Land-Based Evaluations of the Severn Trent De Nora BalPure™ BP-1000 Ballast Water Management System

Maritime Environmental Resource Center

June 2010

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UMCES Technical Report Series: Ref. No. [UMCES]CBL 10-015

Notice

The objective of this Maritime Environmental Resource Center (MERC) evaluation was to provide shipping lines, classification societies, regulators, and flag states with an independent and credible assessment of treatment performance under realistic conditions. Therefore, the ballast water treatment system was tested in accordance with the International Maritime Organization (IMO) International Convention for the Control and Management of Ships' Ballast Water and Sediment (2004), Resolution MEPC.174(58) *Guidelines for Approval of Ballast Water Management Systems (G8)* and Resolution MEPC.169(57) *Procedure for Approval of Ballast Water Management Systems That Make Use of Active Substances (G9)*. The evaluation was conducted under specific, predetermined, agreed-upon protocols, criteria, and quality assurance procedures to assess the treatment system's performance.

MERC does not label or list technologies as acceptable or unacceptable but will present the results in an objective way that can be used to determine regulatory compliance by appropriate administrations, agencies or certification societies. Subsequent data on the technology's performance characteristics is presented to allow for comparison with the IMO Convention discharge standards, Regulation D-2, *Ballast Water Performance Standard*.

MERC and the MERC Advisory Board do not provide certification for technologies, or certify that a technology will always operate as demonstrated. Additionally, no expressed or implied guarantee is provided as to the performance of the technology, or that a technology will always operate at the levels verified. MERC does guarantee the levels verified during the evaluation under the conditions, circumstances, and operations encountered as fully independent and credible.

This report has been reviewed by the MERC Advisory Board and provided to Severn Trent De Nora and MERC funding agencies prior to public release. Mention of trade names or commercial products does not constitute endorsement or recommendation by MERC.

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Note: Detailed Test Plan, Protocols and Standard Operating Procedures (SOPs) can be downloaded from www.maritime-enviro.org.

1. MERC Background and Objectives

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.

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), and University of Maryland (UMD) to provide independent performance testing and to help facilitate the transition of new treatment technologies to shipboard implementation and operations.

This report describes the MERC evaluation of the Severn Trent De Nora BalPure™ BP-1000 Ballast Water Management System through objective and quality assured land-based testing (dockside at a flow rate of 200m^3/hr). The goal of this evaluation was to provide shipping lines, regulators, classification societies, and flag states with an independent and credible assessment of treatment performance under realistic conditions. Therefore, the data and information on performance characteristics covers legitimate information to meet the evaluation's objective, and performance is presented in a way to allow for comparison against the International Maritime Organization (IMO) International Convention for the Control and Management of Ships' Ballast Water and Sediments (2004), Regulation D-2 *Ballast Water Performance Standard*.

2. Description of the Severn Trent De Nora (STDN) Ballast Water Management System

The STDN Ballast Water Treatment System (BWTS) applies established chlorination technology to oxidize and disinfect aquatic invasive species. The BALPURE® BWTS is an electrolytic process for the on-site generation of dilute hypochlorite on demand without storage during the ballasting operation and the neutralization of residual oxidants during the de-ballasting operation. Sodium hypochlorite is generated by means of electrolysis of seawater. In this process, seawater flows through the electrolytic cells in a ratio of 1 part for every 100 parts of ballast water. The system can operate effectively in brackish water to 15 PSU. Because the amount of seawater required to effectively treat ballast water is small, even fresh water ballast can be treated by BALPURE® BWT with supplemental salt or stored seawater in designated ballast tanks such as aft peak tanks. The current passing through the seawater causes the salt (NaCl) and water (H_2O) to form sodium hypochlorite (NaOCl) and hydrogen (H_2) as a secondary by-product. Hypochlorite solution and Hydrogen are produced and separated immediately upon exit from the electrolytic cells. The weak hypochlorite solution (1 g/L) is injected back into the ballast stream. Hydrogen that is separated from the hypochlorite is immediately diluted to less than 1% hydrogen by forced air blowers and discharged to a safe location. The hypochlorite is

generated automatically on demand and is matched to the ballast flow rate and the oxidant demand of the ballast water. Initial oxidant concentration is dosed at 1.5 times the TOC concentration, which remains effective for several days. To minimize potential regrowth of (micro) organisms a background level of chlorine is maintained (1 mg/L) until discharge. To eliminate the release of chlorine, sodium sulfite or bisulfite is added prior to discharge.

For this land-based test, the water is filtered through a 40 micron BallastSafe™ BSFc Automatic Electric Filter, Model BSFc-V-1.6 prior to treatment. The sintered stainless steel screen technology enables it to remove zooplankton. BallastSafe's filter features continuous cleaning of large volumes of dirt during ballasting without interruption, and a reversible screw system for smooth, reliable and rapid cleaning of the entire screen surface.

All test facility equipment and instrumentation was operated by MERC personnel. The ballast water treatment system was operated by STDN.

