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Proceedings of Ballast Water Discharge Standards Compliance Subject Matter Expert Workshop

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Proceedings of BWDS Compliance SME Workshop

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16. Abstract (MAXIMUM 200 WORDS) The USCG is charged with developing protocols and regulations for verifying compliance with the proposed ballast water discharge standards, yet it lacks the tools and methods to quickly and reliably determine vessel compliance with either the proposed Phase One or Phase Two standards. Additionally, there is little information on available compliance technologies or the capabilities (testing limitations) of those technologies to verify the performance of ballast water treatment systems. With the multiple variables for ballast water treatment systems and the vessels on which they will be installed, it is essential to understand and assess the costs, risks, and engineering and market constraints to develop feasible treatment compliance verification tools and methods for Phase One and Phase Two ballast water discharge standards. This report documents the proceedings of the Coast Guard's Ballast Water Discharge Standards Compliance workshop held 28-30 June 2011 in New London, CT. The purpose of the workshop was to develop a compliance monitoring framework and to identify tools and methods that might be used for verification of compliance with the standards. Subject matter experts included representation from the various pertinent governmental bodies like the USCG, EPA, Maritime Administration, and Naval Research Laboratory. Other attendees included leaders in the fields of ballast water treatment and invasive species and related disciplines; such as wastewater and drinking water treatment, aquatic toxicology, water quality instrumentation, genetics and molecular biology, and environmental policy and enforcement.					
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EXECUTIVE SUMMARY

This report describes the proceedings of the Ballast Water Discharge Standards (BWDS) Compliance Subject Matter Expert (SME) Workshop held 28 – 30 June 2011 at the American Bureau of Shipping Group (ABSG) Consulting office in New London, CT in support of HSCG32-11-J-200082, Assessment of Approaches for Compliance and Enforcement of Ballast Water Discharge Regulations. In keeping with the Coast Guard's mission, the report provides a preliminary framework and input from SMEs on verifying compliance with proposed regulation and the proper functioning of ballast water treatment systems (BWTS). It is intended to support a range of U.S. Coast Guard (USCG) decisions regarding potential regulatory steps in support of their marine environmental protection mission. It primarily focuses on the indices and measures that can be utilized to determine compliance with BWDSs.

The USCG is charged with developing protocols and regulations for verifying compliance with BWDSs. It has proposed a two-phase approach for implementing regulation of ballast water, with Phase One that includes a set of discharge standards similar to those found in the International Maritime Organization (IMO) Ballast Water Convention and potential future Phase Two standards approximately 1,000 times stricter than the Phase One standards.

Due to a lack of a compliance monitoring framework and verification tools and methods, the USCG is unable to quickly and reliably determine vessel compliance with the Phase One or the proposed Phase Two BWDS. Additionally, there is little information on available technologies or the capabilities (testing limitations) of those technologies to verify the performance of ballast water treatment systems. With the multiple variables for BWTS and the vessels on which they will be installed, it is essential to understand and assess the costs, risks, and engineering and market constraints to develop feasible treatment verification tools and methods for Phase One and Phase Two ballast water discharge standards.

During this effort, a compliance framework was developed and refined with input from research and SMEs. The framework addresses any methods, technologies or concepts that might be employed in compliance monitoring and verification for vessels' discharge to meet the current Phase One ballast water standard or the other potential standards proposed.

The ABSG Consulting team has developed a compliance monitoring framework to identify the requisite methods, instruments and approaches for determining compliance for the various ballast water treatment types. The compliance framework presented includes three levels, which provide increasing levels of confidence:

1. Measures of treatment performance;
2. Indirect measures of exceedance of the discharge standard; and
3. Direct measures of compliance with the discharge standard.

Compliance Level 1 would utilize indirect or indicative system performance and/or water quality measures that demonstrate appropriate treatment operations, or conditions have been met, for various ballast water treatment approaches. While Level 1 measures may not directly establish compliance with numerical discharge standards, they can determine if equipment is operating as certified or per manufacturer's specifications. While these measures can be collected independently by inspectors, the majority should be integrated into the BWTS themselves, and allow for inspectors to review instrument outputs. Some Level 1



parameters might also be measured independently by inspectors in appropriately collected samples of water before or during discharge.

As a relatively quick estimate of whether discharge from a vessel is significantly above required limits, compliance Level 2 would focus on bulk estimates of biomass. Exceedance can be assessed using various biological, chemical, and optical measures currently available or in development that are related to live organism abundances. Some of these measures might only provide indications of gross exceedance, while others may provide finer resolution. These measures can also be collected autonomously or by inspectors. However, the various indirect measure thresholds, at which the discharge from a vessel is considered out of compliance, will take additional consideration and study to establish.

If it is suspected that a vessel discharge will exceed the discharge standards, compliance Level 3 could be used to make direct measurements of the various regulated biological categories. Compliance Level 3 would involve detailed biological testing for live organisms, similar to evaluations currently conducted under treatment system certification testing and provide the highest level of scientific certainty and statistical confidence. While several of the methods and approaches for Level 3 compliance monitoring currently exist and are employed in full scale shipboard testing for type approval, several logistical challenges remain to be resolved prior to implementation for assessment of vessel compliance.

To further refine the compliance monitoring framework, the ABSG Consulting team has organized, convened, and facilitated a 3-day workshop on 28 – 30 June 2011 with SMEs from the field and representation from the various pertinent governmental bodies like the USCG, Environmental Protection Agency (EPA), Maritime Administration (MARAD), and Naval Research Laboratory (NRL) to provide input on the compliance verification framework and other insights on the subject. For each compliance level, the recommendations are based on either the treatment type for compliance Level 1 or by the organism category being measured for compliance Levels 2 and 3.

Through this workshop, the ABSG Consulting team has listed the discharge category and list the measures to be utilized for each level and has captured the method, instrument or analysis necessary. For each instrument, the participants in the workshop indicated the level of deployment readiness for each technology and the signal relevance of each measurement. In addition, the advantages, disadvantages, and requirements for operational utilization were discussed for each level and for each instrument.



TABLE OF CONTENTS

EXECUTIVE SUMMARY	v
LIST OF TABLES	viii
LIST OF FIGURES	viii
LIST OF ACRONYMS AND ABBREVIATIONS	ix
1 BACKGROUND	1
2 SCOPE	2
3 METHODOLOGY	2
3.1 Literature Review.....	2
3.2 Compliance Monitoring Framework.....	3
3.3 SME Workshop.....	3
3.3.1 Technology Readiness Levels.....	6
4 FINDINGS	8
4.1 Compliance Level 1 – Measures of Treatment Performance.....	8
4.1.1 Approved Treatment Components.....	12
4.1.2 Potential Treatment Components.....	15
4.2 Compliance Level 2 – Indirect Measures of Exceedance	16
4.3 Compliance Level 3 – Direct Measures of Compliance	19
5 CONCLUSIONS AND RECOMMENDATIONS.....	22
APPENDIX A. SUBJECT MATTER EXPERT WORKSHOP AGENDA	A-1
APPENDIX B. SUBJECT MATTER EXPERT WORKSHOP ATTENDEES	B-1
APPENDIX C. TECHNOLOGY READINESS LEVELS.....	C-1
APPENDIX D. MEASUREMENTS	D-1
APPENDIX E. INSTRUMENT OR ANALYSIS OF MEASUREMENT.....	E-1
APPENDIX F. SUBJECT MATTER EXPERT WORKSHOP SPEAKERS.....	F-1
APPENDIX G. SUBJECT MATTER EXPERT WORKSHOP PRESENTATIONS.....	G-1



LIST OF TABLES

Table 1. Ballast water discharge standards. 1

Table 2. Department of Defense TRL levels 7

Table 3. Compliance Level 1: Measures of treatment performance. 9

Table 4. Compliance Level 1: Measures of treatment performance (Potential). 10

Table 5. Compliance Level 1: Recommendations. 11

Table 6. Compliance Level 2: Indirect measures of exceedance. 17

Table 7. Compliance Level 2: Recommendations. 18

Table 8. Compliance Level 3: Direct measures of discharge standard. 19

Table 9. Compliance Level 3: Recommendations. 21

Table B-1. Workshop attendees. B-1

Table C-1. Technology readiness level descriptions. C-2

LIST OF FIGURES

Figure C-1. NASA TRL levels. C-1



LIST OF ACRONYMS AND ABBREVIATIONS

Acronym	Definition
ABSG	American Bureau of Shipping Group
ANS	Aquatic Nuisance Species
ATP	Adenosine Triphosphate
BWDS	Ballast Water Discharge Standard
BWTS	Ballast Water Treatment System
CDOM	Chromophoric Dissolved Oxygen Matter
CFR	Code of Federal Regulations
CFU	Colony Forming Unit
CMFDA	5-Chloromethylfluorescein Diacetate
COTR	Contracting Officer's Technical Representative
CTC	5-Cyano-2,3 Ditolyl Tetrazolium Chloride
CWA	Clean Water Act
DNA	Deoxyribonucleic Acid
DO	Dissolved Oxygen
DoD	Department of Defense
DPD	N,N Diethyl-1,4 Phenylenediamine Sulfate
EPA	Environmental Protection Agency
ETV	Environmental Technology Verification
FDA	Fluorescein Diacetate
GFI	Government Furnished Information
GVP	General Vessel Permit
HPLC	High Performance Liquid Chromatography
IMO	International Maritime Organization
LOPC	Laser Optical Plankton Counter
MARAD	Maritime Administration
MSD	Marine Sanitation Device
m-TEC	Membrane Thermotolerant E. coli
NASA	National Aeronautics and Space Administration
NIS	Non-indigenous Species
NRL	Naval Research Laboratory
ORP	Oxidation-Reduction Potential
OWS	Oily Water Separator
PA	Peracetic Acid
PAM	Pulse Amplitude Modulated
qPCR	Quantitative Polymerase Chain Reaction
R&DC, RDC	Coast Guard Research and Development Center
RNA	Ribonucleic Acid
SME	Subject Matter Expert
SOW	Statement of Work
TRL	Technology Readiness Level
TRO	Total Residual Oxidant



