STEP 2021 Application Package
Revised February 5, 2021

United States Coast Guard
Shipboard Technology Evaluation Program (STEP)

Ship Enrollment Application
for
Ballast Water Management Systems

STEP 2021 Application Package
Revised February 5, 2021

Introduction

The U.S. Coast Guard's Shipboard Technology Evaluation Program (STEP) was developed in 2004 to facilitate the development of prototype technology and design of ballast water treatment systems with demonstrated potential for effective removal or destruction of organisms in ballast water. Since that time, the market has matured, and detailed regulations have been implemented that specify the criteria for designing and testing systems for Coast Guard type approval. For new systems that have not received certification but wish to discharge into waters of the United States, the regulations require that the operating vessel be enrolled in STEP.

The attached form is provided by the Coast Guard's Office of Operating and Environmental Standards (CG-OES) for use as a standardized application form to STEP, in accordance with Navigation and Vessel Inspection Circular (NVIC) 01-04 "Shipboard Technology Evaluation Program (STEP): Experimental Ballast Water Treatment Systems." This form can be used when applying to STEP for an experimental ballast water management system (BWMS) in accordance with the NVIC, and also for a BWMS undergoing Coast Guard type approval (TA) testing in accordance with Title 46 of the Code of Federal Regulations (CFR), Part 162.060. Using this form is not mandatory, but will facilitate review of the STEP application by the Coast Guard.

This application package contains instructions for filling out the form, a checklist to aid in compiling the information, and a copy of the existing "STEP 2010 Application Form," originally developed for prototype systems. Incorporated into this version of the application is a revised checklist that identifies sections of the application form that are also covered by the regulatory type approval process. By doing so, this package abridges the application form to minimize duplicate information and consolidates source data for ease of review.

This guidance is not a substitute for applicable legal requirements, nor is it itself a rule. It is not intended to nor does it impose legally binding requirements on any party. It represents the Coast Guard's current thinking on this topic and may assist industry, mariners, the general public, and the Coast Guard, as well as other federal and state regulators, in applying statutory and regulatory requirements. You can use an alternative approach for complying with these requirements if the approach satisfies the requirements of the applicable statutes and regulations. If you have questions, please send an e-mail to *environmental_standards@uscg.mil*, with ATTN: STEP COORDINATOR in the subject line.

S.T. Brady
Captain, U.S. Coast Guard
Chief, Office of Operating and environmental Standards
February 5, 2021

Instructions

The applicant should provide all requested data in the text boxes and tables. However, the STEP application is flexible, and the applicant may modify it as needed to suit a particular test project. Substantive additions or omissions of information should include a satisfactory explanation of the change. Leaving blanks, or simply citing references to other technical documents in lieu of preparing a response will likely produce a delay while the STEP review team and the applicant correspond to clarify information. The applicant may cite technical references to support responses in the application. Key technical literature, drawings, and other documents cited in the application should be included in the appendices.

Introductory, explanatory, and instructive text in this document are in non-bulleted format. Directions indicating action required of the applicant are bulleted. References in the text to required data tables and figures are highlighted yellow.

Text boxes are provided for the input of information in a narrative format (text box size adjusts automatically). There are also areas of the application that include choosing multiple options; for example, the series of tables in Section 3 requesting information particular to several types of treatment technologies.

This package includes an optional checklist for the applicant to assist in tracking completion of all required components of the application, as well as the 2010 STEP Application Form, which begins with the Table of Contents, identifying all required tables and figures.

NOTE: The Coast Guard and the Volpe Center Review Panel strongly recommend that prospective applicants read the STEP Application Instructions document and NVIC 01-04, with enclosures, before preparing and submitting a STEP application.

Applicant Checklist

This checklist is intended to assist applicants when completing the STEP Application Form. Sections highlighted in **green** can be supported with acceptable U.S. BWMS type approval documents from an independent laboratory (IL), if the shipboard testing is being conducted as part of testing and evaluation for Coast Guard type approval (TA). If the IL believes that one of their internal documents provides the necessary criteria for a section, that IL may submit that documentation with an explanation of why it is acceptable. If the TA documents identified in the checklist are being submitted, this should be stated in the "notes" section of the checklist. Sections highlighted in **blue** do not need to be completed when conducting Coast Guard TA testing in concurrence with STEP. If the system is not being tested for Coast Guard TA (i.e. a prototype), then the application should be completed in its entirety.

Documents Required	Acceptable U.S. BWMS TA documents. If the application is not for a vessel that will be engaged in type approval testing, complete the relevant sections of the application form	Completed: Yes-No-NA	Notes
Is this application for a vessel engaged in testing a BWMS for type approval by the Coast Guard? *Respond Yes or No in the third column.*			
1.0 Applicant's Test Program Organization and Management Plan			
1.1 *STEP's Ballast Water Management System Objective*			
1.2 *Letter of Commitment*			

Documents Required	Acceptable U.S. BWMS TA documents. If the application is not for a vessel that will be engaged in type approval testing, complete the relevant sections of the application form	Completed: Yes-No-NA	Notes
1.3 *Program Organization and Management Plan*			
1.4 *Contact Information*			
1.5 *Proposed Schedule*			
2.0 Vessel and Existing Ballast System Specifications *Vessel applying to STEP and installed Ballast Water Management System (BWMS)*			
2.1 *Vessel and Crew Information*			
2.2 *Existing Ballast System*			
2.3 *Current Ballast/Deballast Practices*			
3.0 Ballast Water Management System	Operation, Maintenance, and Safety Manual (OMSM) 46 CFR 162.060-38		
3.1 *Acceptability Criteria for BWMS*	Design and Construction 46 CFR 162.060-20(a)(4)		
3.2 *Verification of BWMS Claims*	Land-based test results 46 CFR 162.060-26		

Documents Required	Acceptable U.S. BWMS TA documents. If the application is not for a vessel that will be engaged in type approval testing, complete the relevant sections of the application form	Completed: Yes-No-NA	Notes
3.3 ***BWMS Description***	OMSM 46 CFR 162.060-38(a)(3)(i)		
3.4 ***Design Criteria***	OMSM 46 CFR 162.060-38 (a)(3)		
3.5 ***Physical Configuration and Shipboard Installation***	OMSM 46 CFR 162.060-38(a)(5)		
3.6 ***Electrical, Instrumentation and Control Requirements***	OMSM 46 CFR 162.060-38(a)(6)		
3.7 ***Operations and Maintenance Requirements***	OMSM 46 CFR 162.060-38(a)(8)		
3.8 ***Crew Health and Safety***	OMSM 46 CFR 162.060-38(a)(9)		
4.0 Proof of Ballast Water Management System Performance			
Provided shipboard or land-based tests from AMS or conducted under IL under U.S. or IMO protocols (sufficient alone) skip to Section 5; if not available, fill out Subsections 4.1-4.4	Land-based test report 46 CFR 162.060-26(a)		
4.1 ***Summary of Prior Experiments; Bench Tests***			

Documents Required	Acceptable U.S. BWMS TA documents. If the application is not for a vessel that will be engaged in type approval testing, complete the relevant sections of the application form	Completed: Yes-No-NA	Notes
4.2 ***Summary of Prior Experiments; Pilot Scale***			
4.3 ***Literature Summary of Laboratory Experiments; Full Scale***			
4.4 ***Summary of Laboratory Experiments; Full Scale***			
5.0 Step Study Plan *Required of all applicants*	Shipboard test plan 46 CFR 162.060-24		
5.1 ***Test Team Responsibilities*** *List of all team members, expertise and responsibilities in STEP*	Shipboard test plan 46 CFR 162.060-24		
5.2 ***Treatment Performance Goals***	Shipboard test plan 46 CFR 162.060-24		
5.3 ***Ballast Water Management System Experimental Methods***	Shipboard testing 46 CFR 162.060-28(f)		
5.4 ***Sampling and Analysis***	Shipboard testing 46 CFR 162.060-28(f)		
Complete Quality Assurance Project Plan (QAPP) is Provided			

Documents Required	Acceptable U.S. BWMS TA documents. If the application is not for a vessel that will be engaged in type approval testing, complete the relevant sections of the application form	Completed: Yes-No-NA	Notes
5.4.1 Biological Analysis ≥50 µm and <50 - ≥10µm fractions			
5.4.2 Environmental Analysis			
5.5 *Test Voyage(s) Itinerary*	Shipboard testing 46 CFR 162.060(e)(3)		
5.6 *References Used in Section 5*	Optional, may refer to IL or sublab standard operating procedures (SOPs)		
6.0 Long Term Performance Monitoring Plan *This section is applicable to all vessels in STEP until such time as the BWTS is type approved and a request is made to OES to withdraw the vessel from STEP*			
6.1 Personnel Requirements for Long Term Monitoring Plan			
6.2 Operational Performance Monitoring Parameters	OMSM 46 CFR 162.060-38(a)(6)(vii)		

Documents Required	Acceptable U.S. BWMS TA documents. If the application is not for a vessel that will be engaged in type approval testing, complete the relevant sections of the application form	Completed: Yes-No-NA	Notes
6.3 Other Performance Monitoring Parameters	OMSM 46 CFR 162.060-38(a)(6)(vii)		
6.4 Operational Logs for Long-Term Performance Monitoring	Design and Construction 46 CFR 162.060-20 (b)(5)		
6.5 Maintenance Logs for Long-Term Performance Monitoring	Design and Construction 46 CFR 162.060-20 (b)(5)		
6.6 Biological Performance Test Plan, Year 5 *If Vessel's BWMS is type approved before Year 5 testing, an approved request to OES to remove the vessel from STEP voids the need for Year 5 testing.*			
7.0 **Environmental Compliance**			
7.1 ***Description of Routine Operations***			
7.2 ***Water Quality and Discharge of Treated Ballast Water***	Shipboard test report 46 CFR 162.060-28		

Documents Required	Acceptable U.S. BWMS TA documents. If the application is not for a vessel that will be engaged in type approval testing, complete the relevant sections of the application form	Completed: Yes-No-NA	Notes
7.3 *Storage, Handling, and Exposure to Ballast Water Treatment Chemicals*	OMSM 46 CFR 162.060-38(9)(i)		
7.4 *Management of Ballast Water Management System Waste Streams*			
7.5 *Biological Issues*			
Appendix A - Supplemental Information on Proposed BWMS and Shipboard Installation	Not required when conducting U.S. TA testing		
Appendix B - Cited Technical Papers and Test Reports	Not required when conducting U.S. TA testing		
Appendix C - Supplemental Environmental Compliance Information	Not required when conducting U.S. TA testing		

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Section 1.0 Applicant's Test Organization and Management Plan

1.1 STEP's Ballast Water Management System Objective

The STEP policy and guidance in NVIC 01-04 specify treatment performance as 98 percent removal of organisms larger than 50 microns. Acceptance into STEP requires documentation of this level of removal (or inactivation) from a ballast water management system (BWMS) or unambiguous data showing promise of such performance at shipboard scale based on smaller scale experiments.

1.2 Letter of Commitment

Prepare a Letter of Commitment, indicating the identities of the ship operator (if different than owner), the manufacturer or developer of the treatment system, and the principal investigators conducting the biological and engineering tests. Identify the applicant's authorized STEP project manager and state the intent to carry out all components of the study plan for which they are responsible. Submit letter with the application package.

The Government does not specify the form of the Commitment Letter.

1.3 Program Organization and Management Plan

The applicant must provide a clear picture of the management structure of the STEP project team, showing key elements of all organizations involved, and including the owner, operator, treatment system vendor(s), biological test team, and laboratory(ies). It must be stressed that the size of the test team needs to be adequate to complete the sampling and analysis tasks within the sample holding times prescribed in the sampling plan (Section 5). Given the sensitivity of biological tests to sample holding time, there is increased emphasis on sample tracking and analytical quality assurance and quality control (QA/QC), both within STEP and shorebased land-based-test programs following Environmental Technology Verification (ETV) protocols.

Complete Figure 1-3 and use Text Box 1.3a to briefly describe the team structure, including any deviations from the suggested structure in Figure 1-3.

TEXT BOX 1.3a: Team structure description.

Define the role and functions of the **STEP project manager** by checking the boxes below, if they apply in your case. If any do not apply, in use Text Box 1.3b to explain how the proposed team management structure will effectively fulfill that role or function.

☐ Acts with the authority of the applicant, i.e., of the ship owner or operator, especially with regard to financial, operational, and scheduling matters.

☐ Coordinates test plan and shipboard operations, including schedules and activities of the Applicant's operations personnel and subject matter experts (see Figure 1-1) with those of the BWMS vendor(s) and the biological test team.

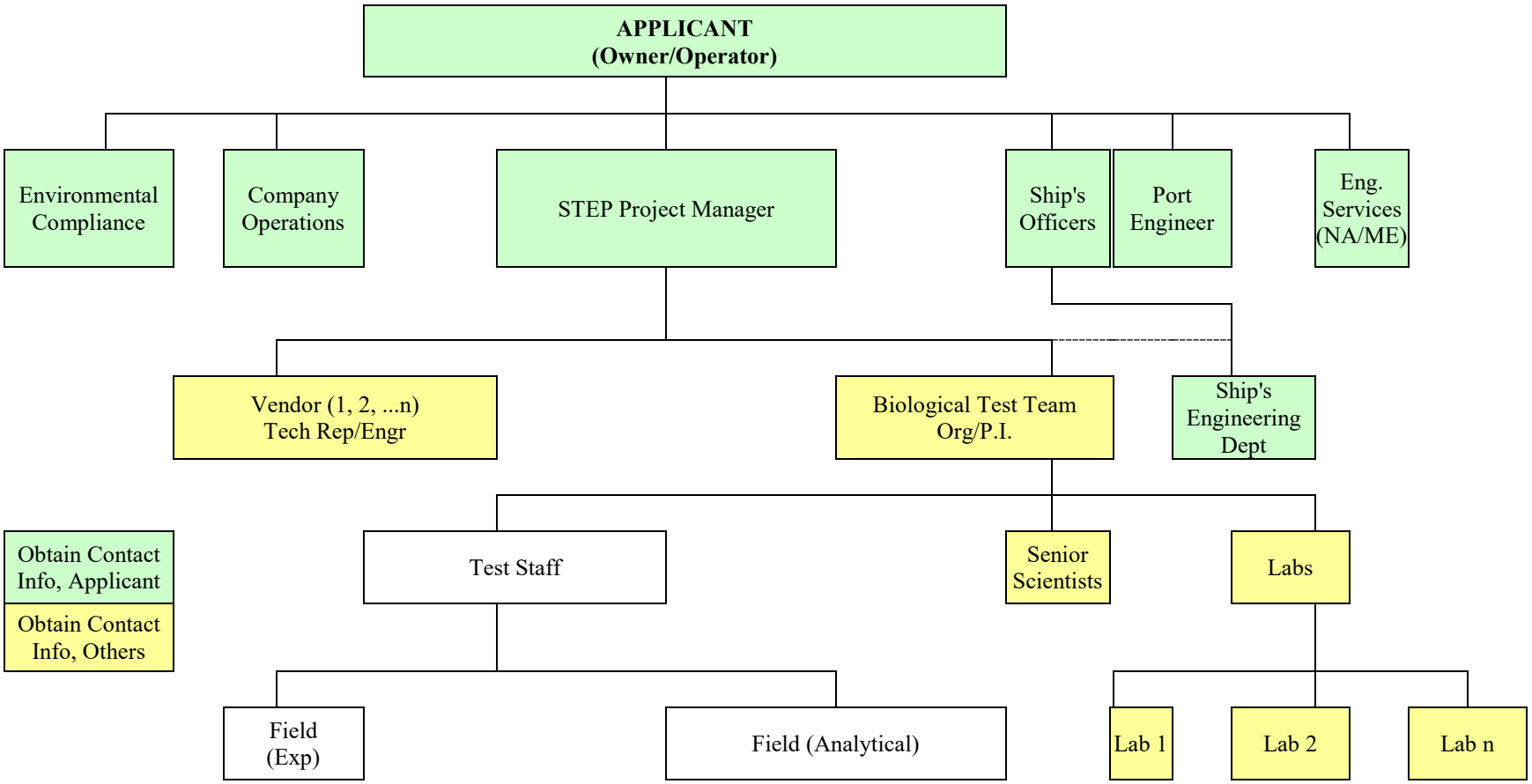
☐ Functions as the primary point of contact with the Coast Guard and Volpe Center Review Panel in matters of scheduling and coordinating STEP review activities, status of application, etc. (Subject matter experts for both parties may correspond directly on technical matters only.)

☐ Directs shipboard BWMS installation and testing activities, with the assistance of the port engineer and the ship's chief engineer.

☐ Oversees preparation of all documents for submission to the Coast Guard, including the STEP Application, application revisions, and long-term performance monitoring reports.

TEXT BOX 1.3b: Description of project manager's role.

Figure 1-3. STEP Project Org. Chart



1.4 Contact Information

Provide contact data for key STEP personnel, in Table 1-4; insert additional rows as necessary.

Table 1-4. Key Points of Contact (pt. 1)

APPLICANT (corporate office data)	Company Name	Street Address	State/Province	Country
Personnel	**Organization**	**Name**	**Phone**	**Email**
APPLICANT				
STEP Project Manager				
Port Engineer				
Company Operations				
Environmental Compliance				
Engineering Services (NA/ME)				
Ship Personnel				
Captain				
Chief Engineer				
Chief Mate				
BW/BWM System Operations				
VENDORS				
Vendor #1, Technical Rep.				
Vendor #1, Sales/Service Rep.				
Vendor #2, Technical Rep.				
Vendor #2, Sales/Service Rep.				

Table 1-4. Key Points of Contact[1] (pt. 2)

[Insert name(s) of individual(s)]	Company Name	Street Address	State/Province	Country
BIOLOGICAL TEST TEAM				
Principal Investigator [ ]				
Field(s) of Expertise				
Senior Scientist #1 [ ]				
Field(s) of Expertise:				
Senior Scientist #2 [ ]				
Field(s) of Expertise:				
Laboratories				
Lab. #1, and Role:				
Director [ ]				
STEP Project PI [ ]				
Lab. #2, and Role:				
Director [ ]				
STEP Project PI [ ]				
Lab. #3, and Role:				
Director [ ]				
STEP Project PI [ ]				

[1] Insert additional rows as necessary.

1.5 Proposed Schedule

Summarize planned activities supporting the installation, test, and operation of the proposed shipboard BWMS.

Complete Table 1-2 "Proposed STEP Project Schedule"

Proposed future dates for system shipboard installation (or date of completed installation, if applicable) and engineering tests both dockside and at sea. Include brief description of the tests to be run, and the results if already completed.

A rough schedule for all work related to the Year 1 shipboard biological experiments. Applicant should make sure to allow time for application review by Review Team and the Coast Guard's environmental review process (see Instructions, Figure 1) and should provide an approximate time frame only, e.g., month or season.

Table 1-5. Proposed STEP Project Schedules (pt. 1)[2]

	Treatment stage(s)	Date(s)	Comments
BWMS, Shipboard Installation and Engineering			***Brief description of test and results, location, organization(s) involved***
Shipboard System Installation			
Dockside Engineering Tests			
Test 1			
Test 2			
Test 3			
Engineering Tests at Sea			
Test 1			
Test 2			
Test 3			

[2] Insert additional rows as necessary.

Table 1-5. Proposed STEP Project Schedule (pt. 2)[3]

Year 1 Shipboard Biological Testing Work)		
	Date(s)	**Description of Work, Organization(s) Involved**
Ship System Modifications (e.g., sampling ports, temporary lab spaces, etc.)		
Biological Testing Plan (coordinated with ship)		
Test Equipment Acquired and Staged		
Test Equipment Onboard the Ship		
Test Equipment Trial Runs		
Biological Sampling #1		
Biological Sampling #2		
Biological Sampling #3		
Biological Sampling #4		
Land Based Laboratory Work		
Test Report to Coast Guard		

[3] Insert additional rows as necessary.

Section 2.0 Vessel and Existing Ballast Water System Specifications

The purpose of Section 2 is to provide information about the engineering and operation of the existing ballast water system on the ship proposed by the applicant for STEP.

2.1 Vessel and Crew Information

Provide essential vessel and service, data. **NOTE:** Table 2-1 requests information for up to two "typical" voyages only; applicant may add additional rows if needed.

Complete Table 2-1 "Vessel and Service Information"

Table 2-1. Vessel and Service Information

Vessel Data	
Name	
IMO# and/or CG-VIN	
Owner	
Operator	
Service Descriptions	
Typical Voyage #1	
Route and Port(s) Served	
Voyage Duration (min # of days)	
Voyage Frequency (# per year)	
Seasonality (if applicable)	
Typical Voyage #2 (if applicable)	
Route and Port(s) Served	
Voyage Duration (min # of days)	
Voyage Frequency (# per year)	
Seasonality (if applicable)	

2.2 Existing Ballast System

In Text Box 2.2a, provide a general description of the operation of the existing ship's ballasting system, including principles of operation, cross-connections with other systems (including purpose and frequency of operations, flushing of piping after use, and how system is segregated), and system monitoring and control at local and remote-control stations.

TEXT BOX 2.2a: Description of operation of ship's ballast system.

Complete Table 2-2.1 "Process/Equipment Design Criteria for Existing Ballast/Deballast System". Provide a brief description of the ballast water system, including piping arrangement, and design criteria on system components such as pumps, sea chests, strainers, and filters. Table 2-2.2 includes a cell for a general description of the system configuration and operation, standard operating procedures, backup arrangements, integration and cross-connections with other systems, and special features such as "mixing manifolds."

Complete Table 2-2.2 "Required Drawings, Ship's Ballast System". Provide a list of ship's drawings showing the existing ballast and associated systems, including components suggested below (submit F or D size drawings). F or D size drawings should be included in Appendix A.

- Ballast system diagrams
- Cross-connected systems' piping diagrams

NOTE: BWMS installation drawings required for Subsection 3.3 presenting adequate space arrangement information are acceptable and do not require duplication with as-built drawings.

Complete Table 2-2.3 "Ship Tank Table," listing all ballast tanks and capacities, including dedicated tanks and other tanks and compartments used as ballast tanks. In the column "Multiple Use," provide information on non-ballast use of the tank or compartment, e.g., for holding black or gray water, even if such use is only occasional.

Table 2-2.1. Process/Equipment Design Criteria for Existing Ballast/Deballast System (pt. 1)

General Description	[Describe configuration and operation, pumps used in SOP, backup arrangements, integration with other systems, use of "mixing manifolds," etc.]		
Cross-connections to Other Systems	**(Y/N)**	**Segregation: Valves & Controls**	**Procedural Description: Line-up of Pumps and Valves, Tanks Used**
Firefighting		[Describe valves and controls related to each cross-tie system]	[Describe line-up of pumps and valves; number of tanks used]
Gray Water		[Describe valves and controls related to each cross-tie system]	[Describe line-up of pumps and valves; number of tanks used]
Bilge		[Describe valves and controls related to each cross-tie system]	[Describe line-up of pumps and valves; number of tanks used]
Back-up General Service Pump		[Describe valves and controls related to each cross-tie system]	[Describe line-up of pumps and valves; number of tanks used]
Other		[Describe valves and controls related to each cross-tie system]	[Describe line-up of pumps and valves; number of tanks used]
Design Parameter	**Units**	**Criteria/Description**	**Comments (optional)**
Ballast Water System Design Flows			
Average Uptake Flow Rate	MT/hour		
Average Discharge Flow Rate	MT/hour		
Ballast Pumps			
Number of Pumps	No.		
Manufacturer and Model #	Name		
Design Flows	MT/hour		
Discharge Pressure	kg/cm^2		
Motor Size	kW		
Motor Type	CS or VFD		

Table 2-2.1. Process/Equipment Design Criteria for Existing Ballast/Deballast System (pt. 2)

Design Parameter	Units	Criteria/Description	Comments (optional)
Sea Chests			
Number	No.		
Size (diameter, or length by width)	meters		
Location(s) (frame and height above baseline)	meters		
Rack/Screen Opening Size Anti-fouling System (if present)	cm No.		
Screen/Filter/Strainer Units			
Number and Type	No.		
Manufacturer and Model	NA		
Capacity	MT/hour		
Maximum Retained Particle Size	mm		
Backwash Type	Manual or Automatic		
Backwash Flow Rate	MT/hour		
Ballast Tank Level Monitors			
Type	NA		
Typical Tank Arrangement	NA		
Ancillary Equipment			
Type	NA		
Number	No.		
Capacity	MT/hour		
Location(s) and Type(s) of Overboard Discharge Openings	NA		

Table 2-2.2. Required Drawings, Ship's Ballast System[4]

Title	Drawing Number	Prepared by (Shipyard or Design Firm)
Ballast System, Piping Diagram		
Cross-connected System(s), Piping Diagram(s)		
1		
2		
3		

[4] Insert additional rows as necessary.

Table 2-2.3. Ship Tank Table[5]

	Compartment Name and Number and Coating (if any)	Ballast Water Capacity (metric tons)	Compartment Type (check one, each row)					Multiple Use
			Dedicated Ballast Tank (or SBT)	Multi-use Tanks	Liquid Cargo Tank (for tank ships)	Dry Cargo Hold	Other	
1								
2								
3								
4								
5								
6								
7								
8								
9								
10								
11								
12								
13								
14								
15								
	Add rows as necessary							

[5] Insert additional rows as necessary. Data are required in all fields, complete as many rows as required for tanks used for ballast at any time.

2.3 Current Ballast/Deballast Practices

The applicant will:

Describe ship's current ballast water management practices, including standing ballast water system operating instructions and normal practices including deck and engineering department responsibilities.

Use Text Box 2.3a to describe for each "typical voyage" in Table 2-1, to the extent possible, uptake and discharge locations, volumes, and frequency. Include bodies of water, names of nearest points of land, distances from shore, depths of water, and type of water body (e.g., ocean, bay, brackish, tidal river, fresh).

TEXT BOX 2.3a: Ballast water management practices, and uptake and discharge locations, volumes, and frequency.

Using Text Box 2.3b to describe typical load/discharge sequence(s). Refer to Table 2-2.3 "Ship Tank Table."

TEXT BOX 2.3b: Typical load and/or discharge sequence(s).

Section 3.0 Ballast Water Management System Description

The applicant must provide a complete description of the ballast water management system (BWMS), including information on treatment processes, design criteria, treatment performance claims and limitations, physical configuration, electrical, instrumentation and control systems, operations and maintenance (O&M) requirements, and health and safety issues. This information should be documented in the text and figure boxes and standard tables provided in this section. The applicant must also identify specific drawings for the BWMS, and provide copies of the drawings in Appendix A of the application. Supplementary information such as product brochures or O&M manuals may be provided in Appendix A as well, but this information shall not replace the information to be provided in the body of the application.

3.1 Acceptability Criteria for Ballast Water Management Systems

The ballast water management system (BWMS) must be designed to meet basic engineering, operations and treatment performance criteria for acceptance into STEP. The vendor's treatment performance claims must be supported by documented results of previous laboratory, pilot-scale (e.g., barge or land-based) or full-scale shipboard testing, as detailed in Section 4. The applicant must also certify that the BWMS meets the following acceptance criteria for entry into STEP and providing any additional clarification, if necessary, in the text boxes that follow.

A. The BWMS is a prefabricated full-scale prototype or commercial-ready treatment system designed to remove, kill or inactivate (prior to discharge) the organism groups of interest in ballast water (zooplankton, phytoplankton, bacteria and viruses).

   Select one: [Yes] [No]

Use Text Box 3.1a to provide additional information.

TEXT BOX 3.1a: Additional clarification if needed.

B. The BWMS is capable of treating the entire ballast water discharge or stored volume for the ship applying for entry into STEP, and for the anticipated range of ballast water quality conditions on the ship's routes.

   Select one: [Yes] [No]

Use Text Box 3.1b to provide additional information.

TEXT BOX 3.1b: Additional clarification if needed.

C. The applicant has completed measurements of biological treatment efficacy for the BWMS in prior experimentation and has provided this information in Section 4.