3. Summary of IMO Standards

This evaluation was designed to determine if the BalPure® BWMS could meet IMO D2 standards in accordance with both the IMO *Guidelines for Approval of Ballast Water Management Systems (G8)* and the *Procedure for Approval of Ballast Water Management Systems that make use of Active Substances (G9)*. The IMO Convention performance standard states that ships must discharge:

1) Less than 10 viable organisms per m^3, greater than or equal to 50 μm in minimum dimension;
2) Less than 10 viable organisms per ml, less than 50 μm in minimum dimension and greater than or equal to 10 μm in minimum dimension and
3) Less than the following concentrations of indicator microbes, as a human health standard:
    1. Toxigenic *Vibrio cholerae* (serogroups O1 and O139), less than 1 colony forming unit (cfu) per 100 ml
    2. *Escherichia coli,* less than 250 cfu per 100 ml;
    3. Intestinal Enterococci, less than 100 cfu per 100 ml.

4. Summary of Test Protocols

The following is a brief summary of the testing approach and methods. For complete details on protocols, data management, and quality control / quality assurance procedures for this MERC evaluation, please refer to the *Test Plan for the Performance Evaluation of the Severn Trent De Nora BalPure™ BP-1000 Ballast Water Management System* (August 2009), available for download at www.maritime-enviro.org.

The protocols described below are based upon the IMO G8/G9 guidelines and the U.S. Coast Guard supported ETV protocols under development. Any deviation from IMO G8/G9 guidelines or draft ETV protocols were explained and justified in the Test Plan. MERC evaluated the biological efficacy of the STDN ballast water treatment system onboard the U.S. Maritime Administration (MARAD) Ro-Ro vessel *MV Cape Washington* while docked in the Port of Baltimore. The ballast system on *MV Cape Washington* was modified to allow for water at a flow rate of 400m^3/hr to be split equally at flow rates of 200 m^3/hr. Just before this split, challenge condition concentrations of total suspended solids (TSS) and particulate organic carbon (POC) were augmented by injecting a concentrated slurry of Arizona test dust and humic

acid (developed and validated by the Naval Research Laboratory, Key West, Florida). The water was then delivered simultaneously to either a "control" (untreated) ballast tank or a "treated" (passing first through the STDN system) ballast tank. These two tanks on the *MV Cape Washington* were used for the required holding time of five days and were essentially identical in size and structure. Each tank was filled to approximately 250 m^3 for each test trial.

Temperature, salinity, dissolved oxygen, chlorophyll fluorescence, turbidity and pH were measured every 15 minutes during the test trials by two identical multi-parameter probes placed, one each, into the control and test tanks. Initial inline samples of ballast water during the filling of the control and test tanks were collected, filtered, and analyzed (using USEPA methods) for the water quality parameters of particulate organic carbon (POC), dissolved organic carbon (DOC) and total suspended solids (TSS) by the CBL/UMCES Nutrient Analytical Services Laboratory (NASL).

A total of 10 identical 1.1 m^3 conical bottom mesocosms were also used for controlled sampling during each trial. Using the mesocosms, five sequential, time-integrated, continuous samples were taken during: (A) initial filling of tanks, just prior to the split of control and treated water (T0 Control), (B) initial filling of test tank, just downstream of the BALPURE system during filling of test tank (T0 Treated), (C) during discharge of control water after a five-day holding time (TF Control), and (D) during discharge of treated water after a five-day holding time (TF Treated).

Immediately after filling, 1.0 m^3 of water in each mesocosm was filtered through a 35 μm plankton net to concentrate the zooplankton for qualitative and quantitative analyses under a dissecting microscope. The proportion and total concentration of live versus dead organisms was determined using standard movement and response-to-stimuli techniques within one hour of collecting the individual samples. Zooplankton samples were also fixed and returned to the laboratory for additional taxonomic evaluations.

Ten liters of well-mixed, but unfiltered, water from each mesocosm were also collected immediately after filling, to determine concentrations of organisms in the 10 to 50 micron size class using four distinct methods: (A) One sub-sample was fixed with standard Lugol's solution to determine total cell abundances under an inverted compound microscope using grid settlement columns and phase contrast lighting. (B) A second sub-sample was stained using CMFDA (5-chloromethylfluorescein diacetate) as a selective live/viable indicator. Stained sub-samples were incubated and observed on a Sedgewick Rafter slide using a Leitz Laborlux S modified for epifluorescence. (C) A third sub-sample was filtered and frozen until analysis of total and active chlorophyll-a by the NASL. (D) Finally, a fourth sub-sample was used to determine chlorophyll-a levels after allowed to regrow under favorable conditions. An increase in chlorophyll, or positive regrowth, indicates that viable phytoplankton were in the samples, whereas chlorophyll levels at or below detection limits of the laboratory analytical method suggests that there was no viable phytoplankton.

Additional subsamples of unfiltered water were also collected from each mesocosm to determine concentrations of total heterotrophic bacteria and three specific indicator pathogens, *E. coli*, intestinal *Enterococci*, and toxigenic *Vibrio cholerae*. Total heterotrophic bacteria were enumerated by spread plate method using NWRI agar. The presence and abundance of intestinal *Enterococci* was determined using a commercially available chromogenic substrate method. Culturable *E. coli* concentrations were determined using a standard USEPA method: membrane filtration on modified mTEC agar. Abundances of total and toxigenic *V. cholerae* were calculated by filtration and selection on TCBS agar and enumerated using a species-specific

RNA colony blot and *ctxA* DNA colony blot hybridization. Viable toxigenic *V. cholerae* was assayed with a commercial DFA kit specific for serogroup O1 using monoclonal antibodies tagged with fluorescein isothiocyanate.

