Land-Based Evaluations of the Maritime Solutions, Inc. Ballast Water Treatment System

Maritime Environmental Resource Center

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c/o Chesapeake Biological Laboratory
PO Box 38, Solomons, Maryland 20688, USA

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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)*. 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 MSI and MERC funding agencies prior to public release. Mention of trade names or commercial products does not constitute endorsement or recommendation by MERC.

Questions and comments should be directed to Dr. Mario Tamburri, tamburri@umces.edu.

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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 Maritime Solutions Inc. (MSI) Ballast Water Treatment System through objective and quality assured land-based testing (dockside at a flow rate of 200 m^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 MSI Ballast Water Management System

The MSI Ballast Water Treatment System (UV), patent pending, is designed to meet IMO D2 discharge standards for low to moderate flow rate shipboard applications. The treatment utilizes Ballast Safe Filtration Company's proprietary self-cleaning filter design to separate the components of the influent ballast water in its primary treatment stage. As a primary treatment, the filter is intended to remove silt and sediment, organic materials and organisms > 25 μm (nominal) in size from the influent ballast water. The remaining filtered water stream is then treated by Hanovia UV In-Line UV units in a secondary treatment stage to address the remaining organisms < 25 μm. In addition to its two treatment stages, the MSI System (UV) has been fully integrated and is controlled by a proprietary ABB Instrumentation water quality monitoring and flow control system designed to assure and document effective treatment by continuously monitoring a number of water quality parameters including total suspended solids (TSS) and UV transmission rate, automatically adjusting flow rate to assure proper treatment, and recording all required water quality and system operation parameters. Because the treatment system was a prototype, it was operated at all times by members of the MSI or ABB staff. All evaluation system equipment and instrumentation, excluding the treatment system, was operated by MERC personnel.

3. Summary of IMO Standards

This evaluation was designed to determine if the MSI system can meet IMO D2 standards in accordance with both the IMO *Guidelines for Approval of Ballast Water Management Systems (G8)*. 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 Plans for the Performance Evaluation of the Maritime solutions, Inc. Ballast Water Treatment System* (April 2008 and March 2009), available for download at www.maritime-enviro.org.

The protocols described below are based upon the IMO G8 guidelines and the U.S. Coast Guard supported ETV protocols under development. Any deviation from IMO G8 or ETV were explained and justified in the Test Plan. MERC evaluated the biological efficacy of the MSI ballast water treatment system onboard the MARAD 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 and delivered simultaneously to either a "control" (untreated) ballast tank or a "treated" (passing first through the MSI system) ballast tank. These two tanks 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 for the water quality parameters of particulate organic carbon (POC), dissolved organic carbon (DOC – only in 2009), and total suspended solids (TSS).

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 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 MSI 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 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 1-liter 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 according to *Standards Methods for the Examination of Water and Wastewater*. The presence and abundance of *E. coli* and intestinal *Enterococci* was 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* were determined using a standard USEPA 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). Abundance of total and toxigenic *V. cholerae* were calculated 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 *V. cholerae* was 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).

5. Summary of Results*

The MSI Ballast Water Treatment System dramatically reduced the numbers of live organisms in ballast water during MERC land-based testing in the Port of Baltimore. For most biological categories, the treatment system consistently met IMO D2 discharge standards. However, while large reductions in the abundances of organisms greater than 50 µm were found, the T-Final average numbers of live zooplankton (> 50 µm) were not consistently below 10 µm in treated water after the 5-day holding time. The MSI system also experienced minor mechanical failures at different points during the testing process (broken in-line sensor housing and tripping of a circuit breaker). However, the failures were easily resolved with small modifications or repairs.