   Select one: [Yes] [No]

Use Text Box 3.1c to provide additional information.

TEXT BOX 3.1c: Additional clarification if needed.

D. The applicant has completed measurements to determine ballast design flows and water quality constraints for the BWMS in prior experimentation and has provided this information in Section 4 or this section.

Select one: [Yes] [No]

Use Text Box 3.1d to provide additional information.

TEXT BOX 3.1d: Additional clarification if needed.

E. The applicant has specified treatment goals based on biological treatment efficacy results in prior experimentation (Section 4), and the goals specified in this section are consistent with the performance results presented in Section 4.

Select one: [Yes] [No]

Use Text Box 3.1e to provide additional information.

TEXT BOX 3.1e: Additional clarification if needed.

F. The applicant has completed measurements of disinfection residual, byproducts and toxicity for BWMS discharges in prior experimentation and has provided this information in Section 4 and this section.

Select one: [Yes] [No]

Use Text Box 3.1f to provide additional information.

TEXT BOX 3.1f: Additional clarification if needed.

G. The applicant has specified operational ranges and setpoints for the BWMS to achieve biological treatment performance goals based on prior experimentation. The operational specifications provided in this section should be consistent with those developed during prior experimentation.

Select one: [Yes] [No]

Use Text Box 3.1g to provide additional information.

TEXT BOX 3.1g: Additional clarification if needed.

3.2 Verification of Treatment Performance Claims

The applicant must establish clear and supportable relationships between biological efficacy testing results, treatment performance goals and critical design and operational parameters for the BWMS, based on prior experimentation results or Type Approval testing. Use Text Box 3.2a to provide a narrative and/or tabular summary that describes how biological efficacy testing results for different ballast water quality conditions (as detailed in Section 4), were used to establish: (1) treatment performance goals, (2) treatment process-train selection, (3) system design capacity, (4)

operational ranges and setpoints for each treatment unit, and (5) design of monitoring and control system.

TEXT BOX 3.2a: Summary of treatment performance claims and linkage to prior experimentation results.

3.3 Ballast Water Management System Description

In this section, the applicant provides a general description of the BWMS, the method of treatment for removal or inactivation of target organisms, potential range of shipboard or land based ballast water treatment applications, and limitations or constraints of the system for these applications.

3.3.1 General Description

Use Text Box 3.3.1a to provide a general description of the BWMS including treatment stages, physical configuration, materials of construction, principles of operation, and integration with the shipboard ballast system. Refer to the process flow diagram to be provided by the applicant under Section 3.1.5 to clarify the written description. Identify each treatment stage by number (i.e., Stage 1, Stage 2, etc.).

TEXT BOX 3.3.1a: General description of BWMS**.**

Use Text Box 3.3.1b to provide information about applications of the treatment technology in ballast water treatment and other applications.

TEXT BOX 3.3.1b: Other applications of treatment technology.

3.3.2 Method of Treatment

Describe the treatment mechanism and target organisms for each treatment stage of the BWMS. **The treatment method should generally be classified as inactivation, sterilization, physical removal or physical disruption**. The target organism group should be classified as zooplankton, phytoplankton, protozoa, bacteria and virus. Define other categories if the treatment mechanism or target organisms do not fall into any of these categories. Add text boxes for additional stages, if necessary.

TEXT BOX 3.3.2a: Method of treatment—stage 1.

TEXT BOX 3.3.2b: Method of treatment—stage 2.

TEXT BOX3.3.2c: Method of treatment—stage 3.

3.3.3 BWMS Shipboard Applications

Use Text Box 3.3.3a to discuss the range of shipboard or land-based applications for the BWMS, including sizes and types of ships for which it would be intended, uptake versus discharge treatment, standard treatment capacities, new or retrofit shipboard applications, etc.

TEXT BOX 3.3.3a: BWMS applications.

3.3.4 BWMS Limitations

Use Text Box 3.3.4a to discuss limitations and/or constraints of the BWMS for shipboard or land-based applications. These limitations may include capacity limits, raw water quality limits, effluent discharge limits, issues regarding chemical residuals, ballast tank corrosion impacts, re-piping requirements, waste stream disposal issues, special storage requirements for hazardous chemicals, etc.

TEXT BOX 3.3.4a: BWMS limitations.

3.3.5 Ballast Water Management Process Flow Diagram

Provide a process flow diagram of the proposed BWMS showing the main treatment processes, chemicals, and monitoring and control devices for the system (Figure 3-3). This figure should only describe the treatment processes for the BWMS; Subsection 3.3 includes all the required information for the onboard system configuration and ship's system interfaces. Provide the following information, properly labeled and located on the diagram:

- Treatment stages (show each stage as a box on the diagram)
- Treatment chemicals and application points
- Recycle streams
- Waste streams
- Monitoring and control devices for measuring pressure, temperature, flow, water quality, power, chemical residuals, etc.
- Utility connections (power, water, compressed air, etc.)

FIGURE 3-3: Ballast Water Management System Process Flow Diagram

3.3.6 Ballast Water Management System Photographs

Provide digital photographs (JPG image format; max 4 inches x 6 inches at a maximum resolution of 100 pixels per inch) of the proposed BWMS in the Photo Box 3-1. All photographs should be labeled, and should include a clear indication of size/scale. A maximum of two pages of photographs may be provided.

PHOTO BOX 3-1: Ballast water management system photographs.

3.4 Design Criteria

In this section, the applicant provides process and equipment design criteria for the BWMS. Standard descriptions, terminology and measurement units must be used so that design criteria can be directly assessed. A series of design criteria tables is provided to cover basic process design criteria for the overall BWMS and more detailed process and equipment design criteria for each treatment stage. In completing the tables, the applicant should identify any process design criteria that were developed from and directly relate to previous experimental testing results, as discussed in Section 4, by providing an explanation in the "Comments" column in each table.

As the tables were developed to cover a variety of treatment types, it is expected that the applicant will delete sections and tables for particular treatment types that are not pertinent to the BWMS being described.

3.4.1 Process Design Criteria

Complete Table 3-4 to define the process design criteria for the BWMS and its treatment stages. The table specifies standard measurement units and terminology. If these units or terms do not apply to the BWMS, the applicant may use different ones, but must provide an explanation.

Terms used in Table 3-4 are explained below:

- *Design Flows*—Indicate design flows for a single BWT module. If higher flows can be handled by manifolding treatment modules (i.e., running multiple modules in parallel), provide an explanation in the "Comments" column.
- *Ballast Water Point of Treatment*—The BWMS is typically designed to provide treatment on ballast water uptake, discharge, in-tank storage or at multiple locations. Identify one or more of these locations for the specific BWMS. If there is a preferred location, provide an explanation in the "Comments" column.
- *Treatment Stage Type*—Provide a short description of the type of treatment process being used for each stage of the BWMS using the standard terms listed in the table. Add any clarifications in the "Comments" column.
- *Treatment Mechanism*— Provide a short description of the treatment mechanism for each stage of the BWMS using the standard terms listed in the table. Add any clarifications in the "Comments" column.
- *Target Organism*—Provide a short description of the target organism(s) or class of organisms used for assessing treatment removal or inactivation, including dose-response, for each stage of the BWMS. Add any clarifications in the "Comments" column.

Table 3-4. Process Design Criteria for Ballast Water Management System[6]

Design Parameter	Units	Criteria/Description	Comments
Ballast Water Management System			
Design Flows			
- Maximum	MT/hr	[Required]	[Optional]
- Average	MT/hr	[Required]	[Optional]
- Minimum	MT/hr	[Required]	[Optional]
Number of Treatment Stages	No.	[Required]	[Optional]
Ballast Water Point of Treatment		[Select one or more of the following locations: in-line at ballast uptake, in-line at ballast discharge, in ballast tanks	[Optional]
Treatment Stage No.1			
Treatment Process		[Select one or more of the following treatment processes: physical separation, physical disruption, thermal inactivation, chemical, hydrocyclone, strainer, cartridge filter, membrane, chlorine, chlorine dioxide, hydrogen peroxide, ozone, UV, heat, pH adjustment, advanced oxidation process (AOP)]	[Optional]
Treatment Mechanism		[Select one or more of the following treatment mechanisms: inactivation, sterilization, removal, disruption, other]	[Optional]
Target Organism(s) or Organism Group(s)		[Select one or more of the following target organism groups: zooplankton, phytoplankton, protozoa, bacteria, virus, other]	[Optional]
Treatment Stage No.2 (if applicable)			
Treatment Process		[Select one or more of the following treatment processes, as above	[Optional]
Treatment Mechanism		[Select one or more of treatment mechanisms, as above	[Optional]
Target Organism(s) or Organism Group(s)		[Select one or more target organism groups, as above	[Optional]
Treatment Stage No.3 (if applicable)			
Treatment Process		[Select one or more of the following treatment processes, as above	[Optional]
Treatment Mechanism		[Select one or more of treatment mechanisms, as above	[Optional]
Target Organism(s) or Organism Group		[Select one or more target organism groups, as above	[Optional]

[6] Insert additional rows as necessary to describe subsequent treatment stages.

3.4.2 Treatment Stage Design Criteria: Physical Removal, Physical Disruption, Thermal Treatment

Complete Table 3-4.2 if the BWMS includes physical removal, physical disruption or thermal treatment as a treatment stage. The design criteria are grouped into eight categories: (1) General, (2) Design Flow, (3) Primary Treatment Unit, (4) Ancillary Treatment Equipment, (5) Treatment Stage Replacement Components, (6) Treated Ballast Water Quality (non-biological), (7) Waste Stream Flows and (8) Waste Stream Quality. If critical design parameters for the proposed treatment stage are missing from the table, the applicant should add additional rows, starting with the row marked "Other." Use the "Comments" column to provide any clarifying comments on particular design criteria values.

Terms used in Table 3-4.2 are explained below:

- *Treatment Stage Number*—use the treatment stage number (as assigned by the applicant, e.g., 1,2, etc.) from Table 3-4 for each treatment stage of the BWMS.
- *Manufacturer/Model Number*—provide the equipment supplier names and model numbers for each major equipment item comprising the treatment stage. If the item is a one-off prototype, indicate such.
- *Ballast Water Design Flows*—indicate design flows for the treatment stage.
- *Primary Treatment Unit*—defined as a treatment unit that provides some level of treatment for the BWMS. For example, a filter strainer is considered a primary treatment unit, but a power supply cabinet is not.
- *Ancillary Treatment Equipment*—defined as equipment or instrumentation that supports the functionality of the primary treatment unit. For example, a chemical feed system used to clean a membrane filter is considered ancillary equipment.
- *Treatment Stage Replacement Components*—defined as components that must be replaced at fixed time intervals during the service life of the BWMS.
- *Expected Service Life*—for treatment stage replacement components, the expected service life is the time interval between replacement events.
- *Treated Ballast Water Quality (non-biological)*—provide information on basic water quality parameters of treated ballast water when discharged from the vessel, for example, characterizing temperature, pH, total suspended solids (TSS), salinity, and dissolved oxygen. These should be presented as ranges to reflect the range of anticipated ballast raw water quality conditions.
- *Waste Stream Flow/Quality*—waste stream flows may occur continuously or intermittently during routine operation of the BWMS. Identify whether waste stream(s) are continuous or intermittent, and whether or not the wastes are generated as byproducts of the treatment process. Examples include backwash water for cleaning particulate filters, concentrate flows from membrane processes, and saturated filter media. The applicant must specify ranges for waste stream flows, durations, volumes and water quality to reflect the range of anticipated ballast raw water quality conditions.

Table 3-4.2. Treatment Stage Design Criteria for BWMS - Physical Removal/Disruption and Thermal Treatment (pt. 1)

Design Parameter	Units	Criteria/Description	Comments
General Information			
Treatment Stage Number	No.	[Use assigned Treatment Stage No. from Table 3-4]	[Optional]
Manufacturer	--	[Required]	[Optional]
Model Number	--	[Required]	[Optional]
Ballast Water Design Flows			
Maximum	MT/hr	[Required]	[Optional]
Average	MT/hr	[Required]	[Optional]
Minimum	MT/hr	[Required]	[Optional]
Primary Treatment Unit			
Type	--	[Describe type of treatment unit and disinfection mechanism]	[Optional]
Method of Removal/Disruption/Inactivation	--	[Describe physical mechanism for achieving disinfection target]	[Optional]
Minimum Particle Size Removed/Disrupted	micron	[Size of largest particle passing through treatment unit]	[Optional]
Number of Units per Treatment Stage	No.	[Required]	[Optional]
Capacity per Unit	MT/hr	[Required]	[Optional]
Materials of Construction	--	[List materials for treatment vessel, seals and wetted parts]	[Optional]
Overall Dimensions (length, width, height)	cm	[Required]	[Optional]
Dry Weight	kg	[Required]	[Optional]
Wet Weight	kg	[Required]	[Optional]
Inlet Pipe Size (diameter)	cm	[Required]	[Optional]
Outlet Pipe Size (diameter)	cm	[Required]	[Optional]
Power Requirements	kW	[Required]	[Optional]
Other	--	[Add additional design criteria if necessary (one criterion per row)]	[Optional]

Table 3-4.2. Treatment Stage Design Criteria for BWMS - Physical Removal/Disruption and Thermal Treatment (pt. 2)

Design Parameter	Units	Criteria/Description	Comments
Ancillary Treatment Equipment			
Type	--	[Describe ancillary equipment items and how they relate to primary treatment unit]	[Optional]
Number of Units per Treatment Stage	No.	[Required]	[Optional]
Capacity per Unit	MT/hr	[Required]	[Optional]
Materials of Construction	--	[List materials for main vessel, seals and wetted parts]	[Optional]
Overall Dimensions (length, width, height)	cm	[Required]	[Optional]
Dry Weight	kg	[Required]	[Optional]
Wet Weight	kg	[Required]	[Optional]
Inlet Pipe Size (diameter)	cm	[Required]	[Optional]
Outlet Pipe Size (diameter)	cm	[Required]	[Optional]
Power Requirements	kW	[Required]	[Optional]
Other	--	[Add additional design criteria if necessary (one criterion per row)]	[Optional]
Treatment Stage Replacement Components			
Type	--	[Describe major replacement components for this treatment stage]	[Optional]
Number of Components per Treatment Stage	No.	[Required]	[Optional]
Expected Service Life	months	[Calculate expected service life assuming 24 BWT operations per year and normal shipboard wear and tear on equipment]	[Optional]
Other	--	[Add additional design criteria to fully specify replacement components (one criterion per row)]	[Optional]

Table 3-4.2. Treatment Stage Design Criteria for BWMS - Physical Removal/Disruption and Thermal Treatment (pt. 3)

Design Parameter	Units	Criteria/Description	Comments
Quality of Treated Ballast Water at Discharge (non-biological)			
Change in Total Suspended Solids (TSS) Concentration (range)	mg/L	[Required]	[Optional]
Change in pH (range)	unit	[Required]	[Optional]
Maximum Water Temperature Rise	deg C	[Required]	[Optional]
Change in Salinity (range)	mg/L		
Change in Dissolved Oxygen (range)	mg/L		
Other Treatment Byproducts	--	[List other by-products generated by treatment stage]	[Optional]
Other	--	[Add additional treated water criteria if needed (one criterion per row)]	[Optional]
Waste Stream Flows			
Type	--	[Describe water/air waste streams generated by this treatment stage, whether continuous or intermittent]	[Optional]
Discharge Flow Range per BWT Event	L/min	[Specify waste stream flow ranges]	[Optional]
Discharge Duration Range per BWT Event	minutes	[Specify waste stream time duration ranges	[Optional]
Total Discharge Volume Range per BWT Event	liters	[Specify total discharge volume ranges for waste stream]	[Optional]
Waste Stream Water Quality			
TSS Concentration Range	mg/L	[Required]	[Optional]
pH Range	unit	[Required]	[Optional]
Maximum Water Temperature Change	deg C	[Required]	[Optional]
Other	--	[Add additional waste stream criteria if needed (one criterion per row)]	[Optional]

3.4.3 Treatment Stage Design Criteria: Chemical Systems

Complete Table 3-4.3 if the BWMS includes a treatment stage that adds or creates chemicals as part of the treatment process. Use separate tables for each treatment chemical. The design criteria are grouped into 11 categories: (1) General, (2) Design Flow, (3) Design Dose, (4) Chemical Properties, (5) Chemical Storage, (6) Chemical Feed Pump and Piping System, (7) Ancillary Treatment Equipment, (8) Treatment Stage Replacement Components, (9) Treated Ballast Water Quality (non-biological), (10) Waste Stream Flows and (11) Waste Stream Quality. If critical design parameters for the proposed treatment stage are missing from the table, the applicant should add additional rows, starting with the row marked "Other." Use the "Comments" column to provide any clarifying comments on particular design criteria values.

With regard to the categories and design parameters in Table 3-4.3, the following explanations are in addition to those discussed under Subsection 3.2.

- *Design Dose*—provide chemical design dose values used for sizing the chemical storage and delivery system (not for a particular ballast water treatment application).
- *Chemical Storage*—the chemical storage design criteria are based on a liquid chemical feed system. If a dry system is used, modify the design criteria accordingly.
- *Chemical Feed Pump and Piping System*—these design criteria assume that chemicals are pumped into the ballast water through a piping system. If a gravity-fed system is used, modify the design criteria accordingly.

Table 3-4.3. Treatment Stage Design Criteria for BWMS - Chemical Systems (pt. 1)

Design Parameter	Units	Criteria/Description	Comments
General			
Treatment Stage Number	No.	[Use assigned Treatment Stage No. from Table 3-4]	[Optional]
Manufacturer	--	[Required]	[Optional]
Model Number	--	[Required]	[Optional]
Ballast Water Design Flows			
Maximum	MT/hr	[Required]	[Optional]
Average	MT/hr	[Required]	[Optional]
Minimum	MT/hr	[Required]	[Optional]
Design Dose			
Maximum	mg/L	[Required]	[Optional]
Average	mg/L	[Required]	[Optional]
Minimum	mg/L	[Required]	[Optional]
Chemical Properties			
Product Name	--	[Specify commercial name of chemical product]	[Optional]
Formula	--	[Specify chemical name or formula]	[Optional]
Product Type	--	[Specify whether chemical is delivered "Dry" or "Wet"]	[Optional]
Active Chemical Ingredients (dry chemical)	% Active Ingredient	[Specify percentage of product containing active ingredient]	[Optional]
Solution Concentration (wet chemical)	% Active Ingredient	[Specify percentage of product containing active ingredient]	[Optional]

Table 3-4.3. Treatment Stage Design Criteria for BWMS - Chemical Systems (pt. 2)

Design Parameter	Units	Criteria/Description	Comments
Chemical Storage			
Chemical Product Name	--	[Required]	[Optional]
Type	--	[Describe tank orientation (vertical or horizontal), materials (FRP, HDPE, steel, etc.)]	[Optional]
Number of Tanks	No.	[Required]	[Optional]
Tank Dimensions (length, width, height)	cm	[Required]	[Optional]
Tank Access	--	[Required]	[Optional]
Storage Volume per Tank	liters	[Required]	[Optional]
Number of Treatment Cycles per Storage Volume	No.	[Calculate number of expected ballast water treatment cycles prior to a tank refill; list assumptions]	[Optional]
Other		[Provide separate chemical storage design criteria for each treatment chemical]	[Optional]
Chemical Feed Pump and Piping System			
Chemical Product Name	--	[Required]	[Optional]
Pump Type	--	[Pump type (e.g., centrifugal, end-suction, vertical turbine, horizontal split-case) and materials of construction for wetted parts]	[Optional]
Number of Duty Pumps	No.	[Required]	[Optional]
Number of Standby Pumps	No.	[Required]	[Optional]
Design Capacity	L/hr	[Required]	[Optional]
Pump Drive	--	[Specify constant-speed (CS) or variable frequency drive (VFD)]	[Optional]
Motor Size	hp	[Fill-in]	[Optional]
Piping System Type		[Describe piping system type and materials of construction]	
Other		[Provide separate chemical feed pump design criteria for each treatment chemical]	[Optional]

Table 3-4.3. Treatment Stage Design Criteria for BWMS - Chemical Systems (pt. 3)

Design Parameter	Units	Criteria/Description	Comments
Ancillary Treatment Equipment			
Type	--	[Describe ancillary equipment items and how they relate to chemical system]	[Optional]
Number of Units per Treatment Stage	No.	[Required]	[Optional]
Capacity per Unit	L/min	[Required]	[Optional]
Materials of Construction	--	[List materials for main vessel, seals and wetted parts]	[Optional]
Overall Dimensions (length, width, height)	cm	[Required]	[Optional]
Dry Weight	kg	[Required]	[Optional]
Wet Weight	kg	[Required]	[Optional]
Inlet Pipe Size (diameter)	cm	[Required]	[Optional]
Outlet Pipe Size (diameter)	cm	[Required]	[Optional]
Power Requirements	kW	[Required]	[Optional]
Other	--	[Add additional design criteria if necessary (one criterion per row)]	[Optional]
Treatment Stage Replacement Components			
Type	--	[Describe major replacement components for this treatment stage]	[Optional]
Number of Components per Treatment Stage	No.	[Required]	[Optional]
Expected Service Life	months	[Calculate expected service life assuming 24 ballast water treatment operations per year and normal shipboard wear and tear on equipment]	[Optional]
Other	--	[Add additional design criteria to fully specify replacement components (one criterion per row)]	[Optional]

Table 3-4.3. Treatment Stage Design Criteria for BWMS - Chemical Systems (pt. 4)

Design Parameter	Units	Criteria/Description	Comments
Quality of Treated Ballast Water at Discharge (non-biological)			
Total Suspended Solids (TSS) Concentration Range	mg/L	[Required]	[Optional]
pH Range	unit	[Required]	[Optional]
Maximum Water Temperature Rise	deg C	[Required]	[Optional]
Treatment Chemical Product Name	--	[Required]	[Optional]
Treatment Chemical Residual Concentration	mg/L	[List expected residual concentration range in treated water]	[Optional]
Treatment Byproduct Type	--	[List byproducts expected to be generated by treatment stage]	[Optional]
Byproduct Concentration Range	mg/L	[List expected DBP concentration range in treated water for each DBP]	[Optional]
Other		[Add additional criteria if needed (one criterion per row)]	[Optional]
Waste Stream Flows			
Type	--	[Describe water/air waste streams generated by this treatment stage, and whether continuous or intermittent]	[Optional]
Discharge Flow Range per BWT Event	L/min	[Specify waste stream flow ranges]	[Optional]
Discharge Duration Range per BWT Event	minutes	[Specify waste stream time duration ranges	[Optional]
Total Discharge Volume Range per BWT Event	Liters	[Specify total discharge volume ranges for waste stream]	[Optional]
Waste Stream Water Quality			
Total Suspended Solids (TSS) Concentration, Range	mg/L	[Required]	[Optional]
pH Range	unit	[Required]	[Optional]
Maximum Water Temperature Rise	deg C	[Required]	[Optional]
Other	--	[Add additional waste stream criteria if needed	[Optional]

3.4.4 Treatment Stage Design Criteria: Ultraviolet (UV) Disinfection

Complete Table 3-4.4 if the BWMS includes a treatment stage with UV -disinfection. The design criteria are grouped into seven categories: (1) General, (2) Design Flow, (3) BWT Water Quality Criteria, (4) UV Design Dose, (5) Ancillary Treatment Equipment, (6) Treatment Stage Replacement Components, and (7) Treated Ballast Water Quality (non-biological). The applicant must fill in the criteria/description cells for each design parameter and category using standard terminology and measurement units to the extent possible. If critical design parameters for the proposed treatment stage are missing from the table, the applicant should add additional rows, starting with the row marked "Other." Use the "Comments" column to provide any clarifying comments on particular design criteria values.

With regard to the categories and design parameters in Table 3-4.4, the following explanations are in addition to those discussed under Subsection 3.2.

- *Ballast Water Quality Criteria*—provide ballast water quality criteria used for designing the UV system.
- *UV Design Dose*—the UV design dose, expressed in mJ/cm^2, should either be a delivered dose based on biodosimetry testing to determine dose-response relationships for the target organism or an average dose based on calculations using a numerical model. Explain how the UV dose was determined under "Comments" column.

Table 3-4.4. Treatment Stage Design Criteria for BWMS - UV Disinfection (pt. 1)

Design Parameter	Units	Criteria/Description	Comments
General Information			
Treatment Stage Number	No.	[Use assigned Treatment Stage No. from Table 3-4]	[Optional]
Manufacturer	--	[Required]	[Optional]
Model Number	--	[Required]	[Optional]
Ballast Water Design Flows			
Maximum	MT/hr	[Required]	[Optional]
Average	MT/hr	[Required]	[Optional]
Minimum	MT/hr	[Required]	[Optional]
Ballast Water Quality Criteria			
Minimum UV Transmittance	%	[Required]	[Optional]
Maximum TSS	mg/L	[Required]	[Optional]
Maximum Turbidity	NTU	[Required]	[Optional]
Maximum Water Temperature	deg C	[Required]	[Optional]
Minimum Water Temperature	deg C	[Required]	[Optional]
UV Design Dose			
UV Design Dose	mJ/cm^2	[Fill-in]	[Optional]
Dose Basis	--	[Select "Delivered Dose," based on biodosimetry testing, "Calculated Dose" based on numerical modeling or Other]	[Optional]

Table 3-4.4. Treatment Stage Design Criteria for BWMS - UV Disinfection (pt. 2)

Design Parameter	Units	Criteria/Description	Comments
UV Reactor Criteria			
Type of Reactor	--	[Select "Closed-Vessel" or "Open-Channel"]	[Optional]
Lamp Orientation	--	[Select "Parallel to Flow" or "Perpendicular to Flow"]	[Optional]
Materials of Construction		[List materials for reactor vessel, seals and wetted parts]	[Optional]
Number of Reactor Units	No.	[Required]	[Optional]
Type of UV Lamps	--	[Select: medium-pressure (MP), low-pressure (LP), low-pressure high-output (LPHO), pulsed (P)]	[Optional]
Number of Lamp Rows per Unit	No.	[Required]	[Optional]
Number of Lamps per Row	No.	[Required]	[Optional]
Total Number of Lamps per Unit	No.	[Required]	[Optional]
Input Power per Lamp	kW	[Required]	[Optional]
Total Operating Electrical Load	kW	[Required]	[Optional]
Total Installed Electrical Load	kW	[Required]	[Optional]
Pressure Drop Through Unit at Design Flow	cm	[Required]	[Optional]
Overall Dimensions (length, width, height)	cm	[Required]	[Optional]
Dry Weight	kg	[Required]	[Optional]
Wet Weight	kg	[Required]	[Optional]
Inlet Pipe Size (diameter)	cm	[Required]	[Optional]
Outlet Pipe Size (diameter)	cm	[Required]	[Optional]
Other	--	[Add additional design criteria if necessary (one criterion per row)]	[Optional]

Table 3-4.4. Treatment Stage Design Criteria for BWMS - UV Disinfection (pt. 3)

Design Parameter	Units	Criteria/Description	Comments
Ancillary Treatment Equipment			
Type	--	[Describe ancillary equipment items and how they relate to UV reactor]	[Optional]
Number of Units per Treatment Stage	No.	[Required]	[Optional]
Capacity per Unit	L/min	[Required]	[Optional]
Materials of Construction	--	[List materials for main vessel, seals and wetted parts]	[Optional]
Overall Dimensions (length, width, height)	cm	[Required]	[Optional]
Dry Weight	kg	[Required]	[Optional]
Wet Weight	kg	[Required]	[Optional]
Inlet Pipe Size (diameter)	cm	[Required]	[Optional]
Outlet Pipe Size (diameter)	cm	[Required]	[Optional]
Power Requirements	kW	[Required]	[Optional]
Other	--	[Add additional design criteria if necessary (one criterion per row)	[Optional]
Treatment Stage Replacement Components			
Type	--	[Describe major replacement components for this treatment stage]	[Optional]
Number of Components per Treatment Stage	No.	[Required]	[Optional]
Expected Service Life	months	[Calculate expected service life assuming 24 ballast water treatment operations per year and normal shipboard wear and tear on equipment]	[Optional]
Other	--	[Add additional design criteria to fully specify replacement components (one criterion per row)]	[Optional]

Table 3-4.4. Treatment Stage Design Criteria for BWMS - UV Disinfection (pt. 4)

Design Parameter	Units	Criteria/Description	Comments
Quality of Treated Ballast Water at Discharge (non-biological)			
Total Suspended Solids (TSS) Concentration Range	mg/L	[Required]	[Optional]
pH Range	unit	[Required]	[Optional]
Maximum Water Temperature Rise	deg C	[Required]	[Optional]
Chemical Byproduct	--	[List expected byproducts to be generated by treatment stage]	[Optional]
DBP Concentration Range	mg/L	[List expected byproduct concentration range in treated water for each byproduct]	[Optional]
Other		[Add additional treated water criteria if needed (one criterion per row)]	[Optional]

3.4.5 Treatment Stage Design Criteria: Ozonation

Complete Table 3-4.5 if the BWMS includes a treatment stage with ozonation. The design criteria are grouped into 10 categories: (1) General, (2) Design Flow, (3) Ozone Design Dose, (4) Ozone Generation Equipment, (5) Ozone Contactor and Dissolution Method, (6) Ancillary Treatment Equipment, (7) Treatment Stage Replacement Components, (8) Treated Ballast Water Quality (non-biological), (9) Waste Stream Flows, and (10) Waste Stream Quality. The applicant must fill in the criteria/description cells for each design parameter and category using standard terminology and measurement units to the extent possible. If critical design parameters for the proposed treatment stage are missing from the table, the applicant should add additional rows, starting with the row marked "Other." Use the "Comments" column to provide any clarifying comments on particular design criteria values.