To evaluate the toxicity of treated water at the completion of each trial, samples from each mesocosm were collected and tested for acute and chronic toxicity, and for total residual chlorine. Filter "backflush" (retenate/filtrate during initial treatment of water) was also tested for total residual chlorine. The toxicity protocols and species used were consistent with the USEPA methods for Whole Effluent Toxicity (WET). The algal species tested was *Isochrysis galbana,* the fish species was the Sheepshead minnow *(Cyprinodon variegatus)* while the invertebrate species was the Mysid shrimp *(Americamysis bahia).* A chlorine concentration in samples was analyzed immediately upon collection to avoid potential loss of oxidant with time. The *Standard Methods for the Examination of Water and Wastewater Low-Level Amperometric Titration* method 4500-Cl D and DPD Colorimetric method 4500-Cl G were used to measure Total Residual Oxidants (TRO) and Total Residual Chlorine (TRC). A Fischer and Porter amperometric titrator was also used for amperometric measurements.

5. Summary of Results

Biological Performance - The Severn Trent De Nora BALPURE Ballast Water Management System dramatically reduced the numbers of live organisms in ballast water during MERC land-based testing in the Port of Baltimore. For all biological categories, the treatment system consistently met IMO D2 discharge standards.

Discharge Toxicity - Treated ballast water upon discharge was not chronically toxic to either mysid shrimp or sheepshead minnows for any of the trials conducted. All five of these samples, however, were chronically toxic to the marine algal species *Isochrysis galbana*. Additional testing of several dilutions of the treated ballast water was conducted on the last three samples (STDN-03 through -05). EC_{50}'s, measured as a reduction in growth, were found at 22%, 42% and 53% for STDN 03, STDN-04 and STDN-05 respectively.

Additional toxicity testing has revealed that under certain circumstances dechlorinated estuarine water remains toxic to some types of algae. The continued toxicity of dechlorinated water is species specific with several golden brown algal species (*Isochrysis galbana* and *Pavlova lutheri*) exhibiting decreased growth after TRO is at or below detection (DPD method). In contrast, similarly treated water was non toxic to the diatom *Phaeodactylum tricornutum*. Toxicity was found in samples that were chlorinated by either electrochlorination or sodium hypochlorite addition; and aged for five days.

Toxicity in high initial TRO samples was found after dechlorination with one of two sulfur-based reducing compounds, sodium thiosulfate or sodium bisulfite. Neither of these reducing compounds alone was found to be toxic at relevant concentrations with NOECs of 100 and 200 mg/l for sodium thiosulfate and sodium bisulfite, respectively. Additional algal toxicity tests are being conducted to investigate the cause, as well as the extent, of toxicity caused by dechlorinated water. Results of this investigation are being submitted to a peer-reviewed journal for publication.

Chemical Analysis of Discharge – Detailed chemical analyses of various consitients (e.g., chloroform, bromoform, trichloroacetic acid, etc.) of treated water upon discharge were conducted by Analytical Laboratory Services, Inc. (www.analyticallab.com). Summary of reuslts can be found in Appendix B.

Mechanical Performance - The BALPURE system itself experienced no mechanical failures during formal test trials.

QA/QC - Finally, the MERC Quality Control and Quality Assurance plan was completed as described in the Test Plan. In summary, there were no adverse findings or problems requiring corrective action in any of the audits. The testing, sampling, analyses and data handling for this evaluation met or exceeded MERC test requirements.

* Additional datasets (e.g., in tank values for temperature, salinity, dissolved oxygen, etc., every 15 minutes during hold time) are available upon request.

6. Results Trial 1 (STDN-CAL): 15-20 October 2009

Physical Parameters

	TSS mg/l		**DOC** mg/l		**POC** mg/l	
T0 Initial	**Ave**	**StDev**	**Ave**	**StDev**	**Ave**	**StDev**
Conditions	20.6	4.1	3.96	0.31	1.997	0.271

	Temp ^{o}C		**Salinity** psu		**Dis. Oxygen** mg/l		**Turbidity** NTU	
	Ave	**StDev**	**Ave**	**StDev**	**Ave**	**StDev**	**Ave**	**StDev**
T0 Control	16.97	0.02	13.26	0.01	7.11	0.06	8.16	0.86
T0 Treated	17.10	0.01	13.21	0.01	7.74	0.09	9.75	0.63
TF Control	16.24	0.02	13.57	0.00	7.09	0.17	1.21	0.09
TF Treated	16.27	0.01	13.36	0.00	8.31	0.01	4.20	0.14

Live Organisms > 50 μm

	T0 Ave #/m^3	**T0 StDev** #/m^3	**TF Ave** #/m^3	**TF StDev** #/m^3
Control	60,000	11,250	72,000	3,000
Treated	ND	ND	1.4	1.7

***TF Control**	***TF Treated**
Spionid polychaete	Rotifera
Copepod nauplii	Spionid polychaete
Rotifera	
Calanoida (*Acartia* sp.)	
Cirripedia nauplii	
Turbellaria	

* Taxa listed in order of abundance.