LIST OF ACRONYMS AND ABBREVIATIONS (CONTINUED)

Acronym	Definition
U.S.	United States
USCG	United States Coast Guard
UV	Ultraviolet
VGP	Vessel General Permit
VHF	Very High Frequency
VOC	Volatile Organic Compound



1 BACKGROUND

Ballasting is a necessary function of vessels. Vessels take on ballast water for a variety of reasons, including, but not limited to, adjusting trim and reversing the effects of discharged cargo on stability. Vessels also discharge ballast water for similar reasons. Longitudinal and transverse loadings must also be kept balanced to ensure stability. Additionally, there are structural requirements for balanced loading to ensure that bending moments and stresses within do not compromise the strength limitations of the ship. Many ships have been lost as a result of uneven loading. As variations occur in weight differentials on a ship, adjusting the volume of ballast water is necessary to ensure an environmentally sound transport. This ballast water, however, has inherent dangers of its own.

Because ballast water systems vary significantly by ship type and even by ship, only a general description is provided here. In general, ballast water systems are comprised of one or more sea chests, from which one or more pumps draw water, typically via filters. These pumps are connected to tanks used for ballast via one or more manifolds, which allow the direction of flow to and from the designated tanks, or overboard. The same systems are used to both fill and drain tanks as needed, with an overboard discharge separate from the sea chest used for draining the tank. As water from one part of the world is transported to another and discharged, the issue of Non-indigenous species (NIS), sometimes known as aquatic nuisance species (ANS) are brought to United States (U.S.) waters. While not all these species survive relocation, many do—and some thrive. Of these, a number have caused significant ecological and economic damage. It has been estimated that NIS may cost the U.S. over one hundred billion dollars per year.

These environmental consequences have resulted in a range of regulations relating to ballast water discharge. The discharge standards utilized for this study are based on the protocols and regulations for verifying compliance proposed by the United States Coast Guard (USCG). The USCG has proposed a two-phase approach for implementing regulation of ballast water, with the Phase One set of standards similar to standards in the IMO Ballast Water Convention and the Phase Two standards, potentially 1,000 times stricter than the Phase One standards (Table 1). To comply with the standards, vessels have begun installing BWTS to treat ballast water to comply with the accepted organism levels at discharge.

Table 1. Ballast water discharge standards.

	Organisms >50 μm in minimum dimension per m³	Organisms 10-50 μm in minimum dimension per ml	Bacteria per ml	Viruses per ml
IMO D-2	10	10	no limit	no limit
USCG Phase 1	10	10	no limit	no limit
USCG Phase 2	0.01	0.01	10	100

Diverse BWTS are being developed to meet the IMO and USCG discharge standard. Prior to use on vessels, each treatment system must go through extensive laboratory, land-based and shipboard testing. Only after a treatment system has been demonstrated to safely, effectively and reliably meet the discharge standards is it provided with a Type Approval certificate for use in routine vessel operations.



This report addresses the monitoring of vessel discharges to estimate or determine compliance using a three level framework. This compliance framework has been developed and refined, with input from research and workshop SMEs. The framework addressed methods, technologies or concepts that might be employed in compliance monitoring and verification for vessels' discharge to meet Phase One ballast water standards or other proposed standards. Input from SMEs was gathered during the SME workshop held 28 – 30 June, 2011 at the ABSG Consulting office in New London, CT.

2 SCOPE

The goal of this workshop was to gather expert insight and advice on methods, instruments, and approaches that can be used in a three level compliance framework. To achieve these goals, the workshop included:

1. A review of the USCG proposed rule making and the EPA Vessel General Permit (VGP);
2. The current state of BWTS;
3. Current ballast water treatment certification testing methods and approaches;
4. Presentation of a proposed three-level compliance monitoring framework;
5. Aggregation of measurements, methods, instruments and analyses per treatment type or regulated discharge category; and
6. Assessment of each method, instrument or analysis.

3 METHODOLOGY

Our technical approach incorporated literature reviews, SME evaluations, and a report. Highlights included the following:

- Coordinating with the Contracting Officer's Technical Representative (COTR) to obtain Government Furnished Information (GFI);
- Conducting a literature review to develop a list of potential ballast water discharge treatment systems and a list of performance factors and measures, instruments and analyses;
- Developing a compliance verification framework to evaluate technologies and to determine what measures to be utilized for ensuring compliance; and
- SME workshop held 28 – 30 June, 2011 in New London, CT at the ABSG Consulting office to elicit feedback on the framework and input into factors and methods. The workshop agenda is included as Appendix A.

3.1 Literature Review

The initial step in the methodology was to obtain and review all literature available relating to ballast water treatment technologies. We reviewed information provided by the Government and from other sources like the EPA Science Advisory Board, American Bureau of Shipping, the Alliance for Coastal Technologies, and from Lloyd's Register. The literature review produced four outputs that were then used for the evaluation:

- List of current and potential ballast water treatment technologies.
- List of standards or regulations that may apply to vessels concerning the discharge of ballast water.



- Performance evaluation criteria, preliminary measures, instruments, and analyses were identified.
- Preliminary criteria for each of the performance measures selected and inputted into the compliance verification framework to evaluate the identified technologies and compliance.

3.2 Compliance Monitoring Framework

The ABSG Consulting team has developed a compliance monitoring framework to identify the requisite methods, instruments and approaches for determining compliance for the various ballast water treatment types. The compliance framework presented includes three levels, which provide increasing levels of confidence:

1. Measures of treatment performance;
2. Indirect measures of exceedance of the discharge standard; and
3. Direct measures of compliance with the discharge standard.

Compliance level 1 would utilize indirect or indicative system performance and/or water quality measures that demonstrate appropriate treatment operations, or conditions have been met, for various ballast water treatment approaches. While Level 1 measures may not directly establish compliance with numerical discharge standards, they can determine if equipment is operating as certified or per manufacturer's specifications. While these measures can be collected independently by inspectors, the majority should be integrated into the BWTS themselves, and allow for inspectors to review instrument outputs. Some Level 1 parameters might also be measured independently by inspectors in appropriately collected samples of water before or during discharge.

As a relatively quick estimate of whether discharge from a vessel is significantly above required limits, compliance Level 2 would focus on bulk estimates of biomass. Exceedance can be assessed using various biological, chemical, and optical measures, currently available or in development, that are related to live organism abundances. Some of these measures might only provide indications of gross exceedance, while others may provide finer resolution. These measures can also be collected autonomously or by inspectors. However, the various indirect measure thresholds, at which the discharge from a vessel is considered out of compliance, will take additional consideration and study to establish.

If it is suspected that a vessel discharge will exceed the discharge standards, compliance Level 3 could be used to make direct measurements of the various regulated biological categories. Compliance Level 3 would involve detailed biological testing for live organisms, similar to evaluations currently conducted under treatment system certification testing, and provides the highest Level of scientific certainty and statistical confidence. While several of the methods and approaches for Level 3 compliance monitoring currently exist and are employed in full scale shipboard testing for type approval, several logistical challenges remain to be resolved prior to implementation for assessment of vessel compliance.

3.3 SME Workshop

The compliance verification framework was presented to SMEs at the workshop held 28 – 30 June, 2011 in the ABSG Consulting office in New London, CT. SMEs included representation from the various pertinent governmental bodies like the USCG, EPA, Maritime Administration (MARAD), and Naval Research Laboratory (NRL). Other SMEs included leaders in the fields of ballast water treatment and invasive



species and related disciplines; such as wastewater and drinking water treatment, aquatic toxicology, water quality instrumentation, genetics and molecular biology, and environmental policy and enforcement.

The discussion on the first day of the workshop, 28 June 2011, began with an overview of current regulatory practices. A representative from the USCG Environmental Standards Division discussed the current status of the ongoing process towards developing a federal ballast water management program and discharge standards. The current USCG proposed rule for the discharge of ballast water involves two phases. Phase One discharge standards would be the same as those the IMO D-2; and Phase Two would, in essence, be 1,000 times more stringent (Table 1).

The ABSG Consulting team has listed the discharge category and the measures to be utilized for each level and has captured the instrument or analysis necessary. For each instrument, the participants in the workshop indicated the level of readiness for each technology and the signal relevance of each measurement based on the 9-point Technology Readiness Levels (TRL) established by the Department of Defense (DoD).¹ See Appendix C for the complete list and description of each level. In addition; the advantages, disadvantages, and requirements for operational utilization were discussed for each level and for each instrument.

A goal of the workshop was to discuss what realistic approaches, methods, and instruments are available or in development that could measure vessel compliance for the Phase One standard. Additional discussion focused on whether Phase One methodology and tools for measuring compliance would be translatable to the standards established for Phase Two. By discussing the confidence intervals of potential methodology and available measurement tools, USCG will be in a better position to determine how the compliance regime should be structured.

