

# **Test Plan for the Performance Evaluation of the BIO-SEA Ballast Water Treatment System**



**Maritime Environmental Resource Center**

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## 1. Background and Objectives of MERC Technology Evaluations

The Maritime Environmental Resource Center (MERC) is a State of Maryland initiative that provides test facilities, information, and decision tools to address key environmental issues facing the international maritime industry. The Center's primary focus is to evaluate the mechanical and biological efficacy, associated costs, and logistical aspects of ballast water treatment systems and the economic impacts of ballast water regulations and management approaches. A full description of MERC's structure, products, and services can be found at [www.maritime-enviro.org](http://www.maritime-enviro.org).

To address the need for effective, safe, and reliable ballast water treatment systems to prevent the introduction of non-native species, MERC has developed as a partnership between the Maryland Port Administration (MPA), Chesapeake Biological Laboratory/ University of Maryland Center for Environmental Science (CBL/UMCES), U.S. Maritime Administration (MARAD), Smithsonian Environmental Research Center (SERC), University of Maryland (UMD), and Old Dominion University to provide independent performance testing and to help facilitate the transition of new treatment technologies to shipboard implementation and operations.

The following protocols describe how MERC will evaluate the performance characteristics of the BIO-UV filter + UV Ballast Water Treatment Systems (BIO-SEA) through objective and quality assured land-based testing (dockside at a flow rate of 100m<sup>3</sup>/hr). The goal of this specific evaluation is to provide shipping lines, regulators, and flag states with an independent and credible assessment of treatment performance under realistic conditions. Therefore, the data and information on performance characteristics will cover legitimate information that users need and will compare performance against the U.S. Coast Guard regulations for discharge of ballast water and similar International Maritime Organization (IMO) D2 regulatory discharge standards.

MERC does not certify technologies nor guarantee that a treatment will always, or under circumstances other than those used in testing, operate at the levels verified. Treatment systems are not labeled or listed as acceptable or unacceptable but tests and results are in a format consistent with that requested by specific regulations (e.g., IMO D2, G8 and G9) so that can be used to determine compliance by Administrations and classification societies. Sampling and analytical procedures utilized by the MERC team also comply with the US Environmental Protection Agency ETV Protocols (2010) in anticipation of the publication of U.S. Federal Standards under the auspices of the U.S. Coast Guard. Final reports on technology performance will be reviewed by the members of the MERC Advisory Board and provided to BIO-UV and the MERC funding agencies prior to public release. All specific terms of a testing program associated with a particular treatment system, including management of test findings, are outlined in a Participation Agreement executed between the treatment developer and MERC/University of Maryland Center for Environmental Science.

While a goal of MERC is to provide independent G8 (G9)/ETV compliant testing of the performance of ballast water treatment systems, it is ultimately up to individual Administrations to decide if this system meets their requirements for Type Approval Certification.

## 2. Introduction to Technology

The BIO-SEA treatment system combines mechanical 40  $\mu\text{m}$  filtration and parallel-mounted UV reactors equipped with one medium pressure lamp each. The treatment system has been designed to be modular and compact, for treating flow rates ranging from 100 to 2000  $\text{m}^3/\text{h}$ . The BIO-SEA system operates automatically and regulates power consumption depending on water quality. The treatment process includes three steps: (1) filtration upon ballasting, (2) followed by UV treatment upon ballasting, and (3) UV treatment on discharge (filter is bypassed).

## 3. Overview of Test Facilities

To take advantage of the diverse physical, chemical and biological conditions found in the Chesapeake Bay, MERC has developed a Mobile Test Platform. With one installation, a test ballast water treatment system can be evaluated with the same protocols, by the same facility and staff, under varying natural salinities and associated ambient communities, by moving the barge-based test facility to different locations.

