Updated on 10 August 2017 to correct an error on figure legends.

PERFORMANCE VERIFICATION STATEMENT
For the Ballast-Check 2 Version: 2015 (Turner Designs)

TECHNOLOGY TYPE:	Ballast water compliance tools
APPLICATION:	Shipboard analysis of ballast water
PARAMETERS EVALUATED:	Response linearity, accuracy, and precision
TYPE OF EVALUATION:	Laboratory and field performance verifications
DATE OF EVALUATION:	Testing conducted from July to September 2015
EVALUATION PERSONNEL:	M.R. First, S.C. Riley, S.H. Robbins-Wamsley, V. Molina, T. Johengen, H. Purcell, G.J. Smith, E. Reavie, K. Carney, C.S. Moser, E.N. Buckley M.N. Tamburri, and L.A. Drake

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BACKGROUND AND OBJECTIVES

In an effort to mitigate the risk of transporting aquatic nuisance species, the United States Coast Guard (USCG) has finalized a rule limiting the concentrations of organisms in ships' ballast water discharged into US ports (US Coast Guard 2012). The specified concentrations are nearly identical (with the exception of not including limits for *Vibrio cholerae* in zooplankton samples) to those in the International Maritime Organization's (IMO) convention (IMO 2004). Further, the limits are consistent with those in the US Environmental Protection Agency's Vessel General Permit (VGP)—regulations on a suite of vessel operations, including the discharge of ballast water (US EPA 2013). In order to meet these limits, most ships will use a ballast water management system (BWMS). These systems incorporate a variety of technologies (including filtration, UV radiation, electrolytic chlorination, and deoxygenation) to ensure that the discharge water meets the specifications.

Determining concentrations of living organisms can require extensive effort and sensitive equipment, especially for sparse populations. For example, direct counts of living organisms ≥10 and <50 μm according to the method stipulated in the US Environmental Technology Verification (ETV) Program Protocol for land-based testing of BWMS requires (1) labeling organisms within a sample with a set of vital fluorophores and (2) tallying the organisms via epifluorescence microscopy (EPA 2010; Steinberg et al. 2011). Direct counts of living organisms yield concentrations comparable to the numerical standard. While this rigorous, complex, and time-consuming analysis is appropriate for verification testing of BWMS, it is typically not feasible to perform this analysis during routine shipboard inspections. Rather, simple, hand-held, field instruments ("compliance tools")—with the ability to rapidly assess that the ballast water *clearly* exceeds the discharge limits—will be of much greater value to the ship owner, the BWMS vendor, and the compliance officer. Compliance tools should immediately produce results that are reliable indicators of the concentrations of living organisms within a regulated size class and predict whether a sample meets or exceeds the discharge standard.

New or refined compliance tools require carefully considered test protocols for evaluating and verifying their performance. The overall goal of this *technology verification* was to evaluate the performance of potential compliance tools designed to rapidly assess ballast water discharge. The outputs of the compliance tools were compared to the standard, validated approach (i.e. epifluorescence microscopy; EPA 2010) used to quantify organisms ≥10 and <50 μm in size during verification testing of BWMS. The objectives outlined below support this goal:

- In a series of laboratory trials to be conducted at the Naval Research Laboratory in Key West, FL (NRL), determine **linearity**, **precision** and **accuracy** of the compliance tool with samples of algal monocultures over a range of concentrations, including concentrations below, equal to, and above the IMO and US discharge standard.
- Evaluate the relationship between numerical concentrations of living organisms ≥10 and <50 μm and the accuracy and precision of the instrument using ambient organisms

collected from natural waters at three various locations (Key West, Chesapeake Bay, and Lake Superior).

INSTRUMENT TECHNOLOGY TESTED

This report describes the test of the Ballast-Check 2 Handheld PAM—pulse amplitude modulation—fluorometer, 2015 version (hereafter, Ballast-Check 2). Ballast-Check 2 is a product of Turner Designs (San Jose, CA). The instrument employs variable fluorescence fluorometry, an approach that measures chlorophyll *a* fluorescence at variable illumination intensities and intervals. These measurements are used to estimate concentrations of living organisms within an aliquot of water. As photosynthetic algae are abundant in the ≥10 and <50 µm size class, the instruments may provide a reasonable determination whether a sample meets the discharge limit of 10 living organisms mL^{-1} in the ≥10 and <50 µm size class.

Upon completion of sample analysis, the Ballast-Check 2 displays the following parameters on a visual display:

- *Abundance:* an initial fluorescence (F_0)-based calculation used to estimate the concentration (mL^{-1}) of algae in a sample
- *Activity*: a decimal value—ranging from 0 to 1—that reflects the photochemical yield (F_V/F_M) of the sample
- *Risk*: a binary outcome determined based upon both *Abundance* and *Activity*, indicating the risk of exceeding the discharge standard: it will be either *Low* or *High*.

Further details of the operation of the Ballast-Check 2 are available in the test plan (**Appendix A**).

PERFORMANCE EVALUATION TEST PLAN

The test protocol for this performance verification was developed at a conference with NRL and the Alliance for Coastal Technologies (ACT) personnel, the participating instrument manufacturers, and a technical advisory committee. The verification of the instrument included both laboratory and field experiments: these tests are summarized briefly in this document and in detail in the test protocol. Experiments were designed to challenge the compliance tool by analyzing ranges of concentrations—spanning from zero to well above the discharge standard. Measurements reported by the instrument were compared to the results of the standard technique, described below. The critical comparison was the agreement on the disposition of the sample: if both the compliance tool and the microscope count indicate concentrations ≥10 mL^{-1}, the methods agree. Likewise, if both methods determine concentrations are <10 mL^{-1}, the methods agree.

Laboratory Experiments

Laboratory tests examined the agreement between cell concentrations measured via microscopy and the compliance tool using two cultured microalgae: *Tetraselmis marina* (cell dimensions: 9-15 µm) and *Prorocentrum micans* (25-50 µm). The organisms represented cell dimensions towards the extremes of the ≥10 and <50 µm size class. For the laboratory experiments with cultured algae, all living cells were counted, even though some individuals may have been slightly larger or smaller than the size limits. Samples with either *T. marina* or *P. micans* were prepared by diluting stock cultures with 0.22-µm filtered seawater (FSW) to yield concentrations of 0, 5, 10, 20, 50, and 100 mL^{-1}. Additionally, two samples were prepared to examine interferences from (1) dissolved and particulate materials and (2) disinfection byproducts (DBP). These samples contained 10 mL^{-1} of either *T. marina* or *P. micans*.

Field Experiments

Instrument performance was also tested in field experiments using ambient water samples collected from three locations representing a range of water temperatures, salinities, and community compositions: The Naval Research Laboratory (NRL; latitude 24.58°N; Longitude: 81.79°W) in Key West, FL represented offshore, high salinity, waters (temperature: 27°C; salinity: 36 psu). The Great Ships Initiative (GSI) in Superior, WI (46.71°N; 92.05°W) represented the Great Lakes (20°C; 0 psu). The Smithsonian Environmental Research Center (SERC; 38.89°N; 76.54°W) in Edgewater, MD, located on the Chesapeake Bay, represented estuarine waters (25°C; 9 psu). Samples with a mixed assemblage of ambient organisms were prepared by either diluting or concentrating natural water from the location: dilution was performed by mixing the sample with FSW (or at GSI, 0.22-µm filtered *lake* water, FLW). Cells were concentrated by screening water through a sieve with mesh netting to retain organisms ≥10 µm. Following these procedures, four samples were generated with different target concentrations:

- 0 mL^{-1}, the 0.22-µm filtered water to be used as a control or blank for fluorescence,
- 5 – 20 mL^{-1}, representing concentrations near the discharge standard (DS),
- 30 – 50 mL^{-1}, representing concentrations above the DS, and
- ≥50 mL^{-1}, representing concentrations well above the DS.

Determining Concentrations of Microalgae by Epifluorescence Microscopy

Organisms ≥10 and <50 µm were quantified using the approach in the Environmental Technology Verification (ETV) Program protocol (EPA, 2010), namely, labeling organisms with a set of vital, fluorescing probes and manually counting fluorescent organisms via microscopy. This is the standard method used in land-based verification of ballast water management systems, and test participants designated this as the reference method for evaluating compliance tools. Fluorophores—chloromethylfluorescein diacetate (CMFDA) and fluorescein diacetate

(FDA)—are added to a water sample. After a brief (10-min) incubation period, the sample is transferred into a gridded counting chamber, and a portion of the chamber is scanned for organisms moving, fluorescing, or both. Fluorescing organisms encountered were identified to general taxonomic group (e.g., dinoflagellates, diatoms, etc.) and manually tallied on a datasheet. At GSI, a validation study demonstrated that a single fluorophore (FDA) yielded equivalent counts of organisms as the dual set, so at this site, only FDA was used to label organisms. The detailed protocol for this approach is in **Appendix A**.