** Complete datasets and further performance information is available upon request.*

6. Results Trial 1 (MSI-05-08): 18-23 September 2008

Physical Parameters

	TSS mg/l		**DOC** mg/l		**POC** mg/l	
T0 Initial	**Ave**	**StDev**	**Ave**	**StDev**	**Ave**	**StDev**
Conditions	5.0	0.5	-	-	1.123	0.129

Note: Did not analyze for DOC in 2008

	Temp ^{o}C		**Salinity** psu		**Dis. Oxygen** mg/l		***Turbidity** NTU	
	Ave	**StDev**	**Ave**	**StDev**	**Ave**	**StDev**	**Ave**	**StDev**
T0 Control	24.5	0.0	11.7	0.0	3.9	.5	-	-
T0 Treated	24.6	0.2	11.6	0.1	3.7	0.3	-	-
TF Control	24.5	0.1	11.7	0.0	3.8	0.1	-	-
TF Treated	24.7	0.1	11.6	0.1	3.3	0.1	-	-

*Data not recorded in 2008

Live Organisms > 50 μm

	T0 Ave #/m^3	**T0 StDev** #/m^3	**TF Ave** #/m^3	**TF StDev** #/m^3
Control	31,175	4,238	55,050	10,828
Treated	107	4.16	6	1.87

***TF Control**	***TF Treated**
Nauplii (copepod)	Nematoda
Copepoda, Harpacticoida	nauplii (copepod)
Copepoda, Cyclopoida	Turbellaria
Copepoda, Calanoida	
Nematoda	
Rotifera	

* Taxa listed in order of abundance.

Live Organisms 10 - 50 μm

Vital Stain Results

Note: the CMFDA stain technique commonly underestimates the abundance of live organisms in this size class because not all live organisms reliably take up this stain.

	T0 Ave #/ml	**T0 StDev** #/ml	**TF Ave** #/ml	**TF StDev** #/ml
Control	530.7	122.5	11.2	2.3
Treated	7.3	0.6	0.6	0.7

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).

		T0 Ave #/ml	**T0 StDev** #/ml	**TF Ave** #/ml	**TF StDev** #/ml
Control	Diatom	7,734	1,280	3,063	457
	Dinoflagellate	578	223	134	49
Treated	Diatom	*		149	52
	Dinoflagellate	*		6	8

* Not yet analyzed

Note: 454/ml = Average counts for T-Final-Treated, for picoplankton <10µm.

Dominant species	**Type**
Leptocylindrus minimum	Diatom
Gyrodinium estuariale	Dino
Eutreptia veridis	Euglenid Flagellate
Thalassiosira sp.	Diatom
Amphora sp.	Diatom
Gonyaulux sp. (danicum?)	Dino
Gyrodinium spirale	Dino
Amphidinium sphenoides	Dino
misc. Tinntinnids	Ciliates

Active Chlorophyll-a

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

	T0 Ave µg/l	**T0 StDev** µg/l	**TF Ave** µg/l	**TF StDev** µg/l
Control	23.70	5.39	3.45	2.19
Treated	*	-	0.46	0.05
Regrowth Control	-	-	11.37	5.33
Regrowth Treated	-	-	0.04	0.01

*No initial treated data collected

Live Indicator Pathogens

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	12.7	0.6	0.89
Treated	*	-	0	0

*No initial treated data collected

Enterococci

	T0 Ave cfu/100ml	**T0 StDev** cfu/100ml	**TF Ave** cfu/100ml	**TF StDev** cfu/100ml
Control	5.66	-	13.58	-
Treated	*	-	0	-

*No initial treated data collected. No StDev reported.

V. cholerae

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

Total Heterotrophic Bacteria

	T0 Ave cfu/100ml	**T0 StDev** cfu/100ml	**TF Ave** cfu/100ml	**TF StDev** cfu/100ml
Control	31,833	7,591	98,288	90,638
Treated	*	-	6,936	6,586

*No initial treated data collected

7. Results Trial 2: (MSI-06-08) 2-7 Oct 2008

Physical Parameters

	TSS mg/l		**DOC** mg/l		**POC** mg/l	
T0 Initial	**Ave**	**StDev**	**Ave**	**StDev**	**Ave**	**StDev**
Conditions	7.4	0.4	-	-	0.474	0.042

