

**10/28/2010 Science Advisory Board (SAB) Ecological Processes and Effects Committee  
Augmented for Ballast Water**

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**EPA SAB Ballast Water Advisory  
Subgroup 3**

**Draft Response to charge question 4: Limitations of existing studies and reports**

*10/28/2010 revised text from Subgroup 3 for consideration during the 11/4/2010 teleconference.*

*This draft supplants the 10/20/2010 annotated outline prepared by Subgroup 3, which was previously posted on the SAB website. Proposed revisions to this draft, primarily for the purpose of simplifying its structure, are briefly described in italicized text following the table of contents, as well as noted throughout in bracketed italicized text. Draft text for onshore treatment (Section VI) is provided as separate document, to be integrated here in next round of revisions.*

Table of contents:

I. Introduction

- A. Charge from EPA and the Focus of this Document.....
- B. Motivation: Standards Drive Technology Development ..... 3

II. Testing Shipboard Treatment Systems: Protocols, Analysis, and Reporting Practices that Could be Improved 5

- A. Major Limitations ..... 5
- B. Lack of Independent Testing ..... 8
- C. Need for Experimental Validation of Methods ..... 8
- D. Lack of Standardized Testing Protocols..... 11
  - 1) *Test Verification Factors* ..... 11
  - 2) *Challenge Conditions* ..... 14
  - 3) *Treatment Facility Experimental Configuration* ..... 18
  - 4) *Verification Testing*..... 18
- E. Compromises Necessary Because of Practical Constraints ..... 20
  - 1) *Standard Test Organisms (Surrogates)*..... 20
  - 2) *Other Potential Surrogates Cannot Reliably Quantify Organisms* ..... 25
  - 3) *Sized-Down Treatments* ..... 28
- F. Incomplete Reporting of Results ..... 29

III. Testing Shipboard Treatment Systems: Inherent Limitations of Direct End-of-the-pipe Sampling 29

- A. Statistical Constraints ..... 30
- B. Mismatch Between the “Living” (Viable) Standard and Practical Protocols..... 38
  - 1) *Rapid concentration of cells from large volumes kills and/or destroys many organisms* 39
  - 2) *Standardized rigorously tested protocols for evaluating organism viability do not yet exist* ..... 40
  - 3) *Resistant stages present special challenges in attempts to assess viability* ..... 41

**10/28/2010 Science Advisory Board (SAB) Ecological Processes and Effects Committee  
Augmented for Ballast Water**

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1 4) Many microbial species are capable of changing their location in counting chambers,  
2 which can bias the count ..... 42  
3 5) Viable when collected (formerly viable) counts should be considered as a practical  
4 alternative for quantifying phytoplankton, other protists, and zooplankton ..... 42  
5 6) Techniques to detect and quantify harmful bacteria are based upon small volumes  
6 relative to ballast tank volumes, and also cannot account for active nonculturable cells .. 43  
7 C. HACCP: An Alternative Approach to Assess Treatment Capabilities ..... 44  
8  
9 IV. Relationship of Testing Protocols to Assuring Feasibility of Voluntary Compliance  
10 Monitoring and Enforceability of Standards 46  
11 A. Statement of Limitation ..... 46  
12 B. Practical Compliance/Enforcement Protocols Needed ..... 46  
13  
14 V. Approaches other than ballast water treatment.  
15 A. Introduction ..... 46  
16 B. Managing Ballast Uptake ..... 47  
17 C. Reducing Invasion Risk from Ballast Discharges..... 50  
18  
19 VI. Onshore Treatment - [see separate document for full text; outline only below]  
20 A. Studies of onshore treatment  
21 B. Advantages of onshore treatment compared to shipboard treatment  
22 C. Operational issues potentially restricting the use of onshore treatment  
23 D. Cost of onshore vs. shipboard treatment  
24 E. Potential effectiveness of onshore treatment  
25  
26 VII. Combined approaches and voyage-based risk management .....51  
27 A. Combined approaches .....52  
28 B. Voyage-based risk assessment to prioritize use of treatment technologies, ballasting, and  
29 deballasting practices, monitoring efforts, and enforcement.....52  
30  
31 VIII. Appendices. [for onshore treatment studies -- see separate document]  
32 Appendix 1: Cost estimate adjustments and calculation on annualized costs  
33 Appendix 2: Adjustment of the cost estimates in Brown and Caldwell, (2007) to the design  
34 criteria in Brown and Caldwell.  
35 Appendix 3: Estimates of treatment plants and treatment capacity needed in onshore and  
36 shipboard treatment approaches for the Port of Milwaukee, Australia, California, and the United  
37 States.  
38  
39 IX. References cited .....53  
40  
41  
42  
43

**10/28/2010 Science Advisory Board (SAB) Ecological Processes and Effects Committee  
Augmented for Ballast Water**

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1 *Likely revisions to this draft include the following:*  
2

- 3 • *The discussion regarding use of “surrogates” will be combined into one portion*  
4 *of the text. In addition, the term “surrogates” as used herein, will be clearly defined to*  
5 *avoid ambiguity.*
- 6 • *Discussions of “compliance monitoring” will be combined in one section.*
- 7 • *Portions of this draft text that summarizes material from the EPA’s 2010*  
8 *Environmental Technology Verification (ETV) report and material from the Lee et al.*  
9 *2010 paper on sampling will be moved into an appendix. The discussion of these*  
10 *documents to remain in the body of the text will clearly state conclusions drawn by the*  
11 *Subgroup that differ from the conclusions of these EPA documents.*
- 12 • *Several sections in this draft need to be completed.*
- 13 • *Several issues require further discussion, including: potential use of surrogates;*  
14 *potential use of HACCP-type analyses; and whether / how to characterize or otherwise*  
15 *distinguish between “fixable problems” and “inherent limitations” associated with*  
16 *current end-of-the-pipe testing.*
- 17 • *Draft text on onshore treatment to be integrated here, apportioned between main*  
18 *body of text and appendices.*  
19  
20

21 **I. Introduction**  
22

23 **A. Charge from EPA and the Focus of this Document**  
24

25 “What are the principal limitations of the available studies and reports on the status of ballast  
26 water treatment technology and system performance and how can these limitations be overcome  
27 or corrected in future assessments of the availability of technology for treating ballast water  
28 onboard vessels?” Charge question 4 (and the other three specific charge questions) are under  
29 the committee’s overall charge to “provide advice on technologies and systems to minimize the  
30 impacts of invasive species in vessel ballast water discharge” (Feb. 2010 Federal Register  
31 notice). While we address Charge Question 4, we also address aspects of the broader charge not  
32 covered by any of the four specific charge questions. Specifically, in later sections of this report,  
33 we address limitations of technology and systems to enable effective compliance and  
34 enforcement, and on-shore treatment systems.  
35

36 **B. Motivation: Standards Drive Technology Development**  
37

38 The interplay between regulatory standards and the technologies employed to meet them creates  
39 a dilemma that regulatory agencies must often have to grapple with. To be useful, the standards  
40 that dischargers are required to meet must be achievable; but to provide an incentive to improve

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1 treatment technology, these standards must be more demanding than can be met by the treatment  
2 systems that are either already in use to treat the discharges or in an advanced stage of  
3 development. Thus if our treatment goals (that is, the level of treatment needed to protect  
4 ecosystems and public health) exceed the technical capabilities of the treatment systems that are  
5 currently deployed or imminently available, then regulators must enact requirements that cannot  
6 be met by the current technology and require improvements in it. Otherwise, there is no  
7 economic incentive—and indeed there is a strong disincentive<sup>1</sup>—to develop or employ  
8 technology that does a better job of treating ballast water.<sup>2</sup>

9  
10 How much improvement over current technology should discharge requirements demand?  
11 Ideally, as much as can be achieved by an appropriate level of effort over the relevant time  
12 period before the discharger is, in practice, substantially subjected to the discharge requirements.  
13 If the requirements are too demanding, dischargers will not be able to meet them and will be  
14 subjected to penalties despite making an appropriate effort to meet the requirements, which is  
15 unfair to the dischargers. If, on the other hand, the requirements are not demanding enough, then  
16 achievable improvements in treatment technology and achievable reductions in pollutant  
17 discharges will not be attained, allowing levels of environmental impact and risks to public  
18 health that could reasonably have been avoided, which is unfair to those affected by these  
19 impacts and risks.

20  
21 In assessing how far beyond the technologies that are currently in use or in development they  
22 should go, regulators should at a minimum consider reasonable improvements in these  
23 technologies; combining technologies to improve treatment levels; and utilizing technologies or  
24 approaches that have been employed in other fields but that have not been applied to treat these  
25 specific discharges. For each of these or for other approaches, the assessment must in significant  
26 part be conceptual or analytical: by definition, none of these improvements over the in-use  
27 technology are currently being applied to these discharges, so there can be no actual test data on  
28 their performance. Professional judgment must be applied, based on whatever relevant data and  
29 analyses are available.

30  
31 Specifically, if we want to have treatment technologies that do a better job than just meeting the

---

<sup>1</sup> This is nothing less than the “invisible hand” of the market. Installing treatment systems that are more effective than is needed to avoid regulatory penalties is economically inefficient. By paying for a better system than is needed, profits are lost, and shipping companies that buy the right system rather than too good a system will outcompete the inefficient companies and ultimately drive them from business. Companies will thus try to avoid buying systems that are more effective than necessary, and manufacturers will avoid designing or building them.

<sup>2</sup> Reeves (1999) makes a related point, that it is a “common fallacy” that “what is feasible and reasonable has to be so for everyone in the business, doing business just as they have always done so before...[T]he feasibility or reasonableness standard becomes a standard based on the lowest common denominator set by the most inefficient or highly polluting segments of the industry. This ignores the basic nature of life in business,” which is that if it is uneconomical for some vessels to make the changes needed to meet strict discharge standards, that “will open up that business for competitors who have found a way to manage their ballast more efficiently. Our public policy should be to encourage that competition to a higher level, rather than protecting all businesses against failure at a low level of efficiency.”

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1 IMO D2 standards, then we will first have to adopt discharge requirements that are more  
2 demanding than the IMO D2 standards. Equipment manufacturers will invest in the development  
3 of more effective treatment systems only if they believe they can make a profit from them. Profit  
4 comes only if ships buy their treatment systems, and ships will only do so if forced by  
5 government regulations. The only way to make progress toward the development of more  
6 effective treatment systems is for regulators to enact discharge requirements that go beyond the  
7 capabilities of the current treatment systems and make it clear that they will vigorously enforce  
8 them. To quote one study of ballast water treatment systems (CAPA 2000), “Standards, in effect,  
9 create the technology to meet those standards.”<sup>3</sup>  
10  
11

12 **II. Testing Shipboard Treatment Systems:**  
13 **Protocols, Analysis, and Reporting Practices that Could be Improved**  
14

15 **A. Major Limitations**  
16

17 *[The description of different discharge standards will be moved to the “background” section of the*  
18 *report. Some other material may be moved to an appendix.]*  
19

20 **Major impediments to evaluating the performance of ballast water treatment technologies**  
21 **include the lack of available data to enable assessment or comparison of most systems; the lack**  
22 **of comprehensive, standardized testing programs; and the lack of standardized, practical**  
23 **protocols for quantifying the removal of some size classes of organisms.**  
24

25 The lack of available data from rigorous scientific testing has prevented assessment of nearly all  
26 ballast water treatment systems (see Section above). The few systems with sufficient available  
27 data have, for the most part, been tested using different scales, experimental protocols, and  
28 statistical approaches. Reporting content also has varied widely. As a more fundamental  
29 concern, most performance testing has not been done by independent, third-party scientific  
30 testing centers.  
31

---

<sup>3</sup> It might be argued that equipment manufacturers are already designing the best possible systems that they can without regard to any particular standard. However, statements made at the July 2010 panel meeting suggest otherwise, that these systems are designed specifically to meet the IMO D2 standards. Kevin Reynolds stated that these treatment systems are “closely tuned” to IMO D2. Edward Lemieux stated that if standards substantially stronger than IMO D2 were to be adopted this might force manufacturers to try completely different treatment approaches, rather than making incremental improvements in the systems currently under development. □□ In his public comments, Jon Stewart (the President of International Maritime Consultants) argued that equipment manufacturers are specifically designing treatment systems to meet IMO D2, and are ignoring the more stringent California and USCG proposed Phase 2 standards because only the global market is large enough to drive the development of treatment systems. Evidence can also be found in the literature (e.g. “The MSI Ballast Water Treatment System (UV), patent pending, is designed to meet IMO D2 discharge standards” (Tamburri 2009)).

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1 There presently is no comprehensive international, federal or state program that includes  
2 performance standards, guidelines, and protocols to verify treatment technology performance,  
3 and no standardized sets of methods for sampling and analysis of ballast water to assess  
4 compliance (U.S. EPA 2010). Treatment evaluations generally are recommended to be designed  
5 to test whether a given technology can meet International Maritime Organization (IMO) D2  
6 standards in accordance with both the IMO *Guidelines for Approval of Ballast Water*  
7 *Management Systems* (G8) and the *Procedure for Approval of Ballast Water Systems that Make*  
8 *Use of Active Substances* (G9) (IMO 2008a,b). The IMO performance standard states that ships  
9 must discharge:

- 10 1) Less than 10 viable organisms per m<sup>3</sup>, greater than or equal to 50 µm in minimum  
11 dimension;
- 12 2) Less than 10 viable organisms per mL, less than 50 µm in maximum dimension and  
13 greater than or equal to 10 µm in minimum dimension; and
- 14 3) Less than the following concentrations of indicator microbes, as a human health standard:  
15 1. Toxigenic *Vibrio cholerae* (serogroups O1 and O139), less than 1 colony-forming unit  
16 (CFU) per 100 mL  
17 2. *Escherichia coli*, less than 250 CFU per 100 mL  
18 3. Intestinal enterococci, less than 100 CFU per 100 mL.

19  
20  
21 The proposed U.S. Negotiating Position (BWM/CONF/13&14) would take this performance  
22 standard to a yet-more stringent level (Albert et al. 2010):

- 23 24 1) Less than 0.01 “living” organism per m<sup>3</sup>, greater than or equal to 50 µm in minimum  
25 dimension;
- 26 2) Less than 0.01 “living” organism per mL, less than 50 µm in maximum dimension and  
27 greater than or equal to 10 µm in minimum dimension;
- 28 3) Less than the following concentrations of indicator microbes:  
29 1. Toxigenic *Vibrio cholerae*, less than 1 CFU per 100 mL  
30 2. *Escherichia coli*, less than 126 CFU per 100 mL  
31 3. Intestinal enterococci, less than 33 CFU per 100 mL.

32  
33 Protocols in the IMO G-8 Guidelines, supported by the new U.S. EPA Environmental  
34 Technology Verification (ETV) Program (U.S. EPA 2010), specify taking whole-water samples  
35 of at least 1 m<sup>3</sup> (1,000 L) for organisms greater than 50 µm, and at least 1 m<sup>3</sup> for organisms  
36 greater than 10 µm but less than or equal to 50 µm.

37  
38 With exception of the USCG’s Shipboard Technology Evaluation Program (STEP), ballast water  
39 treatment systems at present are not approved for use in compliance with federal ballast water  
40 management requirements. Thus, while there are various state ballast water management  
41 requirements, there is no formal environmental assessment approval program for ballast water  
42 treatment systems at the federal level. US EPA has, however, included provisions in the draft  
43 NPDES Vessel General Permit for ships with treatment systems that discharge ballast water  
44 containing biocides or chemical residues. In addition, US EPA’s Environmental Technology  
45 Verification (ETV) Program was created to accelerate the development and marketing of

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1 environmental technologies including ballast water treatment, and recently developed a treatment  
2 technology verification protocol that is available in draft form (U.S. EPA 2010). The state of  
3 California also has developed “Ballast Water Treatment Technology Testing Guidelines that are  
4 intended to provide a standardized approach for evaluating treatment system performance  
5 (Dobroski et al. 2009). Procedures are being developed for verifying vessel compliance with  
6 performance standards as well.  
7

8 An array of ballast treatment technologies have been developed because ballast water exchange  
9 alone cannot accomplish the IMO-D2 (G8) performance standard (hereafter referred to as the  
10 IMO standard). While ballast water exchange is considered an effective means of eliminating  
11 organisms in tanks, its effectiveness has been found to vary substantially, from 50-99% (Cohen  
12 1998, Parsons 1998, Zhang and Dickman 1999, USCG 2001, Wonham et al. 2001, MacIsaac et  
13 al. 2002, Burkholder et al. 2007, Ruiz and Reid 2007). Moreover, the percentage of ballast water  
14 exchanged does not necessarily accomplish a proportional decrease in organism abundance,  
15 since organisms can become concentrated in tank bottom waters or along tank walls and left  
16 behind (Choi et al. 2005, Ruiz and Reid 2007).  
17

18 Treatment technology performance evaluation has lagged behind the rapidly evolving ballast  
19 water treatment industry, in part because shipboard treatment technologies widely vary,  
20 reflecting the broad range of factors involved with ship construction, space constraints, and  
21 voyage duration. Mechanical, chemical, physical, biological treatment approaches, and various  
22 combinations of these are in use or under development. About 30 different treatment  
23 technologies have been produced by manufacturers in ten countries; of these, ~21 use  
24 combination treatments, usually filtration or other mechanical separation together with one or  
25 more other treatment techniques (Dobroski et al. 2009). Chemical treatment is common and may  
26 involve chlorine, ozone, ferrate, a mixture of peracetic acid + acetic acid + hydrogen peroxide, or  
27 advanced oxidation or electrolytic processes that can produce bromine, chlorine, and/or hydroxyl  
28 radicals (Dobroski et al. 2009). Mechanical separation combined with ultraviolet radiation is also  
29 fairly common.  
30

31 Performance standards set requirements for technology to achieve and should help to advance  
32 progress in treatment system designs, but only if a set of standardized, practical, scientifically  
33 rigorous assessment techniques is available to evaluate treatment system performance. The IMO  
34 standards are based upon different size groups of organisms, and the small size groups are  
35 especially problematic in efforts to assess performance (see below). In particular, there presently  
36 are no widely accepted scientific methods to assess the concentrations of viable bacteria and  
37 viruses in ballast water, including harmful species. Assessment has relied upon a subset or  
38 “surrogate” group of organisms as representative of treatment of all bacteria (see Section  
39 below). There is as yet no strong evidence for suitable proxy organisms to represent the virus  
40 size class, and no acceptable methods for verification of compliance with a total virus standard.  
41  
42

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1 **B. Lack of Independent Testing**  
2

3 *[The paragraph on ETV will be moved to an appendix along with other text on the ETV program*  
4 *in sections C and D].*  
5

6 **Testing should be conducted by a party independent from the manufacturer with appropriate,**  
7 **established credentials, approved by EPA/USCG.**  
8