Measuring *Abundance*, *Activity*, and *Risk* using the Ballast-Check 2

The Ballast-Check 2 (Turner Designs; San Jose, CA), when used to evaluate a sample, reports sample *Abundance*, *Activity*, and *Risk* (*High* or *Low*) and cell concentration. The instrument hardware, its software protocols (e.g., setting the instrument gain and scale offset), and its calculations (e.g., determining *Risk*) are proprietary. Sample analysis proceeded according to the protocol provided with the instrument. Briefly, well-mixed samples used drawn into a 60-mL syringe and then into a well-rinsed glass cuvette, and then read on the Ballast-Check 2. If required, a second reading of a 10-µm screened water sample was used by the instrument to calculate the <10 µm fluorescence and adjust the readings of the whole water (i.e., non-screened) sample. Values reported by the instrument were manually recorded on a datasheet, but were also stored in the Ballast-Check 2's memory and could be downloaded to a computer.

RESULTS

Linearity

The linear response of the Ballast-Check 2 was measured by the change in reported *Abundance* relative to the measured concentration of organisms ≥10 and <50 µm. Results of the laboratory and field trials are shown in Figures 1 and 2, respectively. For both laboratory and field trials, linear regression was used to generate a line-of-best-fit describing the relationship between concentration and abundance. A linear relationship indicates the compliance tool's measurements will vary in proportion to the number of organisms in the sample. The strength of that relationship is measured by the coefficient of determination (R^2), a relative measurement (ranging from 0 to 1) that indicates how well the measurement conform to the line-of-best fit. Linear regression was performed on data from all trials for each organism or field site as well as the combined data set (from both organisms and all field sites). Results of linear regression analyses are shown in Table 1.

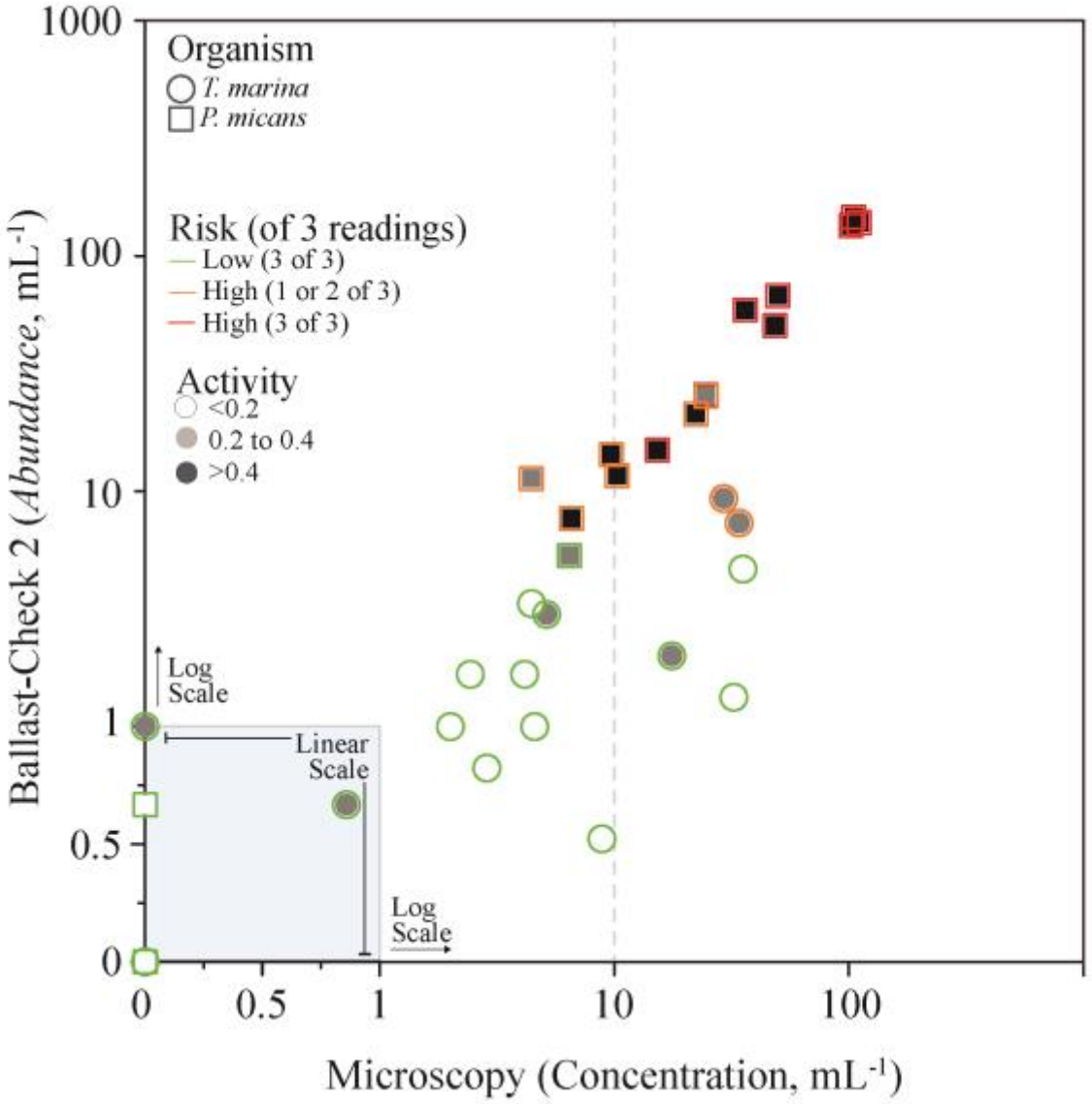

Figure 1. Results of the laboratory experiments. Measurements from the Ballast-Check 2 are compared to concentrations of *Tetraselmis marina* or *Prorocentrum micans*. Symbols mark the mean *Abundance* and colors show the mean *Activity* of three repeated readings. Symbol outlines display the number of repeated readings with low or high *Risk*. The figure inset, with axis scaled from 0 to 1, is a linear scale. The rest of the figure displays data on a logarithmic scale.