Note: Did not analyze for DOC in 2008

	Temp ^{o}C		**Salinity** psu		**Dis. Oxygen** mg/l		***Turbidity** NTU	
	Ave	**StDev**	**Ave**	**StDev**	**Ave**	**StDev**	**Ave**	**StDev**
T0 Control	22.8	0.2	14.9	0.3	1.4	1.0	-	-
T0 Treated	22.6	0.5	14.4	0.1	0.9	0.1	-	-
TF Control	22.1	0.1	15.7	0.1	3.8	0.2	-	-
TF Treated	22.4	0.1	14.7	0.1	4.5	0.8	-	-

*Data not recorded

Live Organisms > 50 µm

	T0 Ave #/m^3	**T0 StDev** #/m^3	**TF Ave** #/m^3	**TF StDev** #/m^3
Control	61,100	8,600	30,625	6,027
Treated	1370	120	2170	523

Note: Increase in numbers for T-Final treated counts likely due to hatch-outs of the egg bearing poicelistome copepod.

*TF Control	*TF Treated
nauplii (copepod)	nauplii (copepod)
Polychaeta (larvae)	Copepoda, Cyclopoida
Copepoda, Cyclopoida	Copepoda, Poicelistomoida
Copepoda, Poicelistomoida	
Copepoda, Calanoida	
Rotifera	
Bivalvia	

* Taxa listed in order of abundance.

Live Organisms 10 - 50 μm

Vital Stain Results

Note: the CMFDA stain technique commonly underestimates the abundance of live organisms in this size class because not all live organisms reliably take up this stain.

	T0 Ave #/ml	**T0 StDev** #/ml	**TF Ave** #/ml	**TF StDev** #/ml
Control	54.7	6.5	2.6	0.3
Treated	1.5	0.1	0.5	0.3

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).

		T0 Ave #/ml	**T0 StDev** #/ml	**TF Ave** #/ml	**TF StDev** #/ml
Control	Diatom	849	176	520	161
	Dinoflagellate	116	39	18	12
Treated	Diatom	*		83	16
	Dinoflagellate	*		4	4

*Not yet analyzed

Note: 365 #/ml = Average counts for T-Final-Treated, for picoplankton <10μm.

Dominant species	**Type**
Leptocylindrus minimum	Diatom
Gyrodinium estuariale	Dino
Chaetoceros socialis	Diatom
Thalassiosira sp.	Diatom
Amphora sp.	Diatom
Navicula didyma	Diatom
misc. Pennates	Diatom
misc. Tinntinnids	Ciliate

Active Chlorophyll-a

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

	T0 Ave µg/l	**T0 StDev** µg/l	**TF Ave** µg/l	**TF StDev** µg/l
Control	1.52	0.10	0.35	0.04
Treated	*	-	0.22	0.02
Regrowth Control	-	-	4.39	0.90
Regrowth Treated	-	-	0.01	0.00

*No initial treated data collected

Live Indicator Pathogens

CFU = colony forming units

E. coli

	T0 Ave cfu/100ml	**T0 StDev** cfu/100ml	**TF Ave** cfu/100ml	**TF StDev** cfu/100ml
Control	10.6	13.56	1.4	1.52
Treated	*	-	0	0

*No initial treated data collected

Enterococci

	T0 Ave cfu/100ml	**T0 StDev** cfu/100ml	**TF Ave** cfu/100ml	**TF StDev** cfu/100ml
Control	24.84	-	14.28	-
Treated	*	-	0	-

*No initial treated data collected. No StDev reported.