9 To ensure insofar as possible that the performance of ballast water treatment systems is  
10 objectively and thoroughly evaluated, experienced specialists in an independent testing  
11 organization should conduct the tests, rather than the system manufacturers. This is important  
12 because science has shown that it is extremely difficult, after the creator of a system has been  
13 constructively designing it, to change h/her perspective and instead approach the treatment  
14 system from a “deconstructive” state of mind to form the necessary mental attitude of wanting to  
15 find flaws and expose weaknesses and limitations (Myers 1979). Thus, verification testing  
16 conducted by independent specialists is critical in accomplishing a scientifically rigorous  
17 assessment of system performance. The testing organization (TO) should provide detailed  
18 information about the expertise of its personnel, and the established credentials of these  
19 personnel should be approved by the U.S. EPA/USCG.  
20

21 The ETV has developed a detailed set of protocols for land-based testing of ballast water  
22 treatment technologies. The focus was on land-based rather than shipboard testing to provide  
23 comparable conditions for verifying treatment performance by independent testing operations  
24 (U.S. EPA 2010). We strongly support most of the detailed protocols and recommendations  
25 outlined by the ETV, with exceptions noted below.  
26

27 **C. Need for Experimental Validation of Methods**  
28

29 *[Text reviewing or summarizing material in the EPA’s 2010 ETV report will be moved to an*  
30 *appendix. The text in this section will focus on any issues or conclusions about validation of*  
31 *methods that differ from what is covered in the ETV report]*  
32

33 **Experimental validation is needed to demonstrate that testing operations can accomplish**  
34 **separation of organisms with quantified uncertainty (e.g. microbead-based experiments to test**  
35 **filtration.**  
36

37 Prior to full-scale standardized testing, validation of ballast water treatment system performance  
38 should be completed by an independent organization using bench-scale testing in controlled  
39 laboratory conditions (see Section ). In land-based, full-scale standardized testing, detection  
40 limits are a major problem for compliance with the IMO standards, especially for small  
41 microorganisms in large volumes. Since biological samples should be continuously acquired on  
42 a time-averaged basis for performance testing, organism abundance can be statistically  
43 represented by the Poisson distribution, so the cumulative or total count is the key test statistic  
44 (Lemieux et al. 2008). A Chi-square transformation can also be used to approximate confidence

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1 intervals. Since the Poisson distribution pools the data to improve measurement precision  
2 (assumption: organisms are randomly distributed – but see Section ), sample replication is not  
3 needed. However, experimental validation must be obtained to ensure that testing organizations  
4 can accomplish separation of organisms with quantified uncertainty because the available  
5 methodologies for testing compliance with the IMO standards for zooplankton and protists  
6 (phytoplankton, protozoans) are at or near the analytic detection limits. The following example  
7 from the ETV illustrates the problem (U.S. EPA 2010):  
8

9 If the desired minimum precision in quantifying organisms  $\geq 50 \mu\text{m}$  is that the upper bound of  
10 the Chi-square statistic should not exceed twice the observed mean (which corresponds to a  
11 coefficient of variation of 40%), then *six* organisms must be counted:  
12

13 Coefficient of variation (CV) = standard deviation (SD) divided by the mean (M).

14 For the Poisson distribution, the variance (V) =  $\text{SD}^2 = M$ .

15 Substituting the critical value of the mean, 6:  $\text{CV} = 6^{.05}/6 \approx 40\%$ .  
16

17 The volume needed to find and quantify six organisms depends on the whole-water sample  
18 volume, the concentration factor, the number of subsamples examined, and the target  
19 concentration. Very large sample volumes (1 m<sup>3</sup> or 1,000 L) are required to quantify  
20 phytoplankton and zooplankton (live/dead), and each sample must be concentrated down to a  
21 volume of 1 L for a concentration factor of 3,000. Based on the Poisson distribution for a 95%  
22 confidence interval (CI) from the Chi-square transformation, 30 m<sup>3</sup> (30,000 L) must be sampled  
23 in order to find and count 10 organisms m<sup>-3</sup> with the desired level of precision. The total sample  
24 volume can be reduced if the concentration factor is  
25 increased, but then the CI is lowered (e.g. from 95% to 90%), or the subsample volume analysed  
26 must be increased (e.g. from 20 mL to 40 mL).  
27

28 The ETV recommends that the sample size should be selected, relative to the targeted  
29 concentration, to provide the level of precision needed to achieve a 95% upper confidence limit  
30 that is no more than twice the observed mean, and does not exceed the targeted concentration  
31 (Tables 1 and 2). If the subsample volume analyzed is increased, then validation experiments  
32 should be conducted to ensure that counting accuracy is high. The problem is worse for  
33 organisms  $\geq 50 \mu\text{m}$  because they are more sparse than organisms in the next smaller size class,  $\geq$   
34 10  $\mu\text{m}$  to  $< 50 \mu\text{m}$ . The Poisson distribution assumption still applies, with a more stringent level  
35 of precision (Table 1).  
36

37  
38 **Table 1.** Sample volume required relative to treatment standards for organisms  $\geq 50 \mu\text{m}$ , assuming that the desired level of  
39 precision of the estimated density is set at the 05% confidence interval of the Poisson distribution (= twice the observed mean and  $\leq$   
40 the standard limit). These are the required whole-water sample volumes that must be concentrated to 1 L as a function of the  
41 number of 1-mL subsamples analyzed (N). From the ETV (U.S. EPA 2010).  
42

Concentration (individuals mL <sup>-1</sup> )	N =			
	2	3	4	
	Sample Volume Required (L) <sup>2</sup>			
0.01	60,000	20,000	12,000	

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1	0.1	6,000	2,000	1,200
2	1	600	200	120
3	10	6	20	12

**Table 2.** Sample volume required relative to treatment standards for organisms  $\geq 10 \mu\text{m}$  and  $< 50 \mu\text{m}$ , assuming that the desired level of precision is set at a CV of  $\leq 10\%$  or the upper confidence limit is  $\leq 20\%$  of the estimated density. These are the required whole-water sample volumes that must be concentrated to 1 L as a function of the number of 1-mL subsamples analyzed (N). From the ETV (U.S. EPA 2010).

Concentration (individuals $\text{mL}^{-1}$ )	N =	2	3	4
	Sample Volume Required (L) <sup>2</sup>			
0.01	6,000	4,000	3,000	
0.1	600	400	300	
1	60	40	30	
10	6	4	3	

The testing facility should conduct validation experiments, such as microbead-based experiments, to test filtration effectiveness, to make ensure that counting accuracy and precision are sufficiently high. The Naval Research Laboratory (Lemieux et al. 2010) tested use of inert, 10- $\mu\text{m}$  standardized microbeads at densities of 1, 5, 10, 50, 100, 500, and 1,000 beads per mL to represent phytoplankton, and use of 100- $\mu\text{m}$  beads at 10, 30, and 60 beads per 500 mL of artificial seawater to represent zooplankton. Such inert, standardized polymer beads are recommended for use rather than organisms to eliminate any potential bias, and artificial rather than natural seawater is recommended to avoid inclusion of various organic particles (e.g. detritus) that could interact with the beads and confound interpretations.

At each bead density, the percent difference of the observed mean from the expected mean was considered to indicate counting accuracy, and the CV was considered as the level of precision. Benchmarks for acceptable accuracy and precision were established at a percent difference of 10% and a CV of 0.2 (20%), respectively. For the “phytoplankton” beads, the 50-1,000 concentrations were not significantly different, with acceptable accuracy and precision below the 10% and 20% benchmarks, respectively. Unfortunately, however, analysis of the “zooplankton” bead populations at all densities showed poor precision, with measurements well above 20%.

From this work, Lemieux et al. (2010) recommended that samples for analysis of the protists size class (more than 10 but less than 50  $\mu\text{m}$ ) should be concentrated by at least a factor of five, and that at least four replicate chambers (e.g. Sedgwick Rafter slides) should be counted for acceptable accuracy and precision, including evaluation of at least 10 random rows (from a total of 20) of the sample slide. Importantly, for the zooplankton size class (larger than 50  $\mu\text{m}$ ) size class, Lemieux et al. (2010) evaluated the ETV protocol recommendations for sample sizes as inadequate to achieve acceptable precision. The data from these microbead experiments

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1 indicated, instead, that this size class requires a sample size of at least 6 m<sup>3</sup>, concentrated to 1 L  
2 (i.e., by a factor of 6,000), and analysis of at least 450 1-mL aliquots in order to attain a CV of  
3 20%. Lemieux et al. (2010) also noted that this analysis represents a “best case” situation  
4 because the study was conducted under simplified, “ideal” conditions rather than with natural  
5 organism assemblages in natural seawater.  
6

7 Overall, these data demonstrate that at present, the IMO G8 guideline for zooplankton at  
8 acceptable precision is practically achievable with great difficulty, at best, using available  
9 methodologies, and that stricter standards are not practically achievable. Additional information  
10 about statistical limitations in testing data are discussed below (Section ).  
11

## 12 **D. Lack of Standardized Testing Protocols**

13  
14 *[As in section C, text reviewing or summarizing material in the EPA’s 2010 ETV report will be*  
15 *moved to an appendix. The text in this section will focus on any issues or conclusions about test*  
16 *protocols that differ from what is covered in the ETV report.*

17  
18 *Also, in this section and in the following section E, the text will be revised to clarify priorities for*  
19 *actions. ]*  
20

### 21 **Testing protocols must be standardized and ideally applied across the full gradient of** 22 **environmental conditions represented by the earth’s ports**

23  
24 Comparison of the performance of different ballast water treatment technologies requires  
25 consistent testing protocols (Phillips 2006). Standardized approaches in evaluating treatment  
26 technologies should include the experimental design, environmental test conditions, sampling  
27 and analytical methods, and replication (Ruiz et al. 2006). All treatment technologies should  
28 function well across the range of physical /chemical conditions and densities/types of biological  
29 organisms that a ship encounters. Thus, ballast water treatment systems should be verified using  
30 a set of standard challenge conditions that ideally should encompass the suite of water quality  
31 conditions that captures the full gradient of environmental conditions represented by major ports,  
32 and the range of densities of the organisms and organism size classes included in the IMO  
33 standard (U.S. EPA 2010). The major assumption in land-based testing is that, although it cannot  
34 mimic shipboard treatment system performance, it can provide enough information to verify  
35 expected shipboard performance.  
36

37 The ETV recommended four key elements of a standardized protocol: test verification factors,  
38 water quality and biological challenge conditions, the treatment facility experimental  
39 configuration, and verification testing including required measurement programs, as follows  
40 (U.S. EPA 2010).

#### 41 *1) Test Verification Factors*

42  
43 All treatment systems should be verified considering the following factors: biological treatment

**10/28/2010 Science Advisory Board (SAB) Ecological Processes and Effects Committee  
Augmented for Ballast Water**

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1 efficacy, operation and maintenance, reliability, cost factors, environmental acceptability, and  
2 safety. Biological treatment efficiency (the removal, inactivation, or death of organisms) should  
3 be measured as the concentration, in the treated ballast water discharge, of the organism size  
4 classes indicated in the IMO standard, comparing the untreated versus treated ballast water.  
5 Other measurements can include organism removal efficiency (the percentage reduction of  
6 organisms that were present in the untreated ballast water), and water quality parameters in  
7 comparison to appropriate water quality standards. Verification protocols should include detailed  
8 descriptions of on-site sampling, sample handling (chain of custody), in-place mechanisms for  
9 selecting independent laboratories with appropriate expertise and certification to conduct the  
10 sample analyses, and requirements for compliance reporting.  
11

12 Operation and Maintenance - Includes the labor expertise, equipment, and supplies required to  
13 operate the ballast water treatment system to achieve the IMO standard. Examples of important  
14 quantitative OM performance indicators are visual observations of treated ballast water quality  
15 (e.g. turbidity, color), treatment conditions (e.g. foaming, floating material), and the inspection  
16 system (e.g. for corrosion, process failures, leaks, etc.); operability (observations about ease of  
17 start-up and operation, and ease of system performance); the utility and quality of the O&M  
18 manual; operator skills (the level of expertise needed for system OM); and system accessibility  
19 (ease of access and required clearances for system OM).  
20

21 Reliability - This term refers to a statistical measure of the number of failures (qualitative or  
22 quantitative) per known number of test cycles. It is calculated by comparing the manufacturer's  
23 projected mean time between failure (MTBF) with the maintenance events during the testing.  
24

25 Cost Factors- These include verifiable factors such as power consumption and labor hours  
26 needed for OM of the treatment system, expendable supplies, replacement parts used during  
27 normal maintenance, quantities of chemicals consumed, and byproduct or waste materials  
28 produced.  
29

30 Residual Toxicity – Tests and species selected for toxicity testing during commissioning need to  
31 be carefully justified and protocols detailed in the Test Plan (see Section , below). Ballast water  
32 treatment systems that involve a chemical mode of action are regulated under the National  
33 Pollutant Discharge Elimination System (NPDES) permit process (Albert et al. 2010), which  
34 requires demonstration of “no adverse effects” as evaluated through chemical-specific  
35 parameters and standardized Whole Effluent Toxicity (WET) testing (U.S. EPA 2002a-c; 40  
36 CFR 136.3, Table 1A; see <http://www.epa.gov/waterscience/WET>). WET experiments are  
37 designed to assess the effects of any residual toxicity on beneficial organisms in receiving  
38 waters.  
39

40 The U.S. EPA has developed standardized acute and chronic toxicity assays for a very limited  
41 number of freshwater and marine species (Table 3). The ETV did not comment on the  
42 freshwater assays, but recommends that toxicity tests for biocide treatments in brackish and  
43 marine waters should include the U.S. EPA acute toxicity assay for mysids (EPA OPPTS  
44 Method 850.1035; [http://www.epa.gov/opptsfrs/OPPTS\\_Harmonized/850\\_Ecological\\_Effects\\_Test\\_Guidelines/Drafts/850-1035.pdf](http://www.epa.gov/opptsfrs/OPPTS_Harmonized/850_Ecological_Effects_Test_Guidelines/Drafts/850-1035.pdf), and the chronic toxicity assays for the inland silverside,  
45

**10/28/2010 Science Advisory Board (SAB) Ecological Processes and Effects Committee  
Augmented for Ballast Water**

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1 *Menidia beryllina* (larval survival and growth, EPA Method 1006.0; <http://www.epa.gov/OST/WET/disk1/ctm13.pdf>) and the sea urchin, *Arbacia punctulata* (fertilization, EPA Method  
2 1008.0; <http://www.epa.gov/OST/WET/disk1/ctm15.pdf>). For additional guidance, U.S. EPA  
3 (2010) refers readers to Klemm et al. (1994) and the American Society of Testing and Materials  
4 (1996a,b). The ETV also includes, as an Appendix, the methods section from an excellent report  
5 by Anderson et al. (2008). This information includes detailed methods for assessment of residual  
6 toxicity to multiple surrogate species of bacteria, protists, zooplankton (see Section ) from use  
7 of chlorine, chlorine dioxide, glutaraldehyde, hydrogen peroxide, and PeraClean Ocean  
8 (hydrogen peroxide + peracetic acid) in ballast water treatment. Zooplankton and the bacterium  
9 *Geobacillus stearothermophilus* were also tested with the commercial product Seakleen™  
10 (active ingredient, vitamin K; see [http://www.hydemarine.com/ballast\\_water/seakleen.htm](http://www.hydemarine.com/ballast_water/seakleen.htm)).  
11  
12

13  
14 **Table 3.** Freshwater and marine species for which the U.S. EPA (<http://www.epa.gov/waterscience/WET>) has  
15 developed standardized acute and chronic toxicity assays.  
16

Habitat	Acute Toxicity	Chronic Toxicity
<u>Freshwaters</u>		
Algae	---	<i>Selenastrum capricornutum</i> (growth)
Zooplankton	<i>Ceriodaphnia dubia</i> <i>Daphnia magna</i> <i>Daphnia pulex</i>	Survival, reproduction --- ---
Fish	Bannerfin shiner ( <i>Cyprinella leedsi</i> ) Brook trout ( <i>Salvelinus fontinalis</i> ) Fathead minnow ( <i>Pimephales promelas</i> )  Rainbow trout ( <i>Oncorhynchus mykiss</i> ) ---	--- --- Larval survival, growth; embryo-larval survival, teratogenicity
<u>Marine</u>		
Mysid shrimp	<i>Americamysis bahia</i>	Survival, growth, fecundity
Sea urchin	---	<i>Arbacia punctulata</i> - fertilization
Fish	Sheepshead minnow ( <i>Cyprinodon variegatus</i> ) Silversides ( <i>Menidia beryllina</i> , <i>M. menidia</i> , <i>M. peninsulae</i> )	Larval survival, growth; embryo-larval survival, teratogenicity <i>M. beryllina</i> - larval survival, growth

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48 Environmental Acceptability – Two performance indicators, water quality and treatment  
49 residuals at the point of discharge, determine the environmental acceptability of a treatment  
50 system. The treated ballast water should be assessed to determine whether it meets acceptable  
51 water quality conditions (range of expected natural conditions), considering parameters such as  
52 temperature, salinity, pH, total suspended solids (TSS), dissolved oxygen (DO), particulate  
53 organic matter (POM), dissolved organic matter (DOM), biochemical oxygen demand (BOD),  
54 nutrients (nitrogen, phosphorus, carbon, silica), and biological efficacy. Assessment should also

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Augmented for Ballast Water**

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1 include treatment residuals such as residual biocides and disinfection products. The treatment  
2 facility should be responsible for obtaining necessary permits (e.g. NPDES), and should ensure  
3 that the discharge is within permitted limits. Toxicity testing of any biocide treatment must also  
4 yield acceptable results before verification testing can begin.  
5

6 Safety - Safety factors considered during verification testing are restricted to any treatment-  
7 specific considerations that may threaten shipboard operations or the health safety of the  
8 treatment system operator. Performance indicators are technology-specific, but should include a  
9 listing of all dangerous or hazardous materials and their potential to impede deballasting, and  
10 visual indicators of potential threats to shipboard operations.  
11

12 Reporting/ Record Keeping – All treatment verification tests should be completed following a  
13 written Test Plan that clearly states the protocol (objectives, goals, scope, and standard operating  
14 procedures used). The Test Plan should also include a Test/Quality Assurance Plan (TQAP, or  
15 Quality Assurance Project Plan - QAPP) that describes quality assurance/ quality control  
16 activities for proper sample handling, care of test instruments, data recording etc. that will be  
17 implemented throughout the test. To facilitate comparison among treatment technologies, it is  
18 recommended that performance test results be reported using standard ETV formats (U.S. EPA  
19 2010). *Importantly, complete results including failures should be reported, which has not*  
20 *previously been done as standard practice.* These data are needed to enable realistic evaluation  
21 of a given ballast water treatment system.  
22

23 A project-specific Quality Assurance Project Plan should be developed which includes all quality  
24 assurance/quality control (QA/QC) records of sample handling and data, records of instrument  
25 calibration, and records of QA/QC activities. Documentation should encompass standard  
26 operating procedures (SOPs) for all routine procedures associated with the testing process; staff  
27 training for technicians, data managers and other personnel; supervision of project personnel;  
28 and routine laboratory activities (e.g. lot numbers of reagents used).  
29

30 Data and operational information should be reported with waterproof ink in laboratory and field  
31 (shipboard) record books that have waterproof pages, and on data sheet chain-of-custody forms  
32 (Ruiz et al. 1996, U.S. EPA 2010). The laboratory notebooks should have permanently bound,  
33 numbered pages. Corrections should be initialed by the person performing the correction,  
34 crossed out only with a line, and dated. Records should be spot-checked by the individual who  
35 entered the data within two weeks of the measurement to make sure that data are correctly  
36 recorded. Electronic data entries should also be checked for obvious errors, and at least 10% of  
37 all records should be checked in detail. Detected errors should be corrected immediately and  
38 initialed. The data generated should also be reviewed by the Program Coordinator before they  
39 are used in calculations or evaluations. See the ETV (U.S. EPA 2010, Chapter 4 and Appendix  
40 A) for further details about reporting.