Additional material on the first day of the workshop included a presentation by an EPA Water Permits Division representative on the current and upcoming modifications to the VGP. The presentation provided background on the Clean Water Act, the EPA's VGP program and the relative ballast water obligations. The Clean Water Act permit effluence limits are technology based (driven by best management and best professional judgment practices) and water quality based. The precedence established by EPA was discussed relative to the methodology for establishing compliance and appropriate measurement technology and methodologies.

Representatives from the USCG gave a presentation outlining vessel onboard inspection procedures. Two regulatory case studies were highlighted: oily water separators and marine sanitation devices. The practice of port state control inspection was brought up as a means to remove substandard shipping practices in domestic waters. Applicability of port state control inspections to ensure ballasting compliance with international standards was discussed. Also of interest was the USCG hierarchical approach to compliance characterized by oily water separator shipboard inspection.

A representative from the Maritime Environmental Resource Center, University of Maryland, presented an overview of current BWTS. The fundamental criteria and challenges of treating ballast water, as well as the different categories of BWTS and the current type approved systems, were specified. The main questions that arose during this presentation were: how to monitor compliance for BWTS; the issue of detecting gross exceedance with discharge regulations; and, how to directly quantify the discharge regulations.

¹ Technology Readiness Levels in the Department of Defense, Source: DOD (2006), Defense Acquisition Guidebook.



The EPA Environmental Technology Verification (ETV) Program generic protocols for verifying ballast water treatment technology was presented by a representative from NRL, Key West. The main discussion was centered on the possibility of adapting methods and approaches from the ETV protocols to verify performance of BWTS for compliance monitoring. The ETV protocols were developed with lengthy stakeholder input and have been validated for their intended purpose.

A review of international testing efforts was also given by a representative from the Royal Netherlands Institute for Sea Research. In this review, the following topics were introduced with an international perspective: common standards and uniform implementation; involvement of public and private stakeholders; encouragement of technological innovation; and practical constraints to compliance enforcements.

The first day of workshop presentations concluded with the EPA Office of Wastewater Management discussion of developing, validating, and approving Clean Water Act and other EPA methods. Analytical methods are used by the EPA to establish regulatory priorities and to develop limitations or standards. The USCG may elect to use an analytical method at the same robust level as the EPA to establish ballast water standards and the framework to monitor and determine compliance. USCG may be able to use EPA methods that are currently in place for wastewater or drinking water with some modification for ballast water. It was suggested that work done in the water security field may also be applicable to ballast water.

The closing discussion for the first day introduced three proposed levels of compliance monitoring: measures of treatment performance, indirect measure of gross exceedance, and direct measures of discharge standard. The indirect and direct measures increase in level of confidence in detecting gross exceedance also increase the logistical constraint levels of effort required and cost. Discussions then began to address in detail the availability, requirements, confidence, limitations, and logistical constraints of instrumentation for indirect measures of treatment performance. There was consensus that the first level of compliance monitoring should be the highest priority for the USCG and that most, if not all, measurements of treatment system performance should be made directly by the treatment systems themselves (as part of normal operational diagnostics) with the data made available to inspectors. In many cases the instruments needed for these measures of treatment performance are off-the-shelf technology, but may need some modification and validation for shipboard use and ballast system application.

On the second day of the workshop, June 29th 2011, discussions began by focusing on indirect measures of gross exceedance. Specifically, biological, chemical, optical estimates of live organism abundances, collected autonomously or by inspectors, for detecting vessels in gross-exceedance of the discharge standards. The discussion also covered the availability, requirements, confidence, limitations, logistic constraints of instrumentation for indirect measures of gross exceedance, and discussions of emerging or promising novel approaches/technologies.

The first presentation of that day was given by a representative of Moss Landing Marine Laboratories as examples of a potential new approach to detect gross exceedance using proxies such as fluorescein diacetate (FDA) stains plus fluorometry, adenosine triphosphate (ATP) kits and in situ / in vivo chlorophyll fluorometers. The presentation emphasized that analytical techniques are needed that: (a) show reliable linear responses to living biomass; (b) have adequate sensitivity; (c) rely on simple biochemical protocol; (d) have rapid analysis time; (e) have predictable temperature response; (f) require only simple portable instrumentation; and, (g) require minimal technical training. Discussion that followed on indirect measures of gross exceedance concluded that analytical kits and instruments (e.g., pulse amplitude modulated (PAM)



fluorometers) have the potential to detect gross exceedance, however, they need additional development for this specific application and rigorous validation before it can be universally employed.

The afternoon of day two focused on direct measures of discharge standards. Specifically, on direct measures of live organism abundances in the various regulated categories, collected autonomously or by inspectors. The discussion also covered the availability, requirements, confidence, limitations, logistic constraints of instrumentation for direct measures, and discussions of emerging or promising novel approaches/technologies. While this level of compliance monitoring would be extremely difficult, time-consuming and costly, the SMEs concluded that most of the approaches and methods currently used in land-based testing of BWTS (and described in the ETV protocols) could be adaptable for shipboard compliance monitoring if this level of confidence is required.

On the third day of the workshop, discussions regarding the matrices resumed. The group strived to gain a general consensus on the most promising approaches and required specifications. SMEs generally believed that the TRL approach should be employed to assess the maturation of the various analyses, methods or instruments towards full operational use in ballast water compliance. It was agreed that the DoD TRL definitions and scale of 1 through 9 was beneficial for this workshop. In general, there was consensus that future work and research is needed to establish the ballast water compliance measurement framework. Though, there was general agreement that existing and in-development instruments, methods and analyses can be adapted and validated to meet the needs of USCG enforcement of ballast water regulations.

3.3.1 Technology Readiness Levels

TRLs together make up a systematic metric, or measurement, system that supports assessments of the maturity of new technologies (materials, components, devices, etc.). TRLs are also used for the consistent comparison of maturity between different types of emerging technology. In general when new technology is invented or conceptualized, it would not fit basic safety regulations to apply it immediately to its planned application. Usually new technology is subjected to experimentation, refinement, and increasingly realistic testing. Once the technology has past enough tests and is proven safe, reliable, and functions by itself in its planned application, it can be incorporated into a system or subsystem.

Each TRL represents a specific timeframe within the overall development process of new technology depicted in Table 2. For the purpose of this workshop, we have taken these TRLs and measured the readiness of the technologies that may be utilized to measure and verify compliance with the discharge standard implemented. The workshop attendees agreed to the understanding that TRL 1 is the initial stage of idea conception while TRL 9 is the stage where technologies were market ready and available. Many of the technologies were market ready, but have not been utilized in a ballast water system context, thus were deemed to be at a TRL 5 or 6. Technologies proven to operate in a ballast water system context were given a TRL of 7 or higher.



Table 2. Department of Defense TRL levels²

TRL	Description
1. Basic principles observed and reported	Lowest level of technology readiness. Scientific research begins to be translated into applied research and development. Example might include paper studies of a technology's basic properties.
2. Technology concept and/or application formulated	Invention begins. Once basic principles are observed, practical applications can be invented. The application is speculative and there is no proof or detailed analysis to support the assumption. Examples are still limited to paper studies.
3. Analytical and experimental critical function and/or characteristic proof of concept	Active research and development is initiated. This includes analytical studies and laboratory studies to physically validate analytical predictions of separate elements of the technology. Examples include components that are not yet integrated or representative.
4. Component and/or breadboard validation in laboratory environment	Basic technological components are integrated to establish that the pieces will work together. This is relatively "low fidelity" compared to the eventual system. Examples include integration of 'ad hoc' hardware in a laboratory.
5. Component and/or breadboard validation in relevant environment	Fidelity of breadboard technology increases significantly. The basic technological components are integrated with reasonably realistic supporting elements so that the technology can be tested in a simulated environment. Examples include 'high fidelity' laboratory integration of components.
6. System/subsystem model or prototype demonstration in a relevant environment	Representative model or prototype system, which is well beyond the breadboard tested for TRL 5, is tested in a relevant environment. Represents a major step up in a technology's demonstrated readiness. Examples include testing a prototype in a high fidelity laboratory environment or in simulated operational environment.
7. System prototype demonstration in an operational environment	Prototype near or at planned operational system. Represents a major step up from TRL 6, requiring the demonstration of an actual system prototype in an operational environment, such as in an aircraft, vehicle or space. Examples include testing the prototype in a test bed aircraft.
8. Actual system completed and 'flight qualified' through test and demonstration	Technology has been proven to work in its final form and under expected conditions. In almost all cases, this TRL represents the end of true system development. Examples include developmental test and evaluation of the system in its intended weapon system to determine if it meets design specifications.
9. Actual system 'flight proven' through successful mission operations	Actual application of the technology in its final form and under mission conditions, such as those encountered in operational test and evaluation. In almost all cases, this is the end of the last "bug fixing" aspects of true system development. Examples include using the system under operational mission conditions.

² DOD (2006), *Defense Acquisition Guidebook*



4 FINDINGS

Chapter 4 presents a high-level summary of proceedings and analysis of the information gathered at the SME Workshop. It includes a thorough compilation of the technologies and the measurements, instruments or analyses and their associated TRL score to ensure compliance with the BWDS.