The barge is 155' x 50' with a draft of 2' when tanks empty and 5' when tanks full. The Mobile Test Platform has two identical steel 310  $\text{m}^3$  test tanks (with typical internal tank coating) and two identical 60 hp centrifugal pumps, with two eight-inch piping systems for versatility in moving ballast water and for tank filling and discharge. Testing flow rates can vary from a minimum of 100  $\text{m}^3/\text{hr}$  and maximum of 350  $\text{m}^3/\text{hr}$  for each pump and flow pressure of up to 60 psi can be achieved. The test facility is operated by an integrated monitoring and control system for remote control of variable speed drives flow rates and pressure, plus data logging of valve positions, tank levels/volume, flow rate, pressure, sampling system operations, treatment system status, water quality parameters, etc. Finally the barge also has onboard office, laboratory, sampling and storage containers.

## 4. Basic Evaluation Approach

Please note that this Test Plan describes the specifics for the MERC evaluation of the BIO-SEA treatment system. Details on program policies and testing approaches/methodologies can be found in the MERC Quality Management Plan (QMP), Quality Assurance Project Plan (QAPP) and various Standard Operating Procedures (SOPs). This Plan also refers to, and incorporates specifics guidelines and requirements found in:

- International Maritime Organization (2008) Resolution MEPC.174 (58) Guidelines for Approval of Ballast Water Management Systems (G8);
- International Maritime Organization (2008) Resolution MEPC.125 (57) Revised Procedure for Approval of Ballast Water Management Systems that Make Use of Active Substances (G9); and
- ETV Generic Protocols for the Verification of Ballast Water Treatment Technologies, (2010) EPA/600/R-10/146.

The fundamental approach of MERC is to conduct independent, scientifically-sound, rigorous, and quality assured evaluations of ballast water treatment systems using the framework provided in the G8/G9 guidelines and specific methodologies found in ETV protocols. As a general rule, MERC relies on challenging ambient conditions found in the Chesapeake Bay, and typically does not artificially augment test waters organisms in most evaluations, to avoid artifacts and the potential for overestimation of treatment system performance (see Table 1). For example, rapid changes in physical conditions (such as salinity or total suspended solids) as

supplemental organisms are being added to influent ballast water may cause significant mortality, independent of treatment. Nevertheless, in cases where natural challenge conditions fall substantially short of the G8 guidelines and/or ETV protocols, MERC has the ability to augment total suspended solids (TSS), particulate organic carbon (POC) and dissolved organic carbon (DOC) and both phytoplankton and zooplankton. While MERC does not make any guarantees on the precise conditions of challenge water, intake water can be modified to more consistently approach the initial challenge conditions described in the G8 guidelines and ETV protocols during testing. Arizona Test Dust, micronized humate (Micromate) and *Camellia sinensis* (tea) (as described in ETV protocols) can be injected inline during initial filling of control and test tanks to increase TSS, POC and DOC levels. Similarly biological conditions can be augmented with native zooplankton and algae. Details on these procedures are available in MERC SOPs. Finally, it is important to note that G8 MEPC 58/23 ANNEX 4, Part 2, Section 2.3.36 is utilized by MERC as the standard for a valid test trial: “If in any test cycle the average discharge results from the control water is a concentration less than or equal to 10 times the values in regulation D-2.1, the test cycle is invalid”.

Table 1. Ranges of various physical and biological parameters in ambient water during the testing season (March/April – October/November) in the Port of Baltimore in comparison to ETV and G8 listed challenge conditions. Port of Baltimore data collected by MERC and various academic and agency studies or monitoring efforts in the general location of the MERC test barge (Patapsco River).