With regard to the categories and design parameters in Table 3-4.5, some of these are further explained in Subsection 3.2.

Table 3-4.5. Treatment Stage Design Criteria for BWMS – Ozone (pt. 1)

Design Parameter	Units	Criteria/Description	Comments
General Information			
Treatment Stage Number	No.	[Use assigned Treatment Stage No. from Table 3-4]	[Optional]
Manufacturer	--	[Required]	[Optional]
Model Number	--	[Required]	[Optional]
Ballast Water Design Flows			
Maximum	MT/hr	[Required]	[Optional]
Average	MT/hr	[Required]	[Optional]
Minimum	MT/hr	[Required]	[Optional]
Ozone Design Dose			
Maximum	mg/L	[Required]	[Optional]
Average	mg/L	[Required]	[Optional]
Minimum	mg/L	[Required]	[Optional]

Table 3-4.5. Treatment Stage Design Criteria for BWMS - Ozone (pt. 2)

Design Parameter	Units	Criteria/Description	Comments
Ozone Generation Equipment			
Type	--	[Describe vessel configuration (horizontal or vertical tubes), type of dielectric (tube or plate) and type of power supply (low-frequency, medium frequency, high frequency)]	[Optional]
Number of Generator Units	--	[Required]	[Optional]
Materials of Construction		[List materials for generator vessel, seals and wetted parts]	[Optional]
Rated Capacity per Unit	grams/hour	[Required]	[Optional]
Ozone-in-Oxygen Concentration	% weight	[Required]	[Optional]
Maximum Cooling Water Temperature	deg C	[Required]	[Optional]
Type of Cooling System	--	[Describe type of generator cooling system (air or water cooled) and main components of cooling system (fan-cooled, water closed-loop with air/water heat exchanger, water closed-loop with chiller, water once-through open-loop, etc.]	[Optional]
Total Operating Electrical Load	kW	[Required]	[Optional]
Total Installed Electrical Load	kW	[Required]	[Optional]
Overall Dimensions (length, width, height)	cm	[Required]	[Optional]
Weight	kg	[Required]	[Optional]
Other	--	[Add additional design criteria if necessary (one criterion per row)]	[Optional]

Table 3-4.5. Treatment Stage Design Criteria for BWMS - Ozone (pt. 3)

Design Parameter	Units	Criteria/Description	Comments
Ozone Contactor and Dissolution Method			
Type of Dissolution Method	--	[Describe type of ozone dissolution method, i.e., bubble diffusers, venturi injection, static mixers, etc.]	[Optional]
Type of Contactor		[Describe type of contactor vessel and whether ballast tankage is used to meet contact time requirements]	
Number of Contactor Trains	No.	[Required]	[Optional]
Materials of Construction	--	[List materials for contactor vessel]	[Optional]
Minimum Contact Time	minutes	[Required]	[Optional]
Overall Dimensions (length, width, height)	cm	[Required]	[Optional]
Weight	kg	[Required]	[Optional]
Other	--	[Add additional design criteria if necessary (one criterion per row)]	[Optional]
Ancillary Treatment Equipment			
Type	--	[Describe ancillary equipment items and how they relate to ozone generator]	[Optional]
No. of Units per Treatment Stage	No.	[Required]	[Optional]
Capacity per Unit	L/min	[Required]	[Optional]
Materials of Construction	--	[List materials for main vessel, seals and wetted parts]	[Optional]
Overall Dimensions (length, width, height)	cm	[Required]	[Optional]
Dry Weight	kg	[Required]	[Optional]
Wet Weight	kg	[Required]	[Optional]
Inlet Pipe Size (diameter)	cm	[Required]	[Optional]
Outlet Pipe Size (diameter)	cm	[Required]	[Optional]
Power Requirements	kW	[Required]	[Optional]
Other	--	[Add additional design criteria (one criterion per row) to fully specify ancillary equipment]	[Optional]

Table 3-4.5. Treatment Stage Design Criteria for BWMS - Ozone (pt. 4)

Design Parameter	Units	Criteria/Description	Comments
Treatment Stage Replacement Components			
Type	--	[Describe major replacement components for this treatment stage]	[Optional]
Number of Components per Treatment Stage	No.	[Required]	[Optional]
Expected Service Life	months	[Calculate expected service life assuming 24 ballast watertreatment operations per year and normal shipboard wear and tear on equipment]	[Optional]
Other	--	[Add additional design criteria to fully specify replacement components (one criterion per row)]	[Optional]
Treatment Stage Replacement Components			
Type	--	[Describe major replacement components for this treatment stage]	[Optional]
Number of Components per Treatment Stage	No.	[Required]	[Optional]
Expected Service Life	months	[Calculate expected service life assuming 24 ballast water treatment operations per year and normal shipboard wear and tear on equipment]	[Optional]
Other	--	[Add additional design criteria to fully specify replacement components (one criterion per row)]	[Optional]

Table 3-4.5. Treatment Stage Design Criteria for BWMS - Ozone (pt. 5)

Design Parameter	Units	Criteria/Description	Comments
Quality of Treated Ballast Water at Discharge (non-biological)			
Total Suspended Solids (TSS) Concentration Range	mg/L	[Required]	[Optional]
pH Range	unit	[Required]	[Optional]
Maximum Water Temperature Rise	deg C	[Required]	[Optional]
Disinfection Byproduct (DBP) Type	--	[List expected DBPs to be generated by treatment stage]	[Optional]
DBP Concentration Range	mg/L	[List expected DBP concentration range in treated water for each DBP]	[Optional]
Other		[Add additional treated water criteria if needed (one criterion per row)]	[Optional]
Waste Stream Flows			
Type	--	[Describe water/air waste streams to be generated by this treatment stage, and whether continuous or intermittent]	[Optional]
Discharge Flow Range per BWT Event	L/min	[Specify waste stream flow ranges]	[Optional]
Discharge Duration Range per BWT Event	minutes	[Specify waste stream time duration ranges	[Optional]
Total Discharge Volume Range per BWT Event	liters	[Specify total discharge volume ranges for waste stream]	[Optional]
Waste Stream Water Quality			
Total Suspended Solids (TSS) Concentration Range	mg/L	[Required]	[Optional]
pH Range	unit	[Required]	[Optional]
Maximum Water Temperature Rise	deg C	[Required]	[Optional]
Other	--	[Add additional waste stream criteria if needed (one criterion per row)]	[Optional]

3.4.6 Treatment Stage Design Criteria: Other Treatment Methods

Complete Table 3-4.6 if the BWMS includes a treatment stage that does not fit into the standard treatment processes covered in Tables 3-4.2 through 3-4.6. The design criteria are grouped into eight categories: (1) General, (2) Design Flow, (3) Primary Treatment Unit, (4) Ancillary Treatment Equipment, (5) Treatment Stage Replacement Components, (6) Treated Ballast Water Quality (non-biological), (7) Waste Stream Flows, and (8) Waste Stream Quality. The applicant must fill in the criteria/description cells for each design parameter and category using standard terminology and measurement units to the extent possible. If critical design parameters for each treatment stage are missing from the table, the applicant should add additional rows, starting with the row marked "Other." Use the "Comments" column to provide any clarifying comments on particular design criteria values.

With regard to the categories and design parameters in Table 3-4.6, some of these are further explained in Subsection 3.2.

Table 3-4.6. Treatment Stage Design Criteria for BWMS - Other Treatment (pt. 1)

Design Parameter	Units	Criteria/Description	Comments
General Information			
Treatment Stage Number	No.	[Use assigned Treatment Stage No. from Table 3-4]	[Optional]
Manufacturer	--	[Required]	[Optional]
Model Number	--	[Required]	[Optional]
Ballast Water Design Flows			
Maximum	MT/hr	[Required]	[Optional]
Average	MT/hr	[Required]	[Optional]
Minimum	MT/hr	[Required]	[Optional]
Primary Treatment Unit			
Type	--	[Describe type of treatment unit and treatment mechanism]	[Optional]
Method of Removal/Inactivation	--	[Describe physical mechanism for achieving treatment target]	[Optional]
Number of Units per Treatment Stage	No.	[Required]	[Optional]
Capacity per Unit	L/min	[Required]	[Optional]
Materials of Construction	--	[List materials for treatment vessel, seals and wetted parts]	[Optional]
Overall Dimensions (length, width, height)	cm	[Required]	[Optional]
Dry Weight	kg	[Required]	[Optional]
Wet Weight	kg	[Required]	[Optional]
Inlet Pipe Size (diameter)	cm	[Required]	[Optional]
Outlet Pipe Size (diameter)	cm	[Required]	[Optional]
Power Requirements	kW	[Required]	[Optional]
Other	--	[Add additional design criteria if necessary (one criterion per row)]	[Optional]

Table 3-4.6. Treatment Stage Design Criteria for BWMS - Other Treatment (pt. 2)

Design Parameter	Units	Criteria/Description	Comments
Ancillary Treatment Equipment			
Type	--	[Describe ancillary equipment items and how they relate to primary treatment unit]	[Optional]
Number of Units per Treatment Stage	No.	[Required]	[Optional]
Capacity per Unit	L/min	[Required]	[Optional]
Materials of Construction	--	[List materials for main vessel, seals and wetted parts]	[Optional]
Overall Dimensions (length, width, height)	cm	[Required]	[Optional]
Dry Weight	kg	[Required]	[Optional]
Wet Weight	kg	[Required]	[Optional]
Inlet Pipe Size (diameter)	cm	[Required]	[Optional]
Outlet Pipe Size (diameter)	cm	[Required]	[Optional]
Power Requirements	kW	[Required]	[Optional]
Other	--	[Add additional design criteria if necessary (one criterion per row)]	[Optional]
Treatment Stage Replacement Components			
Type	--	[Describe major replacement components for this treatment stage]	[Optional]
Number of Components per Treatment Stage	No.	[Required]	[Optional]
Expected Service Life	months	[Calculate expected service life assuming 24 ballast water treatment operations per year and normal shipboard wear and tear on equipment]	[Optional]
Other	--	[Add additional design criteria to fully specify replacement components (one criterion per row)]	[Optional]

Table 3-4.6. Treatment Stage Design Criteria for BWMS - Other Treatment (pt. 3)

Design Parameter	Units	Criteria/Description	Comments
Quality of Treated Ballast Water at Discharge (non-biological)			
Total Suspended Solids (TSS) Concentration Range	mg/L	[Required]	[Optional]
pH Range	unit	[Required]	[Optional]
Maximum Water Temperature Rise	deg C	[Required]	[Optional]
Disinfection Byproduct (DBP) Type	--	[List expected DBPs to be generated by treatment stage]	[Optional]
DBP Concentration Range	mg/L	[List expected DBP concentration range in treated water for each DBP]	[Optional]
Other		[Add additional treated water criteria if needed (one criterion per row)]	[Optional]
Waste Stream Flows			
Type	--	[Describe water/air waste streams to be generated by this treatment stage, and whether continuous or intermittent]	[Optional]
Discharge Flow Range per BWT Event	L/min	[Specify waste stream flow ranges]	[Optional]
Discharge Duration Range per BWT Event	min	[Specify waste stream time duration ranges	[Optional]
Total Discharge Volume Range per BWT Event	liters	[Specify total discharge volume ranges for waste stream]	[Optional]
Waste Stream Water Quality			
Total Suspended Solids (TSS) Concentration Range	mg/L	[Required]	[Optional]
pH Range	unit	[Required]	[Optional]
Maximum Water Temperature Rise	deg C	[Required]	[Optional]
Other	--	[Add additional waste stream criteria if needed (one criterion per row)]	[Optional]

3.5 Physical Configuration and Shipboard Installation

In this section, the applicant must prepare a list of drawings that completely describes the BWMS and its installation on the ship applying for entry into STEP. These shall include treatment system equipment drawings ("cut sheets") and shipboard installation drawings including: general arrangement; electrical, process and instrumentation; all connections with shipboard systems (including piping, air, power, interfaces with monitoring, control, and alarm systems), and foundations. If shipboard installation drawings are not available because the BWMS has not yet been installed on the ship, then the applicant should, at a minimum, provide schematic drawings showing all proposed equipment, piping and instrumentation systems for the proposed shipboard installation. In that case, drawings of the shipboard installation would need to be provided for review prior to a vessel inspection under STEP. The drawings provided with the STEP Application must be referenced in Table 3-5 and included in Appendix A.

3.5.1 General Instructions

- In Table 3-5, list the engineering drawings for the proposed BWMS provided in Appendix A, including titles and numbers of the drawings. Use the following guidelines in compiling the drawing set:

- Confirm that all vendor-supplied drawings have been coordinated with general arrangement and shipboard installation drawings for the BWMS.
- Confirm that all submitted drawings have unique titles, drawing numbers, revision numbers and dates, revisions indicated in the body of the drawing (circled or bubbled to highlight changes), page numbering (if a drawing consists of multiple sheets), and drawing date.
- Provide clean, legible copies in English at an appropriate scale so they can be read.

3.5.2 General Arrangement Drawings

- Provide ballast water treatment equipment general arrangement drawings for the overall BWMS and major equipment components, including individual treatment systems, power panels, pumps, and any other equipment provided by the vendor(s). At a minimum, the drawings should show the following information:

- Plan and section views of major equipment components showing all critical dimensions and minimum clearance requirements on scaled drawings.
- Physical arrangement of skid-mounted or containerized equipment and interconnection piping on scaled drawings.
- Labels on all equipment, piping, instruments, and appurtenances.
- Location and size of piping and utility connections (water, power, air).
- Location and size of power supply panels and enclosures.
- Location of monitoring and control equipment.

3.5.3 Shipboard Installation Drawings

- Provide shipboard installation drawings showing how the BWMS is or will be integrated with existing shipboard equipment. The drawings should clearly identify existing and new equipment and piping systems. At a minimum, the drawings should show the following information:

- Physical arrangement of existing onboard ballast system equipment on scaled drawings, including compartment names and numbers.
- Physical arrangement of new ballast water treatment equipment and integration with existing equipment on scaled drawings, including piping arrangement.
- Piping layouts for entire BWMS showing all connections with shipboard piping, fittings, instrumentation and appurtenances.

- Connections to any shipboard utilities, including ventilation and environmental control, fire/hazard suppression, compressed air, fresh water, salt water, and others.
- Ballast tanks, cargo holds and any other compartments affected by the proposed BWMS.

3.5.4 Electrical, Process and Instrumentation Drawings

- Provide electrical, instrumentation and control (EI&C) drawings for the BWMS showing the functional arrangement and location of the power distribution system, electrical main and local control panels (including programmable logic controllers), valves and field instrumentation. At a minimum, include the following drawings:

- Electrical one-line diagrams.
- Control system architecture block diagram.
- Process and instrumentation diagrams.

Use Text Box 3.5a to provide additional information on support system requirements.

TEXT BOX 3.5a: Support system requirements.

Table 3-5. BWMS and Installation Drawings (to be provided in Appendix A)

Title	Drawing Number and Revision	Date	Prepared by Vendor, Shipyard, Design Firm, etc.
BWMS Equipment Drawings			
Shipboard Installation Drawings			
Electrical, Instrumentation and Control Drawings			

3.6 Electrical, Instrumentation and Control Requirements

In this section, the applicant describes the electrical, instrumentation and control (EI&C) requirements of the BWMS, referencing the EI&C drawings in Appendix A.

3.6.1 General Description

Use Text Box 3.6.1a to describe overall power requirements and EI&C components of the BWMS and how they will be integrated with the existing shipboard ballast system, including:

- System connected load.
- Average power consumption.
- Main and local control panels.
- Power distribution system.
- Power quality equipment.
- Instrumentation and control system architecture.
- Process control description.

TEXT BOX 3.6.1a: EI&C general description.

3.6.2 Ballast Water Monitoring and Control System

Use Text Box 3.6.2a to describe the ballast water monitoring and control system, including control logic, operational setpoints and alarm settings for meeting treatment performance targets during routine operation of the BWMS. Reference the control system architecture drawing provided in Appendix A.

TEXT BOX 3.6.2a: Ballast water monitoring and control system.

3.7 Operations and Maintenance Requirements

In this section, the applicant provides information on O&M requirements for the BWMS. In discussing O&M requirements, the Applicant should assume an average of 24 shipboard ballast water treatment events per year (two per month), unless specific operations data are presented for the ship applying for entry into STEP. The applicant may include an O&M manual for the BWMS in Appendix A to support the summary descriptions to be provided below.

3.7.1 Operating Procedures

Use Text Box 3.7.1a to describe start-up, normal and emergency operating, and shutdown procedures for the BWMS. Discuss power, chemical, and any other "consumable" requirements. Identify all relevant standard operating procedures (SOPs) and provide copies in Appendix A.

TEXT BOX 3.7.1a: Operating procedures.

3.7.2 Maintenance Procedures and Spares

Use Text Box 3.7.2a to describe preventive and corrective maintenance requirements for the proposed BWMS, and requirements for spares carried onboard.

TEXT BOX 3.7.2a: Maintenance requirements.

3.7.3 Personnel Requirements

Use Text Box 3.7.3a to describe expected personnel requirements for the BWMS, including number and types of O&M personnel, labor burden (pro-rated man-hours per ballast water treatment event), and operator training or specialty certification requirements.

TEXT BOX 3.7.3a: Personnel requirements.

3.8 Crew Health and Safety

Use Text Box 3.8a to describe health and safety risks associated with the BWMS, including storage, handling and disposal of any hazardous wastes, and any health and safety certification/training requirements for ship operators. List material safety data sheets (MSDS) for any hazardous chemicals and provide in Appendix A. Provide documentation of the applicable updates to the relevant portions of the ship's crew health and safety plan per the Ships Safety Management System in Appendix A.

NOTE: Ship safety matters relating to the installation of the BWMS are subject to review by the ship's classification society as well as by flag Administration (Coast Guard in the U.S.), and are the responsibility of the applicant.

TEXT BOX 3.8a: Health and safety matters.

Section 4.0 Proof of Ballast Water Treatment Performance

The acceptance of a BWMS into STEP is based upon the ability of the applicant to show supported claims of treatment effectiveness relative to the removal/inactivation of the diversity of taxa present in ballast water. This requires the presentation of test data quantifying the effectiveness of either physical removal or mortality/inactivation of organisms produced by the proposed BWMS. Preventing biological invasions through ballast water discharge focuses on the number of viable organisms discharged per cubic meter of deballasted water. Therefore, biological testing, background data and proposed testing under STEP (Section 5), must address the number of viable organisms across a diversity of taxonomic groups. It is understood that data may not be available for all taxonomic groups at the time of the application; however, taxa specific information is needed for full evaluation of a BWMS. Prior experimental work must demonstrate a strong likelihood that the proposed BWMS can meet the NVIC treatment criterion, that is, "equal to or better than ballast water exchange (defined as 98 percent removal of organisms larger than 50 microns)." Alternatively, applicants may demonstrate the ability of the proposed BWT system to meet the IMO G8 (D2) treatment criterion (less than10 viable organisms per cubic meter for the larger than 50 microns size class [zooplankton]; less than10 viable organisms per milliliter for the 50 to 10 microns size class [phytoplankton, protists]) or propose other treatment performance criteria of their choosing.

The best approach is prior experimentation at smaller scales leading to the full-scale test. The first step is laboratory-scale work on the core treatment processes, resulting in development of optimized dose/response curves, minimum required treatment times, and confirmation of the appropriateness of the selected assays and other metrics. Next, pilot-scale testing (e.g., on a barge or at a land-based site) should address physical scale-up questions such as sufficient mixing, assessment of settling issues, operations and maintenance needs, system controls, and finalization of analytical procedures and sampling protocols for full-scale test programs.

Those steps typically precede full-scale installation and experimentation, which should confirm prior system engineering, operations testing, and biological effectiveness experiments. If either laboratory-scale or pilot-scale steps have not been executed prior to full-scale testing, the applicant should offer a reasoned, scientific rationale for proceeding to full-scale, that is, that the treatment has a high probability of performing effectively, that the test protocols are in-place, and the larger scale experiment(s) will provide meaningful data.

There may be latitude in the matter of small-scale tests for proven water treatment technology, with relevant laboratory results derived from others and the employment of standard experimental procedures at full scale. Such latitude, however, is not appropriate when either the treatment system or the proposed experimental methods for the onboard tests are novel or unproven. In such cases, the applicant must satisfy a higher burden of proof and must demonstrate the feasibility of new technology or experimental methods at small scale. In addition, the applicant must document that the analytical and test protocols are fully operational within the proposed Test Team.

As third-party shore-based test programs are coming on-line, STEP will accept (in most cases) the results of these facilities as evidence of treatment performance, as long as the report documents the methodology and QA/QC, as well as data and conclusions in a manner that allows transferability to shipboard evaluation. For example, a "positive" assessment by independent shore-based test facilities following the ETV ballast water testing protocols would generally be viewed as sufficient documentation of biological treatment performance for application to STEP. As a result, applicants, who have already undergone ETV or equivalent shore-based testing (e.g., type approval testing by other administrations) will not be required to fill-out most of Section 4 of this application (see text box below).

Section 4 requests literature, experimental data, and data available from other sources (technical reports, unpublished manuscripts) in order to gather the background information necessary to evaluate the status of the BWT system being proposed for inclusion into STEP. The subsections are arranged to organize

data on the technology (components and full system) from all sources, regardless of the water treatment application, and to partition these data sources by the three scales of testing typical of technology development: ***bench-scale, pilot-scale, full-scale*** (ship-board and land-based). Within each subsection there are summary tables to be filled out by the applicant. These tables are to reduce the amount of text required, to help reduce redundancy and to allow rapid evaluation of the background data supporting the applicant's BWMS. In the case of well-developed technologies or components, it is important that the applicant include only key literature needed to support claims made.

Complete Text Box 4.0a and proceed.

TEXT BOX 4.0a:

Has ETV Shore-based testing or another third-party shore-based test been conducted on this BWMS?
Select one: **[ ] Yes [ ] No**

If "**Yes**," append the complete test report by the facility and all other primary reports/articles that document the performance of the BWMS in Appendix B and proceed to Subsection 4.4. It is not necessary to fill out the tables in Subsections 4.1 through 4.3.

If this BWMS has not undergone shore-based testing using ETV protocols at a Coast Guard recognized land-based test facility, proceed below.

In Subsections 4.1, 4.2, and 4.3, the applicant provides demonstrative data and information from testing or use of equipment relevant to either the full BWMS or any of its components. Any reference or data source may be included, whether the tests were on ballast water or from other water treatment industries. Treatment performance tests conducted by the applicant's team (or others) will include experimental data exclusively from testing of the specific ballast water treatment components or the full BWMS. Altogether, this section will include sufficient technical literature and experimental data to provide proof that the applicant's BWMS can likely achieve the STEP shipboard treatment criterion. The sub-sections are arranged as follows:

- Subsection 4.1, bench-scale testing
- Subsection 4.2, pilot-scale testing
- Subsection 4.3, full-scale testing (ship-board and land-based)

Relevant experimental work presented should demonstrate that the applicant has:

- Completed measurements to determine ballast design flows and water quality constraints for the BWT system.
- Completed measurements of disinfection residuals, byproducts and toxicity for BWT system discharges.
- Specified operational ranges and setpoints for the BWT system to achieve biological treatment efficacy targets, based on tests of effectiveness over a range of [+/- 20 percent] of key parameter(s) such as biocidal concentration, UV dose, holding time, etc.

The applicant should bear in mind the following instructions for completion of Section 4:

1. Subsections 4-1, 4-2, 4-3 allow inclusion of technical literature regardless of water treatment application as long as it is relevant to the technology proposed for STEP and information from testing on the STEP specific BWMS or its specific components. **It is not expected that all levels of testing will always be available in the literature and in testing of the BWMS, only that sufficient**

information be available to indicate that the performance goals of the proposed BWMS will likely be met under STEP.

2. The application should include any relevant dose/response information, particularly by taxa.
3. Only pre-STEP experimentation for which data are available is relevant, not future experiments or experiments currently underway for which data are not available.
4. Only primary references should be included, that is, those that are the original source of the data rather than those which only reference the primary work or include later reproductions of figures and tables.
5. The application should include all reference identifiers as requested in the tables for each subsection.
6. Appendix B should include hard copies or, if available, electronic (e.g., .pdf) files of all references, annotated with the reference identifiers that the applicant identifies in Subsections 4-1 through 4-3.

4.1 Summary of Prior Experiments, Bench Scale

Bench-scale experiments are meant to establish the abilities of individual treatment components' core technologies to accomplish the desired operations (proof of concept experiments), and to determine the key operational variables (design experiments). The analytical methods, assays, and other metrics employed to measure performance of the core technologies should also be worked out at this stage. For example, bench-scale tests would be used to determine if centrifugal force will sufficiently remove microscopic organisms, the UV dose/inactivation curve for target species, or the chlorine demand and required residual for source waters.

At the benchscale, equipment is generally small in scale, and the experiments are often batch in nature. Data from larger prototype or full-scale equipment testing should be placed in Subsections 4-2 and 4-3, respectively.

If an applicant did not conduct small-scale or bench-scale testing, it is important that a clear rationale is presented offering the assurance that the treatment system has a reasonable chance of performing effectively, and that the larger scale experiment(s) will provide meaningful data. This may include citations of relevant bench-scale work in other applications of the treatment technology. Well-proven treatment technology and/or standard experimental procedures are clearly desirable aspects in such cases, as would be relevant laboratory results from elsewhere, if they exist.

This subsection summarizes bench-scale testing data relevant to either the full BWMS or any of its components as follows:

- From existing literature and other available data sources (technical reports, unpublished manuscripts). "Existing literature" refers to studies of the technology in general, rather than studies on the specific equipment seeking entry into STEP. Any reference or data source may be included, whether the tests were on ballast water or from other water treatment industries or whether tests were conducted by the applicant's team or others. The data and results should be summarized in Table 4-1a, with the applicant entering text in response to specific questions in the provided text boxes.
- Tests for the specific BWMS proposed for STEP or its components, in experiments specific to ballast water treatment. The data, including dose-response data if available, and results should be summarized in Table 4-1b for whole BWMS tests, and in Table 4-1c for its individual components (the applicant can add additional tables 4-1d, 4-1e, etc. for additional components, as needed).