Live Organisms 10 - 50 μm

Vital Stain Results - It is important to note that the specific approach used underestimates the abundances of live organisms in this size class because it has been found that not all live organisms reliably take up the CMFDA stain.

	T0 Ave #/ml	**T0 StDev #/ml**	**TF Ave #/ml**	**TF StDev #/ml**
Control	258	50.6	4	2.4
Treatment	0	0	0	0

Dominant species	**Type**	**Other**
Skeletonema costatum	Diatom	Chain-forming
Thalassiosira sp.	Diatom	Chain-forming
Oxyrrhis marina	Dinoflagellate	

Active Chlorophyll-a - Chlorophyll is used as ancillary data and as a general presence/absence indicator of viable photosynthetic organism (MDL = 0.56 µg/l).

	T0 Ave µg/l	**T0 StDev** µg/l	**TF Ave** µg/l	**TF StDev** µg/l
Control	6.27	0.47	0.88	0.05
Treated	0.56	0.00	0.56	0.00
Regrowth Control			2.61	1.59
Regrowth Treated			0.56	0.00

Live Microbes (cfu = colony forming units)

E. coli	**T0 Ave** cfu/100ml	**T0 StDev** cfu/100ml	**TF Ave** cfu/100ml	**TF StDev** cfu/100ml
Control	13.6	4.93	0.2	0.45
Treated	0	0	0	0

Enterococci	**T0 Ave** cfu/100ml	**T0 StDev** cfu/100ml	**TF Ave** cfu/100ml	**TF StDev** cfu/100ml
Control	11.28	3.05	12.08	5.16
Treated	0	0	0	0

V. cholerae – No detectable culturable toxigenic Vibrio cholerae were detected in any samples during any of the trials.

Heterotrophic Bacteria	**T0 Ave** cfu/100ml	**T0 StDev** cfu/100ml	**TF Ave** cfu/100ml	**TF StDev** cfu/100ml
Control	547	93.24	613	237.51
Treated	0.9	0.78	1.2	2.45

Discharge Toxicity

Residual Chlorine Analysis - Levels of residual chlorine (or total residual oxidants - TRO) were at or below the method detection limit (MDL) of 0.02 ppm in all TF treated samples.

Acute Toxicity - There were no statistically significant survival effects in any test for either acutely tested species (Mysid shrimp or Sheepshead minnows).

Chronic Toxicity - Results showed that ballast water discharge was not chronically toxic to either mysids or sheepshead minnows. All samples, however, were chronically toxic to the marine algal species *Isochrysis galbana* with a statistically significant inhibition in growth in treated ballast discharge compared to controls (see details under Summary of Results on page 4).

7. Results Trial 2 (STDN-01): 22 – 27 October 2009

Physical Parameters

	TSS mg/l		**DOC** mg/l		**POC** mg/l	
T0 Initial	**Ave**	**StDev**	**Ave**	**StDev**	**Ave**	**StDev**
Conditions	28.7	5.1	3.98	0.14	2.912	0.163

	Temp ^{o}C		**Salinity** psu		**Dis. Oxygen** mg/l		**Turbidity** NTU	
	Ave	**StDev**	**Ave**	**StDev**	**Ave**	**StDev**	**Ave**	**StDev**
T0 Control	16.00	0.00	12.14	0.01	8.48	0.04	9.70	0.85
T0 Treated	16.02	0.00	12.17	0.01	8.59	0.05	8.92	0.64
TF Control	15.86	0.01	12.14	0.01	7.34	0.04	1.99	0.12
TF Treated	15.85	0.00	12.23	0.00	8.63	0.02	3.46	0.10

Live Organisms > 50 μm

	T0 Ave #/m^3	**T0 StDev** #/m^3	**TF Ave** #/m^3	**TF StDev** #/m^3
Control	70,000	5,625	30,000	1,500
Treated	No data		0.2	0.4

***TF Control**	***TF Treated**
Copepoda nauplii	Harpacticoida
Spionid polychaete	
Calanoida (*Acartia* sp.)	
Cyclopoida (*Oithona* sp.)	
Harpacticoida	
Nematoda	

* Taxa listed in order of abundance.

Live Organisms 10 - 50 μm

Vital Stain Results - It is important to note that the specific approach used underestimates the abundances of live organisms in this size class because it has been found that not all live organisms reliably take up the CMFDA stain.

	T0 Ave #/ml	**T0 StDev #/ml**	**TF Ave #/ml**	**TF StDev #/ml**
Control	267	14.8	38	25.6
Treatment	0	0	0	0

Lugol's Total Counts - This approach commonly overestimates the abundances of live organisms in this size class because all individuals in fixed samples are counted unless obviously dead (significant damage to organism). Lugol's staining alone does not distinguish live from dead cells.

		T0 Ave #/ml	**T0 StDev** #/ml	**TF Ave** #/ml	**TF StDev** #/ml
Control	Diatom	980		90	
		1,133		60	
Treated	Diatom	758		104	15
	Dinoflagellate	250		19	12

Note: 2,300/ml = Average counts for T-Final-Treated, for picoplankton <10µm.