Parameter	ETV <sup>†</sup>	G8 <sup>‡</sup>	Historic Ranges* Port of Baltimore
Temperature (°C)	4 - 35	No Requirement	4 - 28
Salinity (psu)	0 - 36	Two salinities, >10 psu difference	1 - 18
Total Suspended Solids (mg/L)	Min. 24	> 50	1 – 60
Mineral Matter (mg/L)	Min. 20	No Requirement	< 1 – 50
Particulate Organic Carbon (mg/L)	Min. 4	> 5	<1 – 6.0
Dissolved Organic Carbon (mg/L)	Min. 6	> 5	2 – 10
Live Organisms > 50 µm/m <sup>3</sup>	Min. 100,000	> 100,000	10,000 – 500,000
Live Organisms 10 - 50 µm/ml	Min. 1,000	> 1,000	500 – 15,000
Culturable Bacteria cfu/ml	Min 1,000	>1,000	10,000 – 10,000,000

<sup>†</sup>ETV Generic Protocol for the Verification of Ballast Water Treatment Technologies, 2010. <sup>‡</sup>IMO Guidelines for the Approval of Ballast Water Management Systems (G8), October 2008, Annex 4 Resolution MEPC.174 (58). \*TSS, POC and DOC (2004-2007) MD DNR Chesapeake Bay Water Quality database: [www.chesapeakebay.net/data\\_waterquality.aspx](http://www.chesapeakebay.net/data_waterquality.aspx). Zooplankton (1998–2002) and phytoplankton (2004-2007) Chesapeake Bay Program: [www.chesapeakebay.net/data\\_plankton.aspx](http://www.chesapeakebay.net/data_plankton.aspx). Bacteria (1998-present) Cowell and Huq, University of Maryland; Louis et al. 2003, AEM 69:2773-2785. <sup>‡‡</sup> The dissolved and particulate minimum concentrations are within the salinity range of <3 – 32 psu. The minimum concentrations for the dissolved and particulate parameters at salinities >32 psu are > 1mg/L.

Prior to any formal testing, one mechanical commissioning run of the BIO-SEA system will be provided to assure appropriate treatment operations. This run will identify and correct initial mechanical or operating issues. Any data collected during the commissioning run will only be used for test preparations and will not be provided in the final report.

After treatment system commissioning has been completed and accepted by BIO-UV, MERC will conduct two types of trials to evaluate the BIO-SEA treatment system in Baltimore, MD: (a) operations and maintenance (O&M) verification and (b) logical efficacy evaluations.

(a) O&M Testing - Prior to initiating any biological testing of treatment performance, MERC will evaluate the operational and mechanical reliability of the BIO-SEA system options as it treats at least 1,200 m<sup>3</sup> of water from the Port of Baltimore, at 100 m<sup>3</sup>/hr (two days, 6 hours of operations each). Since an active substance is not used in the BIO-SEA system, water will be treated (passed through the filter + UV system), then discharged directly overboard using a sea-to-sea pumping mode. MERC will monitor power consumption, the various other O&M parameters listed in the ETV protocols, and failures (if any).

(b) Biological Efficacy - MERC will conduct series of up to six biological efficacy tests in Baltimore. See descriptions below and in MERC QAPP and SOPs. The first three test trials will involve 5-day hold times and TSS, POC and DOC levels of approximately 50 mg/L, 5 to 6 mg/L and 4 to 5 mg/L, respectively, in challenge water by adding Arizona Test Dust, micronized humate, and instant ice tea (as described in MERC SOPs and ETV Protocols). The second set of three BE test trials will be determined based on results of first three trials and may include either trials with increasing TSS levels of approximately 60 mg/L, 70 mg/l and 80 mg/l or decreasing hold times of 4 days, 3 days and 2 days (see Table 3).

## **5. Summary of Land-Based Testing and Sampling Design**

The simulated ballast system of the MERC Mobile Test Platform has been designed to allow for water to be split equally, and delivered simultaneously, to a “control” (untreated) tank and a “treated” tank (passing first through the treatment system). BIO-UV has selected a flow rate of 100m<sup>3</sup>/hr for this set of evaluations. The mimic ballast tanks to be used for the five-day holding times are identical in size (310 m<sup>3</sup>) and structure. Each tank will be filled to a minimum of 150 m<sup>3</sup> for each test trial. Water entering the control and test tanks is handled as close to identically as possible, (e.g., passing through similar pumps and piping), aside from treated water passing through the BIO-SEA treatment system. Detailed drawings of the MERC Mobile Test Platform and ballast system can be found in SOPs.