41 *2) Challenge Conditions*  
42

43 The ETV (p.19) defines objectives for challenge conditions are to verify treatment system  
44 performance using a set of “challenging, but not rare, water quality conditions representative of

10/28/2010 Science Advisory Board (SAB) Ecological Processes and Effects Committee  
Augmented for Ballast Water

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1 the natural environment;” and to verify removal or kill of organisms ranging in size from  
2 bacteria to zooplankton, using natural assemblages and appropriate analytical techniques that  
3 enable quantification of densities of live organisms (U.S. EPA 2010). It is important to evaluate  
4 the effectiveness of treatment systems under conditions that challenge the technology because  
5 certain water quality conditions can interfere with some treatment processes. These  
6 environmental conditions are generally understood and relatively few in number (Table 4),  
7 which helps to limit the number of water quality metrics that must be included in the protocol.  
8

9 There are two major practical constraints on such tests: First, alterations to establish the natural  
10 range of physical/chemical conditions should be imposed without affecting the concentrations,  
11 diversity, and viability of the biota present. For that reason, natural water sources should be used  
12 to impose the three levels of salinity recommended, rather than artificially modified salinity. The  
13 ETV recommends adjusting POM by adding commercially available humic materials, plankton  
14 detritus, or ground seaweed; commercially available clays can be added to adjust the MM  
15 concentration (U.S. EPA 2010), but the cation exchange capacity of the dried, then rehydrated  
16 clays can significantly alter plankton communities (Avnimelech et al. 1982, Burkholder 1992,  
17 Cuker and Hudson 1992). Artificial modification of DOC is difficult to achieve without a strong  
18 potential of affecting the biota present, especially the smaller size-fraction components. The  
19 testing organization should be required to verify, insofar as possible, that in preparing the test  
20 water, any materials added had minimal effects on the biota (U.S. EPA 2010); “minimal effects”  
21 should be clearly defined; and the careful protocols for augmentation outlined in the ETV should  
22 be followed. As a second major practical constraint, it would be extremely difficult if not  
23 impossible for a testing organization to include the total global taxonomic biological diversity in  
24 tests of system effectiveness at removing microalgae, other protists, and zooplankton. Therefore,  
25 “representative” global taxonomic diversity is recommended.  
26

27 **Table 4.** Requirements and challenge matrix for verification testing of ballast water treatment systems (BE ≡  
28 biological efficacy; OM ≡ operation and maintenance). Note that relatively few physical/chemical environmental  
29 factors are recommended for inclusion in performance testing, but tests of the full range of these factors (e.g.  
30 temperature, 4-35°C) would be difficult to accomplish. Biological factors are idealized here. Modified from the  
31 ETV (U.S. EPA 2010).<sup>1</sup>  
32  
33

34 Criteria for a Valid BE Test Cycle<sup>3</sup>

35 After 1-day holding time, control tank discharge must have:

36 ≥ 100 organisms m<sup>-3</sup> in size class 1 (≥ 50 μm)

37 ≥ 100 organisms mL<sup>-1</sup> in size class 2 (≥ 10 μm and < 50 μm)

38 500 organisms mL<sup>-1</sup> as culturable aerobic heterotrophic bacteria (size class 3, < 10 μm)  
39

40 Three Water Types (Salinity Groupings)<sup>2</sup>

41 *Fresh (salinity < 1)*

42 *Brackish (estuarine) (salinity 1 to < 28)*

43 *Marine (salinity ≥ 28)*

44 Number of Valid BE Test Cycles: ≥ 3 per salinity regime

45 Volumes Per BE Test Cycle: ≥ 400 m<sup>3</sup> available; ≥ 200 m<sup>3</sup> processed; in addition, OM testing  
46 should distribute ≥ 10,000 m<sup>3</sup> among the BE test cycles (equivalent to  
47  
48  
49  
50

10/28/2010 Science Advisory Board (SAB) Ecological Processes and Effects Committee  
Augmented for Ballast Water

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~50 hr of operation at 200 m<sup>3</sup> hr<sup>-1</sup>, or ~65 hr of operation at 150 m<sup>3</sup> hr<sup>-1</sup>)

Operational Period: ≥ 1 hr for each in-line treatment BE or OM verification cycle; can be extended if flow rates are reduced from 200 m<sup>3</sup> hr<sup>-1</sup>

Holding Time:

In-Line Tests: ≥ 1 day within both control and treatment tanks per BE test cycle (defined operationally - should simulate the time that water would reside in a ballast tank)<sup>4</sup>

In-Tank Tests: ≥ 1 day (cumulative) per BE test cycle

Combination: Test duration = treatment time (in-line + in-tank)

Holding Time (In-Tank Tests): ≥ 1 day (cumulative) per BE test cycle

Parameters

*Voyage Duration:* ≥ 1 day<sup>5</sup>

*Treatment System Flow-Through Rate:* ≥ 200 m<sup>3</sup> hr<sup>-1</sup> (800 gallons per minute)

*Physical/Chemical* (full range)

Environmental: Temperature (4-35°C), DOC, DOC, POC, TSS, MM, pH, DO

Chemicals from Treatment: Chlorine (sodium hypochlorite), H<sub>2</sub>O<sub>2</sub>, ozone, glutaraldehyde, chlorine dioxide (Ecochlor<sup>TM</sup>), mixes such as PeraClean Ocean® (H<sub>2</sub>O<sub>2</sub> + peracetic acid), and residuals

Others of Specific Interest: Example - nutrient concentrations (TN, TP, TKN, NH<sub>x</sub>, NO<sub>x</sub>)

*Biological:* Global taxonomic diversity in size classes from the IMO standard

Maximum biomass/density known to be encountered in ships (from published literature)

Species of interest (e.g. harmful bacteria, harmful microalgae)

<sup>1</sup> Abbreviations: DOC, dissolved organic matter; POM, particulate organic carbon; TSS, total suspended solids; MM, mineral matter (other particulates); DO, dissolved oxygen; total nitrogen; TP, total phosphorus; TKN, total Kjeldahl nitrogen; NH<sub>x</sub>, ammonia + ammonium; NO<sub>x</sub>, nitrate + nitrite.

<sup>2</sup> Intended to ensure that treatment efficacy measurements attributed to the treatment system in any given BE test cycle are not the result of natural or non-treatment system-related effects.

<sup>3</sup> The ETV (p.30) recommends completion of BE tests in at least two of the three salinity ranges (U.S. EPA 2010). We recommend, instead, that the testing should include all three ranges for systems intended for use across the salinity gradient from fresh to marine waters. Our rationale is that if a given ballast water treatment system is planned for use across the salinity gradient, but testing indicates that its efficiency at organism removal is poor under one or more of the salinity groupings, then that system should not be used by ships visiting ports that are characterized by such conditions. Similarly, if a ballast water treatment system is planned for use across other environmental gradients (e.g. temperatures from cold to warm waters), but tests indicate that it has poor efficiency in removing biota under part of the natural temperature range, then that system should not be used by ships visiting ports that have such conditions.

<sup>4</sup> The purpose of the protocol holding time is to provide a conservative assessment of treatment system performance according to the manufacturer's information. Shorter tank holding times may be justified, for example, if populations cannot be sustained in control tanks because of natural mortality.

<sup>5</sup> The voyage duration can be as little as one day (for example, in the Great Lakes), and during shipboard operation, ballasting procedures can occur over periods ranging from minutes to hours (U.S. EPA 2010).

10/28/2010 Science Advisory Board (SAB) Ecological Processes and Effects Committee  
Augmented for Ballast Water

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1

2 To address this issue within practical constraints, the use of standard test organisms has been  
3 described as a “pragmatic and necessary strategy to address the overwhelming diversity of  
4 organisms available in ballasted water” (Ruiz et al. 1996). The ETV recommends use, at  
5 minimum, of representative taxa in three size classes as shown in Table 5; this minimum total  
6 input concentration of viable taxa by size class should be demonstrated for each test cycle at the  
7 influent point of the control tank, and just before the point of treatment.  
8

9 It should be noted that there is no diversity requirement for bacteria. The IMO (2008a,b) uses  
10 the indicator microbes of special interest, *Vibrio cholerae*, *Escherichia coli*, and the grouping  
11 intestinal enterococci, as a “human health standard,” with the implicit assumption that tracking  
12 these taxa of special interest will indicate whether organisms harmful to human health are  
13 effectively removed by the treatment system tested. While the utility of these bacteria is  
14 imperfect as indicators of the presence of other microbes that can adversely affect human health,  
15 the approach is reasonable and practical.  
16

17 On the other hand, the IMO (2008a,b), the ETV (U.S. EPA 2010), and other suggested standards  
18 (e.g. California VGP 401 certification/State regulations (see Albert et al. 2010) make no mention  
19 of organisms in the 0.2 to < 10 µm size range other than those indicator bacteria. Many harmful  
20 organisms occur in this size range (e.g. harmful “brown tide” pelagophytes *Aureococcus* and  
21 *Aureoumbra*, many harmful cyanobacteria, certain potentially toxic dinoflagellates etc. - see  
22 Burkholder 1998, 2009). The selected bacteria presently targeted for standards are not useful as  
23 indicators for the presence of these taxa which, as a general grouping, can adversely affect both  
24 environmental and human health (Burkholder 1998, 2009). Thus, failure to consider this size  
25 range represents a serious omission in efforts to protect U.S. coastal estuarine/marine waters and  
26 the Great Lakes from harmful invasive species introductions. For some of these taxa, such as  
27 toxigenic *Microcystis* spp. affecting the Great Lakes (e.g. Boyer 2007), the tendency of the cells  
28 to aggregate into colonies effectively “boosts” them into the >10 µm size class, but for others  
29 such as the brown tide organisms, such aggregation does not occur. There is a critical need to  
30 include this size class, or at a minimum, harmful representatives from it, in developing protective  
31 ballast water standards. Considerations about this size class, accordingly, are included in some  
32 sections below as appropriate.  
33

34

35

36 **Table 5.** Minimum criteria for total living populations tracked in challenge water, recommended by the ETV (U.S.  
37 EPA 2010).  
38

39

40

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45

Organism Size Class <sup>1</sup>	Total Concentration	Diversity (representative taxa)
≥ 50 µm	10 <sup>5</sup> organisms m <sup>-3</sup>	5 species across 3 phyla
≥ 10 µm and < 50 µm	10 <sup>3</sup> organisms mL <sup>-1</sup>	5 species across 3 phyla

10/28/2010 Science Advisory Board (SAB) Ecological Processes and Effects Committee  
Augmented for Ballast Water

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1 < 10 µm  
2  
3 10<sup>3</sup> organisms mL<sup>-1</sup> as  
4 culturable aerobic  
5 heterotrophic bacteria<sup>2</sup>  
6  
7  
8  
9  
10

<sup>1</sup>Size considers the maximum dimension on the smallest axis.

<sup>2</sup>Effects on culturable aerobic heterotrophic bacteria are assumed to be indicative of effects on all bacteria.

<sup>3</sup>“Global diversity of bacteria” by species or phyla is not applicable; there is no diversity requirement for this size class.

11 3) *Treatment Facility Experimental Configuration*  
12

13 The ETV provides detailed recommendations about the design and installation of appropriate  
14 facilities for testing the biological efficacy of ballast water treatment systems (U.S. EPA 2010).  
15 Validation of the configuration of a test facility should include verification that the sampling  
16 design, geometry, and installation enable collection of representative samples while minimizing  
17 organism mortality.

18 4) *Verification Testing*  
19

20 Testing should determine how treatment efficacy varies over the entire range of conditions under  
21 which a specific treatment will be used. The ETV separates verification testing into three  
22 phases: (Phase 1) treatment system commissioning – tests to validate installation and operation  
23 in accordance with the manufacturer’s requirements; (Phase 2) biological efficacy (BE) tests, and  
24 (Phase 3) Operation/maintenance (OM) tests, conducted under ambient source-water conditions  
25 to check the system engineering performance under realistic physical conditions (U.S. EPA  
26 2010; Table 3). At the end of each BE verification test cycle, the testing facility should verify  
27 that, at a minimum, the following test validity criteria were established and maintained:  
28 operational parameters demonstrating that requisite volumes were transferred and sampled while  
29 maintaining manufacturer-specified flows, pressures etc.; water quality challenge conditions for  
30 uptake and discharge waters were met; and biological challenge conditions for ambient organism  
31 concentrations/ diversity in control and treatment containers were met.  
32

33 The ETV includes specific protocols for collection of water quality and biological samples in  
34 performance testing of ballast water systems (Tables 6 and 7). For zooplankton, phytoplankton  
35 and other protists, it is recommended that a minimum volume of 3-6 m<sup>3</sup> should be collected at  
36 each required sampling location on a time-averaged basis over the testing period. Field quality  
37 control samples and field blanks should be taken under actual field conditions to provide  
38 information on the potential for bias from problems with sample collection, processing, shipping,  
39 and analysis (Ruiz et al. 1996), following protocols in the ETV. Accepted scientific methods  
40 should be used for all analyses (e.g. for water quality parameters, U.S. EPA 1993, 1997;  
41 American Public Health Association et al. 2005). Biological samples should be collected from  
42 the time-integrated sample volumes during the test cycle; sample collection tanks should be  
43 thoroughly mixed prior to sampling to ensure homogeneity. Samples collected from control and  
44 treated tank discharges should be taken upstream from pumps or other apparatus that could cause  
45 mortality or other alterations. Note that analysis of some parameters is extremely time-sensitive

**10/28/2010 Science Advisory Board (SAB) Ecological Processes and Effects Committee  
Augmented for Ballast Water**

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(Table 7).

**Table 6.** Core (required) parameters other than biota, examples of auxiliary parameters, and measurement approaches. Modified from the ETV (U.S. EPA 2010).\*

Parameter	Type	Sample Location and Approach	
		Challenge Water	Post-Treatment
Temperature	Core	<i>In situ</i> , continuous	<i>In situ</i> , continuous
Salinity	Core	<i>In situ</i> or discrete grab	<i>In situ</i> or discrete grab
Total suspended solids (TSS)	Core	Discrete grab	Discrete grab
Particulate organic matter (POM)	Core	Discrete grab	Discrete grab
Dissolved organic matter (DOM)	Core	<i>In situ</i> , continuous or discrete	<i>In situ</i> , continuous or discrete
Dissolved oxygen (DO)	Core	<i>In situ</i> , discrete	<i>In situ</i> , discrete
pH	Core	<i>In situ</i> , continuous	<i>In situ</i> , continuous
Ambient organism concentration	Core	Discrete	Discrete
Ballast system flow rate	Core	<i>In situ</i> , continuous	<i>In situ</i> , continuous
Ballast system pressure	Core	<i>In situ</i> , continuous	<i>In situ</i> , continuous
Sampling flow rates	Core	<i>In situ</i> , continuous	<i>In situ</i> , continuous
Chlorophyll <i>a</i> (algal biomass)	Core	<i>In situ</i> , continuous	<i>In situ</i> , continuous
Dissolved nutrients (N, P, Si)	Auxiliary	Discrete	Discrete
Turbidity	Auxiliary	<i>In situ</i> , continuous	<i>In situ</i> , continuous
ATP (index of living material)	Auxiliary	<i>In situ</i> , continuous	<i>In situ</i> , continuous

\* Auxiliary parameters can include useful indicators of core parameters, or are otherwise advisable to assess system performance. *In situ* ≡ in-line or in-tank measurements; discrete grab ≡ a sample taken at a specific place and time; continuous ≡ measured continuously throughout the period of operation at some defined time interval.

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1 **E. Compromises Necessary Because of Practical Constraints**

2  
3 *[As in sections C & D, text reviewing or summarizing material in the EPA's 2010 ETV report*  
4 *will be moved to an appendix. The text in this section will focus on any issues or conclusions that*  
5 *differ from what is covered in the ETV report. For example, there is a recommendation that*  
6 *filtered natural water be used to prepare treatments rather than seawater; would it be useful to*  
7 *provide some additional explanation and discussion clarifying the advantages/disadvantages of*  
8 *either approach?]*

9  
10 As stated, ideally ballast water treatment systems should be verified using a set of standard  
11 challenge conditions that encompass the full gradient of environmental conditions at major ports,  
12 and the density ranges of organisms across global taxonomic diversity for the size classes  
13 included in the IMO standard (U.S. EPA 2010). Such an ideal goal is impeded by several serious  
14 practical constraints. In fact, Lee et al. (2010, p.19) point out that “perfect compliance and no  
15 failure is practically, if not theoretically, impossible, particularly for microbiological organisms  
16 unless ballast water is discharged into a land-based treatment facility or ships are redesigned to  
17 eliminate the need to discharge ballast water.” This section considers how this ideal can be  
18 modified to accommodate practical considerations while accomplishing a meaningful evaluation  
19 of the efficacy of ballast water treatment systems.

20 *1) Standard Test Organisms (Surrogates)*

21  
22 The ETV (p.xi) defines standard test organisms, or surrogates, as “organisms of known types and  
23 abundance that have been previously evaluated for their level of resistance to physical and/or  
24 chemical stressors representing ballast water technology, ... added to the challenge water during  
25 testing... to determine treatment system effectiveness” (U.S. EPA 2010). Post-treatment viability  
26 of surrogate taxa or life history stages is often used to evaluate the biological effectiveness of  
27 ballast water treatment systems in removing zooplankton, protists (heterotrophic and  
28 phototrophic), and bacteria (e.g. Table 8).