Dominant species	**Type**	**Other**
Skeletonema costatum	Diatom	Chain-forming
Thalassiosira sp.	Diatom	Chain-forming
Oxyrrhis marina	Dinoflagellate	

Active Chlorophyll-a - Chlorophyll is used as ancillary data and as a general presence/absence indicator of viable photosynthetic organisms (MDL = 0.56 µg).

	T0 Ave µg/l	**T0 StDev** µg/l	***TF Ave** µg/l	***TF StDev** µg/l
Control	4.20	0.56	1.39	0.27
Treated	0.56	0.00	0.56	0.00
Regrowth Control			38.23	22.23
Regrowth Treated			0.56	0.00

Live Microbes (cfu = colony forming units)

E. coli	**T0 Ave** cfu/100ml	**T0 StDev** cfu/100ml	**TF Ave** cfu/100ml	**TF StDev** cfu/100ml
Control	30.8	16.29	6.2	3.63
Treated	0	0	0	0

Enterococci	**T0 Ave** cfu/100ml	**T0 StDev** cfu/100ml	**TF Ave** cfu/100ml	**TF StDev** cfu/100ml
Control	31.65	6.38	15.54	1.31
Treated	0	0	0	0

V. cholerae – No detectable culturable toxigenic Vibrio cholerae were detected in any samples during any of the trials.

Heterotrophic Bacteria	**T0 Ave** cfu/100ml	**T0 StDev** cfu/100ml	**TF Ave** cfu/100ml	**TF StDev** cfu/100ml
Control	517	495.26	144.9	81.53
Treated	0	0	0	0

Discharge Toxicity

Residual Chlorine Analysis - Levels of residual chlorine (or total residual oxidants - TRO) were at or below the method detection limit (MDL) of 0.02 ppm in all TF treated samples.

Acute Toxicity - There were no statistically significant survival effects in any test for either acutely tested species (Mysid shrimp or Sheepshead minnows).

Chronic Toxicity - Results showed that ballast water discharge was not chronically toxic to either mysids or sheepshead minnows. All samples, however, were chronically toxic to the marine algal species *Isochrysis galbana* with a statistically significant inhibition in growth in treated ballast discharge compared to controls (see details under Summary of Results on page 4).

8. Results Trial 3 (STDN-02): 29 October – 3 November 2009

Physical Parameters

	TSS mg/l		**DOC** mg/l		**POC** mg/l	
T0 Initial	**Ave**	**StDev**	**Ave**	**StDev**	**Ave**	**StDev**
Conditions	37.0	3.3	3.84	0.24	4.242	0.505

	Temp ^{o}C		**Salinity** psu		**Dis. Oxygen** mg/l		**Turbidity** NTU	
	Ave	**StDev**	**Ave**	**StDev**	**Ave**	**StDev**	**Ave**	**StDev**
T0 Control	15.79	0.02	10.68	0.01	7.69	0.30	6.07	0.91
T0 Treated	15.83	0.01	10.64	0.01	8.66	0.07	7.22	0.39
TF Control	15.69	0.00	10.67	0.00	6.94	0.03	1.69	0.04
TF Treated	15.67	0.01	10.73	0.00	8.61	0.01	4.12	0.12

Live Organisms > 50 μm

	T0 Ave #/m^3	**T0 StDev** #/m^3	**TF Ave** #/m^3	**TF StDev** #/m^3
Control	79,500	3,750	49,500	4,500
Treated	No data		0.4	0.9

***TF Control**	***TF Treated**
Copepoda nauplii	Harpacticoida
Calanoida (*Acartia* sp.)	Spionid polychaete
Spionid polychaete	
Rotifera	
Harpacticoida	
Tintinnid	
Cirripedia nauplii	

* Taxa listed in order of abundance.

Live Organisms 10 - 50 μm

Vital Stain Results - It is important to note that the specific approach used underestimates the abundances of live organisms in this size class because it has been found that not all live organisms reliably take up the CMFDA stain.

	T0 Ave #/ml	**T0 StDev #/ml**	**TF Ave #/ml**	**TF StDev #/ml**
Control	165	75.2	23	9.2
Treatment	0	0	0	0

Lugol's Total Counts - This approach commonly overestimates the abundances of live organisms in this size class because all individuals in fixed samples are counted unless obviously dead (significant damage to organism). Lugol's staining alone does not distinguish live from dead cells.

		T0 Ave #/ml	**T0 StDev** #/ml	**TF Ave** #/ml	**TF StDev** #/ml
Control	Diatom	523		119	
	Dinoflagellate	455		125	
Treated	Diatom	276		55	3
	Dinoflagellate	169		40	19

Note: 3,000/ml = Average counts for T-Final-Treated, for picoplankton <10µm.

Dominant species	**Type**	**Other**
Skeletonema costatum	Diatom	Chain-forming
Thalassiosira sp.	Diatom	Chain-forming
Oxyrrhis marina	Dinoflagellate	
Gyrodinium estuarale	Dinoflagellate	

Active Chlorophyll-a - Chlorophyll is used as ancillary data and as a general presence/absence indicator of viable photosynthetic organisms (MDL = 0.56 µg).