Statistically-validated (Miller et al., 2011), continuous, time-integrated samples will be taken for each of the following: (A) uptake water for both control and treated conditions, (B) control and treated water upon discharge after a five-day holding time. Sample volumes and details of the physical, chemical, and biological analyses for each sample are described below.

All samples collected to quantify live organisms or water quality will be taken by inline sampling of water during the entire filling or discharge of water from the tanks through sample ports located on appropriate filling or discharge pipes. All sample ports include a valve and sample tube with a 90° bend towards the direction of flow, placed in the center of the piping system (based on the design developed and validated by the US Naval Research Laboratory, Key West Florida, see ETV protocols).

Water for biological examination is split for sampling the >50µm size fraction (nominally zooplankton), and the other fractions (10-50 µm size fraction, bacteria, water quality, etc.). See Table 2 for the list of samples to be collected, with corresponding volumes and purpose. At the

completion of each trial, test tanks are thoroughly cleaned by pressure washer, and piping is flushed with fresh municipal water, prior to conducting the subsequent trial. See SOPs for additional details on test operations and sampling.

Table 2. MERC will be collecting a variety of data on physical, chemical, biological, and toxicological parameters during this evaluation. In some case, values will be determined directly for the water using in

Parameter	Sample ID	Purpose	MERC Sample Volume/Time points
Total Suspended Solids (TSS) mg/L	Uptake Treated	Quantify challenge water	3-5 L at initial, mid and final time points
Particulate Organic Matter (as POC) mg/L	Uptake Treated	Quantify challenge water	3-5 L at initial, mid and final time points
Dissolved Organic Matter (as DOC) mg/L	Uptake Treated	Quantify challenge water	3-5 L at initial, mid and final time points
Chlorophyll-a $\mu\text{g/L}$	a. Uptake Control b. Uptake Treated c. Discharge Control d. Discharge Treated	Quantify challenge water	75 L time-integrated sample with 2-L subsamples
Viable Organisms $> 50 \mu\text{m} / \text{m}^3$	a. Uptake Control b. Uptake Treated c. Discharge Control d. Discharge Treated	Quantify live organisms $> 50 \mu\text{m}$	3-10 $\text{m}^3$ time-integrated samples
Viable Organisms 10-50 $\mu\text{m} / \text{ml}$	a. Uptake Control b. Uptake Treated c. Discharge Control d. Discharge Treated	Quantify live organisms 10 – 50 $\mu\text{m}$	75 L time-integrated sample with 2-L subsamples
Bacteria cfu/ml	a. Uptake Control b. Uptake Treated c. Discharge Control d. Discharge Treated	Quantify regulated indicator pathogens and total heterotrophic bacteria	75 L time-integrated sample with 2-L subsamples

situ sensors or instruments. Table 2 describes samples collected and analyzed.

*Viable Organisms >50 $\mu\text{m}$  in size:*

The sampling system consists of two sets of paired canisters, each designed to accommodate a 35  $\mu\text{m}$  (50  $\mu\text{m}$  diagonally) mesh plankton net used to collect the  $>50 \mu\text{m}$  size fraction. One pair handles water from the treated ballast tank and the other pair handles water from the control tank. The paired sampling canister/net arrangement allows for the residual from the cod-end of one net from each pair to be processed for examination while filtration continues via the other net, thereby avoiding clogging. In this way unimpaired filtration back and forth between each pair of nets continues until a total of 3 to 7  $\text{m}^3$  has been processed from each of the treated and control ballast tanks respectively. The sampling canisters are designed to allow complete immersion of each net during the filtration process, thereby minimizing trauma to filtered organisms. At the end of each trial (after five-day hold times), the control and treated ballast tanks are drained and processed as described above, with treated water first passing through the BIO-SEA system for additional UV irradiation (but not filtration) prior to sampling.