4.1 Compliance Level 1 – Measures of Treatment Performance

Compliance Level 1 would utilize indirect or indicative system performance and/or water quality measures that demonstrate appropriate treatment operations or conditions have been met for various ballast water treatment approaches. While Level 1 measures may not directly establish compliance with numerical discharge standards, they can determine if equipment is operating as certified or per manufacturer's specifications. While these measures can be collected independently by inspectors, the majority should be integrated into the BWTS themselves and allow for inspectors to review instrument outputs. Some Level 1 parameters might also be measured independently by inspectors in appropriately collected samples of water before or during discharge.

Most BWTS utilize different and multiple combination of methods to treat ballast water to achieve compliance with the BWDS. Instead of addressing each BWTS and their resulting combinations, this report breaks down the treatment systems into the various treatment components. Most BWTS utilizes one or a combination of the treatment components found in Table 3 below. For Compliance Level 1, the measurements of each component are measured, instead of the different combinations of each BWTS.

In Table 3 below, ballast water treatment components are listed, followed by the indicative measurement to show the proper functioning/operations of the equipment, and then what parameter(s) would be directly measured. The treatment components in Table 3 are methods currently utilized in BWTS that has received Type Approval Certification from an Administration (in accordance with the IMO ballast water convention).

- Column A – Treatment Component: individual process to remove, kill or inactivate organisms in ballast water.
- Column B – Indicative Parameter: the quantification/determination of mechanical, physical or chemical parameters.
- Column C – Instrument or Method: Observation, instrument, sensor, sample, analytical kit, etc. used to make a measurement.
- Column D – Estimated TRL for this specific application.



Table 3. Compliance Level 1: Measures of treatment performance.

Treatment Component	Indicative Parameter	Instrument or Method	TRL
Cavitation	Acoustic	Passive acoustic sensor or acoustic interferometry	7
	Pressure	Pressure sensors (before/after)	9
Coagulation (flocculent)	Dose of coagulant used	System diagnostics	9
	Turbidity	Turbidity sensor	8
Deoxygenation	pH (if CO ₂ is used)	pH sensor	7
	Oxygen sensor	Dissolved Oxygen (DO) sensor	8
Electrochlorination (NaOCl)	Total Residual Oxidant (TRO)	Amperometric sensor	8
	Oxidation-Reduction Potential (ORP)	ORP sensors	8
	Chlorine	Inline N,N Diethyl-1,4 Phenylenediamine Sulfate (DPD) kits, automated	8
	Power supply, voltage, current	System power diagnostics	9
	Conductivity/Salinity	Conductivity and temperature sensor	9
	Chromophoric Dissolved Organic Matter (CDOM) (before /after)	Fluorometer	6
Filters	Water clarity	Sight glass, water sample, turbidity sensor	8
	Flow rate/Pressure	Pressure sensors (before/after)	9
	Back flush frequency	System diagnostics	9
Hydrocyclone	Water clarity of treated water	Sight glass, water sample, turbidity sensor	8
	Filter flush rate	System diagnostics	9
	Power consumption	System diagnostics of voltage and current	9
	Water clarity of waste stream	Sight glass, water sample, turbidity sensor	8
Ozone	TRO	Amperometric sensor	8
	ORP	ORP sensors	8
	Power consumption	System diagnostics of voltage and current	9
	Conductivity/Salinity	Conductivity and temperature sensor	9
	CDOM (before /after)	Fluorometer	6
Peracetic acid	Hydrogen peroxide	Chemical-specific sensor	7
	Peracetic acid	Chemical-specific sensor	7
	pH	pH sensor	7
	CDOM (before /after)	Fluorometer	6
UV, UV+TiO ₂	Power	System diagnostics of voltage and current	9
	Lamp life	System maintenance	9
	Ultraviolet (UV) Output sensor	UV sensor	8
	Rate of flow	Flow meter	9
	CDOM (before /after)	Fluorometer	6

Table 4 lists other proposed treatment components, which are in development.

Table 4. Compliance Level 1: Measures of treatment performance (Potential).

Treatment Component	Indicative Parameter	Instrument or Method	TRL
Alkylamines	pH	pH sensor	7
	Alkylamines	Chemical-specific kit	7
Chlorine dioxide	Chlorine dioxide	In-line chlorine dioxide kit or polarographic sensor	8
Electric pulse	Power consumption	System diagnostics of voltage and current	9
Heat	Temperature	Thermistor	9
Menadione	Menadione	Sample analysis with HPLC	7
Plasma pulse	Power consumption	System diagnostics of voltage and current	9
	Heat	Thermistor	9
Shear	Acoustic	Passive acoustic sensor or acoustic interferometry	7
	Pressure	Pressure sensors (before/after)	9
Ultrasound	Power consumption	System diagnostics of voltage and current	9
	Acoustic sensing	Passive acoustic sensor or acoustic interferometry	7

For each of these technologies, the indicators for each of the treatment types can be incorporated into the BWTS and/or be an inline process for validation and verification of functionality as per manufacturer's specifications. If a BWTS has multiple components, each component would optimally utilize a measure to ensure functionality.

Based on the lists developed in Tables 3 and 4, the SME generated Table 5 that considers the usefulness of the various measurements in compliance monitoring of ballast water discharge and the time required until it has reached true operational readiness for this specific application (i.e., TRL 9). The results of Table 5 can be used to prioritize approaches to compliance Level 1.

Column A – Treatment Type, individual process to remove, kill or inactivate organisms in ballast water.

Column B – Measurement, the quantification/determination of mechanical, physical or chemical parameters.

Column C – BWTS internal (should be replaced with “Instrument or Analysis”), observation, instrument, sensor, sample, analytical kit, etc. used to make a measurement.

Column D – Signal Relevance, describes how pertinent, connected, or applicable the measurement is in determining BWTS effective operations or quantifying fundamental changes to water quality.

Column E - Compliance Operational Readiness, estimates of time required for the Instrument or Analysis to be operational (including specific shipboard ballast water Standard Operating Procedures), validated, and implemented.



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Table 5. Compliance Level 1: Recommendations.

Treatment Type	Measurement	BWTS internal	Signal Relevance	Compliance Operational Readiness Immediate (< 1 yr), medium (2-3 yr), long (> 4 yr)
Cavitation and Shear	Acoustic	Acoustic sensors	medium	medium
	Pressure	Pressure sensors (before/after)	high	Immediate
Coagulation (flocculent)	Sample of water for dose analysis		low	medium
Deoxygenation (inert gas)	Dose of inert gas	treatment monitoring	high	immediate
	pH	pH sensor	high	immediate/medium
	Oxygen sensor	DO sensor	high	immediate/medium
Deoxygenation (nitrogen)	Dose of nitrogen gas	Treatment monitoring	high	immediate/medium
	DO	DO sensor	high	immediate/medium
Electrochlorination (NaOCl)	TRO	Amperometric sensors	medium	immediate/medium
	ORP	ORP sensors	low	immediate/medium
	DPD	Inline DPD kits, automated	high	immediate/medium
	Voltage and current	Power monitoring diagnostics	high	immediate
	Conductivity/salinity	Conductivity, temperature sensors	high	immediate
	CDOM (before /after)	CDOM fluorometers	low	long
Filters	Visual inspection	pipe sight glass or water sample	low	immediate
	Flow rate	Flow meters	medium	immediate
	Pressure	Pressure	high	immediate
	Back flush frequency	treatment monitoring	high	immediate
	Optical clarity of water	turbidity sensors/transmissometers	low	medium
Hydrocyclone	Optical clarity of water	turbidity sensors/transmissometers	low	medium
	Back flush frequency	treatment monitoring	high	immediate
	Voltage and current	Power monitoring diagnostics	high	immediate
	Turbidity of waste stream	turbidity sensor	low	medium
Ozone	TRO	amperometric sensors	medium	immediate/medium
	ORP	ORP sensors	low	immediate/medium
	DPD	Inline DPD kits, automated	high	immediate/medium
	Voltage and current	Power monitoring diagnostics	high	immediate
	Conductivity/salinity	conductivity+temperature sensors	high	immediate
	CDOM (before /after)	CDOM fluorometers	low	long



Table 5. Compliance Level 1: Recommendations (Continued).

Treatment Type	Measurement	BWTS internal	Signal Relevance	Compliance Operational Readiness Immediate (< 1 yr), medium (2-3 yr), long (> 4 yr)
Peracetic acid	TRO	amperometric sensors	medium	medium
	ORP	ORP sensors	medium	medium
	hydrogen peroxide, peracetic acid	peracetic acid sensors	high	medium
	Dose of peracetic acid	treatment monitoring	high	immediate
	pH	pH	medium	immediate/medium
	CDOM (before /after)	CDOM fluorometers	low	long
UV and UV+TiO2	Voltage and current	Power monitoring diagnostics	high	immediate
	Lamp life	treatment monitoring	high	immediate
	UV dose	UV sensor	high	immediate
	Rate of flow	Flow meters	high	immediate
	CDOM (before /after)	CDOM fluorometers	low	long
Alkylamines	pH	pH sensor	medium	medium
	Alkylamines	Alkylamines specific kits	high	?
Chlorine dioxide	Chlorine dioxide	Chlorine dioxide kits	high	medium
Deoxygenation (vacuum)	DO	DO sensor	high	immediate/medium
Electric/Plasma pulse	Voltage and current	Power monitoring diagnostics	high	immediate
Heat	Temperature	Thermistors	high	immediate
Menadione	Menadione	Lab High Performance Liquid Chromatography (HPLC)	high	medium
Ultrasound	Voltage and current	Power monitoring diagnostics	high	immediate
	Acoustic signals	Acoustic sensors	high	immediate

4.1.1 Approved Treatment Components

The following are short summaries of the treatment components outlined in Table 3. These technologies currently are found in BWTS that have received Type Approval Certification from one or more International Administrations in accordance with the IMO ballast water convention. These indicators or measurements are: direct, indirect, or indicative systems operations or water quality measures; collected autonomously and integrated into fundamental BWTS diagnostics; and demonstrates effective and reliable system operations and/or appropriate treatment conditions have been met.