	T0 Ave µg/l	**T0 StDev** µg/l	**TF Ave** µg/l	**TF StDev** µg/l
Control	2.31	0.11	0.56	0.00
Treated	0.56	0.00	0.56	0.00
Regrowth Control			0.56	0.00
Regrowth Treated			0.56	0.00

Live Microbes (cfu = colony forming units)

E. coli	**T0 Ave** cfu/100ml	**T0 StDev** cfu/100ml	**TF Ave** cfu/100ml	**TF StDev** cfu/100ml
Control	19.4	3.16	14.6	3.51
Treated	0	0	0	0

Enterococci	**T0 Ave** cfu/100ml	**T0 StDev** cfu/100ml	**TF Ave** cfu/100ml	**TF StDev** cfu/100ml
Control	14.84	3.11	16.08	7.15
Treated	0	0	0	0

V. cholerae – No detectable culturable toxigenic Vibrio cholerae were detected in any samples during any of the trials.

Heterotrophic Bacteria	**T0 Ave** cfu/100ml	**T0 StDev** cfu/100ml	**TF Ave** cfu/100ml	**TF StDev** cfu/100ml
Control	1,970	214.48	425	118.39
Treated	0.1	0.33	0	0

Discharge Toxicity

Residual Chlorine Analysis - Levels of residual chlorine (or total residual oxidants - TRO) were at or below the method detection limit (MDL) of 0.02 ppm in all TF treated samples.

Acute Toxicity - There were no statistically significant survival effects in any test for either acutely tested species (Mysid shrimp or Sheepshead minnows).

Chronic Toxicity - Results showed that ballast water discharge was not chronically toxic to either mysids or sheepshead minnows. All samples, however, were chronically toxic to the marine algal species *Isochrysis galbana* with a statistically significant inhibition in growth in treated ballast discharge compared to controls (see details under Summary of Results on page 4).

9. Results Trial 4 (STDN-03): 5 – 10 November 2009

Physical Parameters

	TSS mg/l		**DOC** mg/l		**POC** mg/l	
T0 Initial	**Ave**	**StDev**	**Ave**	**StDev**	**Ave**	**StDev**
Conditions	29.4	6.1	3.65	0.10	3.070	0.536

	Temp ^{o}C		**Salinity** psu		**Dis. Oxygen** mg/l		**Turbidity** NTU	
	Ave	**StDev**	**Ave**	**StDev**	**Ave**	**StDev**	**Ave**	**StDev**
T0 Control	15.14	0.03	10.46	0.01	7.28	0.09	7.79	0.74
T0 Treated	15.13	0.01	10.44	0.01	8.13	0.13	8.18	0.74
TF Control	14.50	0.02	10.38	0.00	6.81	0.02	1.71	0.03
TF Treated	14.51	0.01	10.49	0.00	8.15	0.01	4.92	0.17

Live Organisms > 50 µm

	T0 Ave #/m^3	**T0 StDev** #/m^3	**TF Ave** #/m^3	**TF StDev** #/m^3
Control	84,000	1,875	40,500	3,000
Treated	No data		0.8	0.8

***TF Control**	***TF Treated**
Copepoda nauplii	Rotifera
Rotifera	
Spionid polychaete	
Calanoida (*Acartia* sp.)	
Cirripedia nauplii	
Harpacticoida	

* Taxa listed in order of abundance.

Live Organisms 10 - 50 µm

Vital Stain Results - It is important to note that the specific approach used underestimates the abundances of live organisms in this size class because it has been found that not all live organisms reliably take up the CMFDA stain.

	T0 Ave #/ml	**T0 StDev #/ml**	**TF Ave #/ml**	**TF StDev #/ml**
Control	*		28	3.2
Treatment	*		0	0

**No data available at T0*

Lugol's Total Counts - This approach commonly overestimates the abundances of live organisms in this size class because all individuals in fixed samples are counted unless obviously dead (significant damage to organism). Lugol's staining alone does not distinguish live from dead cells.

		T0 Ave #/ml	**T0 StDev** #/ml	**TF Ave** #/ml	**TF StDev** #/ml
Control	Diatom	1,223		128	
	Dinoflagellate	1,463		163	
Treated	Diatom	253		36	25
	Dinoflagellate	408		33	34

Note: 1,200/ml = Average counts for T-Final-Treated, for picoplankton <10µm.

Dominant species	**Type**	**Other**
Gyrodinium estuarale	Dinoflagellate	
Glenodinium danicum	Dinoflagellate	
Skeletonema costatum	Diatom	Chain-forming
Oxyrrhis marina	Dinoflagellate	
Apedinella sp.	Chrysophyceae	
Chaetoceros sp.	Diatom	

Active Chlorophyll-a - Chlorophyll is used as ancillary data and as a general presence/absence indicator of viable photosynthetic organisms (MDL = 0.56 µg).