V. cholerae

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

Total Heterotrophic Bacteria

	T0 Ave cfu/100ml	**T0 StDev** cfu/100ml	**TF Ave** cfu/100ml	**TF StDev** cfu/100ml
Control	12,150	6,954	11,920	6,633
Treated	*	-	7,860	8,308

*No initial treated data collected

8. Results Trial 3: (MSI 01-09) 16-21 April 2009

Physical Parameters

	TSS mg/l		**DOC** mg/l		**POC** mg/l	
T0 Initial	**Ave**	**StDev**	**Ave**	**StDev**	**Ave**	**StDev**
Conditions	7.33	0.50	3.62	0.24	0.84	0.07

	Temp ^{o}C		**Salinity** psu		**Dis. Oxygen** mg/l		**Turbidity** NTU	
	Ave	**StDev**	**Ave**	**StDev**	**Ave**	**StDev**	**Ave**	**StDev**
T0 Control	10.3	0.3	9.8	0.1	8.4	0.1	4.4	0.2
T0 Treated	10.4	0.1	9.8	0.0	8.4	0.5	4.2	0.1
TF Control	13.1	0.1	10.0	0.1	8.6	0.1	1.6	0.1
TF Treated	13.3	0.1	9.3	0.1	9.5	0.1	3.2	0.1

Live Organisms > 50 µm

	T0 Ave #/m^3	**T0 StDev** #/m^3	**TF Ave** #/m^3	**TF StDev** #/m^3
Control	179,000	49,518	127,933	21,624
Treated	200	288	8	5

***TF Control**	***TF Treated**
Calanoida (*Eurytemora affinis*)	Calanoida
Copepoda nauplii	Harpacticoida
various eggs	Copepod nauplii
Cirrepedia nauplii	
Rotifera	
Ploychaeata (Spionidae)	
Harpacticoida	

*****Taxa listed in order of abundance.

Live Organisms 10 - 50 µm

Vital Stain Results

Note: the CMFDA stain technique commonly underestimates the abundance of live organisms in this size class because not all live organisms reliably take up this stain.

	T0 Ave #/ml	**T0 StDev** #/ml	**TF Ave** #/ml	**TF StDev** #/ml
Control	72.7	38.5	22.3	3.7
Treated	10	4	0.4	0.1

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).

		T0 Ave #/ml	**T0 StDev** #/ml	**TF Ave** #/ml	**TF StDev** #/ml
Control	Diatom	41	17	48	18
	Dinoflagellate	21	12	16	4
Treated	Diatom	22	15	3	2
	Dinoflagellate	10	10	1	1

Note: 2552 #/ml = Average counts for T-Final-Treated, for picoplankton <10µm.

Dominant species	**Type**
Amphora sp.	Diatom
Gyrodinium estuariale	Dino
Thalassiosira sp.	Diatom
Heterocapsa rotundatum	Dino

Active Chlorophyll-a

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

	T0 Ave µg/l	**T0 StDev** µg/l	**TF Ave** µg/l	**TF StDev** µg/l
Control	6.20	0.51	2.99	0.38
Treated	5.25	0.46	2.44	0.14
Regrowth Control	-	-	60.00	27.31
Regrowth Treated	-	-	0.07	0.09

Live Indicator Pathogens

CFU = colony forming units

E. coli

	T0 Ave cfu/100ml	**T0 StDev** cfu/100ml	**TF Ave** cfu/100ml	**TF StDev** cfu/100ml
Control	22.40	2.70	0.20	0.45
Treated	0.00	0.00	0.00	0.00

Enterococci

	T0 Ave cfu/100ml	**T0 StDev** cfu/100ml	**TF Ave** cfu/100ml	**TF StDev** cfu/100ml
Control	21.14	8.43	10.12	4.93
Treated	0.2	0.45	0.00	0.00

V. cholerae

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

Total Heterotrophic Bacteria

	T0 Ave cfu/100ml	**T0 StDev** cfu/100ml	**TF Ave** cfu/100ml	**TF StDev** cfu/100ml
Control	1,593.00	4,796.51	556.00	1,672.98
Treated	75.30	22.74	162.00	39.25

9. Trial 4 (MSI-02-09) 23-28 April 2009

Physical Parameters

	TSS mg/l		**DOC** mg/l		**POC** mg/l	
T0 Initial	**Ave**	**StDev**	**Ave**	**StDev**	**Ave**	**StDev**
Conditions	30.13	3.69	3.57	0.34	1.10	0.04