NOTE: Place all relevant bench-scale test literature and technical reports cited in Appendix B.

Complete Text Box 4.1a and proceed.

TEXT BOX 4.1a: Does the application include technical literature on small scale testing for the proposed BWMS or its components? Select one: **[ ] Yes [ ] No**

If the answer is "**No**," proceed to Subsection 4.2.

If "**Yes**," then list information on studies of the general technology in Table 4.1a; if no such studies exist, select: **[ ] No General Studies.**

If the application includes studies specific to ballast water treatment for the proposed STEP system, complete Table 4.1b (for whole system) and Tables 4.1c, 4-1d, etc., as required for individual components.

If no such ballast water treatment studies exist, select: **[ ] No Specific Studies,** and proceed to Subsection 4.2.

Terms used in Table 4-1a are explained below:

- *BWT Unit Tested*— or component(s) tested column, requests specification of the single component or process or multi-component system or process tested, that relates to the BWT components or system in this application.
- *BWT Unit Specifications*—refers to the type of process tested. The request is for information on dose or concentration or filter mesh size, etc.
- *Testing Source Water*—refers to the type of water used in the tests (harbor, coastal, estuarine, lake, ocean, etc.). If possible, descriptors of the actual source water should be noted (e.g., specific location, depth, proximity to port facilities or sensitive environment), as well as any known specifics on water chemistry.
- *Location of Tests*—refers to the test facility or laboratory where the testing was conducted.
- *The "Taxa" Heading*—appears under each of the major taxonomic functional headings (i.e., zooplankton, phytoplankton). The concept is to pair the specific taxonomic level or group tested with the level of removal or inactivation (column to right). For example, a reference may have tested a component of the proposed treatment on zooplankton, only identified to the level of "copepods." The copepods would be entered paired with the appropriate removal or inactivation level. However, referenced work may address several species of phytoplankton separately. In such cases, each species would be individually listed, and each paired with its reported removal or inactivation rate.
- *Literature Reference*—refers to primary references, that is, those sources that include the original data, not reports or papers that merely refer to or reproduce earlier work. The table specifies that the applicant identify these references as alphabetically as "B-a", "B-b", "B-c", etc., indicating a series of bench-scale testing citations. These series are identified as "P" and "F" for pilot-scale and full-scale references, respectively.

Use Text Box 4.1b to provide a brief narrative description (one or two paragraphs each) of bench-scale testing references included in Table 4-1. The purpose is to indicate the applicability of the data to gauging the effectiveness of the proposed BWMS.

TEXT BOX 4.1b: Annotated bibliography**.** If no relevant studies exist, enter "None."

Terms used in the Tables 4-1b and 4-1c are explained below:

- *BWMS*—refers to a multi-component treatment unit, results for which belong in Table 4-1b.
- *Component*—a single treatment process either alone or as experimentally isolated as part of multi-component test. For example, UV is a component within a filtration and UV treatment system. Tests including the combination of filtration and UV would be system tests belonging in Table 4-1b.

Testing that samples the UV unit alone or takes samples just pre- and post- UV are "component tests," which belong in Table 4-1c or 4-1d.

- *Test Taxa*—the organisms tested. The test taxa are partitioned by functional groups (zooplankton, phytoplankton, bacteria, etc.). Under each functional heading, the applicant is to list the taxonomic identifier used in the study referenced. For example, under zooplankton, z1 may be "copepods" in one study or "*Calanus*" (genus) or *Calanus finmarchicus* (species) in another, or even "all zooplankton larger than a given size" in another. Under bacteria, the response may be "heterotrophic bacteria" (trophic designation) or *Vibrio cholera* (species related to IMO standards) or some other taxon of interest.
- *Viability Assay Method*—the method used to determine if an organism is dead or inactivated or unable to reproduce. For example, zooplankton may be assayed using movement or response to physical stimuli; bacteria may be assayed by most probably number (MPN) or plate count culture methods.
- *Test Platform*—the number of the "Bench Test Study#_" filled in by the applicant in the row below the Table Description, which indicates the laboratory where the bench testing was conducted.
- *Treated*—test water that has passed or will pass through the Treatment System (Table 4-4a) or individual treatment component (Table 4-4b and 4-4c).
- *Control*—water that is not treated by the technology, but which has been handled in parallel with the treated water, thus includes all experimental effects exclusive of treatment.
- *Holding*—refers to whether the viability samples were assayed without holding time or were held, for example to simulate holding in a ballast tank.
- *References*—primary references which use the same identifiers in the table and Appendix B.

Use Text Box 4.1c to provide a brief narrative describing the present status of dose and response relative to the proposed ballast water treatment process.

TEXT BOX 4.1c: Only data from bench-scale studies should be discussed. If no relevant studies exist, enter "None."

Use Text Box 4.1d to provide a brief summary (synthesis) of the key findings of all biological testing of removal and/or inactivation from bench scale testing of the ballast water treatment process (system, components, etc.).

TEXT BOX 4.1d: Synthesis should be linked to the references presented in Subsection 4-1.

Table 4-1a. Summary, Generic Results for the Subject Technology in Literature and Studies, Bench Scale

Related to the general technology, rather than studies on the specific equipment proposed for STEP. Summary of literature, reports and website information on the removal or inactivation efficiency of the components or combined systems. Add additional rows as needed.

Treatment Unit Tested (1)	Treatment Unit Specifications (2)	Testing Source Water (3)	Location of Test Lab (4)	Literature Reference (5)	Test Group (6)	Zooplankton (>50µm)		Phytoplankton/Protists (<50µm - >10µm)		Bacteria (<10µm)		Other (9)	
						Taxa (7)	EFF % (8)	Taxa (7)	EFF % (8)	Taxa (7)	EFF % (8)	Taxa (7)	EFF % (8)

Literature and References Cited:

B-a

B-b

B-c

B-d

NOTE: Adjust the column or row dimensions to fit information provided.

(1) Indicate component tested (UV treatment apparatus, filtration apparatus, etc.).

(2) Indicate the specifications of the Treatment Unit/Component dose, filter size, etc.

(3) Source where water used in testing was obtained (Bay water, harbor water, etc.); include salinity if available.

(4) Location of the test facility where experiments were conducted.

(5) Use letter (B-a to B-z) identifiers in this column and list references at bottom (Primary Scientific literature, reports, web sites, etc.). Primary sources should be given, defined as those where the experimental design, methods, analysis and data are presented. Unless absolutely necessary, do not list secondary sources, defined as reports where summaries from primary references are presented.

(6) Relates to who conducted the study. Enter "TT" if experiments conducted by vendor Test Team. Enter "O" for other if experiments were conducted by an external test team.

(7) The major taxa tested. In some studies, may be general categories such as "copepods," "polychaetes," etc., in other studies more specific taxa or even individual species may have been tested, such as Artemia or Fecal coliform bacteria, etc.

(8) The range of removal or inactivation rates (efficiency of system either as % removal/inactivation). If multiple tests or replicates were conducted give the range of removals, such as 80%-95% or 2-3 logs.

(9) Since the treatment unit may have been tested in other applications (wastewater, drinking water) the organism grouping may be very different than typical of ballast water, the table may be amended to capture these grouping or they may be placed in this "other" grouping in which case they need to be listed.

Table 4-1b. Summary of Existing Bench-Scale Test Data on the STEP BWMS (pt. 1)

List primary references that present the experimental design, methods and data, avoid listing multiple references that cite the same data set. As necessary, rows should be added under major taxa heading to provide a full list of the taxonomic breakdown. If tests were conducted by size class, rather than taxonomic grouping, the applicant should alter the heading (for example, zooplankton to >50 μm).

Indicate where the bench test studies were conducted											
Bench Test #1:			Location of Testing (source water):								
Citation/report											
Bench Test #2:			Location of Testing (source water):								
Citation/report											
Bench Test #3:			Location of Testing (source water):								
Citation/report											

Test Taxa (1)	Viability Assay Method (2)	Test Platform (3)	Initial # organisms in Test (4)		Test Duration (5)		% Removal/ Inactivation		# Experiments	# Reps/ Expt.	References
			Treated	Control	No Holding	Holding Time	No Holding	Holding Time			
Zooplankton Taxa (>50μm)											
z1											
z2											
z3											
z_n											
Phytoplankton/Protists (<50μm - >10μm)											
p1											
p2											
p3											
p_n											

Table 4-1b. Summary of Existing Bench-Scale Test Data on the STEP BWMS (pt. 2)

Test Taxa (1)	Viability Assay Method (2)	Test Platform (3)	Initial # organisms in Test (4)		Test Duration (5)		% Removal/ Inactivation		# Experiments	# Reps/ Expt.	References
			Treated	Control	No Holding	Holding Time	No Holding	Holding Time			
Bacteria (<10μm)											
pr1											
pr2											
pr3											
pr_n											
Other											
o1											
o2											
o3											
o_n											
(6) Component #1:											
(6) Component #2:											
(1) Under each major taxonomic grouping add rows to show the taxa tested, (copepods, diatoms, etc., or specific species).											
(2) Give the assay used to determine viability relative to each test taxa (motility for zooplankton, heterotrophic viable plate counts for bacteria, etc.)											
(3) Enter the number of the bench test study indicated above.											
(4) The number of individuals at the start of the viability test for each taxa (# bacteria per mL, # copepods per liter, etc.)											
(5) The number of hours or days that a sample is held after treatment before assay to determine treatment effectiveness											
(6) If the BWMS is comprised of more than one component (filtration+UV or filtration+biocide), and removal effectiveness on individual components has been determined, then fill in the component description and then fill in the appropriate data in Tables 4-4b, etc.											

Table 4-1c. Summary of Existing Bench-Scale Test Data for Individual STEP Ballast Water Treatment Components (pt. 1)

List primary references that present the experimental design, methods and data, and avoid listing multiple references that cite the same data set. As necessary, rows should be added under major taxa heading to provide a full list of the taxonomic breakdown. One copy of this table should be filled out for each component with the appropriate component # and description, that is, Component #1 = Table 4-1c, Component #2 = Table 4-1d, etc. As necessary, rows should be added under major taxa heading to provide a full list of the taxonomic breakdown. If tests were conducted by size class, rather than taxonomic grouping, the applicant should alter the heading (for example, zooplankton to >50 μm).

Indicate where the bench test studies were conducted											
Bench Test #1:					Location of Testing (source water):						
Citation/report											
Bench Test #2:					Location of Testing (source water):						
Citation/report											
Bench Test #3:					Location of Testing (source water):						
Citation/report											
Test Taxa (1)	Viability Assay Method (2)	Test Platform (3)	Initial # organisms in Test (4)		Test Duration (5)		% Removal/ Inactivation		# Experiments	# Reps/ Expt.	References
			Treated	Control	No Holding	Holding Time	No Holding	Holding Time			
Ballast Water Treatment Component #1:											
Zooplankton Taxa (>50μm)											
z1											
z2											
z3											
z_n											
Phytoplankton/Protists (<50μm - >10μm)											
p1											
p2											
p3											
p_n											

Table 4-1c. Summary of Existing Bench-Scale Test Data for Individual STEP BWT Components (pt. 2)

Test Taxa (1)	Viability Assay Method (2)	Test Platform (3)	Initial # organisms in Test (4)		Test Duration (5)		% Removal/ Inactivation		# Experiments	# Reps/ Expt.	References
			Treated	Control	No Holding	Holding Time	No Holding	Holding Time			
Bacteria (<10μm)											
b1											
b2											
b3											
b_n											
Other											
o1											
o2											
o3											
o_n											
(1) Under each major taxonomic grouping add rows to show the taxa tested, (copepods, diatoms, etc., or specific species).											
(2) Give the assay used to determine viability relative to each test taxa (motility for zooplankton, heterotrophic viable plate counts for bacteria, etc.)											
(3) Enter the number of the bench test study indicated above.											
(4) The number of individuals at the start of the viability test for each taxa (# bacteria per mL, # copepods per liter, etc.)											
(5) The number of hours or days that a sample is held after treatment before assay to determine treatment effectiveness											

Create additional tables (Table 4-1d, 4-1e, etc.) as needed.

4.2 Summary of Prior Experiments, Pilot Scale

Pilot-scale experiments are generally conducted on prototype or medium scale technology units or components and are usually performed with higher volumes and frequently under conditions of flow (rather than the batch tests typical of bench-scale tests). These experiments are generally conducted to resolve unforeseen issues encountered in the scale-up of processes, and the conversion of the technology from batch to semi-batch and continuous operations. System controls and materials of construction should be worked out at this stage. Although not necessarily full-scale, the equipment may be large enough to simulate the performance of full-size unit operations, for example, dockside or large tank size. These tests are sometimes associated with mesocosms.

While analytical methods should be consistent with bench-scale tests (assuming they were properly investigated and adapted during bench-scale testing), field sampling techniques (collection, compositing, storage and shipping) should be worked out at this stage.

Complete Text Box 4.2a and proceed.

TEXT BOX 4.2a: Does the application include technical literature on pilot scale testing for the proposed BWMS or its components? Select one: **[ ] Yes [ ] No**

If the answer is "**No**," proceed to Subsection 4.3.

If "**Yes**," then, list information on studies of the general technology in Table 4-2a; if no such studies exist, select: **[ ] No General Studies**

If the application includes pilot scale studies specific to treatment of ballast water associated with the proposed STEP system, complete Table 4-2b (for whole system) and Tables 4-2c, 4-2d, etc., as required for individual components.

If no such pilot scale ballast water treatment studies exist, select: **[ ] No Specific Studies,** and proceed to Subsection 4.3.

Use Text Box 4.2b to providea brief narrative description (one or two paragraphs each) of pilot-scale testing references included in Tables 4-2a, 4-2b, 4-2c, etc. The purpose is to indicate the applicability of the data to gauging the effectiveness of the proposed BWMS.

TEXT BOX 4.2b: Annotated bibliography**.** If no relevant studies exist, enter "None."

Use Text Box 4.2c to provide a brief narrative describing the present status of dose and response relative to the proposed ballast water treatment process.

TEXT BOX 4.2c: Only data from pilot-scale should be discussed. If no relevant studies exist, enter "None."

Use Text Box 4.2d to provide a brief summary (synthesis) of key findings of all biological testing of removal and/or inactivation from pilot scale testing of the ballast water treatment process (system, components, etc.).

TEXT BOX 4.2d: Synthesis should be linked to the references presented in Subsection 4-2.

Table 4-2a. Summary, Prior Laboratory Experiments, Literature and Studies, Pilot Scale

Related to the general technology, rather than studies on the specific equipment proposed for STEP. Summary of literature, reports and web site information on the removal or inactivation efficiency of the components or combined systems. Add additional rows as needed.

Treatment Unit Tested (1)	Treatment Unit Specifications (2)	Testing Source Water (3)	Location of Test Lab (4)	Literature Reference (5)	Test Group (6)	Zooplankton (>50µm)		Phytoplankton/Protist (<50µm - >10µm)		Bacteria (<10µm)		Other (9)	
						Taxa (7)	EFF % (8)	Taxa (7)	EFF % (8)	Taxa (7)	EFF % (8)	Taxa (7)	EFF % (8)

Literature and references cited:

P-a

P-b

P-c

P-d

NOTE: Adjust the Column / Row dimensions to fit information provided.

(1) Indicate component tested (UV treatment apparatus, filtration apparatus, etc.).

(2) Indicate the specifications of the Treatment Unit/Component dose, filter size, etc.

(3) Source where water used in testing was obtained (Bay water, harbor water, etc.); include salinity if available.

(4) Location of the test facility where experiments were conducted.

(5) Use letter (B-a to B-z) identifiers in this column and list references at bottom (Primary Scientific literature, reports, web sites, etc.). Primary sources should be given, defined as those where the experimental design, methods, analysis and data are presented. Unless absolutely necessary, do not list secondary sources, defined as reports where summaries from primary references are presented.

(6) Relates to who conducted the study. Enter "TT" if experiments conducted by vendor Test Team. Enter "O" for other if experiments were conducted by an external test team.

(7) The major taxa tested. In some studies, may be general categories such as "copepods," "polychaetes," etc., in other studies more specific taxa or even individual species may have been tested, such as Artemia or Fecal coliform bacteria, etc.

(8) The range of removal or inactivation rates (efficiency of system either as % removal/inactivation). If multiple tests or replicates were conducted give the range of removals, such as 80%-95% or 2-3 logs.

(9) Since the treatment unit may have been tested in other applications (wastewater, drinking water) the organism grouping may be very different than typical of ballast water, the table may be amended to capture these grouping or they may be placed in this "other" grouping in which case they need to be listed.

Table 4-2b. Summary of Existing Pilot-Scale Test Data on the STEP BWMS (pt. 1)

List primary references that present the experimental design, methods and data, avoid listing multiple references that cite the same data set. As necessary, rows should be added under major taxa heading to provide a full list of the taxonomic breakdown. If tests were conducted by size class, rather than taxonomic grouping, the applicant should alter the heading (for example, zooplankton to >50 μm).

Indicate where the bench test studies were conducted											
Pilot Test #1:					Location of Testing (source water):						
Citation/report											
Pilot Test #2:					Location of Testing (source water):						
Citation/report											
Pilot Test #3:					Location of Testing (source water):						
Citation/report											
Test Taxa (1)	Viability Assay Method (2)	Test Platform (3)	Initial # organisms in Test (4)		Test Duration (5)		% Removal/ Inactivation		# Experiments	# Reps/ Expt.	References
			Treated	Control	No Holding	Holding Time	No Holding	Holding Time			
Zooplankton Taxa (>50μm)											
z1											
z2											
z3											
z_n											
Phytoplankton/Protists (<50μm - >10μm)											
p1											
p2											
p3											
p_n											

Table 4-2b. Summary of Existing Pilot-Scale Test Data on the STEP BWMS (pt. 2)

Test Taxa (1)	Viability Assay Method (2)	Test Platform (3)	Initial # organisms in Test (4)		Test Duration (5)		% Removal/ Inactivation		# Experiments	# Reps/ Expt.	References
			Treated	Control	No Holding	Holding Time	No Holding	Holding Time			
Bacteria (<10μm)											
pr1											
pr2											
pr3											
pr_n											
Other											
o1											
o2											
o3											
o_n											
(6) Component #1:											
(6) Component #2:											
(1) Under each major taxonomic grouping add rows to show the taxa tested, (copepods, diatoms, etc., or specific species).											
(2) Give the assay used to determine viability relative to each test taxa (motility for zooplankton, heterotrophic viable plate counts for bacteria, etc.)											
(3) Enter the number of the bench test study indicated above.											
(4) The number of individuals at the start of the viability test for each taxa (# bacteria per mL, # copepods per liter, etc.)											
(5) The number of hours or days that a sample is held after treatment before assay to determine treatment effectiveness											
(6) If the BWMS is comprised of more than one component (e.g. filtration+UV or filtration+biocide), and removal effectiveness on individual components has been determined, then fill in the component description and then fill in the appropriate data in Tables 4-4b, etc.											

Table 4-2c. Summary of Existing Pilot-Scale Test Data for Individual STEP Ballast Water Management System Components (pt. 1)

List primary references that present the experimental design, methods and data, avoid listing multiple references that cite the same data set. As necessary, rows should be added under major taxa heading to provide a full list of the taxonomic breakdown. One copy of this table should be filled out for each component with the appropriate component # and description, that is, Component #1 = Table 4-2c, Component #2 = Table 4-2d, etc. As necessary, rows should be added under major taxa heading to provide a full list of the taxonomic breakdown. If tests were conducted by size class, rather than taxonomic grouping, the applicant should alter the heading (for example, zooplankton to >50 µm).

Indicate where the bench test studies were conducted											
Pilot Test #1:					Location of Testing (source water):						
Citation/report											
Pilot Test #2:					Location of Testing (source water):						
Citation/report											
Pilot Test #3:					Location of Testing (source water):						
Citation/report											

Test Taxa (1)	Viability Assay Method (2)	Test Platform (3)	Initial # organisms in Test (4)		Test Duration (5)		% Removal/ Inactivation		# Experiments	# Reps/ Expt.	References
			Treated	Control	No Holding	Holding Time	No Holding	Holding Time			
Ballast Water Treatment Component #1:											
Zooplankton Taxa (>50µm)											
z1											
z2											
z3											
z_n											
Phytoplankton/Protists (<50µm - >10µm)											
p1											
p2											
p3											
p_n											

Table 4-2c. Summary of Existing Pilot-Scale Test Data for Individual STEP Ballast Water Management System Components (pt. 2)

Test Taxa (1)	Viability Assay Method (2)	Test Platform (3)	Initial # organisms in Test (4)		Test Duration (5)		% Removal/ Inactivation		# Experiments	# Reps/ Expt.	References
			Treated	Control	No Holding	Holding Time	No Holding	Holding Time			
Bacteria (<10μm)											
b1											
b2											
b3											
b_n											
Other											
o1											
o2											
o3											
o_n											

(1) Under each major taxonomic grouping add rows to show the taxa tested, (copepods, diatoms, etc., or specific species).

(2) Give the assay used to determine viability relative to each test taxa (motility for zooplankton, heterotrophic viable plate counts for bacteria, etc.)

(3) Enter the number of the bench test study indicated above.

(4) The number of individuals at the start of the viability test for each taxa (# bacteria per mL, # copepods per liter, etc.)

(5) The number of hours or days that a sample is held after treatment before assay to determine treatment effectiveness

Create additional tables (Table 4-2d, 4-2e, etc.), as needed.

4.3 Summary of Prior Full-scale Experiments

In this section, the applicant presents information on the full-scale testing of treatment systems or components, in ballast water treatment or other water treatment applications (such as wastewater, water supply, aquaria). Tests referenced may include both shipboard and land-based sites. The concept is to provide data and references that support the biological effectiveness of the technology in tests at the scale of the shipboard application proposed for STEP.

While analytical methods should be consistent with bench-scale and pilot-scale tests (assuming they were properly investigated and adapted during these phases), the approaches to be used under STEP should be documented in this stage.

TEXT BOX 4.3a: Does the application include technical literature on full scale testing of the proposed BWMS or its components? Select one: **[ ] Yes [ ] No**

If the answer is "**No**," proceed to Subsection 4.4.

If "**Yes**," then, list information on studies of the general technology in Table 4.3a; if no such studies exist, select: **[ ] No General Studies**

If the application includes full-scale test studies specific to ballast water treatment proposed for STEP, complete Table 4.3b (for whole system) and Tables 4.3c, 4-3d, etc., as required for individual components.

If no such full scale ballast water treatment studies exist, select: **[ ] No Specific Studies,** and proceed to Subsection 4.4.

Use Text Box 4.3b to provide a brief narrative description (one or two paragraphs each) of full-scale testing references included in Tables 4-3a, 4-3b, 4-3c, etc. The purpose is to indicate the applicability of the data to gauging the effectiveness of the shipboard BWMS.

TEXT BOX 4.3b: Annotated bibliography**.** If no relevant studies exist, enter "None."

Use Text Box 4.3c to provide a brief narrative describing the present status of dose and response relative to the proposed ballast water treatment process.

TEXT BOX 4.3c: Only data from full-scale should be discussed. If no relevant studies exist, enter "None."

Use Text Box 4.3d to provide a brief summary (synthesis) of key findings of all biological testing of removal and/or inactivation from full scale testing of the ballast water treatment process (system, components, etc.).

TEXT BOX 4.3d: Synthesis should be linked to the references presented in Subsection 4-3.

Table 4-3a. Summary, Prior Experiments, Literature, and Studies, Full Scale

Related to the general technology, rather than studies on the specific equipment proposed for STEP. Summary of literature, reports and web site information on the removal/inactivation efficiency of the components or combined systems. Add additional rows as needed.

BWT Unit Tested (1)		Test Program		Literature Reference (5)	Test Group (6)	Zooplankton (>50µm)		Phytoplankton/Protists (<50µm - >10µm)		Bacteria (<10µm)		Other (9)	
Component or system	BWT Unit Spec (2)	Testing Source Water (3)	Location of Test Lab (4)			Taxa (7)	EFF % (8)	Taxa (7)	EFF % (8)	Taxa (7)	EFF % (8)	Taxa (7)	EFF % (8)
Shipboard full-scale studies													
Other types of full-scale studies													

Literature and references cited:

F-b

F-d

NOTE: Adjust the Column / Row dimensions to fit information provided.

(1) Indicate component tested (UV treatment apparatus, filtration apparatus, etc.).

(2) Indicate the specifications of the BWT Unit/Component dose, filter size, etc.

(3) Source where water used in testing was obtained (Bay water, harbor water, etc.), include salinity if available.

(4) Location of the test facility where experiments were conducted.

(5) Use letter (F-a to F-z) identifiers in this column and list references at bottom (Primary Scientific literature, reports, web sites, etc.). Primary sources should be given, defined as those where the experimental design, methods, analysis and data are presented. Unless absolutely necessary, do not list secondary sources, defined as reports where summaries from primary references are presented.

(6) Relates to who conducted the study. Enter "TT" if experiments conducted by vendor Test Team; Enter "O" for other if experiments were conducted by an external test team.

(7) The major taxa tested. In some studies, may be general categories such as "copepods," "polychaetes," etc., in other studies more specific taxa or even individual species may have been tested, such as Artemia or Fecal coliform bacteria, etc.

(8) The range of removal or inactivation rates (efficiency of system either as % removal/inactivation). If multiple tests or replicates were conducted give the range of removals, such as 80%-95% or 2-3 logs.

(9) Since the treatment unit may have been tested in other applications (wastewater, drinking water) the organism grouping may be very different than typical of ballast water, the table may be amended to capture these grouping or they may be placed in this "other" grouping in which case they need to be listed.

Table 4-3b. Summary of Existing Full-Scale Test Data for the STEP BWMS (pt. 1)

List primary references which present the experimental design, methods and data, avoid listing multiple references that cite the same data set. As necessary, rows should be added under major taxa heading to provide a full list of the taxonomic breakdown. If tests were conducted by size class, rather than taxonomic grouping, the applicant should alter the heading (for example, zooplankton to >50 µm).

Indicate where the bench test studies were conducted											
Full-scale Test #1:					Location of Testing (source water):						
Citation/report											
Full-scale Test #2:					Location of Testing (source water):						
Citation/report											
Full-scale Test #3:					Location of Testing (source water):						
Citation/report											

Test Taxa (1)	Viability Assay Method (2)	Test Platform (3)	Initial # organisms in Test (4)		Test Duration (5)		% Removal/ Inactivation		# Experiments	# Reps/ Expt.	References
			Treated	Control	No Holding	Holding Time	No Holding	Holding Time			
Zooplankton Taxa (>50µm)											
z1											
z2											
z3											
z_n											
Phytoplankton/Protists (<50µm - >10µm)											
p1											
p2											
p3											
p_n											

Table 4-3b. Summary of Existing Full-Scale Test Data for the STEP BWMS (pt. 2)

Test Taxa (1)	Viability Assay Method (2)	Test Platform (3)	Initial # organisms in Test (4)		Test Duration (5)		% Removal/ Inactivation		# Experiments	# Reps/ Expt.	References
			Treated	Control	No Holding	Holding Time	No Holding	Holding Time			
Bacteria (<10µm)											
pr1											
pr2											
pr3											
pr_n											
Other											
o1											
o2											
o3											
o_n											
(6) Component #1:											
(6) Component #2:											
(1) Under each major taxonomic grouping add rows to show the taxa tested, (copepods, diatoms, etc., or specific species).											
(2) Give the assay used to determine viability relative to each test taxa (motility for zooplankton, heterotrophic viable plate counts for bacteria, etc.)											
(3) Enter the number of the bench test study indicated above.											
(4) The number of individuals at the start of the viability test for each taxa (# bacteria per mL, # copepods per liter, etc.)											
(5) The number of hours or days that a sample is held after treatment before assay to determine treatment effectiveness											
(6) If the BWMS is comprised of more than one component (filtration+UV or filtration+biocide), and removal effectiveness on individual components has been determined, then fill in the component description and then fill in the appropriate data in Tables 4-4b, etc.											