29  
30  
31 **Table 7.** Sample volumes, containers, and processing for core parameters and auxiliary nutrients (nitrogen, N; phosphorus,  
32 P; silicate, Si; carbon, C). Modified from the ETV (U.S. EPA 2010); note that HDPE ≡ high-density polyethylene, and  
33 POC information is from Baldino (1995).

34  
35

36 Parameter	37 Minimum Sample Volume	38 Containers	39 Processing/Preservation Holding Time	40 Maximum
41 TSS	42 100 mL	43 HDPE or glass	44 Process immediately or store at 4°C	45 1 week
46 DOC	47 25 mL	glass	Pre-combusted GF/F filters; 28 days preserve filtrate with H <sub>3</sub> PO <sub>4</sub> (pH < 2), hold at 4° in darkness	
POC	500 mL	HDPE	Filter (GF/F); store covered	28 days

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1					
2	DO	300 mL	glass BOD	Fix; titrate in 2-24 hours	24 hours
3		or	bottles		
4		<i>in situ</i> sensor		Continuously recording	
5					
6	Chlorophyll <i>a</i> , <sup>1</sup>	400 mL	500-mL dark	Filter (GF/F); fix with saturated	3 weeks
7	pheopigments		HDPE	MgCO <sub>3</sub> solution; freeze filter	
8					
9	Phytoplankton #	200 mL	250-mL dark	Filter (Nuclepore or Anotech);	process
10	(viable, pico-	(concentration	HDPE	assess autofluorescence (e.g.	immediately;
11	plankton; or	not required)		Maclsaac and Stockner 1993)	
12	selected taxa) <sup>2</sup>			<u>or</u> ,	
13				Filter, fix (e.g. 0.2% (v/v)	3-4 weeks
14				formalin), freeze filter	
15				<u>or</u> ,	
16				filter, fix, followed by selected	months
17				molecular techniques (e.g.	
18				Burkholder et al. 2007)	
19					
20					
21					

**Table 7, cont'd.**

Parameter	Minimum Sample Volume	Containers	Processing/Preservation Holding Time	Maximum
Phytoplankton # (viable, nano-/micro-plankton) <sup>3</sup>	3 m <sup>3</sup> (1,000 L) → 1 L	60 mL dark HDPE	No preservative; stain with FDA, CMFDA <u>or</u> , fix with acidic Lugol's solution (Vollenweider 1974), store at preferably 4°C in darkness, and quantify 1 week as viable when collected (formerly viable) <u>and</u> combine with various molecular techniques to confirm harmful taxa of interest (e.g. Burkholder et al. 2007)	process immediately 28 days,
Other protists (#) (viable heterotrophs)	3 m <sup>3</sup> (1,000 L) → 1 L	100 mL, dark HDPE	No preservative; most probable number (MPN) from Anderson et al. (2008); other methods adapted from Petterson et al. (2007) <u>or</u> Filter, freeze, fix as in Sherr and Sherr (1993); or fix, filter, stain (e.g. Sherr et al. 1993, Montagnes and Lynn 1993)	process immediately weeks to months
Zooplankton # (viable)	6 m <sup>3</sup> (6,000 L) <sup>3</sup> → 1 L	1-L flasks	No preservative; subsample into 450 1-mL wells and probe;	Process immediately

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1			fix with buffered formalin and	(< 6 hr) <sup>4</sup>
2			Rose Bengal's solution to	
3			quantify;	
4			or,	
5			fix as above and quantify as	Process within
6			formerly viable (Johnson and	1 month
7			Allen 2005)	
8				
9	Bacteria	≥ 500 mL	sterile HDPE	Process
10	(active culturable,	[1 mL to 500 L] <sup>5</sup>		Immediately
11	selected taxa)		Plate on appropriate media	(< 6 hr; or
12				1-5 days)
13				
14	Total N, P, total	60 mL	polyethylene	28 days
15	Kjeldahl N (TKN)		Preserve with H <sub>2</sub> SO <sub>4</sub> (pH < 2),	
16			hold at 4°C in darkness	
17				
18				
19	Dissolved N, P, Si	60 mL	polyethylene	28 days
20			Filter (Nuclepore membrane);	
21			preserve and hold as above	

---

<sup>1</sup>*In situ* sensors are available for measuring chlorophyll *a* as relative fluorescence units, but not as chlorophyll *a* concentrations.

<sup>2</sup>This size category has not been considered for ballast water treatment standards by IMO (2008a,b), the ETV (U.S. EPA 2010) etc. Because many harmful organisms occur within the 0.2 to < 10 µm grouping, we suggest that at a minimum, harmful representative taxa from the 0.2 to < 10 µm grouping should be included. Accordingly, we provide information on this grouping.

<sup>3</sup> FDA, fluorescein diacetate; CMFDA, 5-chloromethylfluorescein. Delicate protists (e.g. wall-less flagellates) mostly would not be expected to survive the process of rapid concentration of large-volume samples. As a much more practical alternative than attempting to quantify viable algae and other protists from unpreserved samples, we suggest preserving samples immediately upon collection, and then assessing intact organisms as “viable when collected,” based on the fact that protists such as most algae in this general size class are known to lyse and/or decompose rapidly (minutes to several hours) after death, so that the cell contents become distorted or are lost even if the cell coverings remain (Wetzel 2001).

<sup>3</sup> The ETV recommends a sample size for the zooplankton size class of at least 1 m<sup>3</sup> (1,000 L), concentrated to 1 L, and analysis of 20 subsamples (U.S. EPA 2010). However, microbead experiments conducted under “best case” conditions by the Naval Research Laboratory (Lemieux et al. 2010) indicated that the ETV protocol will not achieve acceptable precision.

<sup>4</sup> Research has shown that zooplankton die-off occurs in samples held for 6 hours or more. The approximate maximum hold time should maintain detectable zooplankton mortality over time at ≤ 5% (the ETV - U.S. EPA 2010). As a much more practical alternative than attempting to quantify viable organisms from unpreserved samples, we suggest preserving samples immediately upon collection, and then assessing intact organisms as “viable when collected,” based on the fact that zooplankton are known to decompose rapidly after death (minutes to several hours) (Johnson and Allen 2005).

<sup>5</sup>The volumes used to quantify bacteria vary widely; for example, the ETV recommends techniques that use as little as 1 mL, whereas MERC (2009c) uses 500 L.

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1 **Table 8.** Surrogate species identified and tested by Anderson et al. (2008) with  
2 various ballast water treatment procedures.  
3

4  
5 Bacteria

6 *Enterococcus avium* (Gram-negative pathogen indicator)

7 *Vibrio cholerae* (Gram-negative pathogen indicator)<sup>1</sup>

8 *Geobacillus stearothermophilus* (Gram-positive spore-forming)

9 *Bacillus subtilis* (Gram-positive spore-forming)

10  
11 Heterotrophic Protists

12 *Acanthamoeba* sp. (freshwater amoeba)

13 *Chilomonas* sp. (freshwater flagellate)

14 *Tetrahymena pyriformis* (freshwater ciliate)

15 *Vannella platypodia* (freshwater amoeba)

16  
17 *Rhynchomonas* sp. (marine flagellate)

18 *Vannella anglica* (marine amoeba)

19 *Uronema* sp. (marine ciliate)

20  
21 Photosynthetic Algal Protists

22 *Chlorella* sp. (marine green)

23 *Chaetoceros affinis* (marine diatom)

24 *Skeletonema costatum* (marine diatom)

25 *Scrippsiella trochoidea* (marine dinoflagellate)

26 *Scrippsiella lachrymosa* (marine dinoflagellate - both vegetative and cyst forms)

27  
28 *Prymnesium parvum* (estuarine/ marine, potentially toxic haptophyte)<sup>2</sup>

29  
30 *Microcystis aeruginosa* (potentially toxic freshwater cyanobacterium [blue-green alga])

31 *Fragilaria crotonensis* (freshwater diatom)<sup>3</sup>

32  
33 Zooplankton<sup>4</sup>

34 *Brachionus calyciflorus* (freshwater - resting stage)

35 *Ceriodaphnia lacustris* (freshwater - adult)

36 *Culex* (freshwater - insect larvae)

37 *Daphnia magna* (freshwater)

38 *Daphnia pulex* (freshwater - resting stage)

39 *Diaptomus pallidus* (freshwater - adult)

40  
41 *Acartia hudsonica* (marine, warm waters - resting stage)

42 *Acartia tonsa* (marine, cold waters - resting stage)

43 *Artemia* (marine - brine shrimp)

44 *Brachionus calyciflorus* (marine - adult)

45 Mussel larvae (marine)<sup>5</sup>

46 *Nitokra lacustris* (marine)

47 *Tisbe battagliai* (marine - adult)

48  
49  
50 <sup>1</sup> While it would be impractical for environmental/health safety reasons to use toxic  
51 strains of *V. cholerae* for such tests, caution is warranted in interpreting data from  
52 nontoxic strains because within a given species of toxigenic bacteria or algae, toxic  
53 strains have been shown to respond differently to environmental conditions than  
54 nontoxic strains (e.g. Faruque et al. 1998, Burkholder et al. 2005, Burkholder and  
55 Glibert 2006, Zurawell et al. 2005, Kardinaal et al. 2007, Vézic et al. 2007).

56  
57 <sup>2</sup>*Prymnesium parvum* was tested as a freshwater organism, but it typically is  
58 estuarine/ marine, and is only found in very high-conductivity freshwaters (Burkholder  
59 2009).

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Augmented for Ballast Water

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1  
2  
3<sup>3</sup>*Fragilaria crotonensis* was misspelled as *F. crotensis* in Anderson et al. (2008).

4<sup>4</sup>These zooplankton were suggested as potential surrogates by the earlier ETV  
5 Program; Anderson et al. (2008) substituted the European harpacticoid copepod  
6 *Tisbe battagliai* from European waters with a common, abundant species, *Tisbe*  
7 *cf. furcata*, from the Pacific Northwest. No further specifics (e.g. on life history stage)  
8 for zooplankton were provided by Anderson et al. (2008).

9  
10<sup>5</sup> The species should be selected in each salinity range, for example, Eastern oyster  
11 (*Crassostrea virginica*) which generally is more tolerant of environmental stressors  
12 than other shellfish.

13  
14  
15 The selection and development of surrogate species for use in testing ballast water treatment  
16 system performance is a fertile field of research because of the practical need (Hunt et al. 2005,  
17 Anderson et al. 2008, U.S. EPA 2010). The approach must be applied with caution, however,  
18 since results from a very small number of taxa are broadly applied to all of the organisms in the  
19 same general grouping (e.g. protozoans in a certain size class). An assumption that first must be  
20 validated is that the selected taxa are among the most resistant to treatment, so that most  
21 organisms are eliminated when they are eliminated (Ruiz et al. 1996). The fundamental  
22 challenge is to identify the best species that are “representative” of a broad range of organisms  
23 within a given size class. Good candidates are considered to be easily and economically cultured  
24 in large numbers for future full-scale testing in experimental ballast water tanks, tolerant of a  
25 wide range of environmental conditions, reliable and consistent in their response to treatment  
26 across culture batches, and resilient in withstanding ballast water tests and sampling (Ruiz et al.  
27 1996, Anderson et al. 2008).

28  
29 An obvious risk is spurious results from surrogate taxa that poorly represent the larger group of  
30 organisms. A second risk is that artifactual interactions may occur between biota and artificial  
31 media, for example, artificial seawater prepared with commercially available “sea salts.” For  
32 that reason, we diverge from Anderson et al. (2008) and recommend that filtered natural  
33 freshwater or seawater should be used to prepare treatments insofar as possible. Selection of a  
34 specific combination of surrogate taxa should be based upon extensive testing at bench and  
35 mesocosm scales, preferably by several laboratories located in different geographic regions, of a  
36 wide range of surrogate species, life histories, habitats, and source regions across environmental  
37 gradients (Ruiz et al. 1996). These laboratories should use the same protocols to minimize  
38 confounding factors and strengthen comparability. Several surrogate species or taxonomic  
39 subgroups, including several life stages, should be included in the tests, since confidence in  
40 interpretations can be strengthened by this redundancy. In addition, multiple strains  
41 (populations) of candidate surrogate species from different geographic regions should be  
42 included to account for significant intraspecific variability in response to environmental  
43 conditions that is commonly documented, particularly among protists (Ruiz et al. 1996,  
44 Burkholder and Glibert 2006).

45  
46 Since surrogate species have resilient life history stages, they are expected to be more difficult to  
47 treat and, thus, are potentially more invasive than vegetative stages. Given the premise that if  
48 highly resistant stages or taxa can be killed, then it can readily be assumed that all other

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1 organisms in a given size class are also eliminated (Bolch and Hallegraeff 1993, Hallegraeff et  
2 al. 1997, Anderson et al. 2004), it might seem logical to use cyst life history stages or known  
3 highly resistant taxa as the best surrogate candidates. However, for two reasons the  
4 disadvantages outweigh the advantages: First, a foremost consideration is the disposal of water  
5 volumes from the tests, due to the risk for invasion by these resistant organisms. Second,  
6 because resistant cells often have a low metabolic state and thick, multi-layered walls that are  
7 impermeable to various stains (Romano et al. 1996, Kokinos et al. 1998), their viability can be  
8 difficult to assess and can involve culture analyses that may be weeks to months in duration  
9 (Montresor et al. 2003, Binet and Sauber 2006, U.S. EPA 2010) (see Section below).

10  
11 Two other important general limitations in use of surrogate taxa merit mention: First, native  
12 surrogate taxa are not available for some important organisms, such as for estimating viability of  
13 the pathogenic protozoans *Cryptosporidium* and *Giardia* (Pettersen et al. 2007). Second,  
14 surrogate viruses have not been developed, despite the looming importance of this group of  
15 organisms (Bergh et al. 1989, Fuhrman 1999, Suttle 2005). The recognized limitations in  
16 developing surrogate viruses include safety concerns, and logistical challenges such as producing  
17 them in large quantities (Hunt et al. 2005).

18  
19 If surrogate taxa are used in testing ballast water treatment systems, the ETV recommends that  
20 the test reporting should include a full description of the species; a justification for their use,  
21 including consideration of potential confounding interactions between the surrogates and natural  
22 species; and the percentage ratio of challenge organisms that are surrogates versus naturally  
23 occurring taxa in the challenge water (U.S. EPA 2010).

24 *2) Other Potential Surrogates Cannot Reliably Quantify Organisms*

25  
26 Given the practical/logistical limitations involved in obtaining statistically meaningful estimates  
27 of organism numbers per unit volume, it is tempting to instead focus on parameters that are much  
28 more rapidly and easily assessed. Examples of such “surrogate” parameters are discussed here.  
29 They can be calibrated with organism numbers in laboratory tests on microcosm “ecosystems,”  
30 but would be much more difficult, if not impractical, to calibrate for use with unknown types and  
31 numbers of organisms in ballast tanks. Lacking reliable calibration with natural populations of  
32 ballast water flora and fauna, they cannot be used to evaluate, at the resolution of very low  
33 organism densities needed, whether ballast water treatment systems can meet the IMO standards.

34  
35 Algal pigments -

36  
37 Use of algal pigments, especially chlorophyll *a* and certain “signature” pigments that are  
38 indicators or markers of various algal groups (usually at the class or phylum [eukaryotic  
39 algae]/division [cyanobacteria or blue-green algae] level) allows rapid processing of large  
40 numbers of samples, two advantages over the generally-tedious quantification of algal cell  
41 numbers and biovolumes (Sarmiento and Descy 2008). Chlorophyll *a* is widely used as an  
42 indicator of total algal biomass, and it can be used to assess whether viable phytoplankton are  
43 present at the total assemblage level in treated ballast water and in regrowth experiments. Major  
44 shortcomings are that cell numbers per unit sample volume cannot be discerned from this

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1 measurement (Wetzel 2001, MERC 2009c), and that the techniques available are not sufficiently  
2 sensitive to detect < 10 cells/mL of phytoplankton in small size classes (nanoplankton and  
3 picoplankton, < 20 µm and < 2 µm length in the major cell axis, respectively). A third serious  
4 impediment in attempts to use chlorophyll *a* to test the efficacy of ballast water treatment  
5 systems in removing photosynthetic algae is that cellular chlorophyll content varies greatly (0.1-  
6 9.7% of fresh algal weight) depending upon the algal species (Boyer et al. 2009) and the amount  
7 of available light, so that chlorophyll concentration data cannot be reliably calibrated with algal  
8 cell numbers in natural phytoplankton samples. High chlorophyll levels can be produced by  
9 relatively low algal biomass in low-light environments. Thus, U.S. EPA (2003) and others (e.g.  
10 Buchanan et al. 2005) have recommended that chlorophyll *a* data should be interpreted with  
11 caution as an indicator of algal biomass in habitats characterized by low light. The methodology  
12 for quantifying chlorophyll *a* concentrations is highly variable as well; results depend upon the  
13 solvent used, the extraction period and conditions, and the degree of acidification in attempts to  
14 correct for chlorophyll degradation products or pheophytins (Bowles et al. 1985, Hendry et al.  
15 1987, Porra 1991).

16  
17 “Signature” or marker pigments, unique to certain algal groups at the phylum (division) or class  
18 level, have been suggested as potentially superior to chlorophyll *a* as indicators of algal biomass  
19 (Mackey et al. 1996, Jeffrey and Vest 1997, Schmid et al. 1998, Schlüter et al. 2006). The low  
20 taxonomic resolution provided by the available techniques for analysis and quantification of  
21 signature pigments can sometimes be improved upon by screening samples using inverted  
22 microscopy (Lund et al. 1958) to identify the abundant taxa (Sarmiento and Descy 2008). The  
23 assay techniques must be carefully applied, however, to avoid artifacts and sample bias (Lewitus  
24 et al. 2005). Like chlorophyll *a*, signature pigments cannot be reliably calibrated to indicate  
25 algal cell concentrations (Lewitus et al. 2005).

26  
27 Adenylates

28  
29 ATP (adenosine tri-phosphate) is made by living cells as a form of short-term energy storage and  
30 transfer, and has been used as an indicator of total microbial biomass in sediments and plankton  
31 (Sandrin et al. 2009) since a relatively constant ratio of ATP to total cell carbon has been  
32 reported for all microbial taxa, regardless of metabolic activity or environmental conditions (Karl  
33 1980). ATP is easily extracted from microbial assemblages, assayed fairly easily as well, and is  
34 not associated with dead cells or detritus (Takano et al. 1983).