	T0 Ave µg/l	**T0 StDev** µg/l	**TF Ave** µg/l	**TF StDev** µg/l
Control	6.51	2.24	1.19	0.05
Treated	0.56	0.00	0.56	0.00
Regrowth Control			4.05	4.87
Regrowth Treated			0.56	0.00

Live Microbes (cfu = colony forming units)

E. coli	**T0 Ave** cfu/100ml	**T0 StDev** cfu/100ml	**TF Ave** cfu/100ml	**TF StDev** cfu/100ml
Control	28.8	2.39	2.8	2.77
Treated	0	0	0	0

Enterococci	**T0 Ave** cfu/100ml	**T0 StDev** cfu/100ml	**TF Ave** cfu/100ml	**TF StDev** cfu/100ml
Control	8.7	5.05	4.94	1.35
Treated	0	0	0	0

V. cholerae – No detectable culturable toxigenic Vibrio cholerae were detected in any samples during any of the trials.

Heterotrophic Bacteria	**T0 Ave** cfu/100ml	**T0 StDev** cfu/100ml	**TF Ave** cfu/100ml	**TF StDev** cfu/100ml
Control	712	376.94	287.3	166.88
Treated	0.1	0.33	0	0

Discharge Toxicity

Residual Chlorine Analysis - Levels of residual chlorine (or total residual oxidants - TRO) were at or below the method detection limit (MDL) of 0.02 ppm in all TF treated samples.

Acute Toxicity - There were no statistically significant survival effects in any test for either acutely tested species (Mysid shrimp or Sheepshead minnows).

Chronic Toxicity - Results showed that ballast water discharge was not chronically toxic to either mysids or sheepshead minnows. All samples, however, were chronically toxic to the marine algal species *Isochrysis galbana* with a statistically significant inhibition in growth in treated ballast discharge compared to controls (see details under Summary of Results on page 4).

10. Results Trial 5 (STDN-04): 12 – 17 November 2009

Physical Parameters

	TSS mg/l		**DOC** mg/l		**POC** mg/l	
T0 Initial	**Ave**	**StDev**	**Ave**	**StDev**	**Ave**	**StDev**
Conditions	38.3	5.6	4.04	0.17	7.198	0.369

	Temp ^{o}C		**Salinity** psu		**Dis. Oxygen** mg/l		**Turbidity** NTU	
	Ave	**StDev**	**Ave**	**StDev**	**Ave**	**StDev**	**Ave**	**StDev**
T0 Control	12.90	0.02	9.49	0.04	10.23	0.10	8.96	0.96
T0 Treated	12.95	0.02	9.48	0.01	10.29	0.03	10.00	0.69
TF Control	14.51	0.00	9.44	0.00	4.20	0.07	1.14	0.12
TF Treated	14.52	0.00	9.47	0.00	9.88	0.03	4.40	0.13

Live Organisms > 50 μm

	T0 Ave #/m^3	**T0 StDev** #/m^3	**TF Ave** #/m^3	**TF StDev** #/m^3
Control	92,250	7,125	25,125	1,875
Treated	No data		0	na

***TF Control**	***TF Treated**
Copepoda nauplii	None live observed
Spionid polychaete	
Calanoida (*Acartia* sp.)	
Rotifera	
Cirripedia Nauplii	
Bivalvia veliger	

* Taxa listed in order of abundance.

Live Organisms 10 - 50 μm

Vital Stain Results - It is important to note that the specific approach used underestimates the abundances of live organisms in this size class because it has been found that not all live organisms reliably take up the CMFDA stain.

	T0 Ave #/ml	**T0 StDev #/ml**	**TF Ave #/ml**	**TF StDev #/ml**
Control	6,315	523.1	2,139	158.8
Treatment	6	1.5	0	0

Lugol's Total Counts - This approach commonly overestimates the abundances of live organisms in this size class because all individuals in fixed samples are counted unless obviously dead (significant damage to organism). Lugol's staining alone does not distinguish live from dead cells.

		T0 Ave #/ml	**T0 StDev** #/ml	**TF Ave** #/ml	**TF StDev** #/ml
Control	Diatom	1,886	84	398	87
	Dinoflagellate	16,125	1,715	3,733	100
Treated	Diatom	1,818	272	236	107
	Dinoflagellate	15,437	1,819	812	316

Note: 19,000/ml = Average counts for T-Final-Treated, for picoplankton <10µm.

Dominant species	**Type**	**Other**
Gyrodinium estuarale	Dinoflagellate	
Prorocentrum minimum	Dinoflagellate	
Oxyhrris marina	Dinoflagellate	
Apedinella sp.	Chrysophyceae	
Chaetoceros	Diatom	
Skeletonema costatum	Diatom	Chain-forming
Gymnodinium sp.	Dinoflagellate	
Scenedesmus quadricauda	Chlorophyceae	
Asterionella glacialis	Diatom	

Active Chlorophyll-a - Chlorophyll is used as ancillary data and as a general presence/absence indicator of viable photosynthetic organisms (MDL = 0.56 µg).

	T0 Ave µg/l	**T0 StDev** µg/l	**TF Ave** µg/l	**TF StDev** µg/l
Control	78.28	9.87	12.50	0.86
Treated	0.56	0.00	0.56	0.00
Regrowth Control			41.86	15.67
Regrowth Treated			0.56	0.00

Live Microbes (cfu = colony forming units)

E. coli	**T0 Ave** cfu/100ml	**T0 StDev** cfu/100ml	**TF Ave** cfu/100ml	**TF StDev** cfu/100ml
Control	74.4	40.3	6.8	3.19
Treated	0	0	0	0

Enterococci	**T0 Ave** cfu/100ml	**T0 StDev** cfu/100ml	**TF Ave** cfu/100ml	**TF StDev** cfu/100ml
Control	18.8	6.46	3.7	0.89
Treated	1.33	0.58	0	0

V. cholerae – No detectable culturable toxigenic Vibrio cholerae were detected in any samples during any of the trials.

Heterotrophic Bacteria	**T0 Ave** cfu/100ml	**T0 StDev** cfu/100ml	**TF Ave** cfu/100ml	**TF StDev** cfu/100ml
Control	2,692	408.14	2136	467
Treated	0.1	0.33	0	0

Discharge Toxicity

Residual Chlorine Analysis - Levels of residual chlorine (or total residual oxidants - TRO) were at or below the method detection limit (MDL) of 0.02 ppm in all TF treated samples.