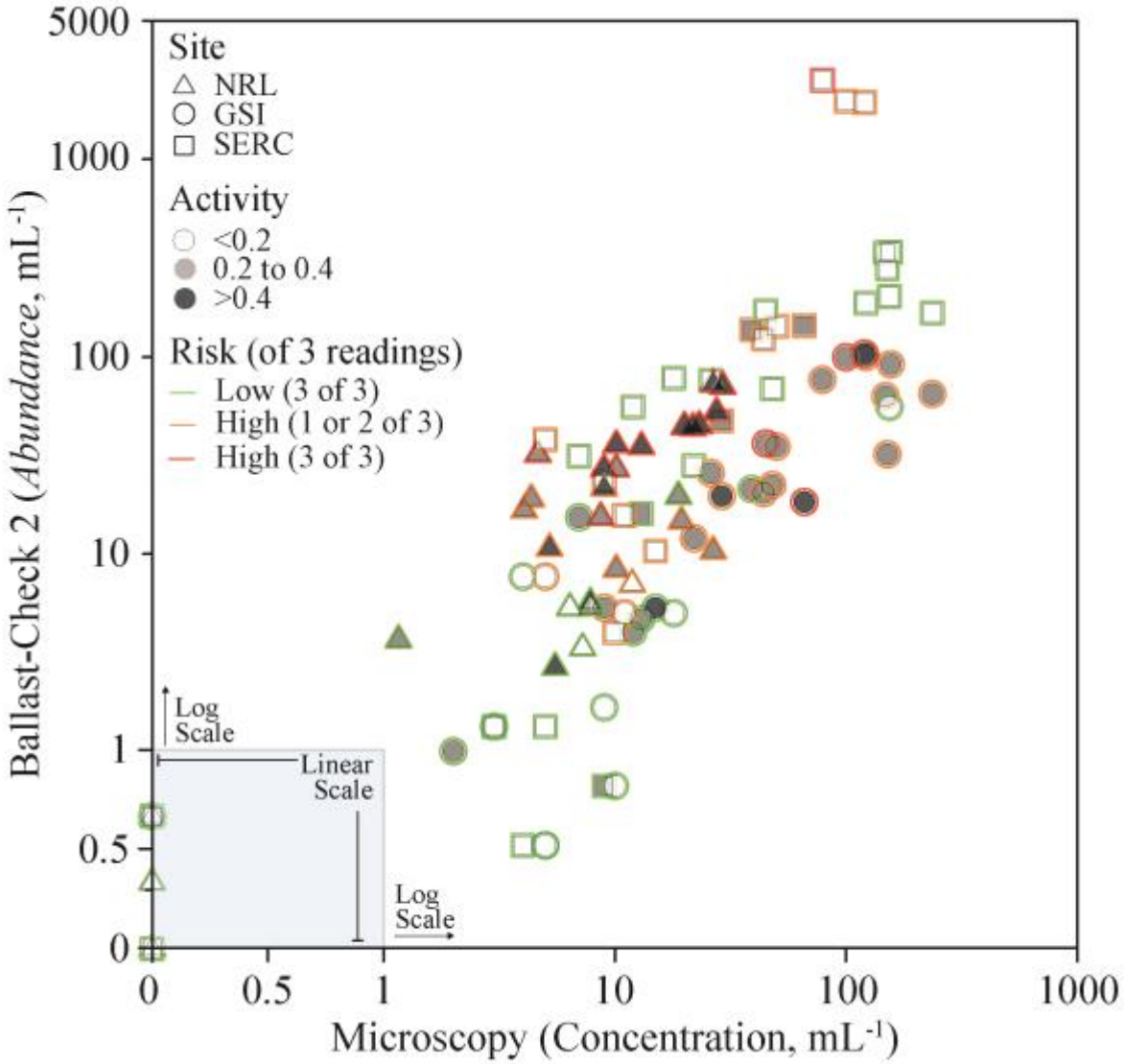

Figure 2. Results of the field experiments. Measurements from the Ballast-Check 2 are compared to concentrations of ambient organisms ≥10 and <50 µm at the three test sites. Symbols mark the mean *Abundance* and colors show the mean *Activity* of three repeated readings. Symbol outlines display the number of repeated readings with low or high *Risk*. The figure inset, with axis scaled from 0 to 1, is a linear scale. The rest of the figure displays data on a logarithmic scale.

Table 1. Results of linear regression analyses for both laboratory and field trials. Values indicate the adjusted (Adj.) R^2 value, the standard error (SE) of the estimates, *F*-values, slopes and y-intercepts (int.) of the relationship between concentration determined by microscopy and *Abundance*.

Data Set	Adj. R^2	R^2 SE	*F*-Value	Slope (±SE)	y-int. (±SE)	n
Laboratory Trials						
All organisms	0.90	12.8	$F_{1,34} = 307$	1.28±0.07	-5.88±2.64	36
T. marina	0.46	1.8	$F_{1,16} = 15.2$	0.14±0.04	0.73±0.59	18
P. micans	0.98	6.2	$F_{1,16} = 1061$	1.33±0.04	-1.74±1.97	18
Field Trials						
All Sites	0.10	338	$F_{1,106} = 12.2$	2.20±39.6	20.8±39.6	108
NRL	0.63	12.3	$F_{1,34} = 59.8$	1.74±0.23	1.44±2.99	36
GSI	0.64	19.6	$F_{1,34} = 63.0$	0.44±0.06	5.25±4.24	36
SERC	0.12	556	$F_{1,34} = 5.54$*	3.69±1.57	72.8±120	36

All p-values for regressions <0.001, except for NRL, where p = 0.021, except *p=0.024

In laboratory trials, readings of *Abundance* were strongly related to cell concentrations of *P. micans* ($R^2 = 0.98$; Figure 1 and Table 1). The relationship was significant for *T. marina*, although the R^2—the coefficient of determination—was lower ($R^2 = 0.46$) than with the *P. micans* samples, indicating a higher variation between observed data and the line-of-best-fit. In the field trials, the linear relationships between *Abundance* and concentrations of organisms ≥10 and <50 µm were significant ($p<0.05$), although the R^2 was low for SERC ($R^2 = 0.12$) and the combined data set of all field site ($R^2 = 0.10$; Table 1).

Precision

Precision is a measure of the variation among repeated analyses. The precision of the instrument was determined by calculating the coefficient of variation (CV, %), a relative measure of the variation among replicate readings. CV is sensitive to small mean values (e.g., mean concentration <10 mL^{-1}): as mean approaches 0, CV approaches infinity. Because of this, the CV of mean concentrations <10 mL^{-1} were reported, but only CV from samples ≥10 mL^{-1} were used to determine the range. In laboratory trials, all samples with *T. marina* (including those with the highest target concentrations of 100 mL^{-1}) did not have mean *Abundance* values ≥10 mL^{-1} (Table 2). Note that *target* concentrations are not the actual concentrations measured by microscopy. For *P. micans*, however, samples with target concentrations >10 mL^{-1} had mean *Abundance* large enough to estimate CV, and CV of three readings ranged from 9 to 70% (32% and 28%, mean and median CV, respectively, n = 13). For field trials, most sample with target concentrations above or well above the discharge standard had mean *Abundance* values ≥10 mL^{-1} (Table 3). From these and other samples, the CV of three subsamples (each with three readings) ranged from 22 to 123% (61% and 58%, mean and median CV, respectively, n = 21).

Table 2. Mean, standard deviation (SD), and coefficient of variation (CV) of *Abundance* measurements in laboratory trials (n = 3 for each sample). Rows show the target cell concentrations: 0, 5, 10, 20, 50, and 100 mL^{-1}. Black circles mark samples with *Abundance* mean values ≥10 mL^{-1}; these values were used in the summary of the CV ranges reported in the text.

			Abundance **(mL^{-1})**	
Target Concentration	**Organism**	**Trial ID**	**Mean ± SD**	**CV**
0 mL^{-1}	*T. marina*	LAB-1	1 ± 1	100%
		LAB-2	0 ± 0	-
		LAB-3	1 ± 1	100%
	P. micans	LAB-1	1 ± 0.6	87%
		LAB-2	0 ± 0	-
		LAB-3	0 ± 0	-
5 mL^{-1}	*T. marina*	LAB-1	1 ± 0.6	87%
		LAB-2	1 ± 1.2	173%
		LAB-3	1 ± 1	100%
	P. micans	LAB-1	8 ± 9.9	129%
		LAB-2	11 ± 6.1 ●	54%
		LAB-3	5 ± 4.7	89%
10 mL^{-1}	*T. marina*	LAB-1	2 ± 2.1	125%
		LAB-2	2 ± 1.5	92%
		LAB-3	3 ± 3.1	92%
	P. micans	LAB-1	14 ± 11 ●	79%
		LAB-2	12 ± 3.2 ●	28%
		LAB-3	15 ± 1.7 ●	12%
20 mL^{-1}	*T. marina*	LAB-1	1 ± 1	100%
		LAB-2	0 ± 0	-
		LAB-3	0 ± 0.6	173%
	P. micans	LAB-1	26 ± 2.3 ●	9%
		LAB-2	25 ± 10 ●	40%
		LAB-3	21 ± 13 ●	61%
50 mL^{-1}	*T. marina*	LAB-1	3 ± 3.6	120%
		LAB-2	7 ± 4.2	57%
		LAB-3	2 ± 3.5	173%
	P. micans	LAB-1	59 ± 6.1 ●	10%
		LAB-2	51 ± 15 ●	30%
		LAB-3	68 ± 23.5 l	35%
100 mL^{-1}	*T. marina*	LAB-1	5 ± 5.5	118%
		LAB-2	1 ± 1.5	115%
		LAB-3	9 ± 4.7	51%
	P. micans	LAB-1	135 ± 32.1 ●	24%
		LAB-2	146 ± 25.8 ●	18%
		LAB-3	139 ± 24.5 ●	18%

Table 3. Mean, standard deviation (SD), and coefficient of variation (CV) of *Abundance* measurements in field trials (n=9 for each sample). Rows show the target sample concentrations: Control (target cell concentration = 0 mL^{-1}), near the discharge standard (Near DS, 5 – 10 mL^{-1}), Above DS (30 – 50 mL^{-1}), or Well Above the DS (>50 mL^{-1}). Black circles mark samples with *Abundance* mean values ≥10 mL^{-1}.