Table 4-3c. Summary of Existing Bench-Scale Test Data for Individual STEP Ballast Water Management System Components (pt. 1)

Study I.D. should refer to the references in Table 4-3 above. One copy of this table should be filled out for each component with the appropriate component # and description. Component #1 = Table 4-3c and Component #2 = Table 4-3d, etc. As necessary, rows should be added under major taxa heading to provide a full list of the taxonomic breakdown. If tests were conducted by size class, rather than taxonomic grouping, the applicant should alter the heading (for example, zooplankton to >50 µm).

Indicate where the bench test studies were conducted											
Full-scale Test #1:					Location of Testing (source water):						
Citation/report											
Full-scale Test #2:					Location of Testing (source water):						
Citation/report											
Full-scale Test #3:					Location of Testing (source water):						
Citation/report											
Test Taxa (1)	Viability Assay Method (2)	Test Platform (3)	Initial # organisms in Test (4)		Test Duration (5)		% Removal/ Inactivation		# Experiments	# Reps/ Expt.	Refs
			Treated	Control	No Holding	Holding Time	No Holding	Holding Time			
BWT Component #1:											
Zooplankton Taxa (>50µm)											
z1											
z2											
z3											
z_n											
Phytoplankton/Protists (<50µm - >10µm)											
p1											
p2											
p3											
p_n											
Protozoa											
pr1											
pr2											
pr_n											

Table 4-3c. Summary of Existing Bench-Scale Test Data for Individual STEP BWMS Components (pt. 2)

Test Taxa (1)	Viability Assay Method (2)	Test Platform (3)	Initial # organisms in Test (4)		Test Duration (5)		% Removal/ Inactivation		# Experiments	# Reps/ Expt.	Referen
			Treated	Control	No Holding	Holding Time	No Holding	Holding Time			
Bacteria (<10μm)											
b1											
b2											
b3											
b_n											
Other											
o1											
o2											
o3											
o_n											

(1) Under each major taxonomic grouping add rows to show the taxa tested, (copepods, diatoms, etc., or specific species).

(2) Give the assay used to determine viability relative to each test taxa (motility for zooplankton, heterotrophic viable plate counts for bacteria, etc.)

(3) Enter the number of the bench test study indicated above.

(4) The number of individuals at the start of the viability test for each taxa (# bacteria per mL, # copepods per liter, etc.)

(5) The number of hours or days that a sample is held after treatment before assay to determine treatment effectiveness

Create additional tables (Table 4-3d, 4-3e, etc.), as needed.

4.4 Program Summary for Ballast Water Management System Experiments at All Scales

Provide a summary of the results of the experimental program at various scales and interpretation establishing the efficacy of the treatment approach (in terms of actual concentrations achieved and the percent difference between controls and treatments), the accuracy of the analytical techniques, appropriateness of the measured variables in predicting the system performance, and the efficacy of achieving similar results with a full-scale shipboard system.

Provide data and interpretation from the experimental program on chemical residuals in the treated water, both for disinfection chemicals and their byproducts. Discussion should include a description of the chemicals (and doses used) and byproducts, the time-course of decay/loss of said chemicals and byproducts, their projected concentration in ballast water upon discharge, and the conditions under which the results were derived (salinity, temperature, etc.), if available.

Include specifics of results at all scales tested, and ensure that references to residuals in Section 3 (Table 3-4.3) and Subsections 3.6.2 and 3.7.2, particularly in regard to BWMS performance targets, are consistent with appropriate interpretation of these results.

Use Text Box 4.4a to summarize the evidence that the proposed BWMS can achieve the selected treatment performance criteria in shipboard testing under STEP. Performance treatment criteria can be the NVIC treatment criterion (98 percent removal effectiveness for organisms $\geq$50 µm), the IMO Ballast Water Management Convention G8 criteria (<10 living organisms per cubic meter for $\geq$50 µm size class; <10 living organisms per mL for <50 µm - $\geq$10 µm class), or other criteria of the applicant's choosing.

TEXT BOX 4.4a:

(A) Summary of experimental results and interpretation.

(B) Summary of evidence that BWMS can satisfy the NVIC (or other) treatment criterion.

Section 5.0 STEP Study Plan

The purpose of this section is to provide detailed information on the experimental program to be implemented in testing the BWMS. The applicant will summarize test program information by filling out provided tables and text boxes relating to experimental design, sampling methods, assays to assess the killing, removal or inactivation of ballast water organisms, the taxa (taxonomic groups, species, etc.) to be assessed, number of experiments to be conducted, replication, etc. We understand that the testing may be designed to meet other purposes as well, but request that only information directly related to the shipboard test program that the applicant will conduct for STEP be included.

The STEP review process has always allowed for innovative approaches and techniques in proposed experimental designs and biological assays, as long as their appropriateness can be justified by data prior to entry into STEP. However, over the past several years, new methods have come on-line, primarily associated with the Environmental Protection Agency (EPA)/Coast Guard Environmental Technology Verification (ETV) program for land-based testing of BWMS. As a result, testing under STEP must use sampling and analytical approaches that have been validated for use in the ETV Test Protocol or which are shown by the applicant to be equivalent.[7] Information for this application is still required on sample volumes, concentration approaches, replicates, etc. for the shipboard experiments, but justification of methods is not required unless any of them have been modified.

All proposed non-ETV methods and assays need to be previously demonstrated and proven to the satisfaction of the STEP Review Team, with all methods and protocols clearly described and cross-referenced to examples of their use in experiments cited in Section 4. Specifically, new methods that are not yet in general use are acceptable as long as there has been sufficient development of said methods and documentation of the results (precision, accuracy, interferences, applications and limitations, etc.) to validate their use and allow their acceptance as part of a STEP Application. Methods not yet developed may be piloted in parallel with approved methods but such arrangements should be disclosed prior to use onboard an enrolled ship.

Within this Application Form, guidance is given on sampling volumes, degree of organism concentration (for biological assays), experimental design, and sampling port design. Test Plans compliant with this guidance need only to indicate the specific guidance will be followed. Non-compliant Test Plans may still be acceptable, but require documentation that the results will be at least as defensible as stipulated in the ETV protocol.

The Study Plan questions are in a sequence starting from general organization and proceeding to specific methods:

1. Subsection 5.1 requests information on the personnel participating in the experimental test program, the management of the test program, and technical personnel executing various components of the test program.
2. Subsection 5.2 requests information on treatment performance goals projected for the BWMS relative to the Coast Guard NVIC criterion, previous system testing, and any other performance criteria specified by the applicant.
3. Subsection 5.3 requests an overview of the test methodologies to be implemented in the test program, including ballast source waters, number of cruises and test runs per cruise, locations within the ballast system where samples are to be taken for analysis, taxa studied, methods used, level of replication, and holding times to be used in tests.

[7] The ETV Protocol (EPA) is set as the testing performance standard, "Generic Protocol for the Verification of Ballast Water Treatment Technologies, Version 4.2, 2010."
Website: http://standards.nsf.org/apps/group_public/document.php?document_id=7597

4. Subsection 5.4 focuses on the sample collection and assay methods implemented for biological (5.4.1) and physical or chemical parameters (5.4.2), the latter of which generally describe the properties of the water to be treated or properties that can directly influence effectiveness of the ballast water treatment process. Quality assurance and control information is presented here, including holding times, analytical replicates (QC) etc.
5. Subsection 5.5 requests information about the test locations, in particular characterizing the ballast source water and discharge locations for ballast water treatment testing, e.g., tropical, temperate, polar, fresh, marine, open-ocean, coastal, harbor and the approximate location and depth of water for the ballast water treatment experiments. It is understood that the precise locations may not be known at the time of the Application. Nevertheless, the general types of waters and typical vessel routes should be indicated.

5.1 Test Team Responsibilities

This section provides an overview of the structure and management of the personnel on the applicant's team conducting the biological experimental program, including the identity, title, and organization with which the individual is affiliated; and activity for which the individual is primarily responsible (e.g., oversight of test program, sample collection, sample handling and transport and assays conducted). For each taxonomic class of organism tested, indicate the responsibility of the individual assigned (sample collection, sample handling, assay, and custody).

As noted in Section 1, it is important that the tests be sufficiently staffed to ensure that the samples and analyses can be properly carried out within the sample holding times specified in the methods. For example, quantitative tests of analytical protocols have indicated that under some conditions, samples of organisms ≥50 µm in minimum dimension (nominally zooplankton) can be held for only 6 hours from time of collection to completion of analysis before organism numbers begin to decline due to holding. Given the critical importance of meeting analytical QA/QC during testing, the application review will examine the level of staffing relative to the demonstrated holding time for each biological and chemical assay to be conducted.

Use Text Box 5.1a to describe test staffing and sample holding times. Please confirm that the level of staffing is adequate to meet state sample holding times as described in Subsection 5.4.1.2.

TEXT BOX 5.1a: Test staffing and sample holding times.

Complete Table 5-1, "Test Team Responsibilities for Ballast Water Management System Testing under STEP."

Terms used in Table 5-1 are explained below:

- *Sample Assay*—provides, in the "Sample Assay" section, information on the assay(s) used by the indicated technician to assess each taxonomic group.
- *Oversight of Test Program*—identifies the person responsible for the oversight functions of management personnel. An entry of "C," "H," and/or "A," for a sample— Collection, Handling, Assay, respectively— is placed in each relevant taxa box for which the indicated oversight person has significant responsibility.

Use Text Box 5.1b to provide a narrative description of the responsibilities of the Biological Test Team Coordinator, particularly his or her role in management of all aspects of the experimental program and the role relative to that of the applicant's STEP project manager (refer to Subsection 1.3). Also, if needed, provide additional information on technical personnel responsibilities.

TEXT BOX 5.1b: Biological test team coordinator.

Additional information required on technical personnel responsibilities.

Table 5-1. Test Team Responsibilities for Ballast Water Management System Testing under STEP

Individual personnel may be responsible for multiple tasks. If person has not yet been identified, then TBA 1, TBA 2 should be entered under "Person." Adjust column widths as needed. Checks in boxes represent significant responsibility under that task category.

Person (1)	Title (2)	Organization (3)	Organisms ≥50 µm (4)(5)	Organisms <50 µm - ≥10 µm (4)(5)	Organisms <10 µm (4)(5)	Other (4)(5)
Oversight of Test Program (6)						
Sample Collection						
Sample Handling and Transport						
Sample Assay						

(1) Name of person responsible for conducting the task, the same person may appear in multiple task lines.

(2) Title of person, Technician, Professor, Principal Investigator, etc.

(3) Name of organization or institution where the person is affiliated

(4) Place a checkmark in the "Sample Collection" and "Sample Handling and Transport" sections under each taxon group that the indicated person has significant responsibility.

(5) In the "Sample Assay" boxes under each size fraction list the assay(s) used for each taxa.

(6) Person responsible for oversight of the sample collection, handling and assay: C-Collection, H for Handling, A for assay.

5.2 Treatment Performance Goals

Subsection 5.2 spells out the Coast Guard's program goal and allows applicants to declare a variety of performance goals. The applicant must clearly delineate the treatment goals for the shipboard BWMS being tested under STEP. Treatment goals that relate to the STEP treatment criteria in the Coast Guard NVIC, the applicant's treatment goals based upon previous testing (per Section 4), and additional standards of the applicant's choosing (e.g., Regulation D-2 of the International Conference on Ballast Water Management) should be indicated.

The applicant should confirm the specified operational ranges and setpoints for the BWMS necessary to achieve the biological treatment performance goals, based on prior experimentation results and in concert with engineering parameters provided in Section 3.

The applicant should note the following key points in regard to completing Table 5-2, "Treatment Performance Goals for Evaluation under STEP."

Treatment goals should be presented by size fractions and must be quantitative. The goals may, in addition, focus on individual taxonomic groups; however, this is not required.

Terms used in Table 5-2 are explained below:

- *Size Fractions*—refer to the partitioning of organisms by size, as specified in some regulatory standards (also referred to in Table 5-2 as "All Taxa Goal"). The organism groups (zooplankton, phytoplankton/protists, bacteria, etc.) which dominate each fraction are listed for reference. It is required to indicate both the removal/inactivation as a percentage (for the NVIC criterion) and the effectiveness as the average and maximum number of viable organisms in discharging ballast waters (for the IMO D-2 criteria). If the applicant has different treatment goals for specific taxa, this information can be added in the rows provided beneath each "All Taxa" row. "Other" refers to additional functional groups like cysts or specific species, *Vibrio cholera, or genera, Enterococci* (species related to IMO standards), or some other taxon of interest.
- *Percent Removal – Inactivation*—is the test team's goal for the ballast water management system's intended performance in either the removal (e.g., via filtration and the like) or reduction in viability of scored organisms, expressed in percent (as for the NVIC treatment criterion).
- *Concentration of Organisms Discharged*—is the test team's estimation of the number of viable organisms that will be discharged into the environment after passage through the treatment system (as for the IMO G8 treatment criteria). Units to be used for each taxonomic group should be as follows:
  - *Size Class, ≥50 µm, nominally Zooplankton*: Number of viable organisms per cubic meter (No./m^3)
  - *Size Class, <50 µm - ≥10 µm, nominally Phytoplankton (protists)*: Number of viable organisms per milliliter (No./mL)
  - *Size Class, <10 µm, nominally bacteria*: Number of viable organisms per milliliter (No./mL).
- *References*—are the "primary" references that support the predicted level of treatment effectiveness. The reference I.D. should be the same as used in Tables 4-1, 4-2 and 4-3

Table 5-2. Treatment Performance Goals for Evaluation Under STEP (1)

	Applicant STEP BWMS Treatment Goals Concentration of Viable Organisms Discharged or Percentage Removal – Inactivation			References (2)
	% Reduction	Average #	Maximum #	
Size Fraction: ≥50 μm (nominally zooplankton)				
All Taxa Goal				
Size Fraction: <50 μm to ≥10 μm (nominally phytoplankton and protists)				
All Taxa Goal				
Size Fraction: <10 μm (nominally bacteria)				
All Taxa Goal				
Other				
All Taxa Goal				
(1) Complete the % and # for each size fraction that the proposed BWMS is targeted to achieve during STEP testing. If specific taxa have been examined, additional rows can be added.				
(2) Include references where data is presented to support the selected "goals." References should be included in Appendix A				

Use Text Box 5.2a to discuss the derivation of numeric treatment goals in Table 5.2, with particular reference to experimental results in Section 4. Clearly show how the data supports the selected numeric treatment performance goals for each size fraction, and add any other organisms that the BWMS testing will examine (*Vibrio cholera*, *Enterococcus*, cysts, etc.).

TEXT BOX 5.2a: Derivation of the numeric treatment goals in Table 5-2.

5.3 Ballast Water Treatment Experimental Methods

Proper evaluation of the applicant's BWMS for entry into STEP requires the use of proper experimental design (sampling locations, replicates, etc.) and assays of removal and viability of organisms. Tables 5-3 and Reference 5-4a address these issues. The purpose of Table 5-3 is to specify the locations within the ballast streams (from raw source water to water discharged following full treatment) where samples will be collected, whether time-course experiments will be conducted, and initial information on replicates. Table 5-3 also correlates with information in Figure 5-3a and 5-3b, which are diagrams of sampling locations for BWMS typically studied.

Key Points to Address: The experimental design and methods must address several criteria:

- The focus of the shipboard testing is to determine the quantitative effectiveness of the applicant's BWMS on organism removal/inactivation within each of 3 size categories across the various taxa in the ballast water biotic assemblage. The system's ability to reduce the number of viable organisms that are discharged is the primary issue. Methods that do not quantitatively yield the number of viable organisms (chlorophyll, ATP, total organism counts, etc.) cannot be used as primary determinants, although they may be included as supporting information.
- The "challenge" or "source" water used for testing should be representative of harbor or coastal waters (see Reference 5-4a), unless the testing is being conducted on and for a specific type of vessel for which the general practice is to ballast at sea, rather than in harbors. Open ocean waters typically do not support sufficient levels of organisms, turbidity, organic matter, and other biological, chemical and physical attributes typical of coastal waters (upon which challenge water criteria are based) to conduct valid testing of ballast water treatment for general application.
- Because it may be difficult to meet minimum required ("challenge") organism densities in the shipboard testing environment, STEP now generally requires that ballasting locations, **for testing purposes**, be in inshore waters or other biologically rich waters. There are many data sources indicating where and in which seasons such waters may be found.

  Vessels where open sea ballasting is the norm are still strongly encouraged to conduct some testing in inshore waters. Further, while minimum organism densities for a valid test are not required for STEP, repeated organism challenge levels significantly below the target densities will drastically reduce the further usefulness of test results and will likely require additional testing if the BWMS is to be used on routes in which ballasting of nearshore or harbor waters is expected.

  In all STEP tests, challenge waters must be analyzed for both ≥50 μm and <50 μm to >10 μm size fractions. However, to simplify the testing program, these challenge water samples need only be collected and properly preserved and transported for counting by trained microscopists in land-based laboratories. The reported data by taxa (to the lowest reasonably lowest taxonomic grouping) will be used to assess the challenge water biological test conditions and to allow a reasonable estimate of organisms entrained in uplifted ballast water that will correspond to the post-treatment samples.

- Complete either Figure 5-3a or Figure 5-3b. These are schematic diagrams of two valid ballast water sampling methods and are visual companions to Table 5-3 and Reference 5-4b representing typical locations for the sampling of ballast systems. The applicant should check all relevant locations in the diagram where samples will be taken. Either Figure 5-3a or Figure 5-3b may be adapted for use by the applicant.
- When ballast tank holding time is part of the treatment process (as for biocides where contact times are specified), the experimental design must ensure that the provenance of the treated ballast water is known (i.e., uptake location where challenge water samples are collected) and that the water is isolated and held for the specified period prior to discharge and measurement. While "isolation" (holding) of the treated ballast water is generally accomplished by filling experimental ballast tanks, holding for a known period, then discharging (with sampling), this is not the only approach. While STEP formerly encouraged use of control ballast tanks for accurate assessment of the BWMS efficacy and validity of test results, as long as the challenge water that is treated and later sampled on discharge is adequately measured, a valid test can still be performed.
- The preferred approach is to track uplift water into pre-determined ballast tanks and sample again on discharge with the full suite of assays, including those for viability. A solid program can be established around sampling uplift (preserved challenge water samples) and discharge related to a single tank on each sampling occasion, as long as it is not the exact same ballast tank on each of the multiple (5 or more test runs, preferred) voyages where testing occurs. This approach will support the statistical evaluation of each individual test run, as well as allowing for a composite analysis of all runs, to increase statistical power (Miller *et al*. 2011). It should be noted that the applicant may wish to include more than 5 test runs to provide additional statistical power to determine if the system is meeting the applicant's stated target (for example, <10 viable org/m^3 for ≥50 μm fraction).

  Alternatively, if challenge waters can be collected and preserved over several days while additional tanks are filled, then all tracked tanks can be sampled in a single discharge event, thus capturing a range of ballast tank holding times and avoiding pseudo-replication issues. Nevertheless, in all tests, the minimum holding time should be used, as it becomes part of the "treatment evaluation process," i.e., if testing is only at X days holding then discharges at <X days holding will have undocumented treatment effectiveness.

Figure 5-3a. Schematic of Required BWMS Sampling

Place a check in boxes where samples will be taken on discharge. The required uplift sample is already checked, as all tests must sample and characterize the challenge water to be treated.

NOTE: Control tank(s) are not required for STEP testing.

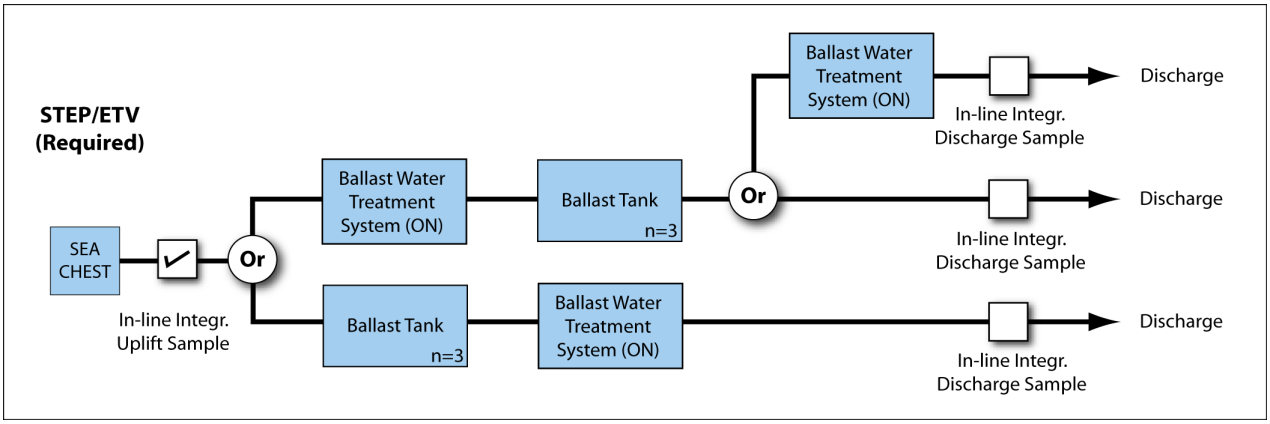
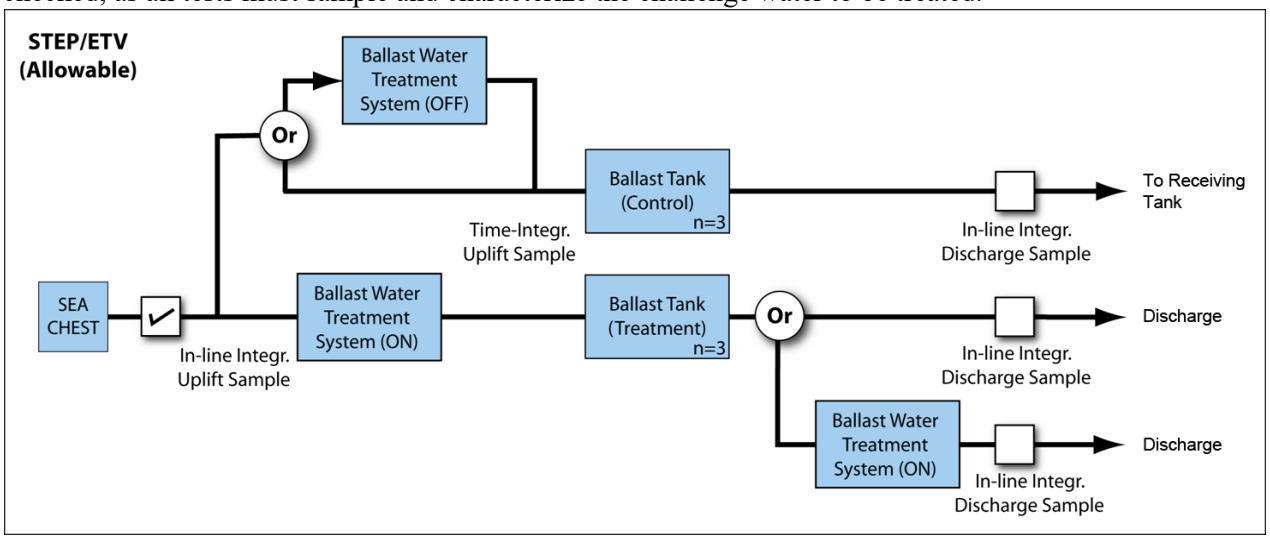


Figure 5-3b. Schematic of an Alternative BWMS Sampling Approach, with Control Tank(s)

Place a check in boxes where samples will be taken on discharge. The required uplift sample is already checked, as all tests must sample and characterize the challenge water to be treated.



Provide a descriptive summary of each type of "experiment" or "test run" to be undertaken in Text Box5.3a. The concept is that there may be experiments of different durations or where only select components are tested, alternated with experiments where only full system tests are undertaken.

TEXT BOX 5.3a: Types of experiments (test runs).

Describe the experimental design. Give particular attention to the location and timing of samples. If treatment includes holding ballast water in ballast tanks, indicate whether replicate ballast tanks will be used and whether there will be control and treated ballast tanks. Indicate how the study ensures that the water held in the tank(s) is the water that is sampled upon discharge.

The use of tank-integrated sampling[8] is required for the collection of valid ballast tank samples. Integrated tank sampling nullifies the effect of biological stratification in tanks, thereby minimizing the analytical effort necessary to meet the statistical requirements for enumerating viable biota.

Use Text Box 5.3b to identify the ballast tanks and/or compartments aboard the ship that are to be used for biological testing. Please use the same nomenclature as used in Table 2-2.3 ("Ship Tank Table").

TEXT BOX 5.3b: Identify ballast tanks used for testing.

Complete Table 5-3, "Ballast Water Treatment Experimental Methods" to provide sample numbers and replicates, challenge water type, etc. associated with the sampling schematic presented in the above figure.

Terms used in Table 5-3 are explained below:

- *Types of Source Water*—delineates the nature of the ballast water tested (e.g., marine harbor water, coastal marine or brackish water, fresh lake water, open ocean water, etc.).
- *Number of Experiments per Cruise*—the number of test runs planned for each cruise (i.e., multiple experiments per cruise).
- *Locations in BWT system where samples/assays to be collected*—is a list indicating how many samples are collected in the ballast system for the assay of the indicated size class (enter number of samples to be collected).
- *Post-BWT time to discharge*—indicates the minimum time ballast water will be held in the ballast tanks between the time of treatment and discharge. If treatment is on discharge, this value will be zero (0). If treatment is on uptake *and* discharge then the value will be entered as: time in ballast tank *and* 0. Because biological and chemical changes occur in ballast water held in ballast tanks, it is important to indicate the required holding time, since it becomes part of the treatment effectiveness. "Replicate tanks" is the number of replicate ballast tanks where water is placed and held prior to discharge measurements. The ballast tanks must be able to isolate the ballast water and hold it for the specified time to ensure that the discharge measurements can be compared to the challenge water measurements.
- *Replicate Assays per Sample*—provides information on the degree of analytical replication used for the assay of each sample taken.
- *Environmental Parameters*—are the physical or chemical variables to be measured at the indicated sampling points in the ballast system, which may have an influence on treatment effectiveness (e.g., TSS, temperature, salinity, pH and the like; also listed in Table 5.5b).

[8] Tank integrated sampling is achieved using a sample port in the ballast line that collects a sample continuously throughout the entire filling or discharge of a ballast tank. Each sample should be collected on a time-integrated basis such that a composite sample of the entire period of tank filling or discharge is acquired.

Table 5-3. Ballast Water Treatment Experimental Methods (pt. 1)

Column widths should be adjusted as necessary. Location of samples within the BWMS should be consistent with locations identified in Figure 5-3a or 5-3b.						
Organism Size Grouping		≥50µm	<50µm-≥10µm	<10µm	Environmental Parameters (7)	Other
Types of Source Water (1)						
# Test Cruises (2)					# Experiments per Cruise (3)	
Location in BWMS of samples/assays to be collected. Enter the number of samples that will be taken during a given experiment at each location for each size class. If there will be different numbers of samples used in different experiments then the box should include the samples for each experiment as (1/2/3) for experiments 1, 2, and 3, respectively.	Source Water from Environment Pre-BWMS					
	Treatment Ballast Tank Discharge					
	Control Ballast Tank Discharge					
	Other					
	Other					

Table 5-3. Ballast Water Treatment Experimental Methods (pt. 2)

Column widths should be adjusted as necessary. Location of samples within the BWMS should be consistent with locations identified in Figure 5-3a or 5.3b.						
Post-BWT Time to Discharge (4)						
# Replicate Tanks per Experiment (5)	Treated					
	Control					
# Replicate Assays per Sample (6)						

(1) Types of ballast water to be tested (harbor, coastal, lake, open ocean, etc.)

(2) Number of Vessel Cruises that testing will have "Full" BWTS Testing and Evaluation

(3) Number of "Test Runs" per Voyage, (e.g., if you are conducting multiple full test runs and ballast tank holding experiments on a single test voyage).