The proportion and total concentration of live versus dead organisms  $> 50 \mu\text{m}$  will be determined using standard movement and response to stimuli techniques and this live/dead

analysis will take place within two hours of collecting the individual samples. Three m<sup>3</sup> is the volume collected for control water upon filling and discharge from test tanks (high numbers of live organisms) and 7 m<sup>3</sup> is collected for treated water on discharge after five-day hold times (presumably very few live organisms). Depending on concentrations, quantification of organisms > 50 µm in initial samples (upon ballasting) and control samples may require analysis of sub-samples and extrapolation to the entire 3 m<sup>3</sup>. The > 50 µm samples will then also be fixed with buffered, 10% formalin in 500ml Nalgene bottles and shipped to the Smithsonian Environmental Research Center (SERC) for additional taxonomic evaluation. Total counts and general taxonomic classification will be conducted under a dissecting microscope at 25X, except for some taxa, which will be removed and identified using a compound microscope. Larval forms of invertebrates will be identified to higher taxonomic levels such as order (e.g., Decapoda) suborder (e.g., Balanomorpha) or class (e.g., Bivalvia). Adults will be identified to species in most cases.

*Viable Organism 10 - 50 µm in size:*

A 75 L integrated sample will be collected as an unfiltered split sample in parallel with the > 50 µm fraction. This sample will be the source water for all other analyses including the 10-50 µm fraction. Two liters from this well-mixed, integrated sample will be subject to three distinct analyses and counts (described briefly below and in detail in SOPs).

All of these live unfiltered samples will be processed or examined within three hours of collection on the MERC Mobile Test Platform or nearby partner laboratories. All preserved samples are also transported to MERC partner laboratories within three hours, for further analyses and taxonomic identification.

One 250 ml sub-sample will be stained using a combination of CMFDA (5-chloromethylfluorescein diacetate) and FDA (fluorescein diacetate) as a selective live/viable indicator. Samples stained with CMFDA+FDA, are incubated and observed on a Sedgewick Rafter slide using a Olympus IX-51 inverted phase/fluorescent microscope. Cells are scored as live when showing strong fluorescence signature under excitation (some cells also show motility). This approach has been validated for use in the Chesapeake Bay (Steinberg et al., 2011) and provides the data for comparison to discharge standards.

As supporting information, two other sub-samples are analyzed. A second 250 ml is collected from the integrated 75 L sample and fixed with standard Lugol's solution in amber Nalgene bottles to estimate total cell abundances (but not live versus dead) and for species identification under an inverted compound microscope using grid settlement columns and phase contrast lighting. A third sub-sample is filtered (Whatman GF/F 0.7 µm pore, 47 mm diameter membrane) and frozen (-20°C) until analysis of total active chlorophyll-a by the CBL/UMCES Nutrient Analytical Services Laboratory using US EPA Methods 445.0 for extractive/fluorometric techniques.

*Viable Bacteria and Indicator Pathogens:*

An unfiltered 1 L sample of water sub-sampled from an integrated 75 L sample will be analyzed to determine concentrations of total heterotrophic bacteria and three specific indicator pathogens, *E. coli*, intestinal *Enterococci*, and toxigenic *Vibrio cholera* (described briefly below and in detail in SOPs).

Total heterotrophic bacteria will be enumerated by spread plate method using MA or R2A agar according to *Standard Methods for the Examination of Water and Wastewater* (21<sup>st</sup> edition, 2005). The presence and abundance of *E. coli* and intestinal *Enterococci* is determined