4.1.1.1 Cavitation

Cavitation is the creation of cavities, or microbubbles, near lower pressure regions in a liquid caused by rapid changes in pressure, typically by subjecting the water to high velocities, followed by the subsequent implosion following the sudden decrease in pressure. Equipment such as high velocity propellers, venture



pipes, or slit plates can generate cavitation bubbles. The creation and rupturing of these cavitation bubbles create hydrodynamic forces and ultrasonic oscillations that can collapse and implode organisms in the water.

Two approaches can be used to quantify a cavitation component of treatment systems, Acoustic sensors may be calibrated to estimate cavitation intensity and pressure sensor before and after the cavitation device could provide indication of the functionality of the system. The instrumentation for these pressure measurements can be incorporated into the overall system design. They are generally currently available, cost effective, in-use on ships, but may require some minimal additional development and validation.

4.1.1.1 Coagulation/flocculation

Coagulation is the utilization of a flocculent to combine with colloids present in the water to come out of suspension. Flocculants aggregate suspended particles to form flocs, increasing the sedimentation or the filterability of small particles. Many flocculants are multivalent cations; such as aluminum, iron, calcium, or magnesium. Flocculants are also manufactured by creating long-chain polymer flocculants like modified polyacrylamides.

Two indicators can be used to indicate treatment performance: dose of coagulant used and water clarity. A dosage meter and a turbidity sensor can be incorporated into the overall treatment system design.

4.1.1.2 Deoxygenation

Deoxygenation via inert gas is the method by which dissolved oxygen is replaced with inactive gases like nitrogen and CO₂. Removing oxygen creates lethal hypoxic conditions; however, typically requires holding times of three or four days for the reduction of organisms to reach discharge standard levels.

To quantify changes produced by deoxygenation systems would simply require the use of DO sensors. DO sensors are currently available, widely used in water quality and oceanography measurements, and may require only minimal additional development and validation. If the deoxygenation systems utilize CO₂ as one of the inert gases, pH sensors can also estimate treatment effectiveness. Similarly pH sensors are currently available, widely used in water quality and oceanography measurements, but will likely require significant additional development and validation.

4.1.1.3 Electrochlorination (NaOCl)

Electrochlorination, or electrolysis, utilizes an electrical current through ballast water in an electrolytic chamber in a manner that creates free chlorine, sodium hypochlorite, hydroxyl radicals, and potentially other unwanted residual compounds. This process is limited to brackish and seawater with salinity above approximately 10 psu but the sodium hypochlorite level produced can effectively kill most aquatic organisms. Depending on initial concentrations of sodium hypochlorite produced, organic content of treated water and holding time, neutralization of treated water prior to discharge may also be required.

For systems that utilize electrochlorination, the following indicators can be used to measure system functionality: TRO, ORP, chlorine levels, power use (voltage and current), water conductivity/salinity, and CDOM. TRO, measured by an amperometric sensor, ORP, measured by an ORP sensor, and chlorine, measured by an inline DPD kit. These approaches are in wide use but likely require some additional development and validation. CDOM can indicate the level of remaining organic matter, which may be utilized in a feedback mechanism.



4.1.1.4 *Filters*

Filters capture and physically remove organisms and particles. The size of the particle captured is determined by the size of the interstitial space of the filter. Filters often utilize a back-flushing cycle to remove and discard captured particles. Filters are often utilized as the first stage of ballast water treatment, typically upon ballast water intake. Filters often create pressure gradients and reduce the flow rate accordingly.

To quantify operations during ballasting, pressure sensors before and after the filter systems can quantify pressure differentials. Back flushing rate can also be logged by the treatment systems and provide indications that filters are operating appropriately. Finally, a variety of water clarity estimates or measures (from a simply sight glass to a turbidity sensor) could provide qualitative estimate of filter effectiveness.

4.1.1.5 *Hydrocyclone (Cyclonic Separation)*

Cyclonic separation utilizes centrifugal forces to separate particles from the water. Particles with a specific gravity greater than water will be separated and removed from the water. Particles and organisms with similar density to water are not easily removed.

Like filters, the operations of hydrocyclones can be examined using back flushing rate logged by the treatment systems water clarity estimates or measures.

4.1.1.6 *Ozone*

Ozone is an oxidizing biocide that oxidizes on contact with an organism. Ozone will also interact with chemicals present in sea water to create bromine compounds, creating additional biocides. Ozone generators utilize ambient air through an electric discharge field to create ozone that is injected into ballast water.

Because ozone is also an oxidizing chemical treatment, several of the approaches used for electrochlorination can be used to quantify ozone treatment performance, including TRO sensors (also called ozone sensors), ORP sensor, power use (voltage and current), water conductivity/salinity, and CDOM. CDOM can indicate the level of remaining organic matter, which may be utilized in a feedback mechanism.

4.1.1.7 *Peracetic Acid*

Peracetic acid is an organic compound ($\text{CH}_3\text{CO}_3\text{H}$) that acts as an oxidizing agent. Peracetic acid can be created in multiple methods, but sold in solution to maintain stability of the chemical. The cell membrane of organisms is disrupted by the hydroxyl radical ($\text{HO}\cdot$).

Sensors and inline monitoring systems (membrane and polarographic sensor) are available to quantify concentrations of peracetic acid and while in wide use would likely require some additional development and validation for this specific application.

4.1.1.8 *Ultraviolet (UV) Light and UV+TiO₂*

Ultraviolet radiation changes and breaks down cellular units like the cell membrane or genetic material and causes cell function failure, which may result in cell death or the disruption of cell reproduction. The utilization of titanium dioxide (TiO_2) as a catalyst increases the oxidation rate by creating hydroxyl radicals and oxide ions. Efficacy is dependent upon variables like the distance from the light source, turbidity of the water, time requirement of treatment and power utilization of the UV light source.



UV treatment performance can be monitored by UV light sensors placed in the treatment chamber and by quantifying system power use. Additionally, routine bulb replacements can be utilized to ensure performance as per manufacturer specifications.

4.1.2 Potential Treatment Components

In this section, the treatment components listed are those that are utilized in a BWTS but have not yet received Type Approval Certification from an Administration (in accordance with the IMO ballast water convention).

4.1.2.1 Alkylamines

An Alkylamine is a compound that has an alkyl group bonded to the nitrogen of an amine. An amine is an organic compound with a nitrogen atom with a lone electron pair. While amines are typically considered a base, alkylamines act as an acid, thus disinfecting ballast water as an oxidizing agent.

Alkylamines can be quantified directly by chemical chromogenic sensors but these are young technologies and will likely require further development and validation for this application. BWTS utilizing alkylamines will also alter pH of treated water so pH sensors may also provide indications of system performance.

4.1.2.1 Chlorine Dioxide

Chlorine dioxide is a strong chemical biocide; and for ballast water treatment is produced by combining sulfuric acid, sodium chlorate and hydrogen peroxide onboard the vessel.

Because chlorine dioxide is also an oxidizing chemical treatment, several of the approaches used for electrochlorination and ozone can be used to quantify ozone treatment performance, including TRO sensors, ORP sensor. CDOM can indicate the level of remaining organic matter, which may be utilized in a feedback mechanism.

4.1.2.2 Electric Pulse

The application of pulsed electric fields, which transmits energy directly into the water, can cause degradation or decomposition of latent organic materials. Exact mechanisms of action vary greatly, dependant on the wide range of power utilized by different pulses systems.

Operational efficiency can be indicated by active monitoring of installed voltmeters connected to the ballast as well as regular power consumption monitoring.

4.1.2.3 Heat

The utilization of increased temperatures in the ballast water over an extended period of time can result in stressing organisms beyond operational levels or denaturing necessary proteins, thus resulting in organism death. Many proposed heating systems utilize the waste heat from the ship's engines, cooling system, or from other heat exchanges. With the heat from these vessel systems, the ballast water may not be sufficiently heated to a temperature high enough to cause death in all organisms, particularly certain types of bacteria. The monitoring of heat BWTS is fairly straight forward, through the use of thermistors.



4.1.2.4 *Menadione (Vitamin K₃)*

Menadione is a synthetic compound that creates a toxic environment for organisms, inhibiting growth of organisms and causes oxidative damage and chloroplast disruption. To measure treatment operations, doses of menadione can be monitored and samples can be collected for laboratory analysis.

4.1.2.5 *Plasma Pulse*

Similar to electric pulse technology, plasma pulse applies short burst of electrical energy to entering water in order to degrade or decompose organic materials. Counter to standard electrical pulse, plasma pulse dramatically increases applied energy density by narrowing the gap between electrodes, causing an intensified electrical arc, simultaneously requiring less treatment duration per effective result.

Due to the higher exothermal release from a pulse resulting in an arc, successful operation could be monitored by temperature differential across the plasma pulse zone of activity, as well as installed ballast voltmeters and general ship power consumption. A functional issue is the amount of energy required to create a plasma pulse onboard.