Acute Toxicity - There were no statistically significant survival effects in any test for either acutely tested species (Mysid shrimp or Sheepshead minnows).

Chronic Toxicity - Results showed that ballast water discharge was not chronically toxic to either mysids or sheepshead minnows. All samples, however, were chronically toxic to the marine algal species *Isochrysis galbana* with a statistically significant inhibition in growth in treated ballast discharge compared to controls (see details under Summary of Results on page 4).

11. Chemical Analysis of Discharge

Summary table for chemical analyses of treated water upon discharge.

	Average Treated BWT Discharge (11-3, 11-10 and 11-17 samples)	Average Control BWT Discharge (11-3, 11-10 and 11-17 samples)	RDL[1] or MRL[2]
Compounds	**µg/L**	**µg/L**	**µg/L**
Chloroform	ND	ND	0.5
Bromoform	706	ND	25.0
Dichlorobromomethane	2.23	ND	0.5
Dibromochloromethane	33.9	ND	0.5
Bromochloroacetic acid	5.1	ND	1.0
Monochloroacetic acid	ND	ND	2.0
Monobromoacetic acid	5.9	ND	1.0
Dichloroacetic acid	1.0	ND	1.0
Dibromoacetic acid	102	ND	5.0
Tribromoacetic acid (ppb) – subcontract to UL	190.0	<4	4.0
Dibromochloroacetic acid (ppb) - subcontract to UL	19.0	<2	2.0
Trichloroacetic acid	3.0	1.3	1.0
Tribromophenol (ppb) - subcontract to UL	<0.25	<0.25	0.25
Sodium Bisulfite (ppb) - subcontract to UL	<5000	<5000	5000
Bromate	<10	<10	
Chlorate	<50	<50	

1) RDL-Reporting Detection Limit (used by ALSI, Analytical Laboratory Services, Inc.)
2) MRL- Minimum Reporting Limit (used by UL, Underwriters Laboratories)

12. Acknowledgments

The MERC Testing Team included J. Barnes, M. Carroll, J. Choi, D. Fisher, C. Grim, B. Haley, A. Huq, R. Kanzleiter, T. Mullady, G. Ruiz, G. Smith, D. Sparks, M. Tamburri. E. Taviani, M. Wilkinson, L. Yonkos, and G. Ziegler. Test protocols were developed in consultation with the Great Ships Initiative (GSI), Bundesamt fuer Seeschifffahrt und Hydrographie (BSH) and Royal Netherlands Institute for Sea Research (NIOZ). We wish thank the crew of the *MV Cape Washington* who provided critical input and support on all aspects of these trials, and the Maryland Port Administration and U.S. Maritime Administration for funding and supporting this ballast water treatment evaluation.

June 7, 2010

Date

Approved By: Dr. Mario Tamburri
MERC Executive Director

June 7, 2010

Date

Approved By: Ross Kanzleiter
MERC Program Coordinator and
Chief Engineer

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12 July 2010

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BalPure BWMS: Testing at MERC test site in Oct.-Nov. 2009

Dear Mario:

Severn Trent De Nora would like to thank the MERC Testing Team for all of their efforts concerning testing of the BalPure Ballast Water Treatment System on board of *MV Cape Washington*. We were pleased with having the opportunity to prove our systems effectiveness under the most challenging and realistic conditions provided for a land based test on board a ship.
The positive results from the testing showed that the BalPure system consistently met IMO D-2 discharge standards for all biological categories and demonstrated reliable mechanical performance.
Additional positive results, concerning discharge toxicity, showed neither acute toxicity nor chronic toxicity in any of the tests to either the Mysid shrimp or Sheephead minnows.
As for discharge chronic toxicity to algae, surprisingly all tests showed chronic toxicity to the marine algal species *Isochrysis galbana*. Chronic toxicity to an algal species in this case may be interpreted as an anomaly due to the particular marine algal species chosen and tested by MERC. Results from treated and neutralized discharge samples taken from trial 4, on November 11th, 2009 were sent to a 3rd party laboratory, Nautilus Environmental (Attachment 1). These results showed no chronic toxicity to the marine algal species *Skeletonoma costatum* which is shown in the MERC report to be the overall predominant species in the seawater tested. Another special consideration to add to this irregularity is that the marine algal species, *Isochrysis galbana*, used in the MERC chronic toxicity tests is not present as a dominant species in any of the seawater tested.

Sincerely,

Lucette Falcon

Lucette Falcon
R&D Engineer II

Correspondence should be addressed to:
Severn Trent De Nora, LLC.