		***Abundance* (mL^{-1})**		
Sample	**Trial ID**	**Mean ± SD**		**CV**
Control	NRL-1	0.1 ± 0.3		300%
	NRL-2	1.7 ± 2.5		1.5
	NRL-3	0.4 ± 0.7		163%
	GSI-1	0.8 ± 1.0		125%
	GSI-2	0.9 ± 0.9		1.04
	GSI-3	3.1 ± 7.6		2.43
	SERC-1	0.7 ± 1.0		150%
	SERC-2	0.4 ± 1.3		300%
	SERC-3	0.4 ± 0.5		119%
Near DS	NRL-1	31 ± 18	●	58%
	NRL-2	9 ± 5		63%
	NRL-3	4 ± 3		76%
	GSI-1	7 ± 6		85%
	GSI-2	9 ± 12		132%
	GSI-3	4 ± 4		110%
	SERC-1	33 ± 21	●	62%
	SERC-2	31 ± 21	●	67%
	SERC-3	10 ± 12	●	120%
Above DS	NRL-1	38 ± 13	●	34%
	NRL-2	18 ± 10	●	0.59
	NRL-3	9 ± 8		82%
	GSI-1	28 ± 16	●	57%
	GSI-2	25 ± 22	●	89%
	GSI-3	15 ± 14	●	92%
	SERC-1	105 ± 62	●	59%
	SERC-2	141 ± 30	●	22%
	SERC-3	83 ± 76	●	92%
Well Above DS	NRL-1	65 ± 26	●	40%
	NRL-2	17 ± 21	●	123%
	NRL-3	25 ± 10	●	41%
	GSI-1	93 ± 35	●	37%
	GSI-2	62 ± 45	●	72%
	GSI-3	73 ± 42	●	57%
	SERC-1	2144 ±740	●	35%
	SERC-2	317 ± 120	●	38%
	SERC-3	185 ± 69	●	37%

Accuracy

Accuracy of the instrument is a measure of the difference between a measurement and the actual or expected value, i.e. how good data are when compared with a recognized standard for measuring organisms ≥10 and <50 µm. (Note: from the Test Protocols "Accuracy is measured as the proportion of samples that correctly assess whether a sample meets the discharge standard"). For each sample read, the instrument reports *Risk* (either Low or High *Risk*), which was calculated using measurements of *Abundance* and *Activity*. As the discharge standard (DS) of organisms in the ≥10 and <50 µm size class is <10 mL^{-1}, samples with concentrations higher than this nominal value would be expected to be rated as High *Risk*. The procedures for determining risk based upon sample measurements were not known, as any calculations, conversions, or variable weighing were considered the manufacturer's proprietary information. Rather, results were categorized either as Low or High *Risk*(as previously mentioned, *Fail* and High *Risk* are both classified as High *Risk*), and a logistical regression analysis was used to determine the probability that the instrument correctly assigns *Risk* as cell concentrations diverge from the DS, whether below the DS (e.g., 0 to 9 mL) or above the *DS*. Concentrations were scaled so that values ≥10 mL^{-1} should be high risk: effectively, 10 was subtracted from all measured concentrations prior to analysis. Results of the logistical regression analyses are shown in Table 4.

Table 4. Logistic regression results for the field trials.

		Constant (*C*)			Coefficient (*x*)			
		Value	**SE**	**p-Value**	**Value**	**SE**	**p-Value**	**n**
Laboratory Trials	Both organisms	-2.38	0.77	0.002	0.110	0.048	0.021	36
	T. marina	-4.48	2.22	0.043	0.165	0.114	0.147	18
	P. micans	-1.73	1.17	0.139	0.185	0.119	0.119	18
Field Trials	All Sites	-1.58	0.30	<0.001	0.021	0.007	0.003	108
	NRL	-0.64	0.41	0.115	0.132	0.049	0.007	36
	GSI	-2.19	0.68	<0.001	0.039	0.014	0.005	36
	SERC	-3.52	1.09	<0.001	0.033	0.018	0.077	36

To visualize the results of this analysis, the resulting values—the constant (*C*) and the coefficient (*x*)—were used to calculate the probability (ρ) of a High *Risk* (*H*) outcome across a range of cell concentrations (*P*):

EQ. 1

$$\boldsymbol{\rho(H) = \frac{1}{(1+e^{(-C+xP)})}}$$

Resulting $\rho(H)$ values across a range of cell concentrations are shown in Figure 3. At an organism concentration of 30 mL^{-1}, which is three times the DS, the probability of High *Risk* ($\rho(H)$) was 0.97, 0.26, 0.07, and 0.28 for NRL, GSI, SERC, and all sites, respectively (Figure

3A). For laboratory trials, $\rho(H)$ was 0.58, 0.98, and 0.69 for *T. marina*, *P. micans*, and the combined data set with both organisms combined, respectively (Figure 3B).

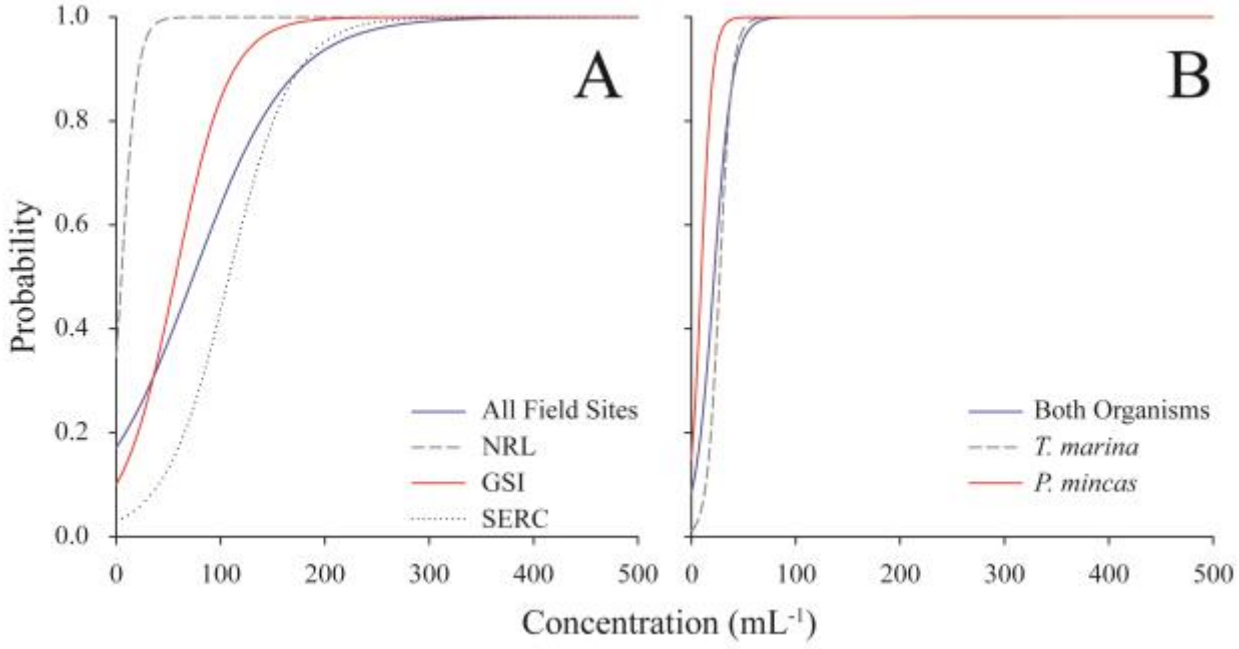

Figure 3. Probability of indicating a sample is High *Risk* based upon cell concentrations in field (**A**) and laboratory trials (**B).**

QUALITY MANAGEMENT

All technical activities conducted by ACT and NRL comply with their respective Quality Management System (QMS), which includes the policies, objectives, procedures, authority, and accountability needed to ensure quality in work processes, products, and services. A QMS provides the framework for quality assurance (QA) functions, which cover planning, implementation, and review of data collection activities and the use of data in decision-making, and quality control. The QMS also ensures that all data collection and processing activities are carried out in a consistent manner, to produce data of known and documented quality that can be used with a high degree of certainty by the intended user to support specific decisions or actions regarding technology performance. Both organizations' QMS meet U.S. Environmental Protection Agency quality standards for environmental data collection, production, and use. The QMS also meets the requirements of General Requirements for the Competence of Testing and Calibration Laboratories (ISO/IEC 17025:2005[E]).