	Temp ^{o}C		**Salinity** psu		**Dis. Oxygen** mg/l		**Turbidity** NTU	
	Ave	**StDev**	**Ave**	**StDev**	**Ave**	**StDev**	**Ave**	**StDev**
T0 Control	11.3	0.1	10.4	0.2	6.4	0.3	8.6	0.3
T0 Treated	11.5	0.1	10.5	0.3	6.4	0.3	8.8	0.3
TF Control	13.8	0.1	10.5	0.0	7.4	0.1	1.3	0.4
TF Treated	13.8	0.1	10.3	0.1	8.4	0.1	5.7	0.2

Live Organisms > 50 μm

	T0 Ave #/m^3	**T0 StDev** #/m^3	**TF Ave** #/m^3	**TF StDev** #/m^3
Control	117,000	32,786	47,167	3,329
Treated	158	53	*516	103

*Increase in treatment numbers due to an increase in newly hatched cirrepedia nauplii.

***TF Control**	***TF Treated**
Calanoida (*Eurytemora affinis*) Copepoda nauplii Cirrepedia nauplii Gastropod larvae various eggs	Calanoida Cirrepedia nauplii Copepoda nauplii Rotifera

* Taxa listed in order of abundance.

Live Organisms 10 - 50 µm

Vital Stain Results

Note: the CMFDA stain technique commonly underestimates the abundance of live organisms in this size class because not all live organisms reliably take up this stain.

	T0 Ave #/ml	**T0 StDev** #/ml	**TF Ave** #/ml	**TF StDev** #/ml
Control	1,259	88	68	20
Treated	91	26	12	5

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).

		T0 Ave #/ml	**T0 StDev** #/ml	**TF Ave** #/ml	**TF StDev** #/ml
Control	Diatom	764	176	211	80
	Dinoflagellate	255	47	106	24
Treated	Diatom	324	137	38	6
	Dinoflagellate	128	46	18	2

Note: 2489 #/ml = Average counts for T-Final-Treated, for picoplankton <10µm.

Dominant species	**Type**
Heterocapsa rotundatum	Dino
Gyrodinium estuariale	Dino
*Prorocentrum minimum**	Dino
Chaetoceros sp.	Diatom
misc. Copepod nauplii	
Microcystis sp.* (4-5 µm)	Cyanobacteria

* Know harmful algal species.

Active Chlorophyll-a

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

	T0 Ave µg/l	**T0 StDev** µg/l	**TF Ave** µg/l	**TF StDev** µg/l
Control	10.22	0.44	1.11	0.17
Treated	8.76	1.41	5.56	0.25
Regrowth Control	-	-	16.39	5.58
Regrowth Treated	-	-	0.12	0.03

Live Indicator Pathogens
CFU = colony forming units

E. coli

	T0 Ave cfu/100ml	**T0 StDev** cfu/100ml	**TF Ave** cfu/100ml	**TF StDev** cfu/100ml
Control	19.40	26.57	1.40	0.89
Treated	0.00	0.00	0.00	0.00

Enterococci

	T0 Ave cfu/100ml	**T0 StDev** cfu/100ml	**TF Ave** cfu/100ml	**TF StDev** cfu/100ml
Control	61.44	11.48	13.72	1.94
Treated	0	0.00	0.00	0.00

V. cholerae

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

Total Heterotrophic Bacteria

	T0 Ave cfu/100ml	**T0 StDev** cfu/100ml	**TF Ave** cfu/100ml	**TF StDev** cfu/100ml
Control	533.00	1,624.82	271.25	947.93
Treated	92.80	18.14	116.88	24.22

10. Trial 5 (real trial MSI -03-09) 30 April – 5 May 2009

Physical Parameters

	TSS mg/l		**DOC** mg/l		**POC** mg/l	
T0 Initial	**Ave**	**StDev**	**Ave**	**StDev**	**Ave**	**StDev**
Conditions	9.27	5.66	3.95	0.10	1.22	0.17