using a commercially available chromogenic substrate method (IDEXX Laboratories, Inc.; Noble et al. 2003) and 10 ml and 100 ml water sample aliquots. Additionally, concentrations of culturable *E. coli* and intestinal *Enterococci* are determined using a standard US EPA 1603 method, namely, membrane filtration on mTEC agar (*E. coli*) (1 ml, 10 ml and 100 ml) and mEA agar (*Enterococcus*) (10 ml and 100 ml). Finally, the abundance of total and toxigenic *V. cholerae* will be determined by filtration and selection on TCBS agar and enumerated using species-specific RNA colony blot (500 µl to 1 ml) and *ctxA* DNA colony blot (1-10 ml). Viable toxigenic cells of *V. cholerae* are assayed with a commercial DFA kit specific for serogroup O1 (New Horizons Diagnostics) using monoclonal antibodies tagged with fluorescein isothiocyanate (FITC) (Hasan et al. 1994).

#### *Quantifying Physical Conditions:*

Temperature, salinity, dissolved oxygen, chlorophyll fluorescence, turbidity (NTU) and pH will be measured every 15 minutes during the test trials using two identical multi-parameter probes (calibrated before each trial according to manufactures specification) deployed into both the control and treated tanks. A third hand-held instrument will be used to measure temperature, salinity, and dissolved oxygen of water (and other parameters as required) during uptake or discharge.

Initial inline samples (three replicates, approximately 500 ml - 2 L each) of ballast water during the filling of the control and treated tanks will be collected, filtered, and analyzed for the water quality parameters of particulate organic carbon (POC), dissolved organic carbon (DOC), and total suspended solids (TSS). Mineral Matter will be calculated. Similarly, inline samples of challenge water will also be analyzed for UV transmittance using a UV254 P200 RealTech field spectrophotometer, with subsamples provided to BIO-UV for their own analysis of UV transmittance. See SOPs for details.

#### *Treatment Toxicity:*

Although the BIO-SEA treatment system does not employ an active substance, MERC will conduct at least one set of toxicity tests during the first biological efficacy trial. The testing is designed to meet IMO G9 requirements and uses test methods and species employed by the EPA for Whole Effluent Toxicity (WET) testing of effluents (EPA 2002 and ASTM 2006).

A fish, an invertebrate and a plant (algae) will be used in all ballast discharge tests. Because the Chesapeake Bay is a mesohaline aquatic environment with salinities ranging from 5 to 25 psu, estuarine organisms will be used in these tests. The fish species used in the test will be the sheepshead minnow (*Cyprinodon variegatus*), invertebrate will be a mysid (*Americamysis bahia*; formerly *Mysidopsis bahia*) and the algal species will be *Isochrysis galbana*, all listed as estuarine test species in EPA's Short-term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Marine and Estuarine Organisms (EPA, 2002).

Both acute and chronic data will be generated for each test. A dilution series, using ambient water, will be run for each species. A total of 38 L samples will be collected at the time of discharge from the treated tank. This includes enough water to do all of the test renewals. Test water will be stored in large HDPE containers and held at 4°C in the dark to retain as much of the initial toxicity as possible. All of the tests will be conducted at the University of Maryland Wye Research and Education Center toxicology laboratory and will be initiated within three to four hours of the completion of a specific trial.

Toxicity endpoints will include survival in acute fish and invertebrate tests, survival and growth in chronic fish and invertebrate tests, and population growth in chronic algal tests as required in Section 5.2.4 of the G9 document (IMO, 2008). Tests are designed with a dilution series to allow calculation of daily LC50 (concentration yielding 50% lethality) values from acute and chronic mortality data. In addition, chronic tests will include sufficient treatment replication to allow calculation of NOEC (no observable effect concentration), LOEC (lowest observable effect concentration) and EC25 (percent concentration yielding a 25% effect) values for all toxicity endpoints as required in Section 5.2.5 of the G9 (IMO, 2008). Statistical analyses will be performed using ToxCalc statistical software (TSS, 2006) according to methods from USEPA (2002) and ASTM (2006) guidance documents. A test trial will be considered a failure on the grounds of residual toxicity upon discharge if acute lethality (as indicated by determination of an LC50 of less than 100%) occurs in any test species.