An effective assessment program is an integral part of a quality system. The ACT Quality Assurance (QA) Manager independently conducted six Technical Systems Audits (TSA, described below) and data quality assessments of all reference data sets for the evaluation.

Technical System Audits

A TSA is a thorough, systematic, on-site qualitative audit of sampling and measurement processes and procedures associated with a specific technology evaluation. The objectives of the TSAs conducted during this evaluation were to assess and document the conformance of on-site testing procedures with the requirements of the Test Protocols and associated Standard Operating Procedures (SOPs).

The TSAs were conducted in accordance with the procedures described in EPA's Guidance on Technical Audits and Related Assessments for Environmental Data Operations (EPA QA/G-7) and ISO 19011, Guidelines for Quality and/or Environmental Management Systems Auditing. A TSA checklist based on the Test Protocols was prepared prior to each audit and reviewed by the respective laboratory's personnel. The TSA assessed the respective laboratories' personnel, the test and analytical facilities, equipment maintenance and calibration procedures, sample collection, analytical activities, record keeping, and QC procedures. The audits were conducted for all field trials and laboratory trials.

During each audit, the auditor met with each person involved in testing and asked that person to describe the procedures. All procedures were observed, and logbooks, data forms, and other records were reviewed.

Key components of each audit included assessments of the following:

Quality Assurance/Quality Control:

- Adequacy of procedures and adherence to procedures
- Chain of command regarding description of assignments and specific duties

Sample System:

- Sample collection
- Analytical procedures
- Analytical equipment maintenance and calibration
- Documentation.

Data and Document Control:

- Chain of custody
- Validation and processing procedures
- Documentation

The findings of the TSA for the four field tests and two laboratory tests were positive. All of these tests were being implemented consistent with the Test Protocols and SOPs. Minor deviations were documented in laboratory records. None of the deviations had an effect on data quality for the evaluation Test Instruments. Failures were due to mechanical problems with the instrument. All phases of the implementation of the test reviewed during the TSAs were acceptable and performed in a manner consistent with ACT/NRL data quality goals. The overall quality assurance objectives of the test were met.

ACT and NRL personnel are well qualified to implement the evaluation and demonstrated expertise in pertinent procedures. Communication and coordination among all personnel was frequent and effective. Internal record keeping and document control was well organized. The ACT and NRL staff understands the need for QC, as shown in the conscientious development and implementation of a variety of QC procedures.

All samples and instrument measurements were collected, analyzed and cataloged as described in the Test Protocols and SOPs. Examination of maintenance and calibration logs provided evidence of recent and suitable calibration of sampling and analytical equipment.

Data Assessments

Data review was conducted to ensure that only sound data that are of known and documented quality and meet quality objectives were used in making decisions about technology performance. Data review processes are based in part on two EPA guidance documents: Guidance on Environmental Data Verification and Data Validation (QA/G-8) (EPA, 2002) and Guidance on Technical Audits and Related Assessments for Environmental Data Operations (QA/G-7) (EPA, 2000).

At the outset of the evaluation, data were verified and validated to evaluate whether data were generated according to the Test Protocols, satisfied acceptance criteria, and were appropriate for their intended use of evaluating the performance of the test instruments. Data verification evaluates the completeness, correctness, and consistency of data sets against the requirements specified in the Test Protocols, measurement quality objectives, and any other analytical process requirements contained in SOPs. The ACT QA Manager reviewed the reference (microscopy) data sets from all field and laboratory tests. Thirty-six (36) reference samples were counted for each field test (total 216 microscopy counts); fifty-six (56) reference samples were counted for each laboratory test (total 112 microscopy counts). The overall reference data set included 328 microscopy counts. Data review verified that the sampling and analysis protocols specified in the Test Protocols were followed, and that the ACT/NRL measurement and analytical systems performed in accordance with approved methods, based on the following criteria:

- The raw data records were complete, understandable, well-labeled, and traceable
- All data identified in the Test Protocols were collected
- QC criteria were achieved
- Data calculations were accurate

Data validation uses the outputs from data verification and included inspection of the verified field and laboratory data to determine the analytical quality of data set. A representative set of approximately 10% of the reference data was traced in detail from 1) raw data from field and laboratory logs, 2) data transcription, 3) data reduction and calculations, to 4) final reported data. Validation of the referenced data set established:

- Required sampling methods were used
- Sampling procedures and field measurements met performance criteria
- Required analytical methods were used

Data validation also confirmed that data were accumulated, transferred, summarized, and reported correctly. There is sufficient documentation of all procedures used in data collection and analysis to validate that data were collected in accordance with the evaluation's quality objectives.

A Data Quality Assessment (DQA) is the third and final process of the overall data assessment. It is a scientific and statistical evaluation of validated data to determine if data are of the right type, quality, and quantity to support conclusions on the performance of the test instruments. The DQA determined that the evaluation's data quality objectives, described in the Test Protocols (Appendix A) were achieved.

REFERENCES

International Maritime Organization; IMO (2004) International convention for the control and management of ships' ballast water and sediments. http://www.imo.org/OurWork/Environment/BallastWaterManagement/Pages/BWMConventionandGuidelines.aspx

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The vendor's formal response letter is appended as a separate document.

APPENDIX A: TEST PLAN

Available for download at www.act-us.info/evaluations.

APPENDIX B: RAW DATA

Table 1. Summary of trials conducted.

Location	Trial Name	Trial Date	Trial Replicate
Naval Research Laboratory (NRL; Key West, FL)	NRL-1	7/14/2015	1 of 3
	NRL-2	7/15/2015	2 of 3
	NRL-3	7/16/2015	3 of 3
Laboratory Trial (LAB; Key West, FL)	LAB-1	6/2/2015	1 of 3
	LAB-2	6/3/2015	2 of 3
	LAB-3	6/4/2015	3 of 3
Smithsonian Environmental Research Center (SERC; Edgewater, MD)	SERC-1	8/7/2015	1 of 3
	SERC-2	8/8/2015	2 of 3
	SERC-3	8/10/2015	3 of 3
Great Ships Initiative (GSI; Superior, WI)	GSI-1	9/1/2015	1 of 3
	GSI-2	9/2/2015	2 of 3
	GSI-3	9/3/2015	3 of 3

Table 2. Concentrations of living organisms ≥10 and <50 µm in samples from field trials. Target concentrations were Control (0 mL^{-1}), near the discharge standard (DS, 5 – 20 mL^{-1}), above the DS (30 – 50 mL^{-1}), and well above the DS (>50 mL^{-1}).

Trial Number	Sample		Concentration (mL^{-1}) NRL	GSI	SERC
1 of 3	Control	A	0	5	0
		B	0	1	0
		C	0	1	0
	Near DS	A	9	9	10
		B	9	23	9
		C	20	16	9
	Above DS	A	23	30	26
		B	13	56	27
		C	10	50	33
	Well Above DS	A	27	110	313
		B	29	143	264
		C	28	164	115
2 of 3	Control	A	0	0	0
		B	0	1	0
		C	0	1	0
	Near DS	A	8	11	10
		B	9	11	7
		C	6	11	10
	Above DS	A	4	69	62
		B	19	25	39
		C	5	51	61
	Well Above DS	A	12	61	66
		B	27	120	73
		C	22	66	73
3 of 3	Control	A	0	0	0
		B	0	0	0
		C	0	0	0
	Near DS	A	7	10	6
		B	1	10	8
		C	8	8	4
	Above DS	A	10	63	26
		B	6	35	29
		C	4	72	41
	Well Above DS	A	5	68	82
		B	10	93	82
		C	19	88	79

Table 3. Concentrations of cultured organisms in samples from laboratory experiments. In two samples, the cultured organisms—*Tetraselmis marina* and *Prorocentrum micans*—were amended with dissolved and particulate materials or disinfection byproducts (DBP). Target concentrations ranged from 0 to 100 mL^{-1}.