	Temp ^{o}C		**Salinity** psu		**Dis. Oxygen** mg/l		**Turbidity** NTU	
	Ave	**StDev**	**Ave**	**StDev**	**Ave**	**StDev**	**Ave**	**StDev**
T0 Control	17.32	0.03	7.41	0.00	8.86	0.13	2.8	0.1
T0 Treated	17.51	0.00	7.34	0.00	9.13	0.01	2.8	0.1
TF Control	15.73	0.01	7.49	0.00	6.77	0.02	1.3	0.1
TF Treated	15.81	0.00	7.33	0.00	7.33	0.09	1.6	0.1

Live Organisms > 50 µm

	T0 Ave #/m^3	**T0 StDev** #/m^3	**TF Ave** #/m^3	**TF StDev** #/m^3
Control	113,500	9,674	51,500	4,515
Treated	38	7	*27	11

*All live individuals found were *Acartia*.

***TF Control**	***TF Treated**
Calanoida (*Acartia tonsa*)	Copepoda nauplii
Cirrepedia nauplii	Cirrepedia nauplii
Copepoda nauplii	Calanoida (*Acartia tonsa)*
Polychaeta (Spionidae)	Polychaeta (Spionidae)
Harpacticoida	Harpacticoida
various eggs	

* Taxa listed in order of abundance.

Live Organisms 10 - 50 µm

Vital Stain Results

Note: the CMFDA stain technique commonly underestimates the abundance of live organisms in this size class because not all live organisms reliably take up this stain.

	T0 Ave #/ml	**T0 StDev** #/ml	**TF Ave** #/ml	**TF StDev** #/ml
Control	1,944	174	280	35
Treated	149	27	11	3

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).

		T0 Ave #/ml	**T0 StDev** #/ml	**TF Ave** #/ml	**TF StDev** #/ml
Control	Diatom	417	186	281	47
	Dinoflagellate	799	173	202	40
Treated	Diatom	134	45	21	0
	Dinoflagellate	285	66	17	7

Note: 1547 #/ml = Average counts for T-Final-Treated, for picoplankton <10µm.

Dominant species	**Type**
Gyrodinium estuariale	Dino
*Prorocentrum minimum**	Dino
Heterocapsa rotundatum	Dino
Gymnodinium sp.	Dino
Gyrodinium sp.	Dino
Amphidinium sp.	Dino
Amphora sp.	Diatom
Microcystis sp.*(4-5 µm)	Cyanobacteria
misc. Tinntinnids	Ciliates

* Know harmful algal species.

Active Chlorophyll-a

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

	T0 Ave µg/l	**T0 StDev** µg/l	**TF Ave** µg/l	**TF StDev** µg/l
Control	23.10	1.84	2.21	0.19
Treated	18.80	1.39	7.23	0.10
Regrowth Control	-	-	11.75	5.53
Regrowth Treated	-	-	0.17	0.12

Live Indicator Pathogens

CFU = colony forming units

E. coli

	T0 Ave cfu/100ml	**T0 StDev** cfu/100ml	**TF Ave** cfu/100ml	**TF StDev** cfu/100ml
Control	2.20	1.10	1.00	1.71
Treated	0.00	0.00	0.00	0.00

Enterococci

	T0 Ave cfu cfu/100ml	**T0 StDev** cfu/100ml	**TF Ave** cfu /100ml	**TF StDev** cfu/100ml
Control	2.62	1.17	3.9	0.84
Treated	0	0.00	0.00	0.00

V. cholerae

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

Total Heterotrophic Bacteria

	T0 Ave cfu/100ml	**T0 StDev** cfu/100ml	**TF Ave** cfu/100ml	**TF StDev** cfu/100ml
Control	208.75	716.73	192.20	33.08
Treated	116.88	24.22	145.30	44.39

11. Acknowledgments

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November 20, 2009

Date

Approved By: Dr. Mario Tamburri
MERC Executive Director

November 20, 2009

Date

Approved By: Ross Kanzleiter
MERC Program Coordinator and
Chief Engineer