(4) Time that ballast water will be "held" from time of treatment until time of discharge (the functional treatment time).

(5) Number of replicate holding tanks where water is held post-treatment.

(6) Number of sub-samples assayed from a single sample bottle to determine analytical variation.

(7) List, in the appropriate sampling points, the names of environmental parameters to be measured (TSS, temperature, salinity, pH, etc.) and analytical methods to be used (see Reference 5-4e).

5.4 Sampling and Analyses

In this section, the applicant provides details of the specific methods to be used for the biological measurements related to treatment effectiveness and physical or chemical measurements related to general ballast water environmental conditions. The study plan will include methods for assays related to viability of each of the size fractions (nominal taxonomic groups), information on sample holding times and capability of staff to conduct assays and other work, chain of custody, calibration of instruments, preservation of samples, and archiving needs. This information is essential to the evaluation of the study plan and therefore is required for entry into STEP. The applicant should follow the table formats for the presentation of as much detailed information as possible.

The application must include a Quality Assurance Project Plan (QAPP) specifically identifying quality assurance issues related to the chosen viability assays, and indicating the quality assurance and quality control (QA/QC) procedures to be used in sample collection, handling, analysis, and data reduction/synthesis. In particular, the QAPP must indicate how the available staff conducting the testing will be sufficient, within the appropriate sample holding time limits, to complete the sampling plan within the QA/QC criteria proposed. Where EPA/Coast Guard ETV protocols are to be used, the approaches, handling, holding times, etc. should be consistent with the ETV[1] (c.f. ETV Appendix A).

Complete Text Box 5.4a and proceed.

TEXT BOX 5.4a: Has a Quality Assurance Project Plan (QAPP) for the STEP testing of the BWT system been developed? Select one: **[ ] Yes [ ] No**

If "**Yes**," append the QAPP to the Application. Is the QAPP appended? Select one: **[ ] Yes [ ] No**

If "**No**," indicate how the QA/QC procedures that are part of a QAPP will be specified.

5.4.1 Biological Analyses

The biological analytical test program is composed of, at a minimum, viability determinations of each of the 3 size fractions (Subsection 5.4.1.1), use of sampling volumes, replicates and holding conditions required to provide the necessary validity, accuracy and precision of the results (Subsection 5.4.1.2), and supporting environmental assays (subsection 5.4.2). STEP requires use of the viability protocols associated with the EPA/Coast Guard ETV (ETV) Program for land-based testing of BWT systems. As a result, testing under STEP must use sampling and analytical approaches that have been validated for use in ETV land-based testing or which are shown to be equivalent.[9] All non-ETV methods and assays need to have been previously documented (SOPs available) and referenced, with evidence that each assay is equivalent to the analogous recommended method.

Throughout this subsection, guidance and recommendations are presented to facilitate the design of the sampling analysis plan by the applicant. Reference 5-4d presents a compilation of information and test conditions necessary for a valid STEP test. This includes the target organism densities in uplift challenge waters, the allowable lower density of organisms in control waters upon discharge, and guidance on screen sizes needed to concentrate organisms from ballast water for analysis.

Reference 5-4d also specifies the minimum sample volumes of ballast water required for each organism size fraction in order to detect low numbers of organisms after treatment. *Sample volume collected is crucial for generating sufficient statistical confidence required for proper analysis.* The test program

[9] EPA "Generic Protocol for the Verification of Ballast Water Treatment Technology, Version 5.1, 2010."

may choose to collect larger sample volumes than the minimum required in order to increase the ability to detect statistically significant differences from a selected target number of organisms (e.g. 10 org/m^3, ≥50 μm fraction).

To accurately quantify small numbers of organisms in large volumes (e.g., 10 organisms/m^3), it is necessary to either a) collect and concentrate moderately large ballast water volumes and count the entire sample or b) collect and concentrate very large volumes (10-60 m^3), and count a specified fraction of the concentrate. The balance between these approaches depends in part on the ballast water being concentrated, e.g. amount of inorganic sediment, organic debris and detritus etc. entrained. Both approaches can produce defensible results. The approach selected depends upon the time of processing the necessary volumes within the QAPP specifications, so that processing occurs without sample degradation. It should be noted that, there is flexibility in approach, as long as the selected approach yields an ETV equivalent result. However, the burden of proof falls upon the applicant to demonstrate that an approach different from that specified in the ETV protocol yields equivalent results. It should be noted that the ETV guidance was developed based upon real-world conditions and likely encompasses the needs of most test programs.

5.4.1.1 Viability Assays

One of the most important and difficult measurements required for the determination of the effectiveness of a BWMS is the number of viable organisms in a unit volume of sample. While measurements of the total number of organisms present in the sample (direct counts) or related bulk parameters (e.g., chlorophyll, ATP, etc.) are relatively straightforward, such measurements do not directly assess the numbers of biota that are "viable", the parameter required for determining efficacy and demonstrating compliance with a discharge standard. The matter is made more difficult when the large number of potential taxa within a single size fraction is considered. The study plan may include assays for organism groups specified by the applicant (e.g., by taxa), but will generally be expected to include assays for each of the size fractions now identified in ETV and the IMO D-2 standard. Guidance for size-fractionated assays follows:

- **Size Class ≥50 μm (zooplankton)** New approaches are available for organisms ≥50 μm, allowing determination of viable organisms across multiple taxa in a single sample. These methods are recommended for use for testing under STEP and are detailed in the paragraphs below. While assays of organisms ≥50 μm continue to rely on movement or internal muscle action (e.g., heartbeat, tentacle retraction, etc.), automated techniques may be available soon to reduce the current reliance on human microscopic examination and elicitation of movement by manual probe (although this remains an acceptable method). If an organism shows no movement when observed for 10 seconds or does not move when physically stimulated, it is scored as "dead."

  In samples that have large numbers of viable organisms present (e.g., when quantifying viable counts in uplift or control waters), one approach for manual viability evaluation is to enumerate and classify only the "dead" organisms within a given well. When the multi-well plate is fully analyzed a chemical preservative is introduced into each well to render "all" the organisms dead. Each well is again enumerated and classified, yielding the "total organism count" and taxonomic distribution. "Viable count" is obtained by difference. When treated water is analyzed, however, the majority of organisms present are likely to be "dead" (e.g., 0-5 living organisms per well). In this case, detection of rare living organisms is the fastest and more accurate approach, avoiding the propagated error of subtracting two rather large numbers to obtain a small number.

  The Test Team is required to identify and record any viable organisms observed in the treated samples within the ≥50 μm size fraction to lowest reasonable taxonomic grouping (and the <50 μm - ≥10 μm fraction, as possible). For uplift or control samples, a record of numbers by taxa is also

required. In most cases uplift ("challenge") water will be collected and preserved for land-based assay, although viability counts of uplift water are also acceptable.

It should be noted that it is essential that the concentrated sample is homogeneous as it is being sub-sampled for counting, so that each aliquot is representative. If this is not achievable, then the approach is invalid. The volumes of required samples and counting volumes are presented in Subsection 5.4.1.2.

- **Size Class <50 µm and ≥10 µm (nominally protists, including phytoplankton and protozoa)** The ETV Protocol for land-based test facilities has determined vital staining to be the best approach for assessing viability of organisms in the <50 µm - ≥10 µm size fraction. The most useful vital stains are those that stain only live organisms across a wide variety of taxa, have sufficient post-staining stability, and work in a variety of matrices. Although there are no "perfect" vital stains and methods for the enumeration and viability analyses of protists remains an active area of investigation, the approach currently shows the most promise for quantifying viable organisms in ballast water. The present ETV Protocol indicates that the:

  "recommended protocol is to use a combination of two vital stains: Fluorescein Diacetate (FDA, Molecular Probes-Invitrogen Carlsbad, CA) and 5-chloromethylfluorescein diacetate (CMFDA, CellTracker™ Green; Molecular Probes-Invitrogen Carlsbad, CA). When non-specific esterases in living cells cleave the stains, the resultant molecules fluoresce green when excited with a blue light (e.g., Selvin et al., 1988[10])."

  Scoring of samples is by "manual epifluorescence microscopy: FDA (final concentration 5 µM) and CMFDA (final concentration 2.5 µM) are added to a 1 mL sample that is incubated in the dark for 10 min, the sample is loaded into a Sedgewick Rafter Counting Chamber, and it is examined under epifluorescence using a Fluorescein Isothiocyanate (FITC) narrow pass filter cube (e.g., excitation 465-495 nm, dichronic mirror wavelength 505 nm, barrier filter 515-555 nm; Steinberg et al., 2011a. Samples should be examined for a maximum of 20 min because the signal fades as stain leaks from the cell. If a cell is labeled by either FDA or CMFDA (as exhibited by a characteristic fluorescent green color) or moves or both, it is scored as viable. It is strongly encouraged to take a photomicrograph of any such cells under fluorescent and brightfield (white light) illumination to create a visual record of viable cells."

  Before this approach is used (the dual-staining method or any other alternative method) preparation, examination, and analysis of challenge water organisms that are killed (i.e., negative controls)[11] must be undertaken. From the perspective of environmental protection, this Type I error (false positives) is conservative, as it overestimates the number of viable organisms. In contrast, Type II errors (false negatives) underestimate the number of viable organisms. Nothwithstanding, in tests on waters at four locations across the U.S., including fresh, brackish, and marine conditions, the Type II error rate was uniformly low across all study sites: 0 percent in three locations and 1 percent at the remaining two locations (*Steinberg et al., 2011a*). Nonetheless, the Type II error rate should also be determined during initial site validation of this method or in alternative method validation, on a seasonal basis, and as part of the on-going QA program.[12]

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[10] Selvin, R., B. Reguera, I. Bravo, and C.M. Yentsch. 1988. Use of Fluorescein Diacetate (FDA) as a single-cell probe of metabolic activity in dinoflagellates cultures. Biol. Oceanogr. 6: 505-511.

[11] Heat-killed, negative control samples: raising sample temperature to 50 ºC; for 15 min Steinberg *et al*., (2011b), cooling to room temperature before staining. Heat killed organisms should not show a green, fluorescent signal; those that do fluoresce represent false positives and indicate the Type I error associated with the dual-stain method.

[12] One approach is to collect ambient protists and place them in one of four categories based on an organism's fluorescence signal and movement: (1) fluorescent and moving, (2) fluorescent and non-moving, (3) non-fluorescent and moving, and (4) non-fluorescent and non-moving (Drake et al., in review; Steinberg et al., in review). Organisms binned as non-fluorescent and

In application of the dual staining technique, STEP **requires** that the protist (*<50 µm and ≥10 µm*) sample is split; half is analyzed as a test sample, and half is heat killed. In the event of high viable counts in the test sample, the heat-killed sample should be immediately processed (stained and counted) to determine if the high counts in the treated discharge sample result from "false positives" (Steinberg *et al*., 2011a, c.f. ETV protocol above). Both results should be reported under these circumstances. This requirement is to ensure that a BWMS is not falsely determined to be ineffective because of analytical issues.

At present, there is no rapid, reliable method to determine viability of cysts.

- **Size Class <10 µm (bacteria)** The classic viable cell count is implemented for enumerating bacteria in uplift and treated water. Because there is no single media for gauging the number of all active bacteria in natural waters, the use of multiple media has been deemed necessary for BWT system testing under STEP. Serial dilutions are prepared from samples, which are then placed onto the surface of agar nutrient media for grow-out of colonies for enumeration (EPA, 2010). Culture under two media is required. Recommended media are 2216 Marine Agar[13] and sodium chloride-amended R2A agar[14] medium for use with marine samples and nutrient agar[15] and Plate Count Agar[16] for freshwater samples. Use of at least two different heterotrophic media provides a broader spectrum of microbes for viability tests and assumes that a BWTS is showing good "inactivation/removal" based upon these media, and likely is effective against the remainder of bacterial assemblage.

NOTE: It is essential that the concentrated sample is homogeneous as it is being sub-sampled, so that each aliquot is representative of the sample. If this is not the case, then the approach is invalid. The volumes of samples and counting volumes are presented in Subsection 5.4.1.2.

Use Text Box 5.4b to describe the biological analysis that is part of the BWMS.

TEXT BOX 5.4b: Biological analysis.

(1) Describe the viability assays that will be used to quantify the organisms within each of the three size classes. Include a description of any supporting biological assays and note any other biological groupings to be assayed in addition to the three primary size fractions.

(2) If non-ETV assays are to be used, demonstrate the equivalence of the result to ETV protocols. Append primary data and reports used to demonstrate equivalence.

(3) Indicate which assays are to be used within the Test Team laboratories. Provide documentation that the viability assays have been verified for the types of test waters to be measured under STEP testing.

5.4.1.2 Sampling

A common problem in determining the effectiveness of BWMS has been the detection of small numbers of viable organisms in large volumes of water. This is particularly the case in the ≥50 µm size fraction where the treatment goal is to discharge very low concentrations of living organisms. For instance, the

moving are obviously viable, but the combination of stains fails to indicate viability, representing the Type II (false negative) error.

[13] http://www.bd.com/ds/technicalCenter/inserts/Marine_Agar_&_Broth_2216.pdf
[14] http://www.bd.com/ds/technicalCenter/inserts/R2A_Agar.pdf
[15] http://www.bd.com/ds/technicalCenter/inserts/Nutrient_Agar.pdf
[16] http://www.bd.com/europe/regulatory/Assets/IFU/Difco_BBL/254483.pdf

IMO BWM Convention's Regulation D-2 lists the discharge standard for this size class is <10 viable organisms per cubic meter, and some U.S. states are attempting to set even lower concentration standards. To accurately detect fewer than 10 organisms per cubic meter, it is necessary to concentrate several cubic meters to 1 liter or less and (e.g. <7 m^3) to count the organisms in the entire volume of the concentrated sample, or in the case of very large original sample volumes (e.g. >10 m^3), which are highly concentrated to 1 liter or less, count subsamples from the homogeneous concentrate. Low or zero counts that result from sample volumes that are too small to be statistically valid likely represent false negatives and are thus the reflection of a faulty protocol rather than a true measure of the effectiveness of the BWMS. To minimize the potential for this artifact in STEP testing, guidance developed for ETV BWMS land-based testing has been modified for shipboard use.

The approach for STEP testing is to collect large enough volumes of water to capture sufficient numbers of viable organisms to ensure statistically adequate numbers in the counting process. For circumstances when low density of organisms is expected, such as for the ≥50 μm fraction of treated water, this always requires a concentration step (filter or net.) However, there are significant constraints on how much concentration can be achieved without introducing an additional source of mortality to the organisms and handling error. In high turbidity waters, such as the amended challenge waters proscribed for land-based tests (EPA, 2010), less concentration can be achieved than in most harbor and near-shore natural waters. The naturally occurring concentrated populations in harbor and near-shore waters (and their lower turbidity than ETV challenge waters for land-based tests) generally results in a reduction of the total sample volume required in shipboard tests (because the volume can be concentrated to a greater degree), as long as the final concentrated volume is reduced proportionally with the reduction in overall sample volume. The following paragraphs (and References 5-4a, 5-4b, 5-4c, and 5-4e,) present guidance on selecting sample volumes for STEP testing. While there is flexibility, the minimum sample volumes, concentrated volumes, and sub-sample volumes to be counted must meet the statistical requirements set forth under the ETV Protocol (EPA, 2010) and presented here.

- ***Sampling Scenarios.*** Two sampling scenarios are permitted within ETV and are illustrated in Figures 5-3a and 5-3b. In both cases, STEP now emphasizes viability assessment on samples of discharge ballast water. The required analyses of uplift samples are to assure that the challenge water meets required ETV conditions in terms of the organism population density (Reference 5-4d); this can be determined from assays of preserved uplift samples paired with the ballasting of the tanks to be assayed upon discharge.

  The scenario in Figure 5-3a does not involve control ballast tanks, and the effects of holding time of treated water within a ballast tank are not assessed independently of the effects of the treatment system. Rather, the combined effects of treatment and holding time are assessed together. In this scenario, ballast water treatment includes "holding" for a specified period, and may not be considered to meet treatment standards in routine operation if the holding times are less than those used in the Year 1 Primary Tests. In other words, the treatment program is evaluated only for holding times greater than or equal to the times used in the experiment and use of these test data to indicate the effectiveness of the BWMS for shorter holding times is invalid. Adequate replication of treated ballast tanks will be a combination of voyages and tanks per voyage; the most statistically powerful approach may be the testing of single tanks over a variety of voyages (recommendation for this approach is five or more voyages).

  Studies making use of control ballast tanks (Figure 5-3b), on the other hand, provide a more robust assessment of performance of the treatment system separate from influences of holding. Study plans using this approach must also demonstrate adequate replication and statistical power. Applicants should note that because of known cross contamination issues, STEP does not require the use of control ballast tanks.

- ***Sample Volumes***. For either sampling scenario the critical issue, identified by the Naval Research Lab (NRL) and other researchers addressed in STEP 2010, is the size of sample volumes required to detect very low densities of viable organisms. Assessment of treatment against the D-2 standard of <10 viable organisms per cubic meter for organisms ≥50 μm requires that large sample volumes be collected and concentrated to small volumes for analysis. To determine if the treatment system is meeting this standard, the sampling program must acquire sample volumes that are sufficiently large to accurately judge that the attainment of low numbers of viable organisms is the result of an effective treatment system rather than a statistical effect of using sample volumes that are too small. Reference 5-4d summarizes the IMO BWM Convention's Regulation D-2 discharge standards and ETV/STEP sampling recommendations.

  Determination of the proper sample volume is based upon several variables:

  - target concentration needed to be detected; e.g., 0.1, 1, 5, 10 organisms per cubic meter
  - sample volume collected; i.e., the volume that is filtered through a plankton net or filter array (e.g. NRL Filter Skid)
  - level of sample concentration; e.g., 5 m^3 concentrated to 1 L
  - volume of concentrate analyzed; e.g., the entire 1 L evaluated

  When the level of sample concentration is increased and the volume of concentrate evaluated is increased, the volume of sample that needs to be collected is reduced. Furthermore, evaluating samples for low target numbers of organisms (e.g., 0.1 vs. 10 org/m^{-3}) require larger sample volumes unless the level of concentration is increased or the volume of concentrate analyzed is increased. A "sample volume calculator" (Reference 5-4a) that has been developed by STEP provides guidance to the applicant regarding the minimum sample volume required to allow detection of the desired concentration of target organisms as a function of concentration factor and volume of concentrate analyzed. The calculator is an active Microsoft Excel file that will compute the required sample volumes to be collected, given various input values (concentration factor, etc.). The applicant is encouraged to determine the best relationship of sample concentration and amount of concentrate analyzed that will allow the applicant to accurately determine if the BWMS is discharging numbers of viable organisms exceeding the set target, while keeping the required sample volumes as low as possible. Some test programs analyze the entire concentrated volume to minimize the sample volume that needs to be collected.

  The calculator is based upon empirical and statistical efforts by Lemieux *et al*., 2008[17] and Miller *et. al*., 2011[18] undertaken to address the sample volume issue for BWMS testing. They found that if in-line, time-averaged sampling is used during emptying of the ballast tanks, enumeration of viable organisms in treated, concentrated samples followed a Poisson distribution, allowing the chi-square to be used to estimate the confidence intervals.[19]

  This approach (Reference 5-4a) allows statistical evaluation of individual test runs to discern that the BWMS does not discharge more than the above-mentioned target number of viable organisms at a probability level of p=0.05. In addition, the approach allows the applicant to composite individual test runs to refine the assessment BWMS performance in borderline cases. Use of this approach greatly reduces the number of replicate tests and analytical costs for the applicant compared to other

[17] Lemieux, E.J., S. Robbins, K. Burns, S. Ratcliff, and P. Herring. 2008b. Evaluation of Representative Sampling for Rare Populations using Microbeads. Report No. CG-D-03-09, United States Coast Guard Research and Development Center, Groton, CT.
[18] Miller, A.W., M. Frazier, G.E. Smith, E.S. Perry, G.M. Ruiz & M.N. Tamburri. 2011. Enumerating sparse organisms in ships' ballast water: Why counting to 10 is not so easy. Environ Sci Technol. 45(8): 3539–3546.
[19] The two statistical approaches yield nearly identical results.

statistical approaches without compromising statistical power. However, for proper application several conditions must be met:

A) The sample must be sufficiently large to prevent artificially low counts due to the failure to capture viable organisms because they are rare (~10 org/m^3), not because they are not present (e.g. too small a volume collected to ensure a statistically valid count of viable organisms).

B) The sample must be representative of the entire ballast tank; this requires time-averaged sampling during complete discharge of the test ballast tank.

C) The losses of organisms due to handling, concentration, and other sampling errors need to small.

The optimal approach would be to determine a minimum sample volume required to enumerate rare organisms with the above-mentioned statistical resolution and to exceed that minimum as much as can be reasonably accommodated. Then concentrate to a volume ≤1000 mL and count as much of the concentrate as possible without exceeding permissible holding times. If large portions of the concentrate are counted, then the minimum acceptable sample volumes are reduced. If the applicant can collect more than the minimum sample volume, then the ability to discriminate a sample count close to the selected target increases. For example, if 1.5 m^3 is collected, concentrated, and the entire concentrate analyzed, an organism count of ~5 is required to be statistically significantly different from 10 organisms per cubic meter ($p<0.05$). But if 7 m^3 is collected, then a count of ~8 organisms per cubic meter is statistically less than 10 organisms per cubic meter (see Reference 5-4c).

In contrast to an approach of counting the entire concentrate, if one were to concentrate the sample to 1000 mL and analyze only a 20 mL sub-fraction of that concentrate, ascertaining that the treated ballast water held <10 viable organisms per cubic meter would require concentrating 60 m^3 of treated ballast water (to 1000 mL). If 100 percent recovery and detection is assumed, this approach provides the statistical resolution to determine if the BWMS was discharging <10 viable organisms per cubic meter (detection of ≤6 viable organisms in the 20 mL subsample shows with 95 percent confidence that the treatment system has <10 viable organisms per cubic meter).

In this example, the sample volume can be reduced from 60 m^3 by either increasing the concentration factor or the volume of subsample analyzed or both. (Reference 5-4a). For example, if a 30 m^3 sample is collected and the organisms concentrated to 500 mL and a single 20 mL aliquot is counted, the same result is achieved. However, the degree of concentration is limited by the potential adverse effects on the organisms due to crowding, grazing, increased turbidity, reduced efficiency of sample recovery, etc. An upper limit to the volume of sub-sample that can be analyzed is determined in significant part by the speed of counting and the allowable holding time of the samples.

NOTE: Allowable holding time is likely to be influenced by many factors and may differ between locations and seasons, due to differences in physical conditions and the composition of the biological assemblage in the sample. For the work reported by Lemieux *et al*. (2008), the allowable holding time was no more than 6 hours, as determined by careful time-series analysis. Use of longer holding times by the applicant will require data illustrating holding time versus viability.

The sample volume calculator (Reference 5-4a) can be manipulated to illustrate a variety of practical sample volumes and concentration factors that can be employed while preserving the desired precision and accuracy for determining that the BWMS achieved endpoint concentrations below selected thresholds (treatment targets or regulatory limits). The required sample volumes decrease as the fraction of the concentrate analyzed increases. For example, based upon the NRL calculations and protocols used during land-based ETV testing, if 20 mL of a 1 L concentrate was analyzed to assess a target of <10 org m^{-3}, a 60 m^3 sample would be required. Merely doubling the volume counted to 40 mL reduced the sample by half (following the formula Sample Vol = 60 X^{-1}, where X = number of replicate 20 mL subsamples analyzed [1, 2, 3, etc.] Reference 5-4e. Note also that a sample

stream with a very low flow rate used to collect a small sample volume may unintentionally result in a sample that is not representative of the entire ballast tank or introduces mortality if the sample is collected over a very long time. Therefore, it must be shown by modeling or empirical study that a small sample volume is representative of the entire tank.

Fortunately, the sample volume required for the <50 μm and ≥10 μm size fractions are substantially smaller than that required for the ≥50 μm fraction. This results from the higher density of organisms likely within the uplift and discharge waters. The sampling volume requirement for the <50 - ≥10 μm (protists) size fraction to detect 10 viable organisms per mL follows the pattern of 6 L concentrated to 1 L and analysis of two 1 mL samples (EPA, 2010). A scenario similar to that shown in Reference 5-4a can be implemented with this size class as well, where a smaller sample volume is offset by a larger sample concentration. The sampling scenario for the analysis of uplift waters and treated waters is diagrammatically illustrated in Reference 5-4b. This figure is provided for guidance, but may be modified and submitted as part of the study plan by the applicant.

While the calculator shows the minimum sample volume required to detect a given discharge target according to the applicant's desired concentration factor, etc., a larger volume may be useful to enhance the detection of slight differences between the discharge target and the density of organisms in the sample (i.e., a larger volume will increase the ability to statistically discriminate between the discharge target and concentrations that are very similar to it). Given the various sources of error and variation, at a low sample volume, a measured density of 8 organisms m^{-3} (≥50 μm) cannot be discriminated from a target of 10 organisms m^{-3}. In general, at volumes of ~1.5 m^3 no more than 5 organisms per cubic meter can be detected to indicate that the sample has <10 org m^{-3} (p=0.05). The relationship of sample volume to the maximum number of organisms detected per cubic meter that still indicates that the sample has <10 org m^{-3} (p=0.05) is shown in Figure 5.-3. This analysis illustrates that increasing the sample volume increases the allowable average number of organisms per cubic meter that can be measured and still meet a standard of <10 μm per cubic meter. However, it also indicates that multiple fold increases in sample volume have a relatively small effect on the allowable number of organisms detected.

Based upon the above relationship, it is possible to conduct the five or more test runs with sample volume based upon the sample calculator and then assess each run as to meeting the applicant stated target number of organisms. Furthermore, all of the individual test runs can be combined to calculate an average number of organisms per cubic meter and sum of sample volumes; in this manner, the additional statistical power is used to evaluate the overall performance of the BWMS. It should be noted that high numbers of organisms detected in a single test cannot be overcome by using the composite approach, since the average number of organisms per cubic meter must still be less than the target value. It is clear, however, that increasing the number of test runs or increasing the sample volume within a test run increases the statistical power for discriminating measured values below, but close to, the applicant's stated discharge target for STEP.

Use Text Box 5.4.1a to indicate the approach used to collect samples and concentrate them for the 3 size classes of biological organisms.

TEXT BOX 5.4.1a: Sample volumes for biological analysis.

(1) ≥*50 μm (zooplankton) size fraction*: State the (a) target concentration of organisms (# m^{-3}), (b) sample volume per test run, (c) the volume of the concentrated sample and (d) the number and volume of sub-samples of concentrate to be assayed. Indicate the approach to be used to collect the sample and concentrate it. Note if this follows the guidance presented. It is understood that the final volumes are determined by the detection limit required by the applicant to determine if the treatment goal has been reached.

(2) <*50 μm and ≥10 μm (protists) size fraction*: State the (a) target concentration (# organisms per mL), (b) sample volume per test run, (c) the volume of the concentrated sample and (d) the number and volume of sub-samples of concentrate to be assayed. Indicate the approach to be used to collect the sample and concentrate it. Note if this is in compliance with the guidance presented. It is understood that the final volumes are determined by the detection limit required by the applicant to determine if the treatment goal has been reached.

(3) <*10 μm size fraction*: State the sample volume and if any concentration step is to be used and the volumes to be assayed using the method presented in the previous text box. Link the volumes to the desired detection limit (i.e., # organisms per mL).

The large volumes of sample water that may require collection for the zooplankton size fraction can cause problems of disposal in the shipboard test environment. The "traditional" method of disposing of processed sample water in the bilge is not practical because of operational constraints associated with bilge water removal and disposal. In addition, ballast water management regulations preclude discharge of untreated ballast water, as from control tanks, if used. It is therefore important that the test plan include the approach for handling and disposing of large volumes of sample water used. While in some cases it may be possible to dispose of the used sample water via the ship's bilge water system, arrangements should be made to pump it directly over the side, back into a ballast tank, or through the BWMS, as necessary to meet applicable regulations.