		Concentration (mL^{-1})	
Trial	**Sample**	***T. marina***	***P. micans***
LAB-1	0 mL^{-1}	0	0
	5 mL^{-1}	0	7
	10 mL^{-1}	2	10
	20 mL^{-1}	5	25
	50 mL^{-1}	5	36
	100 mL^{-1}	35	102
	10 mL^{-1} (Amended)	2	12
	10 mL^{-1} (DBP)	2	9
LAB-2	0 mL^{-1}	0	0
	5 mL^{-1}	3	4
	10 mL^{-1}	4	10
	20 mL^{-1}	10	25
	50 mL^{-1}	34	48
	100 mL^{-1}	32	105
	10 mL^{-1} (Amended)	3	10
	10 mL^{-1} (DBP)	0	6
LAB-3	0 mL^{-1}	0	0
	5 mL^{-1}	2	6
	10 mL^{-1}	4	15
	20 mL^{-1}	9	22
	50 mL^{-1}	18	50
	100 mL^{-1}	29	111
	10 mL^{-1} (Amended)	4	10
	10 mL^{-1} (DBP)	0	6

Table 4. Ballast-Check 2 *Abundance* (mL^{-1}) of samples from field trials at NRL. Red symbols (●) indicate a "high" risk of exceeding the discharge standard (DS).

			Replicate Reading				
Trial	**Sample**		**1 of 3**	**2 of 3**	**3 of 3**	**Mean**	**SD**
NRL-1	Control	A	0	0	0	0.0	0.0
		B	1	0	0	0.3	0.6
		C	0	0	0	0.0	0.0
	Near DS	A	26 ●	10	29 ●	21.7	10.2
		B	20 ●	38 ●	23 ●	27.0	9.6
		C	42 ●	20 ●	71 ●	44.3	25.6
	Above DS	A	47 ●	57 ●	30 ●	44.7	13.7
		B	45 ●	32 ●	28 ●	35.0	8.9
		C	19 ●	55 ●	33 ●	35.7	18.1
	Well Above DS	A	47 ●	69 ●	103 ●	73.0	28.2
		B	112 ●	38 ●	62 ●	70.7	37.8
		C	63 ●	55 ●	39 ●	52.3	12.2
NRL-2	Control	A	1	0	0	0.3	0.6
		B	8	1	1	3.3	4.0
		C	2	2	0	1.3	1.2
	Near DS	A	7	5	4	5.3	1.5
		B	12 ●	15 ●	19 ●	15.3	3.5
		C	4	4	8	5.3	2.3
	Above DS	A	19	25 ●	13	19.0	6.0
		B	13	7	39	19.7	17.0
		C	14 ●	7	11 ●	10.7	3.5
	Well Above DS	A	17 ●	0	4	7.0	8.9
		B	24 ●	7	0	10.3	12.3
		C	50 ●	50 ●	31 ●	43.7	11.0
NRL-3	Control	A	0	0	1	0.3	0.6
		B	0	0	2	0.7	1.2
		C	1	0	0	0.3	0.6
	Near DS	A	1	9	0	3.3	4.9
		B	6	5	0	3.7	3.2
		C	5	5	7	5.7	1.2
	Above DS	A	3	7	15 ●	8.3	6.1
		B	5	2	1	2.7	2.1
		C	24	13 ●	13 ●	16.7	6.4
	Well Above DS	A	26 ●	38 ●	32 ●	32.0	6.0
		B	14 ●	33 ●	34 ●	27.0	11.3
		C	15	18	11 ●	14.7	3.5

Table 5. Ballast-Check 2 *Activity* (no units) of samples from field trials at NRL. Red symbols (●) indicate a "high" risk of exceeding the discharge standard (DS).

Trial	Sample		Replicate Reading 1 of 3	2 of 3	3 of 3	Mean	SD
NRL-1	Control	A	0	0	0	0	0
		B	0	0	0	0	0
		C	0	0	0	0	0
	Near DS	A	0.40 ●	0.51	0.49 ●	0.47	0.06
		B	0.48 ●	0.48 ●	0.42 ●	0.46	0.03
		C	0.44 ●	0.63 ●	0.51 ●	0.53	0.10
	Above DS	A	0.42 ●	0.62 ●	0.54 ●	0.53	0.10
		B	0.33 ●	0.52 ●	0.50 ●	0.45	0.10
		C	0.57 ●	0.43 ●	0.46 ●	0.49	0.07
	Well Above DS	A	0.45 ●	0.45 ●	0.43 ●	0.44	0.01
		B	0.44 ●	0.48 ●	0.41 ●	0.44	0.04
		C	0.47 ●	0.42 ●	0.55 ●	0.48	0.07
NRL-2	Control	A	0.62	0	0	0.21	0.36
		B	0	0	0.27	0.09	0.16
		C	0	0.35	0	0.12	0.20
	Near DS	A	0.47	0	0.65	0.37	0.34
		B	0.32 ●	0.39 ●	0.36 ●	0.36	0.04
		C	0	0	0.37	0.12	0.21
	Above DS	A	0.27	0.35 ●	0.27	0.30	0.05
		B	0.30	0.54	0	0.28	0.27
		C	0.48 ●	0.60	0.44 ●	0.51	0.08
	Well Above DS	A	0.41 ●	0	0	0.14	0.24
		B	0.47 ●	0.63	0	0.37	0.33
		C	0.42 ●	0.41 ●	0.48 ●	0.44	0.04
NRL-3	Control	A	0	0	0	0	0
		B	0	0	0.20	0.07	0.12
		C	0.17	0	0	0.06	0.10
	Near DS	A	0	0.44	0	0.15	0.25
		B	0.37	0.31	0	0.23	0.20
		C	0.48	0.49	0.45	0.47	0.02
	Above DS	A	0.33	0.43	0.41 ●	0.39	0.05
		B	0.47	0.59	0.50	0.52	0.06
		C	0.23	0.43 ●	0.41 ●	0.36	0.11
	Well Above DS	A	0.34 ●	0.42 ●	0.39 ●	0.38	0.04
		B	0.42 ●	0.42 ●	0.31 ●	0.38	0.06
		C	0.26	0.29	0.41 ●	0.32	0.08

Table 6. Ballast-Check 2 *Abundance* (mL^{-1}) of samples from field trials at GSI. Red symbols (●) indicate a "high" risk of exceeding the discharge standard (DS).

			Replicate Reading				
Trial	**Sample**		**1 of 3**	**2 of 3**	**3 of 3**	**Mean**	**SD**
GSI-1	Control	A	1	0	0	0	0.6
		B	0	0	2	1	1.2
		C	0	2	2	1	1.2
	Near DS	A	8	10 ●	18 ●	12	5.3
		B	12	0	0	4	6.9
		C	4	6	4	5	1.2
	Above DS	A	40 ●	0	27 ●	22	20.4
		B	49 ●	17 ●	43 ●	36	17.0
		C	40	20 ●	17 ●	26	12.5
	Well Above DS	A	50	38 ●	141 ●	76	56.3
		B	74 ●	120 ●	117 ●	104	25.7
		C	80 ●	117 ●	101 ●	99	18.6
GSI-2	Control	A	1	2	0	1	1.0
		B	0	2	2	1	1.2
		C	0	1	0	0	0.6
	Near DS	A	40	4	2	15	21.4
		B	10 ●	6	0	5	5.0
		C	0	15 ●	8	8	7.5
	Above DS	A	78 ●	17	10	35	37.4
		B	6	23	35	21	14.6
		C	30 ●	13 ●	12 ●	18	10.1
	Well Above DS	A	41 ●	66	82	63	20.7
		B	0	15 ●	81	32	43.1
		C	68 ●	154	52	91	54.9
GSI-3	Control	A	0	23	0	8	13.3
		B	0	1	4	2	2.1
		C	0	0	0	0	0.0
	Near DS	A	6	7	3	5	2.1
		B	2	0	0	1	1.2
		C	0	3	12 ●	5	6.2
	Above DS	A	20 ●	32 ●	7	20	12.5
		B	0	0	15	5	8.7
		C	29 ●	32 ●	0	20	17.7
	Well Above DS	A	66 ●	90	38 ●	65	26.0
		B	8	67	92	56	43.1
		C	42 ●	112	145 ●	100	52.6

Table 7. Ballast-Check 2 *Activity* (no units) of samples from field trials at GSI. Red symbols (●) indicate a "high" risk of exceeding the discharge standard (DS).