35  
36 In bench-scale, closed-system experiments, a relatively constant ratio of ATP concentration to  
37 total cell counts has also been reported (Takano et al. 1983). However, such an environment  
38 differs markedly from ballast tanks containing natural assemblages from diverse sources, likely  
39 under stressed conditions. The ATP content of a cell has been shown to vary depending upon its  
40 level of activity: rapidly growing cells have higher ATP content than stressed cells, and ATP  
41 content may not be strongly correlated with microbial biomass under environmental stress  
42 (Inubushi et al. 1989, Rosaker and Kleft 1990). Encysted cells with low metabolic activity  
43 would also be expected to have low ATP content. For stressed populations, total adenylates  
44 (ATP + ADP, adenosine diphosphate + AMP, adenosine monophosphate) has been suggested as  
45 a better indicator of microbial biomass (Sandrin et al. 2009). In addition, the adenylates energy

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Augmented for Ballast Water

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1 charge (AEC), determined by measuring all three adenylates, has been used to provide  
2 information about microbial activity; the abundance of ATP in comparison to AMP and ADP  
3 indicates how rapidly ATP, the highest energy state, was formed:  
4

$$5 \quad \text{AEC} = \frac{\text{ATP} + \frac{1}{2} \text{ADP}}{\text{ATP} + \text{ADP} + \text{AMP}} .$$

6  
7  
8 High AEC values (> 0.8) are considered to indicate an active microbial consortium; intermediate  
9 values (0.4 to 0.8) reflect cells in a resting state; and values below 0.4 indicate a high proportion  
10 of dead or moribund cells (Kleft and Rosaker 1991).  
11

12 Assay of total adenylates from size-fractionated ballast water samples could provide information  
13 about the total microbial biomass per unit volume for each size class, and would be of use in  
14 evaluating the efficiency of ballast water treatment systems at biological removal if the data  
15 could be reliably translated to a per-cell basis. While such translation would be readily  
16 accomplished for microbial cultures, for ballast water samples it would involve determining the  
17 mean or median cell size within a given IMO size category from a concentrated subsample, and  
18 then applying that estimate to the total adenylates measurement to estimate cell numbers per unit  
19 volume. Even for relatively small-volume samples, the error factor could be large; extrapolation  
20 to very large volumes could potentially result in much larger overestimates or underestimates of  
21 microbial cell numbers. The AEC for size-fractionated samples would provide information  
22 about the overall metabolic status of a given size category, but detection of relatively few live  
23 cells that might be present in a sample with an AEC < 0.4 – a question of major importance in  
24 evaluating the biological efficiency of ballast water treatment systems – would not be possible.  
25 Thus, while useful from an ecological context (Holm-Hansen 1973), these parameters (total  
26 adenylates, AEC) would not enable accurate assessment of very small numbers of viable  
27 organisms per unit volume in the likely-stressed environmental conditions of ballast water tanks.  
28

29 INT - (2-*p*-iodophenyl)-3-(*p*-nitrophenyl)-5-phenyltetrazolium chloride)  
30

31 A commonly used tetrazolium salt, (2-*p*-iodophenyl)-3-(*p*-nitrophenyl)-5-phenyltetrazolium  
32 chloride) or INT, accepts electrons from dehydrogenase enzymes and is reduced to a reddish-  
33 colored formazan (INTF) which can be quantified by colorimetric analysis (Mosher et al. 2003).  
34 INT has been used to measure microbial activity in surface waters, biofilms, and sediments  
35 across the salinity gradient (Songster-Alpin and Klotz 1995, Posch et al. 1997, Blenkinsopp and  
36 Lock 1998). INT is added to an environmental sample and incubated for as short a time as  
37 possible to minimize changes in the activity of the microbial community. Total cell numbers are  
38 quantified under epifluorescence microscopy using a counter-stain such as acridine orange  
39 (Sandrin et al. 2009). The proportion of the population that is metabolically active is estimated  
40 as the difference between the cells containing INT-formazon and the total cell number. This  
41 sensitive method has been used to detect electron transport chain activity (viable organisms)  
42 from low numbers of microbes, even at low temperatures (Sandrin et al. 2009), with resolution at  
43 the level of individual cells (Posch et al. 1997). Nevertheless, the technique can miss cells with  
44 very low respiration such as cysts, or cells that do not use INT as an electron acceptor (Posch et  
45 al. 1997).

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1 *3) Sized-Down Treatments*  
2

3 Use of full-scale tests has extreme practical and logistical limitations and/or high risk in efforts  
4 to assess the effectiveness of treatment systems in removing maximal densities of harmful  
5 organisms, or mixes of representative organisms within certain density ranges, supporting the  
6 need for sized-down treatments that are larger and therefore more realistic than bench-scale  
7 microcosms, but more manageable in volume than ballast tanks. Sized-down treatments help to  
8 reduce risks to human health safety and receiving aquatic ecosystems for testing treatment  
9 system effectiveness at removing toxic substances and residues that are part of the treatment  
10 process. As Ruiz et al. (1996) stated, “Economy of small scale and ease of manipulating  
11 environmental variables and community assemblage at the laboratory and intermediate scales  
12 make it possible and practical to estimate if a ballast water treatment process and system is likely  
13 to be effective over the full range of physical [, chemical,] and biological conditions expected in  
14 the field;...the same regime on a ship would prove logistically and financially very unwieldy.  
15 Thus, smaller scale tests demonstrate the treatment’s performance and capacity across a wide  
16 range of relevant state variables....” This approach also allows more precise, controlled  
17 sampling during test trials (MERC 2009d). At larger scales, practical limitations restrict the  
18 number of conditions that can reasonably be tested, and testing is directed more toward ensuring  
19 functionality of the engineered system rather than understanding the treatment process under  
20 various conditions.  
21

22 Small-scale (benchtop or laboratory) experiments minimize logistics and expense, and they can  
23 provide proof of concept in assessing whether a given treatment meets expectations (Ruiz et al.  
24 1996). For example, if a ballast water treatment system is planned for use across the salinity  
25 gradient, then its efficacy should be tested across all three salinity ranges (Table 4). Logistically,  
26 however, it may be feasible to test two salinity ranges at full scale, but not the third. In such cases,  
27 small scale and intermediate scale (see below) tests could be completed using the third salinity  
28 range.  
29

30 The test results may indicate limitations, or critical flaws that can be resolved with design  
31 changes and other improvements before scaling up. These experiments are conducted with  
32 flasks, other small containers, or microcosms (volume generally  $\leq 5$  liters) are used to rigorously,  
33 quantitatively assess organism response to treatment under controlled conditions. The tests  
34 conducted at this scale should include an array of organisms, a range of densities including the  
35 maximal expected density, and a range of environmental conditions (temperature, pH, light,  
36 salinity), including, importantly, the challenging conditions that test the limits of the treatment  
37 process (Ruiz et al. 1996). Sufficient numbers of replicates should be used to ensure that the  
38 results are consistent, repeatable, and predictable. The ETV recommends use of standard test  
39 organisms (surrogates) from specified functional groups (zooplankton, protists, bacteria), size  
40 classes, and salinities in these tests, following protocols of Anderson et al. (2008). The ETV  
41 further stipulated that tests should be run at least in triplicate for zooplankton, and in  
42 quadruplicate for protists and bacteria; and that the standard test organisms should have been  
43 thoroughly evaluated as broadly resistant to treatments (U.S. EPA 2010; see above SubSection).  
44

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1 Mesocosm-(intermediate-) scale tests include more realistic conditions, closer to volume and  
2 tank-like characteristics than bench-scale comparison. Mesocosms are containers that  
3 accommodate hundreds to thousands of liters (Ruiz et al. 1996). The experiments are conducted  
4 over a narrower range of conditions, especially targeting challenging or limiting conditions, and  
5 include more resistant organisms. The model system and engineering should be similar to that of  
6 the full-scale version to check treatment performance with increasing scale and engineering  
7 design. Successful tests of ballast water treatment efficiency at this scale provide confidence that  
8 the full-scale system will be effective.  
9

10 Land-based, full-scale testing, using hundreds of tons of volume and relevant flow rates  
11 applicable to actual treatment, can also include mesocosms to improve sampling in evaluations  
12 of treatment efficiency in biota removal. As an example, the State of Maryland's Maritime  
13 Environmental Resource Center (MERC 2009a-c) uses a set of ten identical, conical bottom  
14 mesocosms installed on the test ship in its land-based evaluations of the effectiveness of biota  
15 removal by ballast water filtration systems (Figure 1). Following calibration trials, triplicate  
16 independent test trials are conducted at least twice during different seasons to capture a range of  
17 natural challenge water conditions. Each test trial is completed over a single ballasting event;  
18 each filter system is operated for 5-6 hours continuously during each test trial, with individual  
19 trials separated by at least one day. The test trial duration produces a specific filtered amount of  
20 1,000 m<sup>3</sup> of treated water. Five mesocosms are used to sample initial challenge conditions prior  
21 to splitting the water; the second five are used to sample after the water passes through the  
22 control line versus the treatment line. At each sampling time, the designated mesocosms are  
23 filled to ~1.05 m<sup>3</sup> in sequence, drawing continuously over about 80% of the total 90 minutes  
24 required to fill or drain a ballast tank.  
25

## 26 **F. Incomplete Reporting of Results**

### 27 **Complete test results, including failures, must be reported.**

28 *[Should this section be expanded to include a description of how results are reported under the*  
29 *existing IMO D8 testing program?]*  
30  
31  
32

## 33 **III. Testing Shipboard Treatment Systems:** 34 **Inherent Limitations of Direct End-of-the-pipe Sampling**

35  
36 *[Subgroup 3 is still discussing whether there is a useful distinction to be drawn between, on the*  
37 *one hand, difficulties or problems in the current program of testing treatment system prototypes*  
38 *by sampling and directly enumerating viable organisms in different size or taxonomic groups*  
39 *that could be fixed or improved upon (such as not reporting full results), and on the other hand,*  
40 *problems that are currently insurmountable (such as impractically large sampling volumes*  
41 *needed to assess extremely low organism concentrations, or possibly the problems in assessing*

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1 *viability of some types of organisms). We have tentatively sorted these issues into Section II*  
2 *(practices that could be improved) and Section III (inherent limitations)].*

3 **A. Statistical Constraints**

4  
5 **Tests should be designed and the data analyzed using appropriate volumes and statistical**  
6 **methods, including explicit quantification of statistical power (Lee et al. 2010).**

7  
8 *[Most of the text summarizing the analysis in Lee et al. 2010 will be moved to an appendix].*  
9

10 This section mainly highlights the excellent analysis of Lee et al. (2010). Detection of  
11 microorganisms at very low concentrations, required to assess performance and compliance, is a  
12 major practical and statistical challenge, partly because of the inherent stochasticity of sampling.  
13 Due to random chance, the number of organisms in multiple samples taken from the same  
14 population will vary. In addition, very large volumes of water must be sampled in order to  
15 estimate the organism densities:  
16

17 “If a small volume is used to evaluate whether the discharge meets a standard, the sample may  
18 contain zero detectable organisms, but the true concentration of organisms may be quite  
19 high...For example, even with a relatively high concentration of 100 organisms m<sup>-3</sup>, only about  
20 10% of 1-L samples will contain one or more organisms. Furthermore, even if zero organisms  
21 are detected in a 1-L sample, the upper possible concentration, based on a 95% confidence  
22 interval, is about 3,000 organisms m<sup>-3</sup>. The general point is that more organisms may be released  
23 in ballast discharge using a stringent standard paired with a poor sampling protocol than a more  
24 lenient standard paired with a stringent sampling protocol” (Lee et al. 2010, p.72). Thus, when  
25 dealing with small numbers of organisms in large volumes of ballast tanks, the true degree of  
26 protection depends on the sample volume, regardless of how strict the standard might be.  
27

28 **Remainder of this page intentionally left blank**  
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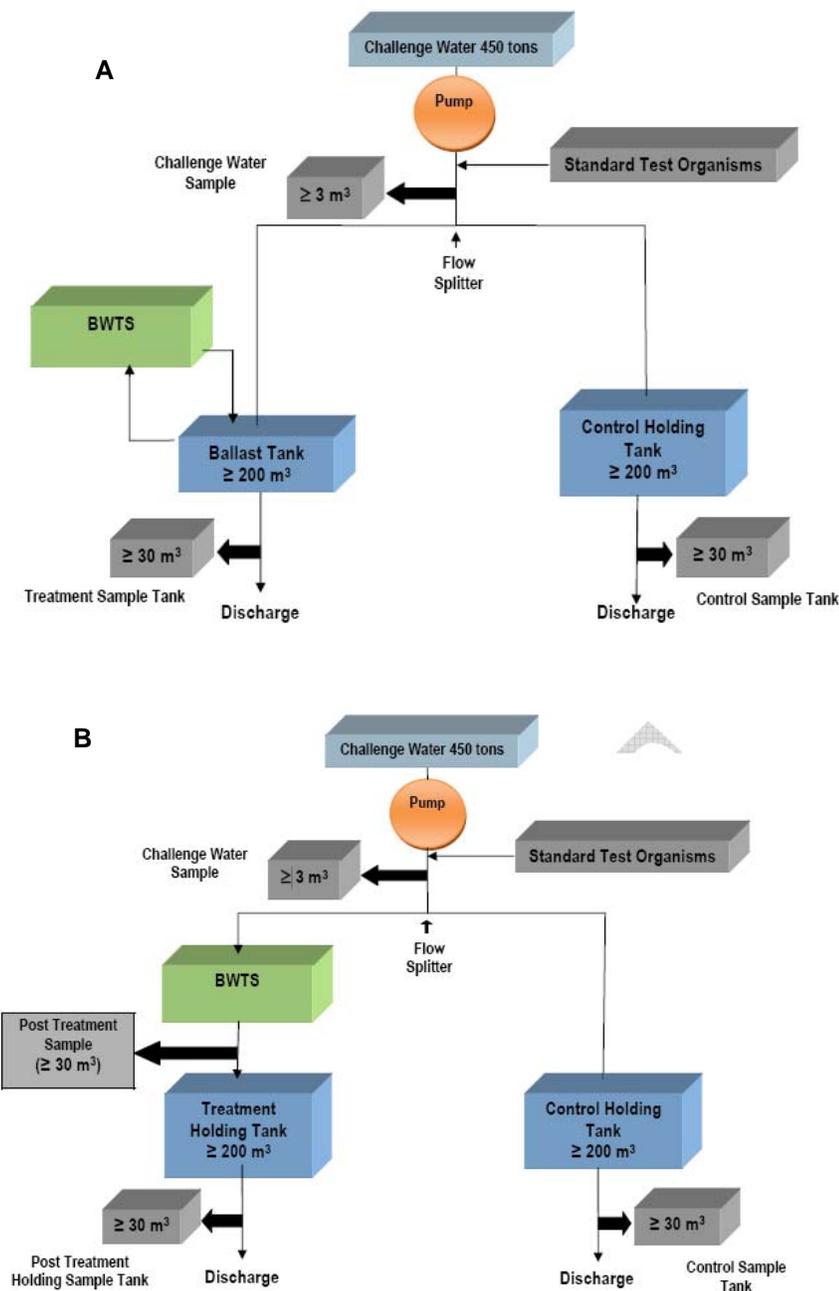


Figure 1. Sampling design example for (A) In-Tank treatment and (B) In-Line treatment. From ETV (2010).

1  
2 Statistical approaches in assessing treatment performance generally rest upon the premise that  
3 the samples realistically represent the actual concentrations of organisms discharged which, in  
4 turn, is based on two assumptions, namely, no human or equipment error that would fail to detect  
5 organisms in a sample volume, and random distribution of organisms in ballast tanks and  
6 discharge water. Neither assumption is true: Human and equipment errors are realities, and  
7 organisms are typically “patchy” or non-random in the water column of a tank or the stream of a  
8 large-volume discharge (Murphy et al. 2002, U.S. EPA 2010). The assumptions are made for

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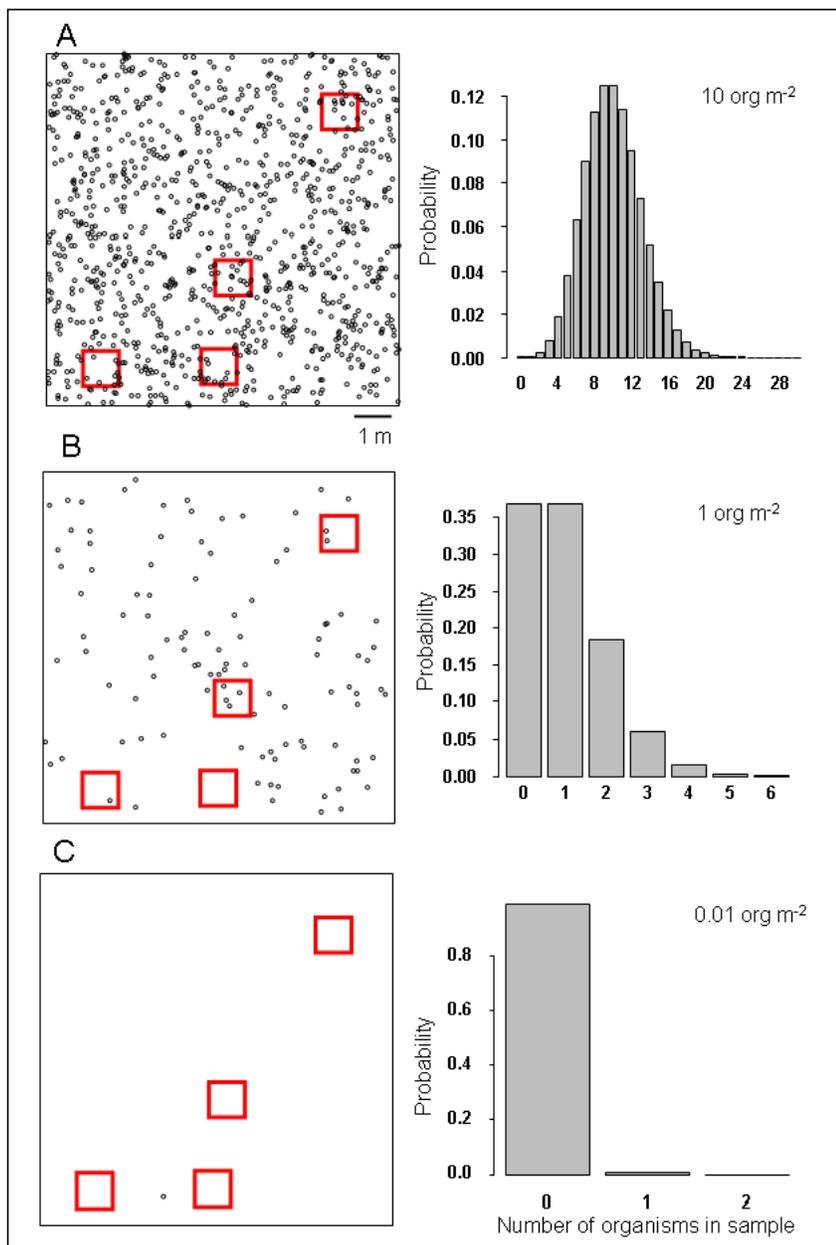
1 practical reasons; if appropriate quality control/assurance was used in collecting the data, then  
2 human error and equipment malfunction would have been accounted for. Regarding the second  
3 assumption, data are usually lacking to estimate aggregation in ballast water.  
4