Use Text Box 5.4.1b to describe the disposal of processed water collected for biological analysis.

TEXT BOX 5.4.1b: Disposal of processed water collected for biological analysis.

(1) Indicate the approximate volume of water generated in sampling that will need disposal post-processing and the approach that will be employed for disposal.

(2) If control (untreated) water will be collected, indicate how it will be discharged post-processing to ensure that it meets the need for STEP vessels to "treat all ballast water" before discharge.

Reference 5-4a. Volume Calculator

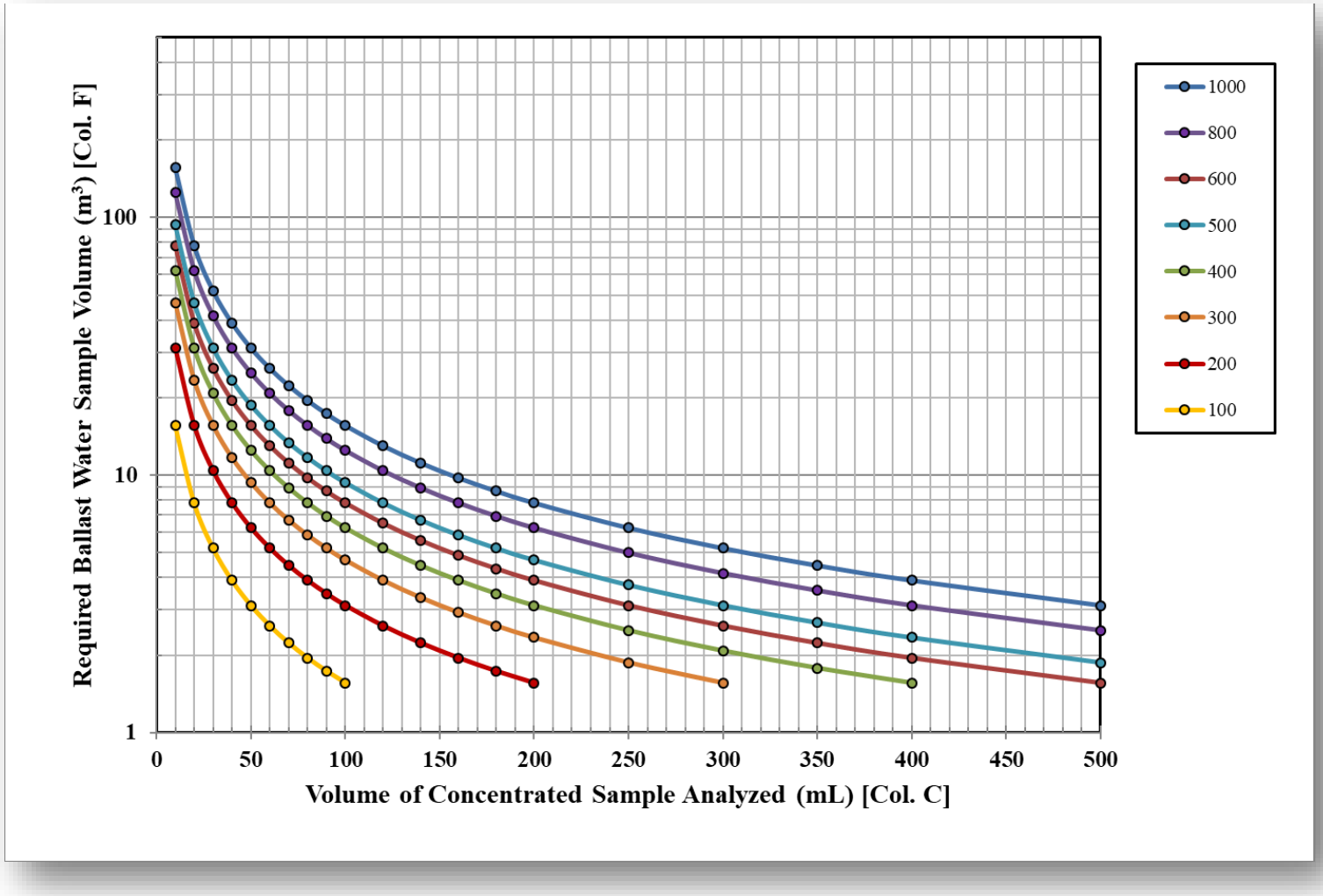
This table computes the minimum volume of ballast water that must be collected and analyzed for viability of the >50 µm zooplankton size fraction with enough statistical power to validly discern that the treatment system does not allow more than the target number of organisms per m3 to be discharged. Note that the computed sample volume represents the minimum value and that increasing the sample volume increases the ability to discriminate between the experimental results and the selected target number of organims [Col. A] as presented in Reference 5-6.4.

Instructions:

1) *Double click on the table to activate for making entries. Click on desired cells A-D and enter numbers as described below.* ***DO NOT*** *make entries into cells E and F, as values shown are computed. When finished click outside the table to return to text.*
2) **COLUMN A:** Enter target number of organisms per m^3 (i.e., 10 org/m^3, 1 org/m^3, etc.).
3) **COLUMN B:** Enter volume that the sample will be concentrated to (mL).
4) **COLUMN C:** Enter the volume (mL) of the concentrated sample that will be analyzed for viability.
5) **COLUMN D:** Enter the number of consecutive samples collected over the entire deballesting event that will be combined in a given experimental run. On occasions where the time required for obtaining time-integrated samples during complete emptying of the ballast tank exceeds recommend sample holding times, it may be necessary to sequentially collect a number of equal volume samples during deballasting so that viability analysis of a given sample can begin before maximum holding times are exceeded. In this case, divide the total time required for deballasting the test ballast tank by the desired maximum holding times to acquire the nearest integer estimate of the number of sequentially collected samples that will be required. For example, 8 hours deballisting time per 2 hours maximum holding = 4 sequentially collected samples; enter the value "4" in column D.
6) **COLUMN E (Computed):** Required volume (m^3) of each ballast water sample to be combined into a single experimental event.
7) **COLUMN F (Computed):** Required total sample volume (m^3) collected from a single experimental event (i.e., to be used in statistical analysis for comparison to the target [Col. A].

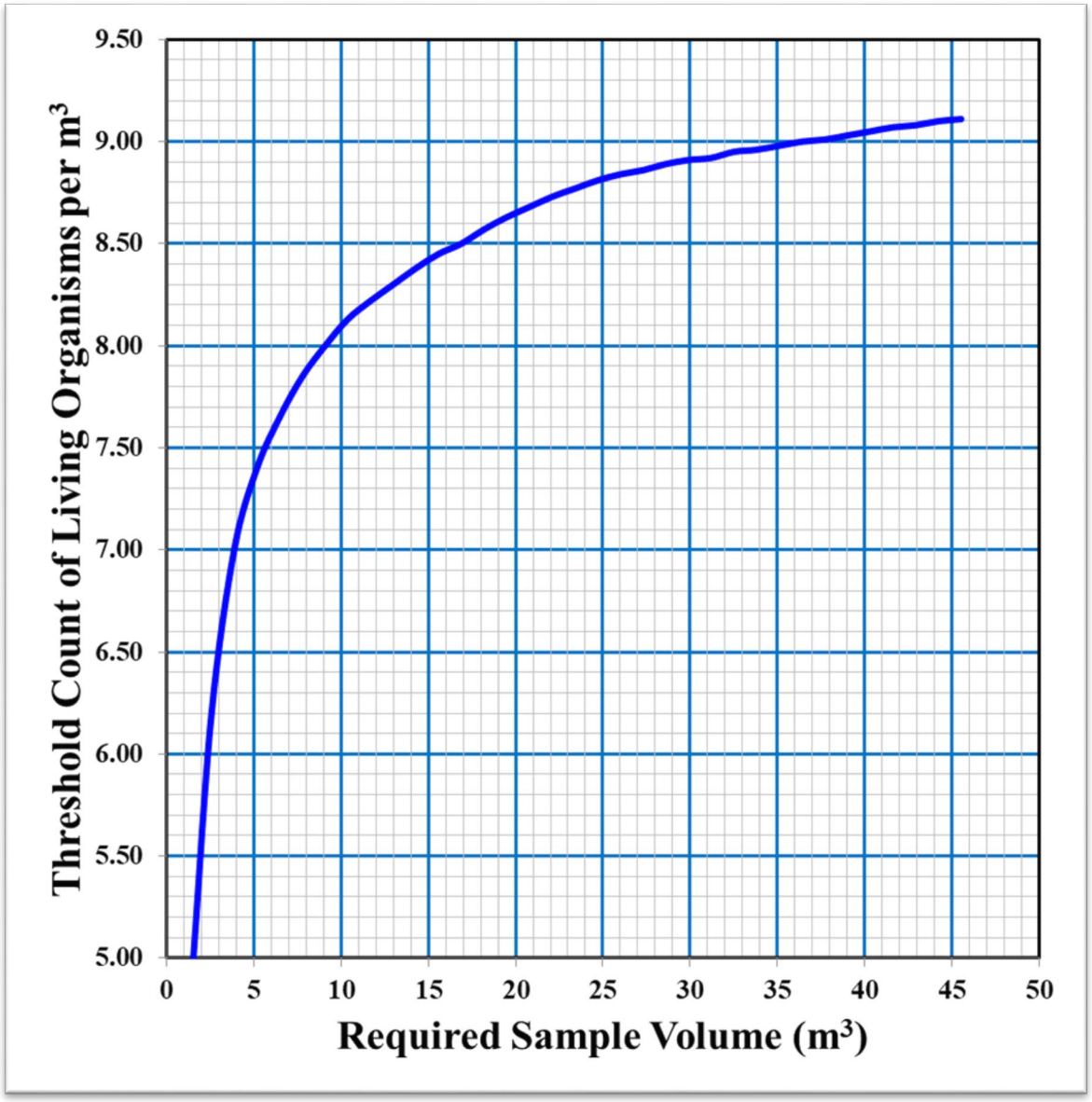
A	B	C	D	E	F
Target # organisms per m^3	**Concentrated Volume (mL)**	**Volume of Concentrated Sample Analyzed (mL)**	**Number of Samples to be Combined in a Single Run**	**Required Volume of Each Sample to be Combined into a Single Run [Col. D] (m^3)**	**Required Total Volume Analyzed in a Single Run (m^3)**
10	**1000**	**100**	**1**	**15.60**	**15.60**

Reference 5-4b. Graphical Representation of the Volume Calculator (Reference 5-4a).



Assumptions: Target number of organisms, 10 per m^3 [Col. A in Calculator]; number of samples combined in a single run, 1 [Col. D]. The volume of concentrated sample [Col. B] is indicated in the legend.

Reference 5-4c. Threshold Numbers of Living Organisms per Cubic Meter at the Indicated Sample Volumes



Threshold numbers of living organisms per cubic meter at the indicated sample volumes analyzed that must not be exceeded for the BWMS to have demonstrated that <10 living organisms per cubic meter are being discharged. The analysis is based upon a calculated value of 40% and a combined sampling error, loss of organisms during holding error, and other unaccounted for errors totaling 30%.

The required total volume of ballast water to be analyzed presented in ***Column F*** of the ***Volume calculator*** (sample processed according to Columns B, C & D) is selected on the "X" axis of this graph to find the threshold total count of living organisms measured that must not be exceeded to guarantee that ≤10 viable organisms are released into the environment.

Reference 5-4d. Components of Viability Assays to be Conducted

Note that the sample volume, concentration and concentrate sub-sample volume counted can be altered in a balanced fashion following ETV guidelines (i.e., lower sample volume requires higher concentration or more volume analyzed, see Reference 5-4a). Dilution of samples may be required for counting in some instances. In all cases, sample preparation and counting must be completed within an empirically determined maximum allowable processing time.

Organism Class	IMO D-2 Standard (No. of Living Organisms on Discharge) [C]	Target Minimum Number of Viable Organisms in Challenge Water (uplift)	Required Mesh to Concentrate Sample
≥50 µm (Zooplankton)	<10 per m^3	≥ 1 x10^4 per m^3 [B]	50 µm diagonal
<50 - ≥10 µm - (Phytoplankton, Protists)	<10 per mL	≥ 5 x 10^2 per mL	10 µm diagonal
<10 µm (Bacteria)	N/A (CFU aerobic heterotrophs)	≥ 5 x 10^2 per mL	10 µm diagonal
	V. cholerae <1 CFU per 100 mL; gm Zooplankton.	---	
	E. coli, <250 CFU per 100 mL	---	
	Enterococci, <100 CFU / 100 mL	---	

[A] Minimum sample volume collected, volume of concentrated sample and volume of concentrated sample analyzed for enumerating number of viable organisms is calculated from Reference 5-4a.

[B] This is the average of all shipboard tests for a system, to allow testing periodically to occur at slightly lower densities. However, if the number of organisms in the ≥50 µm size class is less than 5 x 10^4 m^{-3}, the test will be discarded and the results will not be accepted and the test will need to be repeated for fulfillment of STEP testing requirements.

[C] IMO Standard for treated discharge water is used here as an example.

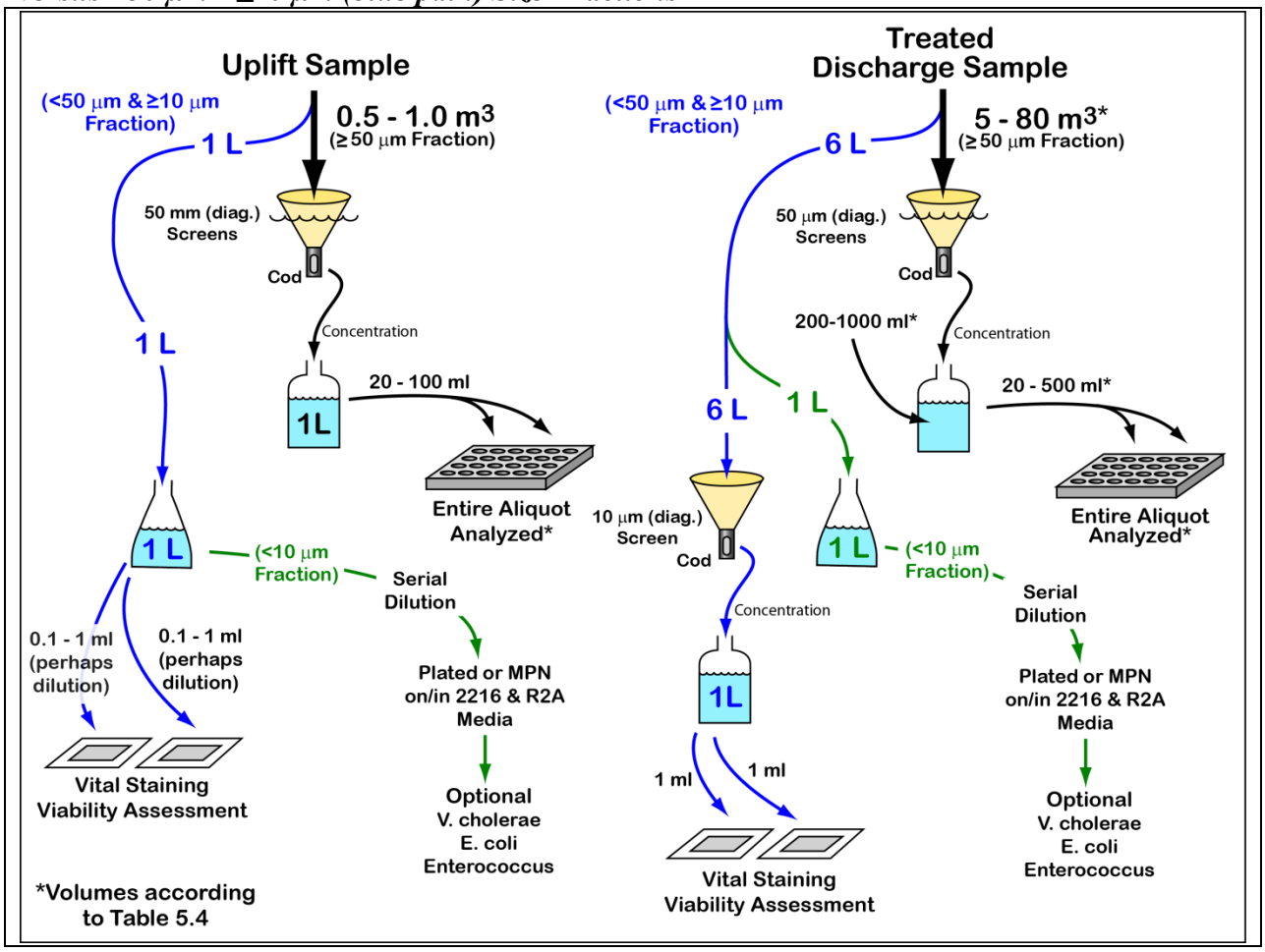
[D] Untreated water does not require concentration, although test programs may decide to concentrate for analytical reasons.

Reference 5-4e. Relationship of Number of Concentrated Subsamples Analyzed to Total Required Sample Volume, in order to Assess if Different Standard Thresholds (0.1, 1.0, 10 org/m^3) Are Met for Organisms ≥50 µm

For example, to detect (with 95 percent confidence) 10 organisms m^{-3}, a single sample of 60 m^3 (concentrated to 1L) is required if one 20 mL subsample of the concentrate is analyzed, whereas a single sample of 12 m^3 (concentrated to 1 L) is required if five 20 mL subsamples are analyzed. The choice to concentrate less volume and analyze more 20 mL sub-samples is generally limited by the allowable holding time for viability analysis. Table modified from ETV Protocol.[2]

Detection Limit (Org/m^3)	Volume of Each Sample with *n* Replicate 20-mL Subsamples of a 1 L Concentrate and Counting the Whole 1 L Concentrate			
	1	**3**	**5**	**Whole 1 L**
	Required Sample Vol. (m^3)	**Required Sample Vol. (m^3)**	**Required Sample Vol. (m^3)**	**Required Sample Vol. (m^3)**
0.1	6000	2000	1200	120
1	600	200	120	12
10	60	20	12	1.2

Reference 5-4f Generalized Sampling Plan for Untreated (uplift) and Treated Samples, Showing Approximate Volumes and Concentration Approaches for the ≥50 µm (black path) versus <50 µm - ≥10 µm (blue path) Size Fractions



Complete Table 5-4, "Biological Sampling and Analysis" which is an expansion of the information entered into Reference 5-4b and more specifically focuses on the detail of the assays implemented for each taxonomic group assessed. If the taxonomic group measured is not presented in the table fill in the taxa under "other."

Terms used in Table 5-4 are explained below:

- *Number of Replicate Assays*—the number of sub-samples of concentrate (e.g., 1, 2, or *n* subsamples) assayed of the concentrate of the total sample volume collected (m^3) for each size fraction that are taken from each concentrated sample of the discharge (Reference 5-4b).
- *Subsample Collection Methods*—provides information on the approach used to collect the above subsamples from the sample or its concentrate for each size category (nominal taxonomic grouping) assessed.
- *Holding Time Implemented for Viability Assays*—the time between when the sample is concentrated from the in-line sampling port and the subsamples of the sample or its concentrate have been analyzed (scored). This is **not** the holding time in a ballast tank prior to sampling, nor is it the holding time between taking a subsample and beginning the analysis of that subsample. It is the

entire time a sample or its concentrate is held during the analysis, and represents the time during which concentration and holding effects might be hastening the degradation of the subsample. The plan must demonstrate that sample holding times do not result in lower counts in viability assays.

- *Subsample Holding Conditions*—the conditions under which concentrated samples or subsamples are held before and during analysis (i.e., temperature, method and duration of shipping, preservatives used, light conditions, etc.). Also, information on "Chain of Custody" of the subsamples to be analyzed and the Quality Control measures implemented to assure and document their integrity prior to analysis.
- *Sample Holding for Total Count Assays*—relates to total counts independent of viability, such as for preserved phytoplankton samples.
- *Sample Archiving*—the conditions for long-term storage of samples e.g., preservative used, where samples are stored, and number of samples archived.

Provide additional explanatory detail as needed on biological sample collection, holding, analysis and archiving

Use Text Box 5.4.1 c to describe details of biological sample handling, custody and holding times and conditions linking to Reference 5-4a. Append chain of custody forms and indicate any special handling requirements. Sample holding conditions are of particular importance. Indicate the sample holding time for each of the three organism size classes (time from sample collection to viability assay) and provide references or documentation that this holding time does not cause significant change in the number of viable organisms in the sample prior to "counting." Documentation can take the form of showing the number of viable organisms in a sample over time, where the time selected as "acceptable" is prior to the decline in viable counts. Note that the number of viable organisms in treated water is anticipated to be small, making predation in concentrated samples less than in concentrated natural waters.

TEXT BOX 5.4.1c:

Table 5-4. Biological Sampling and Analysis

Collection, analytical methods, holding, etc. for the BWMS for evaluation under STEP.

Size Class -->		≥50µm	<50µm - ≥10µm	<10µm	Other	Other
# Replicate Assays per Sample (1)						
Sample Collection Method (2)						
Sample Holding Time for Viability Assays (3)						
Sample Holding Conditions (between sampling and analysis)	Preservative					
	Temperature					
	Method and Duration of Shipping					
	Chain of Custody					
	Quality Control (4)					
Holding Time: Total Count Assays						
Sample Archiving (long-term storage)	Preservation					
	Location					
	# Samples					

(1) Number of sub-samples assay from a single mesocosm to determine analytical variation.

(2) The method used to determine the removal or inactivation of organisms within each taxa (for example: motility in zooplankton).

(3) Time from sample collection to assay for viability assays.

(4) How will integrity of "held" samples be documented.

- **Sampling Port Design.** Because in-line, time integrated sampling is required for valid adherence to Poisson statistics discussed above, it is critical that "flow proportional" samples for analysis be obtained during the entire filling and emptying of the ballast tanks under study. Such a time integrated-sample is appropriately free from influences of "patchiness" in organism distribution within the period of sampling. On the other hand, discrete sampling within the tank can be dramatically influenced by such heterogeneities and is not an acceptable sampling approach. Note that if holding time becomes an issue, consecutive integrated samples collected over the deballasting, allows for processing to begin as deballasting continues. This is the basis for Column "D" in the calculator (Reference 5-4a).

  To obtain samples that are truly representative of the ballast flow stream at the site of sampling, reasonable care in the design of the sampling port is necessary. "*To achieve isokinetic* [flow proportional] *sampling conditions, a sampler* [must be] *designed to separate a subsection of the total flow-stream in a manner that does not encourage or discourage water entry other than that which is otherwise in the cross-section of the sampler opening. In other words, flow streams in the main flow of the pipe should not diverge or converge as they approach the opening of the sampler.*[2]" Significant deviation from these flow conditions can cause "biases" in the captured organism population because the "effective" diameter of the sample port is in reality then slightly smaller or larger (respectively) than the true physical diameter of the sampling port.

  Recent fluid dynamics studies at NRL[20] discuss these issues in detail and have defined the type of in-line sampling port that should be used. The ratio of the diameters of the sample port to ballast pipe diameters are important variables for minimizing biases mentioned above and are discussed in those documents. It has been shown that the sampling port isokinetic diameter (D_{iso})[21] calculation:

$$D_{iso} = D_m \sqrt{\frac{Q_{iso}}{Q_m}}$$

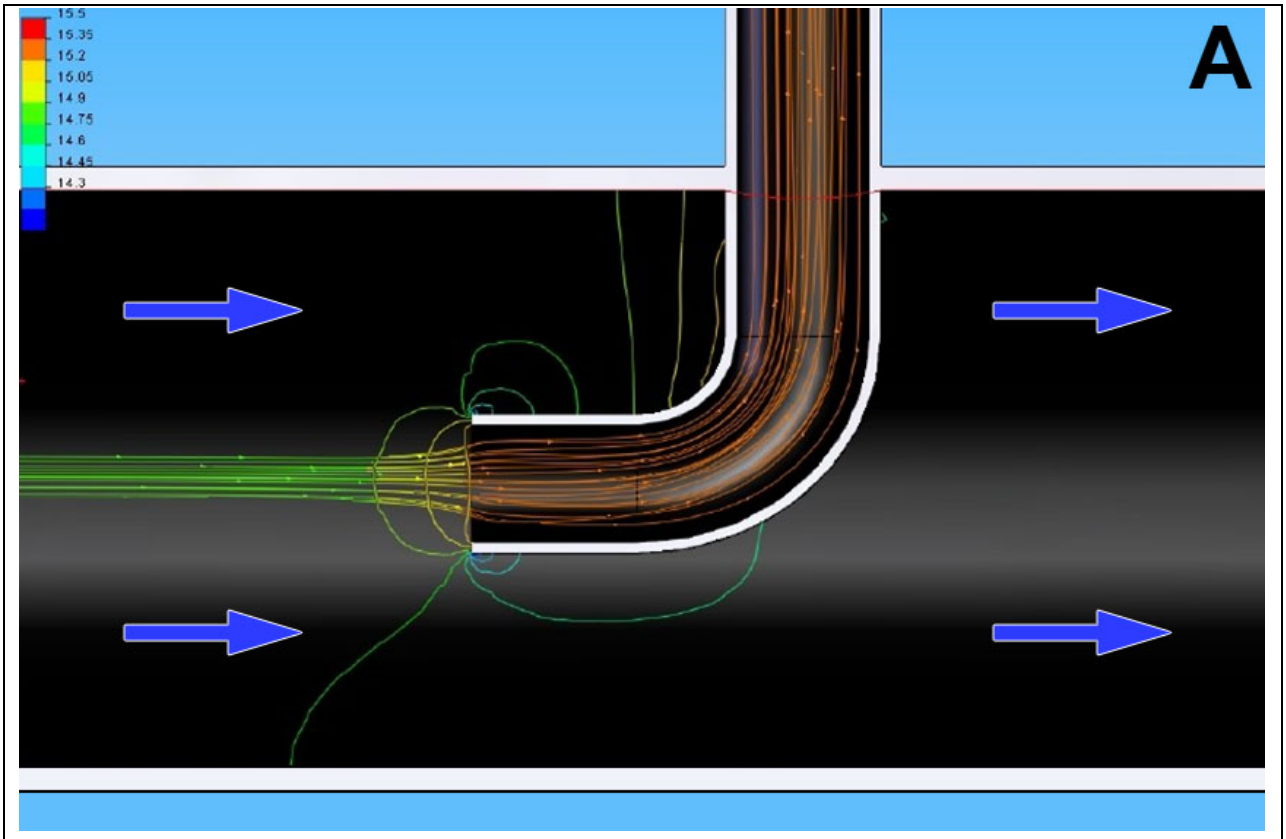
  This equation provides useful guidance for the sizing of sampling ports, where D_m is the inside diameter of the ballast line being sampled, Q_{iso} and Q_m are the volumetric flow rates within the sampling port and ballast line, respectively. Model simulations showed that for sampling neutrally buoyant ballast organisms, sample port diameters between 1.5 and 2.0 times the isokinetic diameter were best.

  In general, the sample port should penetrate through the main pipe wall and gradually curve into the central flow within the main pipe as illustrated in Figure 5-3a. The sampling port should ideally be scaled so that the desired sample flows are obtained with the on or off valve fully open, minimizing sheer and pressure differentials. The entrance into the sample port should face into the flow stream within the ballast line and be chamfered to minimize local disturbances in the flow field near the sample inlet. If minor adjustments of the sample flow rate are required, the flow should be controlled by a "diaphragm valve;" ball valves, gate valves, and butterfly valves should be avoided as they cause damaging shear in the sample flow stream. An effective port design is also shown in Figure 5-3b, where the sampling port enters the ballast line from an elbow. The length of the internal sampling pipe must extend beyond the distortion of the flow field caused by the elbow but short enough to not vibrate in the ballast line flows. See Richard *et al*., 2008 for detailed explanation and guidance details on the design and positioning of sample ports.