Trial	Sample		Replicate Reading 1 of 3	Replicate Reading 2 of 3	Replicate Reading 3 of 3	Mean	SD
GSI-1	Control	A	0	0	0	0	0
		B	0	0	0	0	0
		C	0	0.29	0	0.10	0.17
	Near DS	A	0.41	0.36 ●	0.34 ●	0.37	0.04
		B	0.26	0.50	0	0.25	0.25
		C	0.45	0	0.37	0.27	0.24
	Above DS	A	0.48 ●	0	0.34 ●	0.27	0.25
		B	0.32 ●	0.41 ●	0.42 ●	0.38	0.06
		C	0.30	0.43 ●	0.31 ●	0.35	0.07
	Well Above DS	A	0.25	0.35 ●	0.40 ●	0.33	0.08
		B	0.42 ●	0.44 ●	0.38 ●	0.41	0.03
		C	0.41 ●	0.31 ●	0.35 ●	0.36	0.05
GSI-2	Control	A	0	0.73	0	0.24	0.42
		B	0	0.56	0	0.19	0.32
		C	0	0	0	0	0
	Near DS	A	0.26	0.62	0	0.29	0.31
		B	0.32 ●	0.58	0	0.30	0.29
		C	0	0.42 ●	0	0.14	0.24
	Above DS	A	0.34 ●	0.25	0.42	0.34	0.09
		B	0.62	0.30	0.13	0.35	0.25
		C	0.48 ●	0.51 ●	0.42 ●	0.47	0.05
	Well Above DS	A	0.54 ●	0.06	0.08	0.23	0.27
		B	0	0.55 ●	0.17	0.24	0.28
		C	0.31 ●	0.29	0.29	0.30	0.01
GSI-3	Control	A	0	0.17	0	0.06	0.10
		B	0	0	0	0	0
		C	0	0	0	0	0
	Near DS	A	0.30	0.43	0.55	0.43	0.13
		B	0	0	0	0	0
		C	0	0	0.35 ●	0.12	0.20
	Above DS	A	0.45 ●	0.40 ●	0.61	0.49	0.11
		B	0	0	0.29	0.10	0.17
		C	0.49 ●	0.53 ●	0	0.34	0.30
	Well Above DS	A	0.33 ●	0.24	0.56 ●	0.38	0.17
		B	0	0.20	0.19	0.13	0.11
		C	0.49 ●	0.30	0.38 ●	0.39	0.10

Table 8. Ballast-Check 2 *Abundance* (mL^{-1}) of samples from field trials at SERC. Red symbols (●) indicate a "high" risk of exceeding the discharge standard (DS).

			Replicate Reading				
Trial	**Sample**		**1 of 3**	**2 of 3**	**3 of 3**	**Mean**	**SD**
SERC-1	Control	A	1	0	3	1.3	1.5
		B	1	0	1	0.7	0.6
		C	0	0	0	0.0	0.0
	Near DS	A	25	26	33	28.0	4.4
		B	59	36	72	55.7	18.2
		C	25	5	18	16.0	10.1
	Above DS	A	104	54	48	68.7	30.7
		B	202	108	203	171	54.6
		C	117	68	42	75.7	38.1
	Well Above DS	A	2517 ●	2517 ●	2517 ●	2517	0.0
		B	2517 ●	798	2517 ●	1944	993
		C	2517 ●	2517 ●	879	1971	946
SERC-2	Control	A	0	0	0	0.0	0.0
		B	4	0	0	1.3	2.3
		C	0	0	0	0.0	0.0
	Near DS	A	32	30	32	31.3	1.2
		B	0	43	27 ●	23.3	21.7
		C	28	11	75 ●	38.0	33.2
	Above DS	A	139	189	98 ●	142	45.6
		B	156 ●	128	126	136	16.8
		C	181	109 ●	141 ●	144	36.1
	Well Above DS	A	266	490	249	335	135
		B	413	270	151	278	131
		C	494	292	229	338	138
SERC-3	Control	A	1	0	0	0.3	0.6
		B	1	0	1	0.7	0.6
		C	0	1	0	0.3	0.6
	Near DS	A	14	0	17 ●	10.3	9.1
		B	12 ●	0	0	4.0	6.9
		C	11	36 ●	0	15.7	18.4
	Above DS	A	7	101	34 ●	47.3	48.4
		B	0	120	113	77.7	67.4
		C	206 ●	0	162	123	109
	Well Above DS	A	166	221	112	166	54.5
		B	145	288	172	202	76.0
		C	81	267	213	187	95.7

Table 9. Ballast-Check 2 *Activity* (no units) of samples from field trials at SERC. Red symbols (●) indicate a "high" risk of exceeding the discharge standard (DS).

Trial	Sample		Replicate Reading 1 of 3	2 of 3	3 of 3	Mean	SD
SERC-1	Control	A	0	0	0.05	0.02	0.03
		B	0	0	0.29	0.10	0.17
		C	0	0	0	0	0
	Near DS	A	0.18	0	0	0.06	0.10
		B	0.14	0.20	0.15	0.16	0.03
		C	0.17	0.50	0.18	0.28	0.19
	Above DS	A	0.03	0.02	0.04	0.03	0.01
		B	0.10	0.17	0.10	0.12	0.04
		C	0	0	0	0	0
	Well Above DS	A	0 ●	0 ●	0 ●	0	0
		B	0 ●	0	0 ●	0	0
		C	0 ●	0 ●	0	0	0
SERC-2	Control	A	0	0	0	0	0
		B	0	0	0	0	0
		C	0	0	0.40	0.13	0.23
	Near DS	A	0	0	0.08	0.03	0.05
		B	0	0.13	0.40 ●	0.18	0.20
		C	0.06	0	0.32 ●	0.13	0.17
	Above DS	A	0.06	0.10	0.33 ●	0.16	0.15
		B	0.30 ●	0.27	0.16	0.24	0.07
		C	0.16	0.34 ●	0.35 ●	0.28	0.11
	Well Above DS	A	0	0	0	0	0
		B	0	0	0	0	0
		C	0	0	0	0	0
SERC-3	Control	A	0	0	0	0	0
		B	0	0.75	0	0.25	0.43
		C	0	0.20	0	0.07	0.12
	Near DS	A	0.06	0	0.44 ●	0.17	0.24
		B	0.55 ●	0	0	0.18	0.32
		C	0.05	0.31 ●	0	0.12	0.17
	Above DS	A	0	0.21	0.57 ●	0.26	0.29
		B	0	0.24	0.15	0.13	0.12
		C	0.33 ●	0	0.24	0.19	0.17
	Well Above DS	A	0	0	0	0	0
		B	0	0	0	0	0
		C	0	0	0	0	0

Table 10. Ballast-Check 2 *Abundance* (mL^{-1}) of *T. marina* in samples from laboratory trials. In two samples, cultures were amended with dissolved and particulate materials or disinfection byproducts (DBP). Red symbols (●) indicate a "high" risk of exceeding the discharge standard.

		Replicate Reading				
Trial	***T. marina* sample**	**1 of 3**	**2 of 3**	**3 of 3**	**Mean**	**SD**
LAB-1	0 mL^{-1}	1	0	2	1.0	1.0
	5 mL^{-1}	1	1	0	0.7	0.6
	10 mL^{-1}	1	0	4	1.7	2.1
	20 mL^{-1}	0	2	1	1.0	1.0
	50 mL^{-1}	7	0	2	3.0	3.6
	100 mL^{-1}	11	2	1	4.7	5.5
	10 mL^{-1} (Amended)	0	1	0	0.3	0.6
	10 mL^{-1} (DBP)	1	1	2	1.3	0.6
LAB-2	0 mL^{-1}	0	0	0	0.0	0.0
	5 mL^{-1}	0	2	0	0.7	1.2
	10 mL^{-1}	2	3	0	1.7	1.5
	20 mL^{-1}	0	0	0	0.0	0.0
	50 mL^{-1}	12 ●	6	4	7.3	4.2
	100 mL^{-1}	3	0	1	1.3	1.5
	10 mL^{-1} (Amended)	3	1	2	2.0	1.0
	10 mL^{-1} (DBP)	1	0	1	0.7	0.6
LAB-3	0 mL^{-1}	2	1	0	1.0	2
	5 mL^{-1}	0	1	2	1.0	0
	10 mL^{-1}	4	6	0	3.3	4
	20 mL^{-1}	0	1	0	0.3	0
	50 mL^{-1}	6	0	0	2.0	6
	100 mL^{-1}	13 ●	4	11 ●	9.3	13
	10 mL^{-1} (Amended)	1	2	0	1.0	1
	10 mL^{-1} (DBP)	0	2	0	0.7	0

Table 11. Ballast-Check 2 *Activity* (no units) of *T. marina* in samples from laboratory trials. In two samples, cultures were amended with dissolved and particulate materials or disinfection byproducts (DBP). Red symbols (●) indicate a "high" risk of exceeding the discharge standard.