## 6. Test Trials

After the successful commissioning (see SOPs and ETV Protocols), and successful completion of the O&M verification trials (with no more than one mechanical failure, described above), MERC will conduct a maximum of six test trials of the BIO-SEA treatment system to assess its ability to meet IMO D2 standards, under various conditions, in land-based testing during the summer of 2012. For any test that is considered valid (and for which the facility testing system functioned properly and the average discharge from the control water is greater or equal to 10 times D2), an inability to: (a) successfully treat ballast water without interruption and/or (b) to meet D2 discharge standards upon discharge, will be considered a “failure”. Results of tests regarded as failures will be noted and included in the final report. Two failures on the part of the treatment system may result in the termination of testing prior to the maximum of six test trials depending on the nature of the failures. MERC Senior Management will make a final decision on early termination of the tests, in consultation with BIO-UV representatives.

This evaluation will be based on physical and biological characterization of water upon ballasting (uptake of water) and comparisons of organisms in control versus treated water after a five-day, in-tank holding time for the different D2 biological categories. Results will also be presented as concentration of viable organisms per biological category in treated water upon discharge versus D2 standards.

Table 3. A summary of the trials to be conducted, including target TSS and percent UV transmittance levels. TSS and UVT levels listed are only targeted estimates. Precise level will be quantified but can not be guaranteed because of variability in ambient water. Targets for Trials 6 – 8 will be established based on results from Trials 3 – 5. Two options under consideration for trials 6 – 8 are to conduct identical trials but with increasing TSS levels (e.g., 60 mg/L, 70 mg/l and 80 mg/l) or decreasing hold times (4 days, 3 days and 2 days).

Trail #	Treatment	Trial Type	Target TSS	UVT
1	BIO-SEA 40 µm filter + mono-lamp UVs	Commissioning	~ 50 mg/L	~ 70%
2	BIO-SEA 40 µm filter + mono-lamp UVs	O&M	Ambient	Ambient
3	BIO-SEA 40 µm filter + mono-lamp UVs	Biological	~ 50 mg/L	~ 70%
4	BIO-SEA 40 µm filter + mono-lamp UVs	Biological	~ 50 mg/L	~ 70%
5	BIO-SEA 40 µm filter + mono-lamp UVs	Biological	~ 50 mg/L	~ 70%
6	BIO-SEA 40 µm filter + mono-lamp UVs	Biological	TBD	TBD
7	BIO-SEA 40 µm filter + mono-lamp UVs	Biological	TBD	TBD
8	BIO-SEA 40 µm filter + mono-lamp UVs	Biological	TBD	TBD

## 7. Data Analysis

As noted above, continuous time-integrated samples and measures from each tank will be taken. Consequently please note that although certain assays employ replicates or sub-samples during analysis, to avoid pseudo-replication, the unit of replication for statistical analyses is each trial ( $n = 5$  or  $6$ ). We assume that all measures for a single trial provide one estimate of treatment efficacy. Thus, treatment efficacy for any biological parameter is estimated as changes found before and after trial (percent reduction), and as the difference in concentration between treated water and IMO standards. This approach controls for variation due to temporal changes in environmental conditions.

Quality Assurance and Quality Control policies and procedures, data recording processing and storage, and detailed roles and responsibilities can be found in the MERC QMP, QAPP and SOPs.

## 8. Evaluation Schedule (planned dates based on current plan and may vary):

- MERC Test Plan for BIO-SEA finalized and Evaluation Agreement signed by June 15, 2012
- Delivery and installation of BIO-SEA system, July 5 – 13, 2012
- MERC evaluation of BIO-SEA system in Baltimore MD initiated by July 16, 2012
- MERC will complete sample analysis and compile data from the evolution by November 2012
- MERC will distribute a draft report on the performance of the BIO-SEA system for review by the MERC Advisory Board and BIO-UV representatives by December 2012
- MERC will submit a final report to MPA, MARAD and BIO-UV by January 2013

## 9. References

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