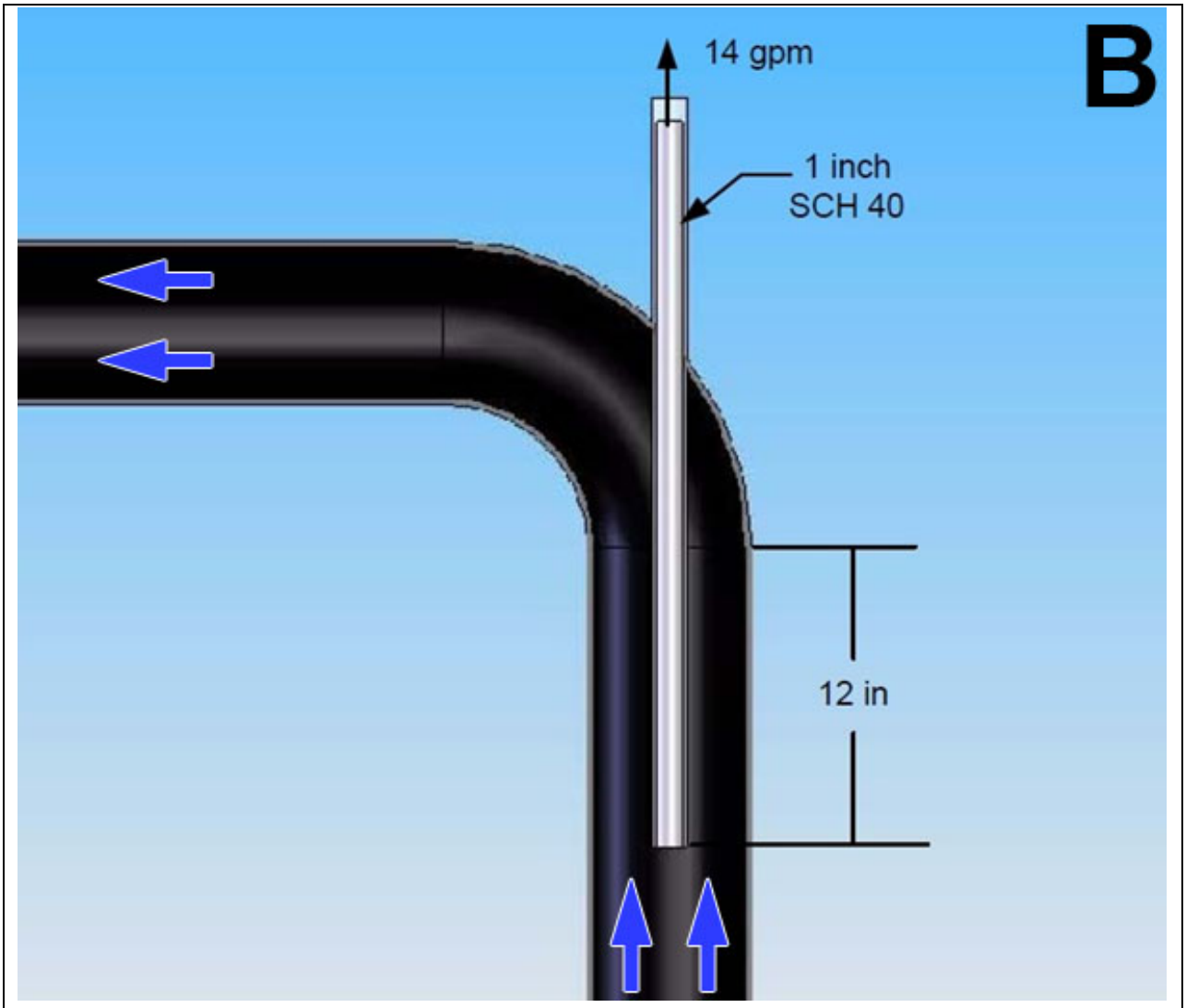
[20] Ibid.

[21] The diameter of the sampling port permitting "isokinetic sampling," sampling where the linear velocity of the fluid within the sampling port is equal to that of the fluid flowing within the ballast line.

Figure 5-4. Examples of the Two "Best" Sampling Port Designs Modeled by Richard* et al.*, 2008



Panel A: Sampling port within a straight stretch of ballast line. Arrows indicate direction of ballast flow.



Panel B: Sampling port at a 90-degree elbow in the ballast line. Arrows indicate direction of ballast flow.

Use Text Box 5.4.1d to provide additional explanatory information on the sample port design to be used to test the BWMS proposed in this Application (e.g., explanation of key components in the sampling port diagram).

TEXT BOX 5.4.1d: Provide a drawing and explanatory information on the sample port design to be used in testing under STEP. Indicate how the design and placement of the sampling port(s) are consistent with STEP guidelines discussed above.

5.4.2 Environmental Analyses

The environmental analysis section provides data on various water quality variables that generally describe the properties of the water to be treated (e.g., temperature, salinity, pH, oxygen content, nutrient and chlorophyll *a* content and the like) or properties that can directly influence effectiveness of the BWT process (e.g., parameters that affect the optical properties of water, such as TSS, turbidity, water color that can affect UV treatment systems; variables that relate to organic matter content of the water that may influence the concentration and half-life of chemical used for treatment, etc.).

Enter requested data for each environmental parameter analyzed in the appropriate column. If the variable measured is not listed in the table write in the variable under "other." Complete Table 5-4.2a "Environmental Parameters and Water Chemistry". Chemical residuals and byproducts (Table 5-4.2b) must be measured as part of STEP testing, and sampling needs to allow assessment of the discharge of these constituents. Doing so can also allow the ship to meet its Vessel General Permit (as required by the EPA) water quality discharge monitoring requirements if these discharge tests are set-up accordingly.

Terms used in Table 5-4.2a and Table 5-4.2b are explained below:

- *Method of Sample Collection*—explains how samples were collected for water quality measurements. If the assays are measured *in situ* (e.g., inserting a probe in the environment, ballast tank or in line near a sampling port) enter "*in situ*"; if samples were obtained and placed in a container for subsequent analysis aboard the ship then enter sampling method used.
- *Number of Replicate Assays*—the number of sub-samples assayed from a single sample bottle to determine analytical variation.
- *Analytical Method Used*—the method used for the assay of the environmental parameter; include the reference to *Standard Methods* as appropriate.
- *Sample Holding Time for Assays*—the time between sample collection into a container and analysis. If an *in-situ* method of analysis is employed (e.g., use of a probe, etc.) enter "0."
- *Sample Holding Conditions*—the conditions under which samples and any subsamples are held before analysis (i.e., temperature, method and duration of shipping, preservatives used). Also, information on "Chain of Custody" of the samples and subsamples to be analyzed and the Quality Control measures implemented to assure and document the integrity of the samples and subsamples prior to analysis.
- *Sample Archiving*—the conditions for longer term storage of samples, as might be employed when samples are analyzed in a shore-based laboratory after the cruise and/or samples preserved for archival storage. For example, preservative used, where samples are stored, the conditions of storage, and the number of samples archived, etc.
- Provide additional explanatory detail as needed on sampling approaches, sample holding, preservation and archiving, measurements of water quality parameters.
- *Chemical Residuals*—the potential residuals of chemicals used for treatment or byproducts resulting from treatment of ballast water.

Use Text Box 5.4.2a to describewater quality sampling and analysis in addition to information in Table 5-8a if needed.

TEXT BOX 5.4.2a: Water quality sampling and analysis.

Use Text Box 5.4.2b to describe disinfection by-product and residuals sampling and analysis in addition to information in Table 5.8b if needed.

TEXT BOX 5.4.2b: Disinfection byproduct and residuals sampling and analysis.

Table 5-4.2a. Environmental Parameters and Water Chemistry

Sample collection, analytical methods, holding, etc.									
Water Quality/Environmental Parameters		Turbidity	TSS	Chlorophyll *a*	Total Organic Carbon	Temperature	Salinity	pH	Dissolved Oxygen (6)
Sample Collection Method (1)									
# Replicate Assays per Sample (2)									
Analytical Method (3)									
Sample Holding Time for Assays (4)									
Sample Holding Conditions (between sampling and analysis)	Preservative								
	Temperature								
	Shipping								
	Chain of Custody								
	Quality Control (5)								
Sample Archiving (Long-term Storage)	Preservation								
	Location								
	# Samples								

(1) Assays are "*in situ*" if inserting a probe in the environment or ballast tank; if grab samples are taken and analyzed separately then enter sampling method.

(2) Number of sub-samples drawn from a single sample bottle to determine analytical variation.

(3) The method used for analysis, include reference to Standard Methods as appropriate.

(4) Time from sample collection to analysis.

(5) How will integrity of "held" samples be documented.

(6) Identify chemical(s) used for the ballast water treatment or created during treatment and indicate details for analysis of the residual concentration released to the environment. Add extra columns as needed.

Table 5-4.2b. Water Chemistry- Disinfection Byproducts and Residuals

Sample collection, analytical methods, holding, etc.								
Water Chemistry: Parameter		Disinfection Byproducts				Residuals			
Chemical Species or Group Name:		1_____ (7)	2_____	3_____	4_____	1_____	2_____	3_____	4_____
Sample Collection Method (1)									
# Replicate Assays per Sample (2)									
Analytical Method (3)									
Sample Holding Time for Assays (4)									
Sample Holding Conditions (between sampling and analysis)	Preservative								
	Temperature								
	Shipping								
	Chain of Custody								
	Quality Control (5)								

(1) Assays are "*in situ*" if inserting a probe in the environment or ballast tank; if grab samples are taken and analyzed separately then enter sampling method.

(2) Number of sub-samples drawn from a single sample bottle to determine analytical variation.

(3) The method used for analysis, include reference to Standard Methods as appropriate

(4)Time from sample collection to analysis.

(5) How will integrity of "held" samples be documented.

(6) Identify chemical(s) used for the BWT or created during treatment and indicate details for analysis of the residual concentration released to the environment. Add extra columns as needed.

(7) Fill in the names of the chemical species or groups assayed for disinfection by-products or residuals. Add columns as necessary.

5.5 Test Voyage(s) Itinerary

- Provide information on test locations for each planned test voyage and experiment, in particular characterizing the ballast water uptake and discharge locations for BWMS testing, e.g., tropical, temperate, polar, fresh, marine, open ocean, coastal, estuarine, or harbor.
- Provide a test voyage schedule for BWMS experiments, preferably in tabular format. It is understood that the test locations may not have been selected at the time of the filing of the STEP Application. In these cases, the experimental test schedule for the typical (or one of the typical) voyage for the ship should be shown. If available, information provided should include testing locations (ports/waters) and season(s).

Insert Table 5-5 "Test Voyage(s) Schedule" (format to be selected by the applicant).
Present evidence that the challenge waters will meet the minimum criteria (see Reference 5-4a)

Use Text Box 5.5a to provide additional information in the text box below if needed.

TEXT BOX 5.5a: Information on vessel routes and likely test schedule.

5.6 References

Steinberg, MK and others. 2011a. Determining the viability of marine protists using a combination of vital, fluorescent stains. Marine Biology 158:1431-37.

Steinberg, MK and others. 2011b. Continuing validation of a method for determining viability in protists using two vital, fluorescent stains. Letter report 6130/1113. Published by the U.S. Naval Research Laboratory. Washington DC.

U.S. Environmental Protection Agency. 2010. Environmental Technology Verification (ETV) Program Generic Protocol for the Verification of Ballast Water Treatment Technology. Version 5.1. Report No. EPA/600/R-10/146. Developed in cooperation with the U.S. Coast Guard Environmental Standards Division (CG-5224) and the U.S. Naval Research Laboratory. September.

Section 6.0 Long-Term Performance Monitoring Plan

The applicant **must** prepare a preliminary long-term monitoring plan for shipboard testing of the BWMS as a condition of entry into STEP. The plan covers years 1 – 5 in STEP and addresses monitoring and reporting of:

- Experimental results
- Operational data for routine system operation (key engineering parameters and setpoints)
- Chemical residuals and by-products, if necessary
- Changes in system operation or configuration

The plan submittal requirements are:

- Preliminary plan, with STEP Application
- Final plan following Year 1 primary biological experiments, including any changes to system design or operation based on experimental results, subject to Coast Guard review prior to proceeding with the long-term monitoring program
- Subsequent revisions to plan for changes in system operation or configuration, if and as needed, during Years 2 through 5

The long-term reporting requirements are summarized in Table 6-0, which shows the monitoring and reporting requirements, as specified in NVIC 01-04 and modified per Coast Guard guidance. The applicant should note that the Coast Guard will make available standard templates for quarterly and annual reports and will require reports submitted in those formats, with provisions for modification by the applicant to allow vessel- and BWMS-specific reporting.

Generally, the plan addresses monitoring of ballasting operations, routine operation and maintenance of the BWMS, test results, unanticipated problems, and discharge water quality. In particular, the preliminary plan should document whether the BWMS is operated within treatment limits established during the primary biological experiments, and whether treatment performance goals are achieved over the long term.

It is of particular importance that the engineering monitoring performance parameters in this plan are consistent with the engineering parameters shown in Section 3, the experimental results presented in Section 4, and the study plan in Section 5. The final monitoring plan must clearly demonstrate that the routine performance monitoring methods and performance targets are linked to the results of the Year 1 primary biological experiments.

Table 6-0. Quarterly and Annual Reporting Requirements

Report	Essential Requirements
Quarterly Reports, Years 1-5 ("Experimental Phase")	• Study plan tasks completed during the reporting period • Unanticipated problems with the BWMS, such as unscheduled or emergency maintenance, shutdowns or repairs, during the reporting period • Identification and description of all ballast/deballast events that occurred during the reporting period, including confirmation that ballast water was treated or otherwise properly managed in accordance with regulations
Annual Report, Year 1	• Operational logs of BWMS, including key performance parameters for all routine ballast/deballast operations • Results of discharge water quality monitoring, including concentrations of chemical residuals and treatment by-products • Maintenance and repair logs of BWMS, and any modifications of system hardware or operating procedures • Summary of quarterly reports • Results of Year 1 primary experiments
Annual Reports, Years 2-5	• Operational logs of BWMS, including key performance parameters for all routine ballast/deballast operations • Results of discharge water quality monitoring, including concentrations of chemical residuals and treatment byproducts • Maintenance and repair logs of BWMS, and any modifications of system hardware or operating procedures • Summary of all biological and engineering performance results for the entire experimental phase to date • Summary of all monitoring work and treatment performance results to date
Five-year Report, Year 5	• Study plan tasks completed and unanticipated problems • Results of Year 5 performance validation experiments • Summary of the biological and BWMS performance results for the entire experimental phase

NOTE: NVIC 01-04 defines the "first year" as the "12-month period following acceptance into the STEP", during which the applicant must install the BWMS, if not done previously. For these purposes, Year 1 is the year following installation, in which the first round of primary experiments takes place.

The NVIC states that annual reports will be submitted within four months following the end of the year; however, it is silent on the submittal deadline for quarterly reports. Annual reports submitted for subsequent years will summarize the treatment performance of the BWMS.

6.1 Personnel Requirements for Long-Term Monitoring Program

Describe personnel requirements for the long-term monitoring program in Text Box 6.1a. Define the person, title, organization and responsibility of individual team members. Team members should include the Long-Term Monitoring Program Coordinator and those responsible for daily record keeping, operation and maintenance tasks, instrument calibration, etc. Where a specific person cannot be identified, identify the position (e.g., "2nd engineer", 1st mate, etc.).

TEXT BOX 6.1a: Personnel requirements for long-term monitoring program.

Describe the responsibilities of the Long-Term Monitoring Program Coordinator, particularly his or her role in management of all aspects of the monitoring program, in Text Box 6.1b.

TEXT BOX 6.1b: Responsibilities of Long-Term Monitoring Program Coordinator.

6.2 Operational Performance Monitoring Parameters

Describe the critical operational parameters that will be used in the primary biological experiments, and subsequently in routine operation of the BWMS. Define the operational setpoints and alarm settings for each parameter to meet treatment performance goals (e.g., disinfectant dose, residual concentration, contact time, power consumption, etc.). Indicate how the operational ranges and setpoints for these parameters were selected. These parameters will typically be established first as a result of prior experiments (Section 4) or type approval testing and then confirmed or modified as appropriate according to results and analysis of Year 1 primary experiments.

Performance monitoring parameters may include, but are not required to include, simplified biological tests of the applicant's choosing.

Complete the Text Box 6.2a and Table 6-2 to specify the ballast water management system's operational parameters that will be monitored and their relationship to prior treatment performance testing results. The table does not accommodate information on biological monitoring methods; for these the applicant may add rows or a new table suitable to the purpose.

TEXT BOX 6.2a: Summary of operational performance monitoring parameters.

Table 6-2. Treatment Performance Parameters for Long-Term Monitoring Program[22]

	Description	Comments
Performance Metric #1:		
Parameter to be measured	[Specify operational or biological parameter]	[Optional]
Unit of measurement	[Specify measurement units for selected parameter]	[Optional]
Treatment stage to be monitored	[Specify treatment stage associated with parameter]	[Optional]
Treatment performance goal	[Specify treatment stage performance goal]	Explain how treatment performance goal was correlated with biological efficacy testing results
Operational setpoint range	Specify min/max setpoint value for treatment stage]	Explain how setpoint values were correlated to biological efficacy testing results
Alarm conditions	[Specify min/max alarm conditions indicating system failure]	Explain how setpoint values were correlated to biological efficacy testing results
Data collection requirements	[Describe data collection method (on-line, grab, manual recording, etc.) and frequency]	[Optional]
Instrument calibration requirements	[Describe method and frequency of instrument calibration for on-line data collection]	[Optional]

[22] Insert additional rows as needed.

6.3 Other Performance Monitoring Parameters

Testing for discharged ballast water quality is required during the Year 1 experiments (see Table 5-8) and will be repeated during Year 5. The monitoring plan must include all relevant water quality parameters and test methods. The tests should confirm results of prior proof of performance experiments and demonstrate that chemical residuals and disinfection by-products (DPBs) do not exceed regulated limits or others specified in the applicant's treatment performance goals. It is intended that these water quality tests required for STEP will also meet the ships requirements for discharge monitoring under the VGP.

Use Text Box 6.3a to describe other types of performance monitoring parameters for the BWMS that impact the overall operation of the BWMS but do not directly impact capability to meet treatment performance goals (e.g., power, air and water consumption, instrument calibration checks, frequency of component replacement, predicted mean time between failure events (MTBF), etc.).

TEXT BOX 6.3a: Summary of other performance monitoring parameters.

6.4 Operational Logs for Long-Term Performance Monitoring

Table 6-4 is a suggested format for logging the ballast water management system's engineering performance parameters, first during the Year 1 primary experiments, and then during routine ballasting events. Applicants may propose modified or entirely different formats of their choosing, but must in any case present data tracking of routine engineering performance against criteria established during testing.

Table 6-4. BWMS Operational Log for Long Term Performance Monitoring

Ballast Water Treatment Component (1)	Operational Parameter (2)	Units	Event Type (3)	Number Events (4)	Target Value (5)	Average Value (6)	Maximum Value (7)	Standard Deviation % (8)
Primary Biological Experiment #1								
Primary Biological Experiment #2								
Primary Biological Experiment #3								
Primary Biological Experiment #4								

(1) List each ballast water treatment component that contributes to the overall treatment performance, pre-filters, primary treatment units, secondary treatment units, etc.

(2) For each ballast water treatment component list operational parameters or performance metrics that impact treatment performance. More than one parameter may be listed for each component.

(3) Identify whether the recorded data pertains to ballast (B), deballast (D), or a combine (C) test or routine treatment event.

(4) Record the number of replicate test runs for the primary biological experiments or number of routine treatment events for the first year of operation.

(5) List the target or setpoint value for each operational parameter or metric based on previous biological efficacy tests completed by the applicant, or updated values determined during the first year biological experiments.

(6) Calculate the average value for each operational parameter or metric based on the number of replicate tests run for the primary biological experiments or first year routine treatment events.

(7) Record the maximum value for each operational parameter or metric based on the number of replicate tests run for the primary biological experiments or first year routine treatment events.

(8) Record the standard deviation, expressed as a plus or minus percentage, for each operational parameter or metric based on the number of replicate tests run for the primary biological experiments or first year routine treatment events.

6.5 Maintenance, Repair and Equipment Modification Logs for Long-Term Performance Monitoring

Use Text Box 6.5a to describe the type of information to be included on maintenance and repair logs to document the history of preventive and corrective maintenance on the BWMS.

Note that any changes in the physical configuration or operational procedures, setpoints and engineering parameters must be noted in the quarterly and annual reports.

TEXT BOX 6.5a: Description of maintenance logs.

6.6 Biological Performance Tests, Year 5

It will be mandatory for the applicant to conduct discharge sampling and biological performance testing in Year 5 to confirm the results of the Year 1 primary experiments. It is not necessary to describe the Year 5 tests in the application; however, the Year 4 Annual Report must include the particulars of the planned tests.

It is expected that the Year 5 tests will be similar in type and scope to the Year 1 treatment performance experiments. However, the Coast Guard allows for the possibility that new or improved test methods may be proposed.

Section 7.0 Environmental Compliance

7.1 Description of Routine Operations

Use Text Box 7.1a to provide a detailed description of ballasting procedures, including approximate intake and discharge locations, number and frequency of ballasting operations per year, minimum ballast water hold time in ballast tanks, and the approximate volumes of water used during ballasting (refer to Table 2-1 and Subsection 2.3). One or more narratives of ballast water operations on a typical voyage or typical voyages should be included, with relevant copies of ballast logs or of the Coast Guard's ballast water report forms submitted to the National Ballast Information Clearinghouse (NBIC).

TEXT BOX 7.1a: Ballasting operations information.

7.2 Water Quality and Discharge of Treated Ballast Water

Use Text Box 7.2a to provide a description of chemical and physical water quality characteristics, and depth of the local waters, at both the intake and discharge points for ballast water. This description pertains to the "typical voyages(s)" identified in Section 2. If the ship's route changes in the course of the application review or after acceptance into STEP, the Applicant is expected to provide them to the STEP Review Team. The table should include the ballast water applicable VGP water quality standards.

TEXT BOX 7.2a: Water chemistry data. Refer also to Subsection 5.5 for water characteristics at uptake and discharge locations, if the primary experiments will occur in the same waters as the ship's normal operations do.

Complete Table 7-2, "Environmental Compliance: Water Quality and Discharge, Water Quality", and address all relevant local, state, or Federal clean water regulations for discharge of treated ballast water into the receiving waters. It is expected that, if any chemicals are used or generated by the BWT system or waste streams discharged, the relevant regulations, standards and agencies have been identified. A copy of the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) registration letter (or pending application and experimental use permit) is required. Completing the table is important even if the applicant has deemed that these discharges do not require permits or approvals.

Provide copies of any correspondence the applicant has had with Federal, State or local environmental resource and compliance agencies, in regard to operation of the BWMS and discharge of treated water in their jurisdictions.

If chemicals are added to the ballast water and/or if chemical byproducts are generated as part of the treatment process, use Text Box 7.2b to provide the following data and information (refer to Table 3-4.3):

a. Quantitative data on chemical residuals, including peak concentrations in ballast water, concentrations at discharge, and a time-course of chemical breakdown or dissipation (i.e., concentration versus time). The change in chemical concentration over time needs to be documented, with appended data. For many chemicals, this time-course of breakdown needs to be documented for different waters and temperatures.

b. Appropriate toxicity test data

c. Comparison of concentrations against relevant (e.g., EPA) standards for seawater quality

d. A conclusion as to the fate of the chemicals:

   That they will degrade, e.g., in the tanks, to acceptable levels and pose no environmental threat upon discharge, or

   That the treated ballast water must be conditioned prior to discharge to reduce concentrations of said chemicals to acceptable levels

e. Description of how discharge concentrations will be monitored and reported (refer to Table 6-1)

TEXT BOX 7.2b: Treatment chemicals and/or chemical byproducts residuals and, if necessary, a description of the conditioning of treated ballast water.

Table 7-2. Environmental Compliance: Water Quality and Discharge of Treated Ballast Water

EPA and state regulations and standards	
Contact initiation with local, state, and federal agencies (include correspondence in Appendix C)	
Permits, approvals, etc. from applicable jurisdictions for the discharge of water and effluent (list here and include copies in Appendix C)	

7.3 Storage, Handling, and Exposure to Ballast Water Treatment Chemicals

Use Text Box 7.3a to provide documentation via ships safety management system of how the crew will handle the components of the system, including appropriate levels of personal protective equipment and other safety precautions (consistent with all applicable safety requirements) for the handling of ballast water treatment chemicals or protecting against exposure to the active components of the ballast water treatment process (refer to Subsection 3.8).

TEXT BOX 7.3a: Handling of treatment chemicals.

Use Text Box 7.3b to provide a description of appropriate methods (that meet all applicable local, state, and Federal requirements) for the storage of primary treatment chemicals or chemicals that occur as byproducts of the ballast water treatment process, if applicable (refer to Subsection 3.8).

TEXT BOX 7.3b: Storage of treatment chemicals.

7.4 Management of BWMS Waste Streams

Use Text Box 7.4a to provide identification and characterization of any ballast water treatment system side streams, for example filtered organic material (i.e., remains of organisms), centrifugal concentrate, consumable hardware (e.g.- lamps) and waste or residual chemical, as appropriate.

TEXT BOX 7.4a: Ballast water treatment system waste streams.

Complete Text Box 7.4b. Show the planned methodology to properly manage and dispose of waste.

TEXT BOX 7.4b: Ballast water treatment waste stream disposal.

Complete Table 7-4, identifying all applicable local, state, and Federal requirements, points of contact with resource protection agencies, and required permits as appropriate. Include copies of waste management permits and approvals in Appendix C.

If chemicals are added to any waste stream or if chemical byproducts generated as part of the treatment process occur in a waste stream, the applicant must use Text Box 7.4c to provide adequate data and information as follows:

- Quantitative data on chemical residuals, including peak concentrations in the waste stream, concentrations at discharge, and a time-course of chemical breakdown or dissipation (i.e., concentration versus time)
- Appropriate toxicity test data
- A conclusion as to the fate of the chemicals:
  - That they will degrade to acceptable levels and pose no environmental threat upon discharge, or
  - That the waste stream must be conditioned prior to discharge to reduce concentrations of said chemicals to acceptable levels
- Description of how discharge concentrations will be monitored and reported (ref: Table 6-1)

TEXT BOX 7.4c: Chemicals and chemical byproducts in ballast waste stream(s) and, if necessary, a description of the conditioning of ballast treatment waste streams.

Table 7-4. Regulations and Agency Checklist, BWMS Waste Streams

Contact initiation with local, state, and federal agencies	
Correspondence with applicable agencies appended	
Information on regulatory criteria or standards for any disposed waste, if applicable	
Copies of permits, approvals, etc. from applicable jurisdictions for the discharge of water and effluent obtained and appended	
Documentation on integration of the new equipment and related processes into the vessel safety management system. Should include	

Flag administration attestation per Owner/operator Safety Management Certificate.	

7.5 Biological Issues

The Federal Government needs to assess the potential environmental effects and possible mitigative measures of experimental treatment systems accepted into STEP. Any relevant information the applicant can provide will expedite the Coast Guard's environmental review process. Applicants should be familiar with the potential for their BWMS to cause adverse environmental effects, and with the options for prevention or mitigation of such effects, as a result of the research and development work on the treatment technology.

Provision of this information is helpful, but not required.

If available, use Text Box 7.5a to provide the results of a literature search of published and peer-reviewed articles or 'gray literature' reports that address the potential environmental effects of the proposed BWMS, or its component treatment processes, on the marine, estuarine and freshwater environments as appropriate, and preventive and mitigative measures (attach full cited documents and geographic information on potentially affected areas, in Appendix C).

TEXT BOX 7.5a: Environmental effects.

Use Text Box 7.5b to provide detailed descriptions of appropriate measures to mitigate any impacts identified.

TEXT BOX 7.5b: Preventive and mitigative measures.