		Replicate Reading				
Trial	***T. marina* sample**	**1 of 3**	**2 of 3**	**3 of 3**	**Mean**	**SD**
LAB-1	0 mL^{-1}	0	0	0	0	0
	5 mL^{-1}	0.31	0.55	0	0.29	0.28
	10 mL^{-1}	0.45	0	0.04	0.16	0.25
	20 mL^{-1}	0	0	0	0	0
	50 mL^{-1}	0.34	0	0.51	0.28	0.26
	100 mL^{-1}	0.25	0.35	0	0.20	0.18
	10 mL^{-1} (Amended)	0	0.42	0	0.14	0.24
	10 mL^{-1} (DBP)	0	0.60	0.35	0.32	0.30
LAB-2	0 mL^{-1}	0	0	0	0	0
	5 mL^{-1}	0	0	0	0	0
	10 mL^{-1}	0	0.07	0	0.02	0.04
	20 mL^{-1}	0	0	0	0	0
	50 mL^{-1}	0.34 ●	0.08	0.48	0.30	0.20
	100 mL^{-1}	0	0	0	0	0
	10 mL^{-1} (Amended)	0.19	0.60	0	0.26	0.31
	10 mL^{-1} (DBP)	0.44	0.74	0.37	0.52	0.20
LAB-3	0 mL^{-1}	0	0.64	0	0.21	0.37
	5 mL^{-1}	0.37	0	0	0.12	0.21
	10 mL^{-1}	0.33	0.14	0	0.16	0.17
	20 mL^{-1}	0	0.42	0	0.14	0.24
	50 mL^{-1}	0.28	0	0.73	0.34	0.37
	100 mL^{-1}	0.31 ●	0.45	0.41 ●	0.39	0.07
	10 mL^{-1} (Amended)	0	0	0	0	0
	10 mL^{-1} (DBP)	0	0.15	0	0.05	0.09

Table 12. Ballast-Check 2 *Abundance* (mL^{-1}) of *P. micans* in samples from laboratory trials. In two samples, cultures were amended with dissolved and particulate materials or disinfection byproducts (DBP). Red symbols (●) indicate a "high" risk of exceeding the discharge standard.

		Replicate Reading				
Trial	***P. micans* sample**	**1 of 3**	**2 of 3**	**3 of 3**	**Mean**	**SD**
LAB-1	0 mL^{-1}	1	1	0	0.7	0.6
	5 mL^{-1}	1	19 ●	3	7.7	9.9
	10 mL^{-1}	11	27 ●	5	14.3	11.4
	20 mL^{-1}	23 ●	27 ●	27 ●	25.7	2.3
	50 mL^{-1}	62 ●	52 ●	63 ●	59.0	6.1
	100 mL^{-1}	98 ●	158 ●	148 ●	134.7	32.1
	10 mL^{-1} (Amended)	18	21 ●	17 ●	18.7	2.1
	10 mL^{-1} (DBP)	16 ●	23 ●	5	14.7	9.1
LAB-2	0 mL^{-1}	0	0	0	0.0	0.0
	5 mL^{-1}	18 ●	6	10	11.3	6.1
	10 mL^{-1}	13 ●	8	14 ●	11.7	3.2
	20 mL^{-1}	29	33 ●	14 ●	25.3	10.0
	50 mL^{-1}	42 ●	68 ●	42 ●	50.7	15.0
	100 mL^{-1}	125 ●	139 ●	175 ●	146.3	25.8
	10 mL^{-1} (Amended)	19 ●	7	8	11.3	6.7
	10 mL^{-1} (DBP)	7	5	17 ●	9.7	6.4
LAB-3	0 mL^{-1}	0	0	0	0.0	0.0
	5 mL^{-1}	9	0	7	5.3	4.7
	10 mL^{-1}	13 ●	16 ●	16 ●	15.0	1.7
	20 mL^{-1}	8	34 ●	22 ●	21.3	13.0
	50 mL^{-1}	45 ●	67 ●	92 ●	68.0	23.5
	100 mL^{-1}	140 ●	163 ●	114 ●	139.0	24.5
	10 mL^{-1} (Amended)	16 ●	28 ●	3	15.7	12.5
	10 mL^{-1} (DBP)	12	2	24	12.7	11.0

Table 13. Ballast-Check 2 *Activity* (no units) of *P. micans* in samples from laboratory trials. In two samples, cultures were amended with dissolved and particulate materials or disinfection byproducts (DBP). Red symbols (●) indicate a "high" risk of exceeding the discharge standard.

		Replicate Reading				
Trial	***P. micans* sample**	**1 of 3**	**2 of 3**	**3 of 3**	**Mean**	**SD**
LAB-1	0 mL^{-1}	0.04	0	0	0.01	0.02
	5 mL^{-1}	0.58	0.39 ●	0.50	0.49	0.10
	10 mL^{-1}	0.26	0.42 ●	0.69	0.46	0.22
	20 mL^{-1}	0.36 ●	0.51 ●	0.33 ●	0.40	0.10
	50 mL^{-1}	0.40 ●	0.52 ●	0.53 ●	0.48	0.07
	100 mL^{-1}	0.44 ●	0.49 ●	0.47 ●	0.47	0.03
	10 mL^{-1} (Amended)	0.10	0.51 ●	0.47 ●	0.36	0.23
	10 mL^{-1} (DBP)	0.40 ●	0.47 ●	0.34	0.40	0.07
LAB-2	0 mL^{-1}	0	0	0.40	0.13	0.23
	5 mL^{-1}	0.32 ●	0.42	0.36	0.37	0.05
	10 mL^{-1}	0.41 ●	0.42	0.46 ●	0.43	0.03
	20 mL^{-1}	0.28	0.47 ●	0.40 ●	0.38	0.10
	50 mL^{-1}	0.41 ●	0.45 ●	0.48 ●	0.45	0.04
	100 mL^{-1}	0.44 ●	0.41 ●	0.42 ●	0.42	0.02
	10 mL^{-1} (Amended)	0.41 ●	0.53	0.33	0.42	0.10
	10 mL^{-1} (DBP)	0.28	0.47	0.51 ●	0.42	0.12
LAB-3	0 mL^{-1}	0	0	0	0	0
	5 mL^{-1}	0.33	0	0.45	0.26	0.23
	10 mL^{-1}	0.46 ●	0.51 ●	0.55 ●	0.51	0.05
	20 mL^{-1}	0.49	0.44 ●	0.46 ●	0.46	0.03
	50 mL^{-1}	0.43 ●	0.47 ●	0.46 ●	0.45	0.02
	100 mL^{-1}	0.46 ●	0.44 ●	0.45 ●	0.45	0.01
	10 mL^{-1} (Amended)	0.38 ●	0.48 ●	0.12	0.33	0.19
	10 mL^{-1} (DBP)	0.10	0.02	0.02	0.05	0.05

Ballast-Check 2 is used for quick indicative compliance checks by providing an indication of risk for exceedance of ballast water regulations. Precision and accuracy are optimized for the ballast water regulation which states, "Not more than 10 living organisms within the 10 – 50 µm size class of algae". Any tests performed outside of the limits of this regulation in an effort to evaluate performance of the Ballast-Check 2 for this application may not accurately reflect this instrument's capability for this analysis.

Prorocentrum micans can range in size from 10 to 50 µm, within the size class specified for this application by current regulations. Accordingly, *P. micans* would be the best candidate for evaluating discharge standard limits and testing accuracy, precision, and linearity of Ballast-Check 2 for use with ballast water applications. Results from laboratory testing using *P. micans* would accurately reflect performance of Ballast-Check 2 in this evaluation.

Conversely, *Tetraselmis marina* is a less than ideal candidate for testing Ballast-Check 2 as its size range includes <10 µm cells. The target size class specified for this application is 10 to 50 µm, so using *T. marina* will introduce error in the measurement by potentially underestimating cell concentrations when selecting for cell sizes specific to this evaluation, as was observed in laboratory results where *T. marina* was used.

In addition, the *RISK* assessment is a binary outcome determined by *Abundance* and *Activity* results together to generate a *RISK* factor of either low or high risk of exceeding the discharge standard. *RISK* cannot be determined using either *Abundance* or *Activity* alone, both results are required to assess risk.

Given the points mentioned in this note above, Turner Designs is very pleased with the results of this evaluation which showed how well the Ballast-Check 2 performed in both laboratory and field tests.

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