5 The Poisson distribution can be used to determine the probability that a given number of  
6 individuals in a randomly distributed population will occur in a sample given the true  
7 concentration of organisms. In a Poisson distribution, the variance is considered to be  
8 approximately equal to the mean ( $\sigma^2 = \mu$ ). It has been argued that an assumption of random  
9 distribution is reasonable as an initial approach because the Poisson is the default (null)  
10 hypothesis and should be considered until rejected by testing (Elliott 1971, in Lee et al. 2010).  
11 Since the Poisson distribution pools the data to improve measurement precision and assumes  
12 random distribution, sample replication is unnecessary if samples are continuously taken on a  
13 time-averaged basis.  
14

15 A major challenge in sampling at low organism concentrations is that a large number of samples  
16 of typical sample volume (~1 L or less) will contain no organisms; thus, the sample volume  
17 collected will have to be extremely, impractically large, together with excellent detection  
18 techniques, to enable detection of organisms in low abundance (Figure 2). Consider the  
19 examples given in Lee et al. (2010): From the Poisson distribution, if 1 m<sup>3</sup> of ballast water was  
20 sampled from a discharge that had a concentration of 10 organisms m<sup>-3</sup>, about 95% of the  
21 samples will contain 4-17 organisms. As the concentration of organisms decreases, the frequency  
22 distribution becomes increasingly skewed, and there is a high probability of obtaining a sample  
23 with zero organisms. Thus, if the sample concentration is 1 organism m<sup>-3</sup>, the probability of a 1  
24 m<sup>3</sup> sample containing zero organisms is 36.8%. If the sample concentration is only 0.01  
25 organism m<sup>-3</sup>, or 1 organism in 100 cubic meters of ballast water, the probability of obtaining a  
26 sample with zero organisms is ~99%. The large sampling containers, sample transport costs  
27 (since samples usually are not processed aboard ship), analytical supplies, and personnel time  
28 constraints would make it impractical, if not impossible, to process all of the volume of even  
29 one 100 m<sup>3</sup> sample, much less multiple samples. This problem becomes yet more intractable in  
30 efforts to assess compliance with IMO standards for microorganisms.  
31  
32

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**Figure 2.** Illustration of the need to sample very large volumes to detect low concentrations of organisms present, assuming random distribution: Probability distributions for random samples of 1 m<sup>2</sup> for a randomly distributed population with 10 (A), 1 (B), or 0.01 (C) organisms m<sup>-2</sup>. Red squares represent random samples. The data are displayed in terms of area with units of m<sup>2</sup>, but the probabilities are the same for volumes. Plots on the right indicate the probability that a 1 m<sup>2</sup> sample will contain a given number of organisms. At low concentrations, the concentration of organisms is likely to be estimated as 0 organisms m<sup>-2</sup>, unless very large volumes are sampled. From Lee et al. (2010), with permission.

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1  
2  
3 Lee et al. (2010) calculated the probability of finding 1 or more organisms in a sample as  $1 - e^{-cv}$   
4 (1 minus the probability of finding no organisms) for a series of organism concentrations and  
5 sample volumes, where  $e \equiv$  the natural log,  $c \equiv$  the true concentration of organisms, and  $v \equiv$  the  
6 sample volume (Table 9). They used the following assumptions:  
7  
8 (i) discharge standards are for the concentration of organisms in the ballast discharge (rather than  
9 the maximum number of organisms), so that the purpose of sampling is to estimate the “true”  
10 concentration of organisms in the discharge, referred to as average-based sampling;  
11  
12 (ii) the organisms are randomly distributed and therefore amenable to modeling with the Poisson  
13 distribution, as above;  
14  
15 (iii) all organisms are counted, with no human or instrumentation errors, so that any variation  
16 among samples for a given population (species) is from the natural stochasticity of sampling;  
17  
18 (iv) the sample volume is calculated from the total volume of ballast water filtered (concentrated)  
19 and the filtrate volume that is subsampled, for example, following Lemieux et al. (2008):  $100 \text{ m}^3$   
20 of ballast water is filtered through a net to retain the  $> 50 \mu\text{m}$  size class; the organisms are rinsed  
21 from the net, collected, and diluted up to 1 L of water; and the organisms from 20 1-mL  
22 subsamples are counted: Total sample volume =  $20 \text{ mL subsamples} / 1000 \text{ mL concentrated}$   
23  $\text{sample} \times 100 \text{ m}^3 \text{ ballast water filtered} = 2 \text{ m}^3$ .  
24  
25 As Table 9 illustrates, about 100 L of ballast must be sampled to have a 95% probability of  
26 detecting at least 1 organism. The probability of detecting an organism is low even at relatively  
27 high organism concentrations when small sample volumes are collected; for example, organisms  
28 will be detected in fewer than 10% of subsamples if a 1-L sample is taken and the “true”  
29 concentration is  $100 \text{ organisms m}^{-3}$ . This analysis also illustrates that when no organisms are  
30 detected from a ~small sample, the true concentration in the ballast tank may be large – it  
31 depends on the sample volume collected.  
32  
33

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**Table 9.** Probability of detecting  $\geq 1$  organism for various sample volumes (100 mL to 300 m<sup>3</sup>) and ballast water concentrations (0 to 100 organisms m<sup>-3</sup>). Gray boxes indicate probabilities of detection  $\geq 0.95$ . From Lee et al. (2010), with permission.

Sample volume, m <sup>3</sup>	True concentration (organisms per m <sup>3</sup> )						
	0	0.001	0.01	0.1	1	10	100
0.0001 (100 mL)	0	<0.001	<0.001	<0.001	<0.001	0.001	0.01
0.001 (1 L)	0	<0.001	<0.001	<0.001	0.001	0.01	0.095
0.01 (10 L)	0	<0.001	<0.001	0.001	0.01	0.095	0.632
0.1 (100 L)	0	<0.001	0.001	0.01	0.095	0.632	>0.99
1	0	0.001	0.01	0.095	0.632	>0.99	>0.99
5	0	0.005	0.049	0.393	>0.99	>0.99	>0.99
10	0	0.010	0.095	0.632	>0.99	>0.99	>0.99
25	0	0.025	0.221	0.918	>0.99	>0.99	>0.99
50	0	0.049	0.393	>0.99	>0.99	>0.99	>0.99
100	0	0.095	0.632	>0.99	>0.99	>0.99	>0.99
300	0	0.259	0.950	>0.99	>0.99	>0.99	>0.99

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Lee et al. (2010) then estimated the upper possible concentration (UPC, upper 95% CI) of organisms actually present in ballast water from the number of organisms in a sample volume (range, 100 mL to 100 m<sup>3</sup>), based on the Poisson distribution. As Table 10 shows, 0 organisms detected in 1 m<sup>3</sup> of sample could correspond to a true concentration of organisms in the ballast tank of up to ~3.7 organisms m<sup>-3</sup>. The error is much larger for a small sample volume of 1 L; 0 organisms detected could correspond to a true concentration of ~3,700 organisms m<sup>-3</sup>.

**Table 10.** Upper possible concentration (UPC) of organisms based on one and two tailed 95% exact confidence intervals when zero organisms are detected in a range of sample volumes. From Lee et al. (2010), with permission.

Sample volume, m <sup>3</sup>	Upper possible concentration, org m <sup>-3</sup>	
	one-tailed	two-tailed
0.0001 m <sup>3</sup> (100 mL)	29,960	36,890
0.001 m <sup>3</sup> (1 L)	2,996	3,689
0.01 m <sup>3</sup> (10 L)	299.6	368.9
0.1 m <sup>3</sup> (100 L)	29.96	36.89
0.5 m <sup>3</sup> (500 L)	5.992	7.378
1 m <sup>3</sup>	2.996	3.689
10 m <sup>3</sup>	0.300	0.369
100 m <sup>3</sup>	0.030	0.037

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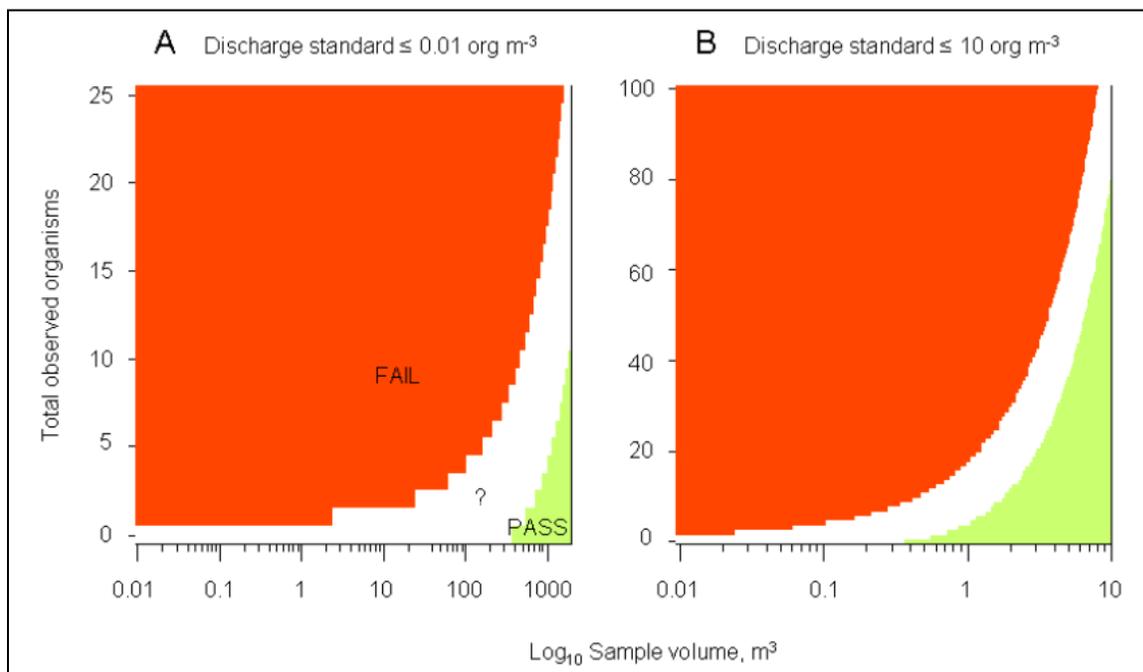
In the above analyses, the true organism concentrations are known. The goal in sampling unknown concentrations of organisms in ballast water is to accurately assess whether a given ballast water treatment system produces treated water with true organism concentrations that pass versus fail to

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1 meet a set discharge standard. Inherent stochasticity of sampling may result in an indeterminate  
2 category, as well, and the probability of obtaining an indeterminate evaluation increases with  
3 decreasing sample volume and increasing stringency of the ballast water standard (Figure 3). Based  
4 on this analysis, it would be necessary to sample  $\sim 0.4 \text{ m}^3$  of ballast water to determine whether the  
5 IMO standard of less than  $10 \text{ organisms m}^{-3}$  was met (Figure 3B).  
6  
7



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**Figure 3.** Determining whether ballast water discharge exceeds or meets a discharge standard of  $<0.01$  (A) and  $<10$  (B) organisms  $\text{m}^{-3}$  (note: axes have different scales). Red regions indicate total organism counts that exceed the standard. Green regions indicate total organism counts that meet the standard. White regions indicate indeterminate results; counts in this region do not pass or fail inspection based on two-tailed 95% confidence intervals. From Lee et al. (2010), with permission.

What if, as in reality, the organisms are aggregated (i.e., clumped or contagious populations) rather than randomly distributed in the ballast tank being sampled? For aggregated populations, the variance exceeds the mean ( $\sigma^2 > \mu$ ); thus, as the variance increases, the number of organisms in a random sample is increasingly unpredictable. Because it is more difficult to accurately estimate the true concentration, more intensive sampling is required. Lee et al. (2010) recommend use of the negative binomial distribution to model aggregated populations. This distribution can be used to predict the probability of finding a certain number of organisms in a sample. It is defined by the mean ( $\mu$ ) and the dispersion or size parameter ( $\theta = \mu^2 / (\sigma^2 - \mu)$ ); the smaller the dispersion parameter, the more aggregated the population.

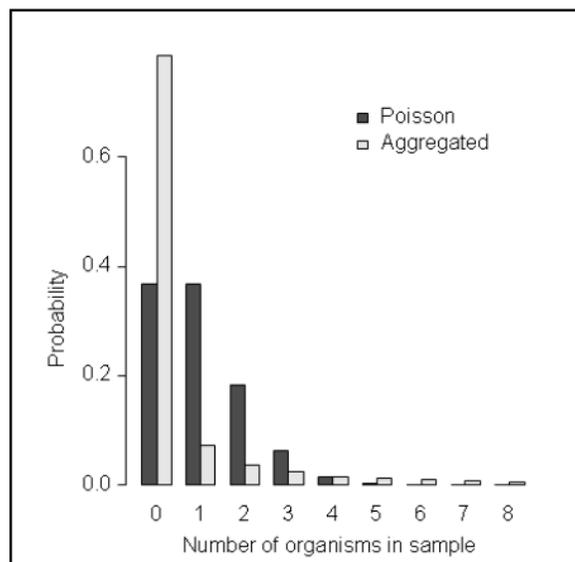
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1 The problem of having to sample multiple subsamples from large volumes to accurately assess  
2 low densities of organisms is compounded by aggregated distributions (Figure 4). In the  
3 comparison given in Lee et al. (2010), for a randomly distributed population with a true  
4 concentration of 1 organism  $m^{-3}$ , ~37% of the subsamples from a 1  $m^3$  sample of treated ballast  
5 water would contain zero organisms. For an aggregated population with a dispersion parameter  
6 of 0.1, however, ~79% of the subsamples would contain zero organisms (Figure 4). The  
7 probability of samples containing large numbers of organisms relative to the true concentration  
8 also increases. Thus, large numbers of subsamples from large sample volumes must be taken to  
9 account for aggregated populations; otherwise, there will be a high probability that the  
10 concentration estimates from sample analyses will be either much lower or much higher than the  
11 true concentration.  
12  
13

**Figure 4.** Comparison of sample probabilities from a randomly distributed population (Poisson distribution) vs. an aggregated population with a dispersion parameter of 0.1 (negative binomial distribution) for a sample volume of 1  $m^3$  and concentration of 1 organism  $m^{-3}$ . For low organism numbers (3 or fewer  $m^{-3}$ ), the probability that a sample will contain zero organisms tends to be much greater for the aggregated population. From Lee et al. (2010), with permission.

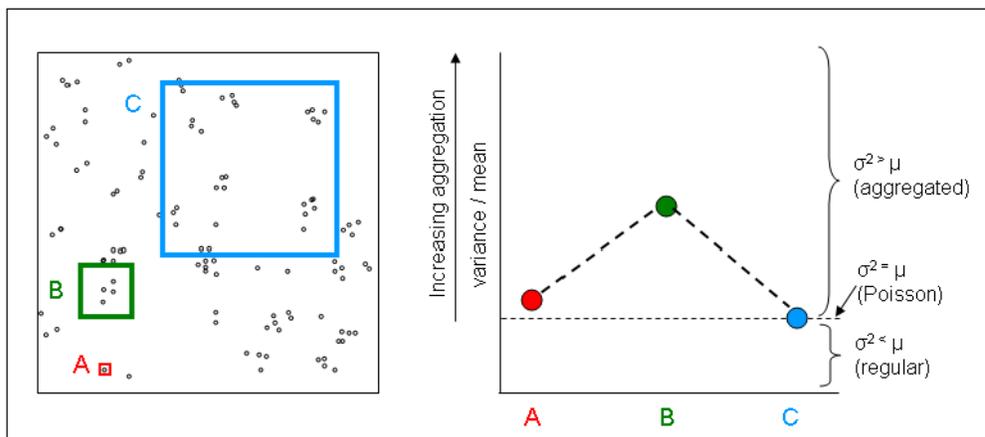


14  
15  
16  
17 Lee et al. (2010) refer readers to Elliott (1971) for how to determine whether a population is  
18 aggregated. Analysis is complicated since the scale of the aggregation pattern in comparison to  
19 the size of the sampling unit largely controls estimates of aggregation (Figure 5). If organisms  
20 form clumps that are randomly distributed, the population may be highly aggregated but in a  
21 ~small sample volume containing 0 or 1 organisms, the population will appear randomly  
22 distributed or slightly aggregated. With increasing sample volume, the variance in the number of  
23 organisms increases in comparison to the mean, and maximum variance is encountered when the  
24 sample volume is ~equal to the volume of a single cluster of organisms. For larger sample  
25 volumes, a sample unit will include several clusters, so the variance decreases in comparison to  
26 the mean. Lee et al. (2010) recommend the Taylor power law (Taylor 1961) as an alternative to  
27 the negative binomial because it can accommodate a wider range of aggregated distributions than  
28 the negative binomial.

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**Figure 5.** Theoretical example of how the apparent aggregation in the population will differ based on the scale of aggregation relative to the size of the sample unit. Green region  $\equiv$  acceptable counts; red region  $\equiv$  unacceptable counts; white region  $\equiv$  indeterminate result (ambiguous – may be considered as unacceptable if a high degree of confidence is needed). From Lee et al. (2010), with permission.

3  
4  
5

6 Overall, these analyses indicate that it will be a major challenge to sample sufficiently large  
7 volumes to determine whether a given ballast water treatment system passes versus fails to meet  
8 standards more stringent than the present IMO guidelines, even if the true concentrations of  
9 organisms are 10- to 1,000-fold higher than the discharge standard. Therefore, considering  
10 compliance issues, we support Lee et al.'s (2010) recommendation that the quality control  
11 needed to assure that treatment systems adequately minimize organism concentrations “may best  
12 be achieved through rigorous type-approval of ballast water treatment systems in controlled  
13 testing facilities, rather than from after-the-fact compliance shipboard sampling.”

14

15 A final consideration regarding statistical analysis concern the potential for covariance, or  
16 interactive effects among environmental conditions - for example, a treatment system that  
17 performs well under high-temperature or high-biomass conditions, but not both (Ruiz et al.  
18 1996). To help address this problem, covariate measurements should be addressed in  
19 experiments, and treatment evaluations should consider the potential for interactions and target  
20 tests of especially challenging combinations.

