, forms, and reports as indicated by the data sheets provided in SOP 7 - Quality Control Checklist for Cycle (Shipboard). In addition, Quality will communicate with the Operator and Scientist to identify any automation system errors. Automation errors found independently or highlighted by team members will be documented and made part of the automation and online system status report. Where automation errors occur, hand logs of the monitored parameter will replace the automated entry. An action plan for hand monitoring and logging of non-functional items will be submitted. Quality will scan all hand logs into the online information system.

At the start of a test event, before pumping activities, Quality will initiate the automation system. The automation system will automatically generate a unique test event number and begin logging all monitored parameters. The test event number will be matched to all monitored data and all online data sheets in the online information system. The test event number will be based on the start date and time of the event. For example, if initiation occurs at 1:32 pm on March 14, 2012, the test number would be “12-03-14-1332.”

At the conclusion of the test event, Quality will gain confirmation from the Operator and stop the automatic collection of data.

Red-lining of Procedures

As testing efforts progress, it may be advantageous or imperative to change procedures due to the complexity of the operations and variability of the equipment and challenge water conditions.

For example, the BWTS may fail to open a valve imperative to its operation. In this case, the Operator may use ordinary means, such as manually opening the valve, to allow the test to continue.
An additional example may include a failed sampling net that has allowed some part of its contents to spill into the sample tub. In this case, the Scientist might continue sampling with that net, analyze its contents separately from the other two nets, and not average results until it is determined that all results are consistent.

In any case, where there is a deviation from the test procedures, protocol, or plan, the person-in-charge of that operation must “red-line” that document. The red-lining procedure is as follows:

1. Strike a single line using indelible ink through the affected text, figure, chart, or other item.
2. Initial adjacent to each and every strike.
3. Provide any required correction in the form of text, figure, chart, or other item.
4. Provide the reason for the change in the “notes” section of the document.
5. Date and sign each affected page.

**Hand Log Tracking and Filing**

At the end of each test cycle, Quality will collect all hand logs from the team and scan the hand logs into the online information system. Each hand log will be assigned the test event number and an associated identifier. For example, a biological efficacy (BE) sampling log for a test event that occurs at 1:32 pm on March 14, 2012 would be “2012-03-14-1332-BESAMPLING.”

**Online Log Tracking and Filing**

During each test cycle, Quality will view automated reports and online data logs daily to confirm that the systems are working correctly. A copy of completed logs will be copied to a secure location on the online information system and named using the same convention as the hand logs.

**Verification Data Record**

Following each test cycle, Quality will make two (2) copies of the automation system database, scanned hand logs, and online data forms on a secure read-only DVD. Each DVD will be scribed with test cycle name, date, and copy recipient.

For example, a DVD for a test initiated on March 14, 2012 and sampled on March 16, 2012 would be scribed with “Test Cycle 12-03-14-1332, Data Recorded 12-03-16, Director Copy.” Quality will review all the automation system database, scanned hand logs, and online data forms for conformance with the Verification Data Record DVDs. One DVD copy will be immediately distributed to the Director for off-site storage and the second copy kept on-site by Quality.
Section 8  Assessments

Per the BWTS specific Project Plan, the VO for this project will audit project documentation and performance for compliance with the TQAP, IMO G8, and USCG Final Rule.
Appendix A  Project Training Requirements

Quality is responsible to ensure that each person is trained as appropriate to their tasks on the project, as follows:

Facility Training: This training covers all information provided in the TQAP, along with specialized training relevant to their role per the test plan; for example GBF personnel may take training course on Confined Spaced Entry Procedures and Documentation and Safety Reviews.

Equipment Training: This training covers all information provided by the equipment manufacturer, along with specialized training relevant to the role in testing; for example, GBF personnel may take training on System Installation Requirements Review (particularly USCG and ABS requirements for electrical connections and ground protection), and proper Lock-Out/Tag-Out Procedures.

SOP Training: This training ensures that personnel can successfully complete their assigned tasks using the appropriate SOP.

Quality is also responsible for documenting and maintaining training program records. The Director, Operator and Scientist will conduct the training unless otherwise designated by Quality. The logs on the following pages are for documentation of the project training completed by all GBF personnel before the start of testing. A copy of these training records will be filed onboard the GBF, and a summary will be included in the final testing report.

Training Logs

Ballast Facility: William (Bill) Davidson is the chief engineer of the T/S Golden Bear; has been the lead engineer during the construction of the GBF; and has become expert with all of the technical aspects of the ballast treatment piping and testing system. Bill is the trainer for those GBF personnel who operate the pumps, valves, and piping system.

List of personnel trained to operate the ballast pump and piping system:

- Dan Lintz, trained November 2010
- John Coyle, trained November 2010
- Dan Weinstock, trained May 2010 and updated November 2010
- David Coleman, trained May 2010 and updated November 2010
- Bill Schmidt, trained July 2010 and updated November 2010
- Nic Shields, trained February 2012

Water Quality: Richard Muller has installed and maintained in situ water quality sensors for a majority of his career as a technician onboard various oceanographic research vessels during the past 20 years. His training has been primarily through manufacturer interaction and at-sea experience troubleshooting instrumentation.
Biological: Dr. Nicholas Welschmeyer is professor and research scientist at the Moss Landing Marine Laboratories and has performed research on ballast water and ballast treatment systems for the past seven years with 31 years of field experience in biological oceanography. All biological analysts are trained within the laboratory of Nicholas Welschmeyer. First, analysts are given the full set of standard operating procedures verifying that they have a command of the techniques. Second, for subjective analyses, analysts count trial samples simultaneously to determine comparability between analysts. Third, all biological analysts are familiarized with instrumentation used for US EPA Environmental Technology Verification (ETV) *Generic Protocol for the Verification of Ballast Water Treatment Technology*. 

List of scientific technicians are:

- Erin Jensen;
- Julie Kuo;
- Brian Maurer; and
- Jeff Johnsen.
Log Sheet 1: Facility Training

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<th>Role/Position</th>
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## Log Sheet 2: Equipment Training

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Log Sheet 3: SOP Training

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**DATE / TIME**  **RECEIVED BY**  **SAMPLE CONDITION:**

**SAMPLE TYPE CODES:**
- AC = Aqueous
- NA = Non-Aqueous
- SL = Sludge
- DW = Drinking Water
- PW = Public Water
- EW = Effluent Water
- NW = Non-Potable Water
- GP = Ground Water

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