4.1.2.6 *Shear*

BWTS that may utilize shear uses shear stress to cause organism death. The proposed techniques utilize high levels of pressure changes to stress organisms, due to the rapid change in pressure resulting in the rupture of the cell membrane.

Like cavitation, two approaches can be used to quantify a shear. Acoustic sensors may be calibrated to estimate shear intensity and pressure sensor before and after the cavitation device could provide indication of the functionality of the system.

4.1.2.7 *Ultrasound*

Like cavitation, ultrasound treatment creates microbubbles to rupture cell membranes that result in organism death. The microbubbles are created via high frequency vibration. The efficacy of ultrasound varies based on the intensity of vibration and length of exposure. To measure functionality, acoustic sensing can measure the output of an ultrasound system and operational efficiency can be monitored by power consumption monitoring.

4.2 Compliance Level 2 – Indirect Measures of Exceedance

As a relatively quick estimate of whether discharge from a vessel is significantly above required limits, compliance Level 2 would focus on bulk estimates of biomass. Exceedance can be assessed using various biological, chemical, and optical measures, currently available or in development, that are related to live organism abundances. Some of these measures might only provide indications of gross exceedance, while others may provide finer resolution. These measures can also be collected autonomously or by inspectors. However, the various indirect measure thresholds, at which the discharge from a vessel is considered out of compliance, will take additional consideration and study to establish.

Table 6 provides a high level summary of: discharge standard categories and associated estimate and indicators; various measurements for the different categories; instruments and analyses that can be employed as measures of exceedance; and their estimated TRL. TRL levels also consider sample collection and processing as part of the analysis. See Appendix D and E for further descriptions of instrument and analyses.



Table 6. Compliance Level 2: Indirect measures of exceedance.

Discharge Standard Category	Measurement	Instrument or Analysis	TRL
Biomass Estimates	Enzymatic activity	FDA + fluorometer kit	5
	ATP	Luminometer reagent kit	6
	Chlorophyll fluorescence	Chlorophyll fluorometer	8
	Variable fluorescence	PAM fluorometer	8
	DNA/RNA	Lab analysis	4
	Respiration rate	Biochemical Oxygen Demand/DO sensor Electron Transport Systems	4
Live/Dead Indicators	Membrane potential	3,3'-dihexyloxacarbocyanine (DiOC6(3))	5
	Variable fluorescence	PAM fluorometer	8
	Dead stain	Green nucleic acid stain	8
	Live stain	FDA/CMFDA	8
	General stain	Neutral red	8
	Movement	Visual inspection	8
	Movement	Video	7
	Metabolic activity, enzyme	CTC	4
	Swimming organisms in sample	Visual inspection	8
	Direct count	Flow cytometers (imaging)	8
Live organisms > 50 µm	Direct count	Coulter counter	8
	Direct count	Laser Optical Plankton Counter	7
	Variable fluorescence	PAM fluorometer	8
	Chlorophyll fluorescence	Chlorophyll fluorometer	8
Live organisms 10 - 50 µm			
Total Live Bacteria	CFU	Plate counts	9
<i>E. coli</i>	CFU	Selective substrate and most probable number	9
<i>Enterococci</i>	CFU	Selective substrate and most probable number	9
<i>V. cholerae</i> (toxigenic)	CFU	Colorimetric immunoassay kit	8

Based on the lists developed in Table 6, the SME generated Table 7 that considers the usefulness of the various measurements in compliance monitoring of ballast water discharge and the time required until it has reached true operational readiness for this specific application (i.e., TRL 9). The results of Table 7 can be used to prioritize approaches to compliance Level 2.

Column A – Discharge Standard Category

- Biomass estimates are quantifications of biological material from living organisms, which are in turn correlated to numbers of planktonic organisms for estimates of abundance.
- Live/dead indicators are instruments, assays, stains, and analyses that distinguish between live and dead organisms.

Column B – Measurement, the quantification/determination of physical, chemical or biological parameters as an estimate of organism abundances.

Column C – Instrument of Analysis, observation, instrument, sensor, sample, analytical kit, etc. used to measure or estimate organism abundances.

Column D – Signal Relevance, describes how pertinent, connected, or applicable the measurement is in determining live organism abundances.



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Column E - Compliance Operational Readiness, estimate of time required for the Instrument or Analysis to be operational (including specific shipboard ballast water Standard Operating Procedures), validated, and implemented.

Table 7. Compliance Level 2: Recommendations.

Discharge Standard Category	Measurement	Instrument or Analysis	Signal Relevance	Compliance Operational Readiness Immediate (< 1 yr), medium (2-3 yr), long (> 4yr)
Biomass estimates	Enzymatic activity	FDA fluorometer kit	high	long
	ATP	Luminometer reagent kit	high	medium
	Chlorophyll fluorescence	Chlorophyll fluorometer	medium (specific to subset)	medium
	Variable fluorescence	PAM fluorometer	high (specific to subset)	immediate/medium
	DNA/RNA	Lab analysis	medium	long
	Respiration rate	Biochemical Oxygen Demand/DO sensor Electron Transport Systems	medium	long
	Membrane potential	3,3'-dihexyloxacarbocyanine (DiOC6(3))	low	long
Live/dead indicators	Variable fluorescence	PAM fluorometer	high (specific to subset)	immediate/medium
	Dead stain	Green nucleic acid stain (Sytox)	low	medium
	Live stain	FDA/CMFDA	high (specific to subset)	immediate/medium
	General stain	Neutral red	medium	immediate/medium
	Movement	Visual	high	immediate/medium
	Movement	Video	high	medium
	Metabolic activity, enzyme	CTC	medium	long
Live organisms > 50 µm	Swimming organisms in small volume	Visual	high	immediate/medium
	Direct count	flow cytometers (imaging)	high (with live stain)	long
	Direct count	Coulter counter	low	medium
	Direct count	Laser Optical Plankton Counter	low	long
Live organisms 10 - 50 µm	Variable fluorescence	PAM fluorometer	high (specific to subset)	immediate/medium
	Chlorophyll fluorescence	Chlorophyll fluorometer	medium (specific to subset)	medium
Total live bacteria	CFU	Plate counts	medium	immediate/medium
<i>E. coli</i>	CFU	Selective substrate most probable number and other EPA methods	high	immediate
<i>Enterococci</i>	CFU	Selective substrate most probable number and other EPA methods	high	immediate
<i>V. cholerae</i> (toxigenic)	CFU	Colorimetric immunoassay kit	high	medium
Viruses Virus-like particles	Direct count	Electron microscopy	high* (phase II)	



4.3 Compliance Level 3 – Direct Measures of Compliance

If it is suspected that a vessel discharge will exceed the discharge standards, compliance Level 3 could be used to make direct measurements of the various regulated biological categories. Compliance Level 3 would involve detailed biological testing for live organisms, similar to evaluations currently conducted under treatment system certification testing, and provide the highest level of scientific certainty and statistical confidence. While several of the methods and approaches for Level 3 compliance monitoring currently exist and are employed in full scale shipboard testing for type approval, several logistic challenges remain to be resolved prior to implementation for assessment of vessel compliance.

Table 8 provides a high level summary of: discharge standard categories; various measurement for the different categories; instruments and analyses that can be employed as measures of compliance; and their estimated TRL. TRL levels also consider sample collection and processing as part of the analysis. See Appendix D and E for further descriptions of instrument and analyses.

Table 8. Compliance Level 3: Direct measures of discharge standard.

Discharge Standard Category	Measurement approach	Instrument or Analysis	TRL
Live organisms > 50 µm	Visual count	Microscope	8
		Automated analysis	5
		Stain (neutral red)	7
Live organisms 10 - 50 µm	PAM fluorometer		8
	FDA and/or Sytox plus chlorophyll autofluorescence	Flow cytometer	8
	FDA/CMFDA and/or Sytox	Epifluorescent Microscope	8
	Rose Bengal (heterotrophs)	Microscope	8
Total live bacteria	Plate counts	CFU	8
	Stains	Epifluorescent Microscope	8
	Stains	Flow cytometry	8
<i>E. coli</i>	CFU	Selective substrate and most probable number	8
	mTEC		6
	qPCR		6
<i>Enterococci</i>	Defined media, Most Probable Number		8
	qPCR		6
<i>V. cholerae</i> (toxigenic)	CFU	Colorimetric immunoassay kit	8
	Direct count	Fluorescent antibodies	7
Viruses and Virus-like Particles	Nucleic acid Stains	Epifluorescent microscope	7
	Nucleic acid Stains	Electron microscopy	7
	Nucleic acid stains	Flow cytometry	8

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Based on the lists developed in Table 8, the SME generated Table 9 that considers the usefulness of the various measurements in compliance monitoring of ballast water discharge and the time require until it has reached true operational readiness for this specific application (i.e., TRL 9). The results of Table 9 can be used to prioritize approaches to compliance Level 2.

Several of these analyses are likely going to be conducted in a laboratory setting. Issues with how to sample, who will sample, standardization, laboratory verification, and procedural issues of testing and sampling are issues that were raised during the workshop, however would need to be addressed in a different venue.

Column A – Discharge Standard Category

Column B – Measurement, the quantification/determination of physical, chemical or biological parameters as a direct indication of organism abundances.

Column C – Instrument of Analysis, observation, instrument, sensor, sample, analytical kit, etc. used to measure organism abundances.