21

## 22 **B. Mismatch Between the “Living” (Viable) Standard and Practical Protocols**

23

24 **Testing protocols for determining “viability” and/or “living” must be standardized, and the**  
25 **limitations of these protocols must be assessed.**

26

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1 *[Although there are difficulties with assessing viability in any class of small aquatic organisms,*  
2 *some Subgroup 3 members felt that viruses and nonculturable bacteria pose a relatively*  
3 *intractable problem, possibly constituting inherent limits to our ability to enumerate viable*  
4 *organisms in samples taken from the effluent from a prototype treatment system. This is in*  
5 *comparison to zooplankton and phytoplankton, for which we think we can more readily identify*  
6 *acceptable methods for enumerating viable organisms. Other Subgroup 3 members weren't so*  
7 *sure. Is this a useful distinction? If so, text on issues related to the difficulties of assessing*  
8 *viability in zooplankton and phytoplankton should be moved to Section II.*

9  
10 *Should subsection 1) below (on the concentration of samples killing organisms) be moved to*  
11 *III.A above, as an issue complicating “the premise that the samples realistically represent the*  
12 *actual concentrations of organisms discharged”?*

13  
14 *Some discussion of viruses should be included in this section].*

15  
16  
17 As Lee et al. (2010, p. 72) aptly state, “A discharge standard of ‘zero detectable organisms’ may  
18 **appear** [emphasis added] very protective; however, the true degree of protection depends on the  
19 sampling protocol.” Here, a viable or living organism is defined as in U.S. EPA (1999), namely,  
20 as an organism that has the ability to pass genetic material on to the next generation. The  
21 percentage of non-viable cells has been shown to vary markedly, for example, from 5-60%  
22 among phytoplankton taxa, and in general, non-viable organisms are believed to represent a  
23 substantial component of the total plankton (Agusti and Sánchez 2002). There are several  
24 fundamental problems confronted in present attempts to quantify viable organisms to evaluate  
25 ballast water treatment efficiency, outlined as follows.

26 **1) Rapid concentration of cells from large volumes kills and/or destroys many organisms**

27  
28 Performance evaluation of biological efficacy in ballast water treatment systems requires  
29 sampling viable (living) cells concentrated to maintain viability, into a 1-liter container. Yet this  
30 concentration step must also be accomplished quickly because quantification of viable cells must  
31 be completed within a short time of sample collection – for example, within 6 hours for  
32 zooplankton. There is a fundamental disconnect in these requirements: It is difficult if not  
33 impossible to rapidly concentrate microflora and microfauna from very large volumes (hundreds  
34 of liters) by available filtration or centrifugation techniques without killing many of the  
35 organisms (e.g. Turner 1978, Cangelosi et al. 2007). Such rapid concentration techniques can  
36 cause the loss of a major fraction of the viable cells, even when dealing with small sample  
37 volumes such as a liter (Darzynkiewicz et al. 1994). This problem affects zooplankton and  
38 protist size classes, especially delicate species such as wall-less algal flagellates. ETV (2010,  
39 p.45), for example, described one way of concentrating protists (10 to <50 µm size class) by  
40 using a sieve with mesh size  $\leq 10$  µm in the diagonal, and noted that “Care should be taken to  
41 gently sieve organisms to ensure [that] they are not killed in the process.” Sufficient care may  
42 not be possible, however, given the need for rapid concentration. It should be noted that

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1 concentration-related losses do not affect the smallest size class in the present IMO standard,  
2 bacteria, because they are so abundant in most fresh, estuarine, and marine waters that it usually  
3 is not necessary to concentrate them from whole water samples prior to analysis by standard  
4 microbial techniques (U.S. EPA 2010; see below).

5 **2) Standardized rigorously tested protocols for evaluating organism viability do not yet exist**  
6

7 Cell viability and cell death are not easily detected by a single morphological or physiological  
8 parameter, making it advantageous to use more than one approach (Brussaard et al. 2001).  
9 Moreover, the procedures used have varying degrees of uncertainty in categorizing live versus  
10 dead, and even the recommended procedures have practical limitations because of time  
11 constraints. For example, ETV (2010) defines dead zooplankton operationally as individuals that  
12 do not visibly move during an observation time of at least ten seconds. Since live zooplankton  
13 may not move over that short period, death is verified by gently touching the organism with the  
14 point of a fine dissecting needle to elicit movement. However, ETV acknowledges that if every  
15 apparently dead zooplankton in a concentrated subsample was probed and monitored for at least  
16 10 seconds, analysis of the sample could be extended enough to increase the potential for sample  
17 bias due to death of some individuals.  
18

19 Cell death is accompanied by loss of membrane integrity (Veldhuis et al. 2001), a feature that  
20 has been used to indicate live versus dead cells, usually with fluorescent tags under  
21 epifluorescence microscopy. The nucleic acid SYTOX-Green (excitation 504 nm, emission 523  
22 nm; Molecular Probes Inc.) is used as an indicator of dead cells (Roth et al. 1997, Lebaron et al.  
23 1998). This high-affinity probe easily penetrates cells with compromised plasma membranes, but  
24 it does not cross intact membranes of viable cells (Roth et al. 1997, Veldhuis et al. 1997, 2001).  
25 Detection of viable cells frequently is done with electrically neutral or near-neutral substrate  
26 molecules that passively move into cells, where the nonfluorescent substrates are hydrolyzed by  
27 enzymes to become polar fluorescent substances that are retained by cells with intact plasma  
28 membranes.  
29

30 For example, the fluorogenic substrate Calcein-AM (Molecular Probes Inc.) is used to stain live  
31 cells with metabolic esterase activity (Kaneshiro et al. 1993, Porter et al. 1995). Once the  
32 colorless, nonfluorescent substrate is inside the living cell, its lipophilic blocking groups are  
33 cleaved by nonspecific esterases to a charged green fluorescent (excitation 496 nm, emission 520  
34 nm) product that cannot pass across the plasma membrane. Dead cells, in contrast, cannot  
35 hydrolyze the Calcein-AM or retain the fluorescent product. Use of fluorescein diacetate (FDA),  
36 another vital stain, is based on the measurement of intracellular esterase, which colors viable  
37 cells green under blue light excitation. Fluorescein accumulates in cells when nonspecific  
38 esterases are present and when intact cell membranes allow transport, criteria that are met when  
39 the algal cell is alive (Laabir and Gentien 1999, Hampel et al. 2001). Reavie et al. (2010)  
40 reported that FDA was a reliable, efficient method for assessing densities of concentrated viable  
41 freshwater organisms in the 10- to < 50  $\mu\text{m}$  size class from ballast discharge. These authors also  
42 tested various other techniques – digestion with enzymes, flow cytometry, and various vital and  
43 mortal stains – and found that all of them produced inconsistent or ambiguous results.

10/28/2010 Science Advisory Board (SAB) Ecological Processes and Effects Committee  
Augmented for Ballast Water

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1 Nevertheless, FDA and CMFDA have limitations because various algal species differ in their  
2 uptake of the markers, cells are scored as “live” if they show strong fluorescence, and other  
3 particles in a given sample can fluoresce (Garvey et al. 2007, MERC 2009c). The vital stain  
4 propidium iodide (PI), in combination with molecular probes, has been used to discern live  
5 versus dead (including recently dead) bacteria (Williams et al. 1998). However, PI cannot be  
6 used to assess viability in phytoplankton because its emission spectrum overlaps with that of  
7 chlorophyll (Veldhuis et al. 2001).  
8

9 At this juncture, while recognizing the technique limitations (below), the ETV recommends the  
10 combination of two vital stains, FDA and CMFDA (5-chloromethylfluor-escein diacetate), to  
11 detect vital protists, including resistant cysts (below): Non-specific esterases in live cells cleave  
12 the stains, resulting in green fluorescence when excited with a blue light (Selvin et al. 1988). If a  
13 cell is labeled by either stain and/or if a cell moves, it is scored as viable. ETV’s protocol  
14 includes taking a photomicrograph of every viable cell to create a visual record, which could be  
15 unacceptably time-consuming for dense samples. Moreover, research at marine and estuarine  
16 sites has shown that this method tends to overestimate rather than underestimate viable  
17 organisms (Type I and Type II errors, respectively). The number of false positives or  
18 overestimates of viable organisms has varied from 3% to nearly 40% (Steinberg et al. 2010).  
19 Therefore, the ETV recommends that before using any viability method, on-site validation  
20 should be completed by preparing and analyzing negative controls, i.e. samples that are killed  
21 (U.S. EPA 2010). The technique should also be evaluated for false negatives. Other limitations  
22 identified by the ETV in using manual epifluorescence microscopy with vital stains are that  
23 errors can occur in the counts because of operator-specific biases and operator fatigue during  
24 extended observation periods.

25 **3) *Resistant stages present special challenges in attempts to assess viability***  
26

27 The protist size class (10 to < 50  $\mu\text{m}$ ) includes many species (microalgae, heterotrophic protists,  
28 metazoans) that form dormant cells or resting stages, or cysts (Matsuoka and Fukuyo 2000,  
29 Marrett and Zonneveld 2003). For example, cysts from potentially toxic dinoflagellates are  
30 commonly found in ballast waters and sediments (Hallegraeff and Bolch 1992). These cysts  
31 have been used as model indicator organisms to assess ballast water treatment efficiency  
32 (Anderson et al. 2004, Stevens et al. 2004), based on the premise that treatments which can  
33 eliminate the cysts likely also eliminate other, less resistant organisms (Bolch and Hallegraeff  
34 1993, Hallegraeff et al. 1997).  
35

36 Unfortunately, the resistant outer coverings of dormant cells such as cysts limit the utility of  
37 most vital stains and also can require application of multiple stains. For example, viability of the  
38 pathogenic protozoans *Cryptosporidium parvum* and *Giardia lamblia* has been assessed using  
39 vital fluorogenic dyes DAPI (4',6'-diamidino-2-phenyl-indole) and PI (propidium iodide)  
40 (Connelly et al. 2007). Oocysts that are intact and viable stain with DAPI but are impermeable  
41 to PI, whereas damaged cyst walls will allow uptake of PI and staining of the nucleic acids inside  
42 the cysts with both DAPI and PI. This assay can be affected by the pH of the medium, however:  
43 fluorescent crystals of the stains form at neutral to alkaline pH, which can lead to oocyst

**10/28/2010 Science Advisory Board (SAB) Ecological Processes and Effects Committee  
Augmented for Ballast Water**

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1 occlusion that makes viability assessment difficult or subjective (Bukhari et al. 1999). Other  
2 dormant, resistant structures such as dinoflagellate cysts have thick walls through which many  
3 vital stains cannot reliably penetrate. Therefore, these structures can be missed in viable  
4 analyses with live staining techniques, yet the cells within the resistant outer coverings can form  
5 inocula for nonindigenous species invasions (Dobbs and Rogerson 2005, Doblin and Dobbs  
6 2006, and references therein).

7  
8 Because resistant cells often have a low metabolic state and thick, multi-layered walls that are  
9 impermeable to many stains (Romano et al. 1996, Kokinos et al. 1998), their viability can be  
10 difficult to assess without culture analyses that may require weeks to months (Montresor et al.  
11 2003, U.S. EPA 2010). As the ETV (pp.46-47) states, “At present, no rapid, reliable method to  
12 determine cysts’ viability is in widespread use, and the FDA-CMFDA method has yielded  
13 variable results with dinoflagellates and cyst-like objects.” The ETV recommends use of this  
14 method as a “place holder” until more effective methods become available. Such improved  
15 methods have, in fact, been developed that work well across some algal groups: As examples,  
16 Sytox®Green does not penetrate intact cysts of some dinoflagellate species, but causes cysts  
17 with compromised cell walls to fluoresce yellow-green when excited with a 450-490 nm light  
18 source (Binet and Stauber 2006, Gregg and Hallegraeff 2007).

19 ***4) Many microbial species are capable of changing their location in counting chambers,***  
20 ***which can bias the count***

21  
22 Another “disconnect” in protocols that involve quantifying live (unpreserved) cells is that they  
23 are quantified in counting chambers, based upon an underlying premise that the cells do not  
24 change their location in the chamber. However, many protists move rapidly by means of flagella  
25 or other structures. Because they do not maintain their position in a counting chamber, as live  
26 cells they could be counted multiple times. Moreover, their sudden movement can disrupt the  
27 locations of other cells in the chamber, mixing cells that may have been counted with other that  
28 have not yet.

29 ***5) Viable when collected (formerly viable) counts should be considered as a practical***  
30 ***alternative for quantifying phytoplankton, other protists, and zooplankton***

31  
32 Consideration of the above points – lack of reliable procedures to assess viability across taxa  
33 within a given size class (e.g. phytoplankton), movement of live organisms in counting chambers  
34 that can result in serious quantification errors, etc. – has led to an alternate approach that may be  
35 more reliable than attempting to quantify living cells from unpreserved, concentrated samples:  
36 Quantification of algae, other protists, and zooplankton from *preserved* samples taken from the  
37 concentrated material, and assessment as *viable when collected (formerly viable)*. This approach  
38 is commonly used in characterizations of microflora and microfauna assemblages in the peer-  
39 reviewed, published literature. It is based on the fact that protists and zooplankton deteriorate  
40 quickly once dead (within minutes to hours; e.g. Wetzel 2001, Johnson and Allen 2005). Thus, if  
41 effective, “fast-kill” preservatives are used that cause death before distortion can occur, it is  
42 assumed that whole organisms with intact cellular contents were viable when collected.

10/28/2010 Science Advisory Board (SAB) Ecological Processes and Effects Committee  
Augmented for Ballast Water

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1 Obviously dead organisms are omitted from the count; for example, dead diatom cells are  
2 identified from the presence of empty silicon valves (cell walls (Knoechel and Kalff 1978). As  
3 shortcomings to this approach, dying organisms that still contain apparently intact cellular  
4 contents would be included in the “viable” estimate; and, as for counts based on unpreserved  
5 material, it is difficult to assess whether some resistant structures such as thick, opaque cysts  
6 contain organisms with intact cell contents. Because of practical and environmental health/safety  
7 constraints, neither approach avoids the problem of likely-major losses of viable organisms that  
8 occur during rapid concentration of large sample volumes.  
9

10 Rigorous comparative tests thus far are lacking to demonstrate whether the “viable counts”  
11 approach from unpreserved samples, with the practical “disconnects” discussed above, yields a  
12 more accurate count of organisms that actually were viable when collected than assessments of  
13 intact organisms in samples that were immediately preserved after the concentration step. While  
14 the ideal would be to quantify viable organisms using standardized, accepted techniques, such  
15 techniques do not yet exist, and the serious practical limitations in presently available approaches  
16 for testing the efficiency of ballast water treatment systems make the ideal far from reality. In  
17 contrast, standardized, accepted techniques *are* presently available for quantifying “viable when  
18 collected” protists and zooplankton from preserved material (e.g. Lund et al. 1958, Wetzel and  
19 Likens 2000, Johnson and Allen 2005).  
20

21 Series of tests be completed in testing facilities from different geographic regions to compare  
22 “viable versus viable when collected” data. It is expected that especially for zooplankton and for  
23 delicate groups of phytoplankton, viable-when-collected data from preserved samples will be  
24 superior to counts from unpreserved samples in quantifying viable organisms. In contrast,  
25 unpreserved samples prepared with “best available” viable stains will likely yield more reliable  
26 counts of viable picoplankton (very small photosynthetic organisms, ~2-4 µm bacteria-sized or  
27 slightly larger) than estimates of viable-when-collected cells from preserved samples. Very  
28 likely, a combination of the two approaches will prove to be the most advantageous in  
29 quantifying viable organisms from ballast water.

30 ***6) Techniques to detect and quantify harmful bacteria are based upon small volumes relative***  
31 ***to ballast tank volumes, and also cannot account for active nonculturable cells***  
32

33 Sample volumes used to quantify bacteria remaining after ballast water treatment range from 1  
34 mL to 500 L (Table 7) (MERC 2009a-d, U.S. EPA 2010). Considering the known, substantial  
35 heterogeneity of organism distributions throughout the water column of ballast tanks, the use of  
36 very small sample volumes could easily misrepresent the bacterial density unless the tank was  
37 well mixed, which would be difficult to accomplish. Thus, the use of mesocosms that can be  
38 well mixed, such as the MERC (2009a-d) approach, would be advantageous.  
39

40 A second difficulty especially pertains to techniques used to quantify certain indicator bacterial  
41 pathogens, such as harmful strains of *Escherichia coli*, intestinal enterococci, and toxigenic  
42 *Vibrio cholerae*. The effectiveness of ballast water treatment in removing viable bacteria is  
43 commonly evaluated by using multiple bacterial media in combination with taxon-specific  
44 molecular techniques (MERC 2009c, U.S. EPA 2010, and references therein). Colonies are

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1 monitored and quantified after ~1 to 5 days, depending upon the organism and its growth. These  
2 methods enable detection and quantification of viable, culturable cells. However, it has been  
3 repeatedly demonstrated that bacterial consortia across aquatic ecosystems commonly have a  
4 substantial proportion of cells which are active (viable) but nonculturable (Oliver 1993, Barcina  
5 et al. 1997 and references therein). These cells obviously would be overlooked in culturing  
6 techniques, a problem that would result in failure to detect viable cells of bacterial pathogens in  
7 treated ballast water. Under some conditions, the nonculturable organisms can regain activity  
8 and virulence (Barcina et al. 1997 and references therein).  
9

### 10 C. HACCP: An Alternative Approach to Assess Treatment Capabilities

11  
12 *[Subgroup 3 members feel that issues related to HACCP or “HACCP-like” analyses need to be*  
13 *more fully discussed in order to assess the role such analyses may have in assessing treatment*  
14 *system capabilities and, thus, how such analyses might be discussed in this report. An*  
15 *alternative way of describing such assessments might be “process-based” analysis as contrasted*  
16 *with “end product-based analysis that involves sampling effluent from prototype treatment*  
17 *systems.*  
18

19 As discussed in the preceding sections, there are inherent limits to our ability to evaluate, by end-  
20 of-the-pipe testing, the level of risk reduction that can be achieved by available technology; and  
21 on top of those inherent limits, there are major problems with the way end-of-the-pipe testing of  
22 ballast water treatment systems has been implemented through the IMO G8 process. An  
23 alternative approach is to base the assessment on an analysis of the engineering and operational  
24 design of the treatment system and the individual processes used in the treatment system. This is  
25 the approach employed when Hazard Analysis and Critical Control Points (HACCP) methods are  
26 used to set regulatory and permit requirements.  
27

28 [NOTE: INSERT a 1-3 paragraph history of HACCP and its employment in the food, bottled  
29 water, pharmaceutical and cosmetics industries, biosolids management, the treatment of  
30 wastewater for recycled use, drinking water treatment, the management of watersheds and water  
31 distribution systems, and reducing invasion risk in aquaculture, the baitfish trade and natural  
32 resource management and restoration activities.]  
33