Column D – Signal Relevance, describes how pertinent, connected, or applicable the measurement is in determining live organism abundances.

Column E - Compliance Operational Readiness, estimates of time required for the Instrument or Analysis to be operational (including specific shipboard ballast water Standard Operating Procedures), validated, and implemented.



Table 9. Compliance Level 3: Recommendations.

Discharge Standard Category	Measurement	Instrument or Analysis	Signal Relevance	Compliance Operational Readiness
Live organisms > 50 µm	Direct count and movement	Microscope	high	immediate
	Direct count and movement	Automated video analysis	high	medium
	Direct count	Live stain and flow cytometry	high	medium
Live organisms 10 - 50 µm	Variable fluorescence	PAM fluorometer	low	medium
	Live stain direct count	FDA/CMFDA epifluorescent microscopy or flow cytometry	high	medium
	Live and dead stain direct count	Green nucleic acid stain + FDA/CMFDA epifluorescent microscopy or flow cytometry	high	medium
Total live bacteria	CFU	Plate counts	low	immediate
	live stains	Epifluorescent Microscope	high	immediate
	live stains	Flow cytometry	high	immediate
<i>E. coli</i>	CFU	Selective substrate most probable number kit	high	immediate
	CFU	m-TEC	high	immediate
	Direct count	Other EPA-approved methods	high	immediate
	Abundance estimates	qPCR	high	long
<i>Enterococci</i>	Selective substrate most probable number	Selective substrate most probable number kit	high	immediate
	CFU	Other EPA-approved methods	high	immediate
	Abundance estimates	qPCR	high	long
<i>V. cholerae</i> (toxigenic)	Direct counts	Colorimetric immunoassay kit	high	immediate
	Direct counts	Lab fluorescent antibodies	high	immediate
Viruses Virus-like Particles	Direct count	Nucleic acid stains and epifluorescence microscope		
	Direct count	Electron microscopy		
	Nucleic acid stains	Nucleic acid stains and flow cytometry		



5 CONCLUSIONS AND RECOMMENDATIONS

At the conclusion of the workshop, the SMEs proposed a host of specific recommendations to the USCG for a ballast water discharge compliance and enforcement framework. The chief recommendation was that treatment performance should be built into BWTS. This recommendation is tied directly to the approaches outlined in Level 1 compliance monitoring measures. By building treatment performance into the actual ballast water systems, readily available technology can be leveraged quickly, and comparatively cost effectively. Any data collected by the installed performance measure can then be provided to the USCG for verification. It was clear to the SMEs that of the three levels of compliance monitoring, treatment performance measures (Level 1) were considered the highest priority for USCG to take action on.

However, the SMEs did propose additional recommendations that were given lower priority than the Level 1 compliance monitoring measures. One of the lower priority recommendations was for further development and validation of bulk biomass estimates. In particular, FDA stains with hand-held fluorometers, commercial ATP kits, and in situ PAM fluorometry were approaches and technology earmarked as requiring further consideration through research, development and testing. Another lower priority recommendation was for increased investments in transitioning/maturation activities for those Level 1 and Level 2 indicator instruments with TRL rankings of 6 – 8. The goal of these investments was to bring those indicator instruments from a TRL 6-8 to a TRL 9 where they are validated and truly operational for this specific application. TRL 9 instruments must be developed specifically for shipboard, ballast water discharge compliance monitoring. Therefore, related to the need for investments in sub 9 TRL instruments was also the need for clear criteria to dictate method and/or instrument validation and recognition.

The SMEs strongly recommended that a second workshop be held at some point in the future. The purpose of the second workshop would be to take the items discussed in the first workshop and shape them into components that contribute to a practical compliance and enforcement framework or strategy for the USCG to consider. Particular needs for discussion include but are not limited to: defining key terminology (i.e.: “gross exceedance”) and identifying appropriate approaches and methodologies for employment.



APPENDIX A. SUBJECT MATTER EXPERT WORKSHOP AGENDA

**Tuesday, 28 June 2011
USCG R&D Center**

Part 1: Overview of Current Regulatory Practices

- | | |
|--------------------------------|--|
| 8:30 a.m. – 9:00 a.m. | Welcome, Workshop Outline and Objectives, Participant Introductions (Roderick, Schnappinger USCG) |
| 9:00 a.m. – 9:30 a.m. | Review of the USCG (Proposed) ballast water discharge regulations (Everett USCG) |
| 9:30 a.m. – 10:00 a.m. | Mandatory Reporting: Review of EPA General Vessel Permit in relation to ballast water discharge (Albert EPA) |
| 10:00 a.m. – 10:10 a.m. | Break |
| 10:10 a.m. – 10:30 a.m. | Onboard Inspections: Regulatory case study – USCG compliance and enforcement of oily water discharge and separators (Allain/Fluke USCG) |
| 10:30 a.m. – 10:50 a.m. | Onboard Inspections: Regulatory case study – USCG compliance and enforcement of Marine Sanitation Devices (MSD) (Allain/Fluke USCG) |

Part 2: Overview of Current Technology and Verification of Systems

- | | |
|--------------------------------|--|
| 10:50 a.m. – 11:10 a.m. | Review of currently available ballast water treatment systems (Tamburri UMCES) |
| 11:10 a.m. – 11:30 a.m. | Overview of the ETV Generic Protocols for the Verification of Ballast Water Treatment Technology (Drake NRL) |
| 11:30 a.m. – 11:45 a.m. | Review of International Testing Efforts (Veldhuis NIOZ) |
| 11:45 a.m. – 1:15 p.m. | Lunch |
| 1:15 p.m. – 2:00 p.m. | Overview of EPA water quality testing in regards to the Clean Water and Safe Drinking Water Acts and related system verification (Danesi EPA) |

Part 3: Overview of Measurable Water Quality Parameters and the Availability of Tools and Methods

- | | |
|------------------------------|---|
| 2:00 p.m. – 3:30 p.m. | Measures of treatment performance – Discussions of indirect or indicative water quality measures, collected autonomously or by inspectors, that could demonstrate appropriate treatment conditions have been met for various ballast water treatment approaches. |
| 3:30 p.m. – 3:45 p.m. | Break |
| 3:45 p.m. – 4:45 p.m. | Measures of treatment performance – Discussions of availability, requirements, confidence, limitations, logistic constraints of instrumentation for indirect measures of treatment performance. |
| 4:45 p.m. – 5:00 p.m. | Summary (Schnappinger USCG) |



Proceedings of BWDS Compliance SME Workshop

Wednesday, 29 June 2011
USCG R&D Center

8:30 a.m. – 8:45 a.m.	Introduction (Schnappinger USCG)
8:45 a.m. – 10:30 a.m.	Indirect measures of non-compliance – Discussions of indirect biological, chemical, optical measures of live organism abundances, collected autonomously or by inspectors, for indications of clear/gross non-compliance.
10:30 a.m. – 10:45 a.m.	Break
10:45 a.m. – 12:00 p.m.	Indirect measures of non-compliance – Discussion of availability, requirements, confidence, limitations, logistic constraints of instrumentation for indirect measures of non-compliance, and discussions of emerging or promising novel approaches/technologies.
12:00 p.m. – 1:15 p.m.	Lunch
1:15 p.m. – 1:30 p.m.	Travel reimbursement procedures – (Murphy, USCG)
1:30 p.m. – 3:30 p.m.	Direct measures of discharge standard – Discussions of direct measures for concentration of live organisms in the various regulated categories – adapting current certification testing methods to compliance monitoring.
3:30 p.m. – 3:45 p.m.	Break
3:45 p.m. – 4:45 p.m.	Direct measures of discharge standard - Discussions of requirements, confidence, limitations, and logistic constraints for direct measures of concentration of live organisms in the various regulated categories.
4:45 p.m. – 5:00 p.m.	Summary (Schnappinger USCG)

Thursday, 30 June 2011
USCG R&D Center

8:30 a.m. – 8:45 a.m.	Introduction (Schnappinger USCG)
8:45 a.m. – 10:30 a.m.	Consensus on most promising approaches and required specifications: Measures of treatment performance Indirect measures of non-compliance Direct measures of discharge standard
10:30 a.m. – 10:45 a.m.	Break
10:45 a.m. – 11:30 p.m.	Consensus on future work/research needed
11:30 a.m. – 12:00 p.m.	Recommendations for a ballast water discharge compliance and enforcement framework (Schnappinger USCG)
12:00 p.m.	Lunch and adjourn workshop



APPENDIX B. SUBJECT MATTER EXPERT WORKSHOP ATTENDEES

On 28-30 June 2011, an SME meeting was held in the ABSG Consulting office in New London, CT, with 32 in attendance. The table below lists the workshop attendees and their affiliation or organization.

Table B-1. Workshop attendees.