34 A HACCP-type analysis or approach could enter into a ballast water regulatory regime in at least  
35 four ways. First, the responsible regulatory agency could conduct an analysis of the most  
36 promising conceptual treatment systems employing available technology organized in a series of  
37 processes or steps. The analysis would be based on the available data on the capability of each  
38 process or step to remove or kill exotic organisms, and would estimate the maximum level of  
39 risk reduction that could feasibly be achieved by these systems. The achievable risk reduction  
40 goal could be expressed as an end-of-the-pipe number or concentration of viable exotic  
41 organisms in various size or taxonomic classes, or as a specified log reduction in the number or  
42 concentration of such organisms. The results of this analysis would support a determination by

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Augmented for Ballast Water**

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1 the responsible agency of the appropriate treatment standards or discharge requirements to set.  
2

3 Second, the manufacturer of a shipboard treatment system or the proponent of an onshore  
4 treatment facility could develop a formal HACCP plan for the system or proposed facility—  
5 including hazard analysis, identification of critical control points, specification of critical limits,  
6 and the other HACCP elements—and would submit this to the responsible agency for approval.  
7

8 Third, the agency would review the submission, and either approve it, reject it, or reject it with  
9 suggested modifications (such as different critical limits or changes in monitoring requirements  
10 or corrective actions). A manufacturer/proponent could resubmit after modifying the plan. In  
11 addition to the HACCP plan itself, the agency would consider any available and relevant end-of-  
12 the-pipe testing data, or could require end-of-the-pipe testing in bench-top, test-facility, or  
13 shipboard conditions, though depending on the risk reduction goal, the organism type or size  
14 class, and the test conditions such data might be of limited resolution, as discussed in the  
15 preceding sections.  
16

17 Fourth, the agency could assess compliance by verifying that equipment is installed, maintained  
18 and operated and that processes are monitored as described in the manufacturer's or proponent's  
19 approved HACCP plan; by checking the ship's or facility's monitoring data and records to verify  
20 that critical limits are met and that the requisite corrective actions are taken when needed; and,  
21 where feasible, by independently checking whether critical limits are met. Some end-of-the-pipe  
22 compliance testing could also be conducted; though subject to limits in resolution as discussed  
23 earlier, such testing might nonetheless be able to detect flagrant (e.g. orders-of-magnitude)  
24 violations, which could be of value in a compliance monitoring setting as a check on records  
25 falsification.  
26

27 The first use of a HACCP-type analysis described above (to support the setting of a treatment  
28 standard or discharge requirements) is separable from the latter three uses (to implement the  
29 standard or requirement). That is, a standard or requirement could be set based on data from  
30 sources other than a HACCP-type analysis, such as data from end-of-the-pipe testing, if such  
31 data were adequate to the task. Once set, the standard or requirement could then be implemented  
32 either through a HACCP approach, or by monitoring compliance with end-of-the-pipe testing, or  
33 by a combination of these.  
34

35 Alternately, a numeric discharge standard or requirement could be set based on a HACCP-type  
36 analysis, and then implemented either through a HACCP approach, by end-of-the-pipe  
37 compliance monitoring, or by both. Given the limitations and problems of the end-of-the-pipe  
38 testing program as discussed earlier, using a HACCP-type analysis to develop standards might be  
39 most effective, regardless of what approach is used to implement them.  
40

1 **IV. Relationship of Testing Protocols to Assuring Feasibility of Voluntary**  
2 **Compliance Monitoring and Enforceability of Standards**  
3

4 *[This section on compliance monitoring will be moved to and combined with the compliance*  
5 *earlier discussion on monitoring].*

6 **A. Statement of Limitation**  
7

8 Both initial testing of technology protocols (section above) and potential compliance and  
9 enforcement monitoring suffer from slow and expensive methods. Statistical limitations related  
10 to sample volumes may, in practice, mean that it is impossible to directly assess whether a  
11 treatment system can meet all the numerical standards for viable organisms. Thus even for on-  
12 shore testing and certification of treatment systems, it may be necessary to adopt the use of  
13 surrogate metrics, including preserved organisms as an index of viable organisms and/or indirect  
14 metrics of organism abundance or metabolic activity (see previous sections).  
15

16 Furthermore, the same challenges will apply with much greater force with respect to future  
17 compliance monitoring and enforcement of ballast water discharge standards (King and  
18 Tamburri 2010). No information was provided (does any exist?) on whether protocols and  
19 systems for compliance monitoring (whether voluntary by ship operator or legally required) and  
20 enforcement were being considered alongside the development and testing of technology. The  
21 committee feels that it is essential that these be developed in concert with technology testing to  
22 avoid a situation where the creation of enforceable policy or rules is difficult or impossible.  
23

24 **B. Practical Compliance/Enforcement Protocols Needed**  
25

26 Full protocols for initial evaluation of a technology (previous sections) are not practical for  
27 routine inspections (either self-inspections or regulatory inspections) (King and Tamburri 2010).  
28 Protocols assessing surrogate parameters (including perhaps chlorophyll, DNA, ATP) for  
29 concentration standards should be developed that can be easily and quickly measured on board  
30 ship or nearby. If sufficient foundation of rigorous studies demonstrate the relationship between  
31 surrogate variables and the numerical standards for living organisms (specified in policy) then  
32 such surrogates could be used not only in future compliance and enforcement testing but also in  
33 initial testing of technology systems. [NOTE: This section could be developed further once we  
34 decide how to square with earlier text on surrogate species, surrogate procedures (live vs  
35 preserved counting), and surrogate metrics (e.g., ATP, DNA).]V. Approaches other than Ballast  
36 Water Treatment  
37

38 **V. Approaches Other Than Ballast Water Treatment**

39 **A. Introduction**  
40

41 Several approaches other than the treatment of ballast water could help to reduce the risk of

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Augmented for Ballast Water**

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1 biological invasions from ballast water discharges, and contribute to the achievability of  
2 discharge standards and permit requirements. These approaches include ballasting practices to  
3 reduce the uptake of organisms, ballast water exchange to reduce the concentration of exotic  
4 organisms, reductions in the volume of ballast water discharged in U.S. waters, and management  
5 of the rate, pattern or location of ballast water discharge to reduce the risk of  
6 establishment. Although the committee's charge questions focused on shipboard treatment, we  
7 consider these other approaches because in combination with shipboard treatment they appear to  
8 be capable of achieving a greater level of risk reduction than shipboard treatment alone.  
9

10 **B. Managing Ballast Uptake**

11  
12 Several studies have recommended various ballasting practices—sometimes referred to as ballast  
13 micro-management (Carlton et al. 1995; Oemke 1999; Dames & Moore 1998, 1999; Cohen &  
14 Foster 2000), shipboard management measures (Gauthier & Steel 1996), or precautionary  
15 management measures (Rigby & Taylor 2001a,b)—to reduce the number of organisms, or the  
16 number of harmful or potentially harmful organisms (such as bloom-forming algae and human  
17 pathogens found in sewage), that are taken up with ballast water (Table V.B-1). It is suggested  
18 that this can be accomplished by managing the time, place and depth of ballasting. Some of these  
19 measures have been included in laws, regulations or guidelines, including International Maritime  
20 Organization guidelines and the U.S. Coast Guard rules implementing the National Invasive  
21 Species Act (Table V.B-2).<sup>4</sup> Although some of these regulations or guidelines have been in  
22 effect for nearly 20 years, there appear to be no data at all on levels of compliance and no studies  
23 of the effectiveness of any of these measures in reducing the uptake of organisms.  
24

25 **Remainder of this page intentionally left blank**  
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<sup>4</sup> Some of these measures have also been included in state laws and regulations (e.g. California Public Resources Code 71204(b)).

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**1** **Table V.B-1. Ballasting practices to reduce the uptake of organisms in ballast water cited by different studies.**  
**2**

Measure	Hallegraeff & Bolch 1992	AQIS 1993b	Carlton et al. 1995	NRC 1996	Gauthier & Steel 1996	Reeves 1998	Cohen 1998	Dames & Moore 1998	Oemke 1999	Dames & Moore 1999	Cohen & Foster 2000	Rigby & Taylor 2001a,b
<b>AVOID HIGH ORGANISM CONCENTRATIONS OR ORGANISMS OF CONCERN</b>												
Not in "global hot spots" [1]			X	X		X		X	X	X		X
Not during seasons when organisms of concern are dense in the water column			X	X	X			X	X	X	X	X
Not during (toxic) phytoplankton blooms	X		X		X		X				X	X
<b>AVOID HUMAN PATHOGENS</b>												
Not near sewer discharges			X	X	X	X	X	X	X	X	X	X
Not where there are known incidences of water-borne human disease			X	X			X				X	
<b>AVOID SUSPENDED SEDIMENT AND NEAR-BOTTOM BALLASTING</b>												
Not in sites with high sediment loads			X	X	X	X			X		X	
Not near dredging activities						X	X		X	X	X	X
Not in fast, turbid ebb tides								X		X		
Not in turbid rivers									X			
Not where propeller wash stirs up sediment								X		X	X	
Not in shallow water		X					X		X		X	X
Not at low spring tide, when ballast intakes are closer to the bottom								X		X		
Use intakes that are higher in the water column		X	X				X		X		X	X
<b>OTHER</b>												
Not in areas with industrial discharges												X
Not in areas with poor tidal flushing											X	
Not at night			X	X	X	X	X	X	X	X	X	X
[1] "Hot spots" are defined as regions known to contain local outbreaks of infectious diseases or water-borne organisms, or known for the existence of problem species, including local outbreaks of phytoplankton blooms.												

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Implementation of this measure depends on the development of a global hot spot reporting system (Carlton et al. 1995).

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2  
3  
4  
5

**Table V.B-2. Ballasting practices to reduce the uptake of organisms in ballast water included in laws, regulations or guidelines.**

<b>Measure</b>	<b>IMO 1991, 1993</b>	<b>AQIS 1992</b>	<b>IMO 1997</b>	<b>USCG 1999</b>	<b>IMO 2004</b>	<b>IMO 2005a</b>	<b>IMO 2005b</b>
<b>AVOID HIGH ORGANISM CONCENTRATIONS OR ORGANISMS OF CONCERN</b>							
Not in areas known to have infestations of harmful organisms	X		X	X	X	X	X
Not during (toxic) phytoplankton blooms	X	X	X	X	X	X	
Not near aquaculture areas						X	
<b>AVOID HUMAN PATHOGENS</b>							
Not near sewer discharges			X	X	X	X	
Not where there are known incidences of water-borne human disease	X	X					
<b>AVOID SUSPENDED SEDIMENT AND NEAR-BOTTOM BALLASTING</b>							
Not near dredging activities	X	X	X	X			X
Not in turbid tidal streams			X	X	X	X	
Not where propeller wash stirs up sediment			X	X		X	X
Not in shallow water	X	X	X			X	X
<b>OTHER</b>							
Not in areas with poor tidal flushing			X	X	X	X	
Not at night			X	X		X	X

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19

While there may be reasons for skepticism regarding the effectiveness or feasibility of several of these measures (AQIS 1993b; Cohen 1998; Dames & Moore 1998, 1999; Cohen & Foster 2000; Rigby & Taylor 2001b), some could be helpful in meeting stringent standards if vessels had sufficient incentive to implement them. For example...[NOTE: discuss the potential value of avoiding phytoplankton blooms when ballasting in helping to meet standards limiting the concentration of phytoplankton in discharges].

The value of such practices could be evaluated with models using currently available data on organism distributions or by experimental approaches. To the extent that these practices would reduce the uptake of organisms, they are available to vessels to help them meet any discharge standards that might be adopted and therefore, from the perspective of technical limitations on the feasibility of different discharge standards, would allow the adoption of, and vessel

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1 compliance with, more stringent standards than would otherwise be achievable. Thus there are  
2 valid reasons for the US EPA to consider the potential for employing these practices in  
3 combination with ballast treatment to further reduce the risk of releasing exotic organisms in  
4 U.S. waters.  
5

### 6 **C. Reducing Invasion Risk from Ballast Discharges** 7

8 Mid-ocean ballast water exchange has the potential, in combination with the other approaches  
9 discussed here, to further reduce the concentration of exotic organisms (though not necessarily  
10 reduce the concentration of all organisms) in ballast discharges. There is general agreement that  
11 when properly done ballast water exchange can reduce the concentration of initially-loaded  
12 organisms by about an order of magnitude on average (Minton et al. 2005).  
13

14 Invasion risk is positively related to the total number of propagules released in a given time and  
15 place. Thus, risk is positively related to the concentration of propagules times the volume of the  
16 discharge. Even if the concentration of propagules is unmanaged, reducing discharge volumes  
17 will reduce invasion risk in ways that are predictable across taxa (Drake et al. 2005).  
18

19 Technologies and practices to reduce the volume of ballast water discharged in US waters could  
20 include:

- 21 • operational adjustments;
- 22 • systems that allow shifting of ballast water between tanks;
- 23 • larger, wider vessels that require less ballast water per unit of cargo; and
- 24 • the potential development of ballastless vessels.

25 The second and third bullets describe changes in ship design that are already occurring and are  
26 driven by economic factors. Ballast regulations that address not only the concentration of  
27 organisms in ballast discharges but also the volume of ballast water discharged could further  
28 encourage these developments.  
29

30 Generally, the amount of ballast water taken up and discharged by vessels has increased with  
31 increased cargo volumes, but changes in shipping methods, vessel construction, navigational  
32 improvements and possibly vessel operations have had substantial impacts. Among these factors  
33 are the shift to container shipping changing ballasting patterns; the size of the Panama Canal  
34 locks limiting the width of vessels (to just under 106') leading to the construction of narrow  
35 "Panamax" size vessels that piled containers high relative to their width and therefore required  
36 large quantities of ballast to stabilize the vessels; the shift in the world's cargo fleet in recent  
37 decades to a larger proportion of beamier post-Panamax vessels (which cannot fit though the  
38 canal; the first of these was built in 1992 (Tagg 1999)) that carry less ballast water per unit of  
39 cargo; and the development of internal piping and pump systems that allow vessels to adjust  
40 ballast water by pumping it from one part of the vessel to another, rather than discharging it from  
41 one part while loading in another. The current enlargement of the Panama Canal may have  
42 further impacts on the transport of ballast water. There has also been interesting work on

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1 designing ballast-less vessels (e.g. Parsons & Kotinis 2008).  
2

3 Independent of practices of ballast water uptake (previous section) and total volume of a given  
4 discharge, operational adjustments that modify the temporal and spatial patterns of ballast water  
5 discharge may also reduce the probability that discharged propagules will found a self-sustaining  
6 population (Drake et al. 2005). At least for sexually reproducing populations of planktonic  
7 species, for a given concentration of a given species in ballast discharge, the greater the volume  
8 discharged in a given time at a given location, the greater the probability of population  
9 establishment. If a total discharge volume for a given port of call can be broken up in space or  
10 time, invasion risk will be lowered. This is the straightforward outcome of the well known  
11 principles of population biology (CITE) in which the more likely that individuals are to  
12 encounter each other, the more likely the rate of reproduction will overcome the rate of diffusion  
13 of organisms away from each other. Thus, if a given discharge volume can be spread over space  
14 (e.g., has a vessel approaches harbor), be discontinuous in time (with scheduled breaks in  
15 discharge), or be discharged in a mixing environment (to dilute the concentration of propagules),  
16 the risk of invasion will be lowered (Drake et al. 2005).  
17

18 For the same reasons, infrastructure modifications within ports that increase the rate and/or  
19 magnitude of dilution of discharged propagules would also decrease the risk of population  
20 establishment by discharged propagules. If discharges could be made in or piped to locations of  
21 greatest mixing within the harbor (e.g., closer to the tidal channels instead of in partially  
22 enclosed ship slips), then the rate of diffusion would be more likely to overcome the rate of  
23 reproduction. For example, low velocity, low energy propellers, oloid mixers, or other mixing  
24 methods are routinely used in sewage treatment plants (CITE), industrial applications (CITE),  
25 and lakes (CITE from Imberger). Such devices could be used in ports to increase the severity of  
26 Allee effects and other population hurdles faced by newly discharged propagules to minimize the  
27 probability of population establishment.  
28 **VII. Combined Approaches and Voyage-based Risk  
Management**  
29

30 **VII. Combined Approaches and Voyage-based Risk Assessment**

31 **A. Combined Approaches** *[this section is not yet complete]*  
32

33 It may be possible to meet more stringent discharge standards, or otherwise reduce the risk of  
34 invasions from ballast water discharges, by combining the approaches discussed in sections V  
35 (technologies and procedures involved with ballast uptake and discharge) with either shipboard  
36 or onshore treatment.  
37

38 **Remainder of page intentionally left blank**  
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1 **B. Voyage-based Risk Assessment to Prioritize Use of Treatment Technologies,**  
2 **Ballasting and Deballasting Practices, Monitoring Efforts, and Enforcement**  
3

4 Most current ballast water treatment technologies (reviewed by other subgroups), and the current  
5 and proposed policies that motivated them, are built with a one-size-fits-all approach and  
6 designed to be adopted by hundreds of ships at some (possibly distant) future time. There are  
7 defensible reasons for this one-size-fits-all approach, but as we argued in the previous  
8 subsection, additional reasons exist to consider more flexible and combination approaches. This  
9 is especially true in the face of tight budgets and the constant need to prioritize spending on the  
10 most cost effective strategies to reduce invasion risk. Furthermore, invasion risk clearly differs  
11 among ships, voyages, and ports in ways that are predictable, and that could provide a basis for  
12 guiding the deployment of combinations of technologies and practices now and in the future  
13 (Keller et al. 2010). For example, to most cost effectively minimize invasion risk while ballast  
14 water treatment systems are being phased in, the highest risk ships that conduct the highest risk  
15 voyages should be retrofitted first. Likewise, ship-voyage specific risk assessments could guide  
16 the schedules for compliance monitoring of the operation and condition of installed water  
17 treatment systems.

18  
19 The important components of ship-voyage based risk assessments include the frequency and  
20 volume of ballast water discharged (e.g., as indicated by the frequency of visits by ships of  
21 different types and cargoes); the route of voyages by ships with respect to the similarity of  
22 aquatic environments linked by the voyages; and specific information on the occurrence of  
23 invasive species in one or more ports linked by the voyages, where such species data exists  
24 (CITES, Keller et al. 2010). Recent network analyses of global shipping demonstrate that all the  
25 ports on earth are remarkably closely connected by shipping (Keller et al. 2010). However, they  
26 also demonstrate that some ports are much more frequently connected than others and that the  
27 environmental similarity (considering water temperature and salinity) differs markedly among  
28 pairs of closely connected ports. Using data on temperature and salinity estimated and reported  
29 by Keller et al. (2010) for every port globally, any nation or port authority can conduct their own  
30 voyage-specific risk assessments to guide risk management. Thus, such risk assessments and  
31 future refinements of them could be used to more cost effectively prioritize ships for early  
32 adoption of combinations of risk management technologies and practices, and to prioritize ships  
33 for compliance monitoring and enforcement efforts.

34  
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36

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