Name	Affiliation/Organization
Ryan Albert, Ph.D.	EPA
LCDR Ryan Allain	CG-5431/5224
Sarah A. Bailey, Ph.D.	Fisheries and Oceans Canada
Alex Balsley	R&D Center
Allegra Cangelosi	Great Ships Initiative
Robin Danesi	EPA Office of Wastewater Management
Charles Dorchak	American Bureau Of Shipping
Lisa A. Drake, Ph.D.	Naval Research Laboratory Key West, Code 6136
Rich Everett, Ph.D.	CG-5224
Ray Finley, Ph.D.	Sandia National Laboratories
Michele Fitzpatrick, Ph.D.	PMG/ATSC
Jim Fletcher	R&D Center
LCDR Chuck Fluke	CG-5432
Ray Fredrick	EPA
John Griffith, Ph.D.	Southern California Coastal Water Research Project
Thomas H. Johengen, Ph.D.	University of Michigan
Carolyn E. Junemann, Ph.D.	MARAD
Sue Kim	ABS Consulting
Dennis M. King, Ph.D.	University of Maryland
Brandon Lee	ABS Consulting
Edward Lemieux	Naval Research Laboratory, Code 6130
M. J. Lewandowski	R&D Center
Gary Logsdon, Ph.D.	Black & Veatch
Gail Roderick	R&D Center
Craig Schnappinger	ABS Consulting
Mike Sprague	ABS Consulting
Tom Stevens	NSFI
Lynn Takata Ph.D.	California State Lands Commission
Mario Tamburri, Ph.D.	ABS Consulting/University of Maryland
Marcel J.W. Veldhuis, Ph.D.	Royal Netherlands Institute for Sea Research
Nick Welschmeyer, Ph.D.	Moss Landing Marine Laboratories
Greg Ziegler, Ph.D.	Wye Research and Education Center, UMD



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APPENDIX C. TECHNOLOGY READINESS LEVELS

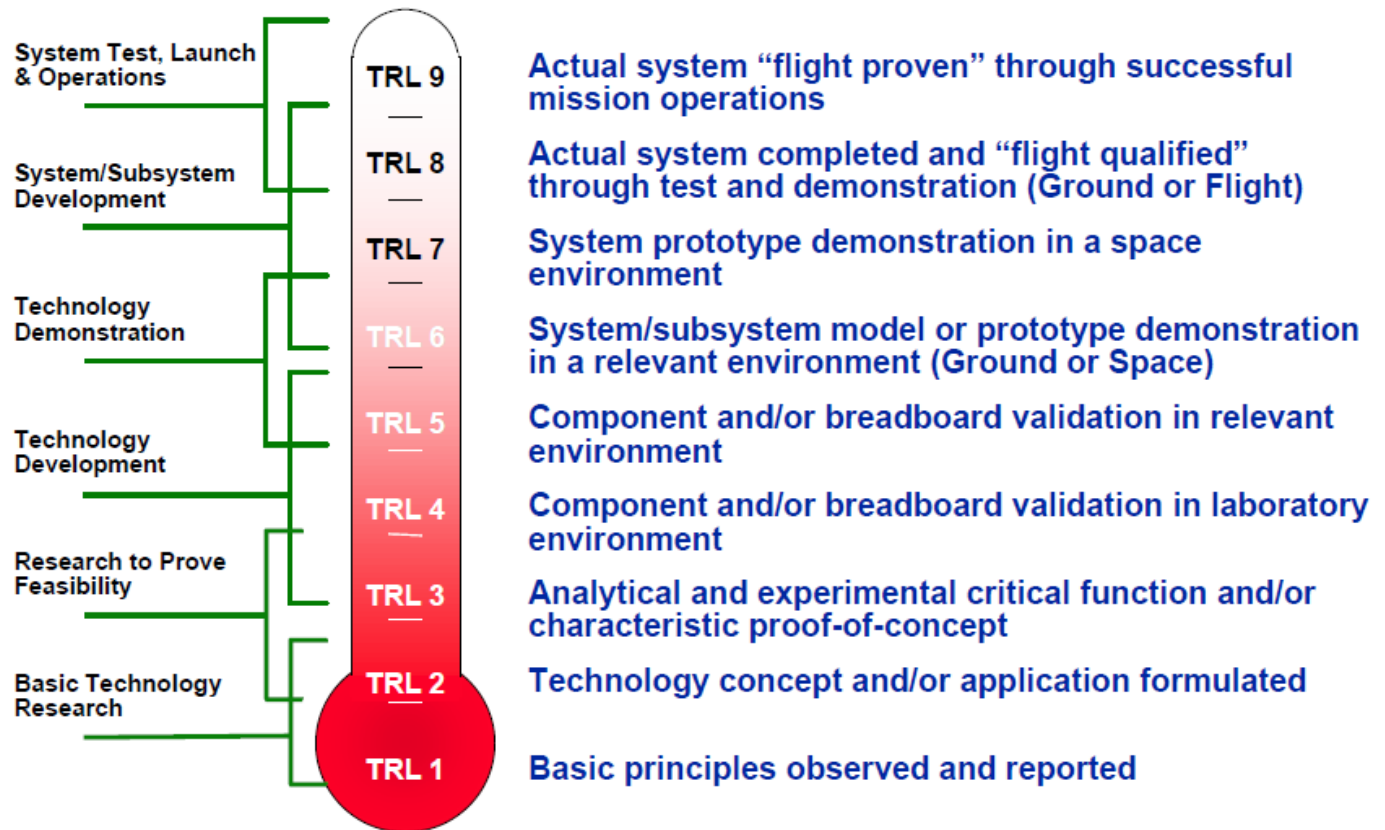


Figure C-1. NASA TRL levels³.

³ “Technology Readiness Levels.” National Aeronautics and Space Administration, www.hq.nasa.gov/office/codeq/trl/trlchrt.pdf.



Table C-1. Technology readiness level descriptions.

TRL	Description
1. Basic principles observed and reported	Lowest level of technology readiness. Scientific research begins to be translated into applied research and development. Example might include paper studies of a technology's basic properties.
2. Technology concept and/or application formulated	Invention begins. Once basic principles are observed, practical applications can be invented. The application is speculative and there is no proof or detailed analysis to support the assumption. Examples are still limited to paper studies.
3. Analytical and experimental critical function and/or characteristic proof of concept	Active research and development is initiated. This includes analytical studies and laboratory studies to physically validate analytical predictions of separate elements of the technology. Examples include components that are not yet integrated or representative.
4. Component and/or breadboard validation in laboratory environment	Basic technological components are integrated to establish that the pieces will work together. This is relatively "low fidelity" compared to the eventual system. Examples include integration of 'ad hoc' hardware in a laboratory.
5. Component and/or breadboard validation in relevant environment	Fidelity of breadboard technology increases significantly. The basic technological components are integrated with reasonably realistic supporting elements so that the technology can be tested in a simulated environment. Examples include 'high fidelity' laboratory integration of components.
6. System/subsystem model or prototype demonstration in a relevant environment	Representative model or prototype system, which is well beyond the breadboard tested for TRL 5, is tested in a relevant environment. Represents a major step up in a technology's demonstrated readiness. Examples include testing a prototype in a high fidelity laboratory environment or in simulated operational environment.
7. System prototype demonstration in an operational environment	Prototype near or at planned operational system. Represents a major step up from TRL 6, requiring the demonstration of an actual system prototype in an operational environment, such as in an aircraft, vehicle or space. Examples include testing the prototype in a test bed aircraft.
8. Actual system completed and 'flight qualified' through test and demonstration	Technology has been proven to work in its final form and under expected conditions. In almost all cases, this TRL represents the end of true system development. Examples include developmental test and evaluation of the system in its intended weapon system to determine if it meets design specifications.
9. Actual system 'flight proven' through successful mission operations	Actual application of the technology in its final form and under mission conditions, such as those encountered in operational test and evaluation. In almost all cases, this is the end of the last "bug fixing" aspects of true system development. Examples include using the system under operational mission conditions.

(Source: DOD (2006), *Defense Acquisition Guidebook*)



APPENDIX D. MEASUREMENTS

For Compliance Levels 2 and 3, the count or estimation of organisms is done to measure whether the vessel is in compliance with the specific discharge standard. These measurements are listed below.

Abundance estimates

Abundance estimates are calculated quantities of biomass based on indicators and measurements taken. Total tank biomass is often inferred from smaller sample populations taken, and then extrapolated using statistics. Population quantities can either be directly counted or inferred from detected amounts of biological activity characteristic of population sizes.

ATP

A well developed system for monitoring general biological activity is monitoring of Adenosine Triphosphate (ATP). This molecule is utilized virtually across all domains of life, thereby providing a universal indicator of the presence or absence of biological activity. A unique characteristic of ATP is the immediate degradation (within minutes) following cell death, thereby lowering risk of false positives post sterilization. Dormant forms of life such as bacterial spore or viral capsids in the absence of host cells may not be detected by this method. ATP is commonly measured relatively cheaply using a combination of chemical reagent kits, and a photometer (i.e. luminometer). ATP must be extracted from living cells using a combination strong acid with ATP optimized buffer solution. Once extracted, the product is mixed with an ATP reactive chemical which emits light in the presence of ATP. The light is measured by the photometer in relation to the calibrated biomass to produce a validation of presence or absence of biological activity. Modern photometers are generally palm sized, portable, and inexpensive.

CFU

Colony forming Bacteria and Fungi are often reported using Colony Forming Units (CFUs). A sample containing these organisms is incubated on a culture plate, which form small visible clusters of genetically identical specimens. Due to localized nutrient restriction these colonies generally grow to approximately the same size, before spreading elsewhere on the plate to form additional colonies. The advantage of organisms growing this way is that from a given dilution of sample, the counted CFUs can be used to infer their concentration in tested ballast tank. The two main disadvantages of this measure, are the incubation time (hours to days) and the limited detectable species. Culture plates work for detecting commonly cultured bacteria such as *Escherichia coli* (E. Coli); however, it is estimated that less than 0.2% of all microbes have been successfully grown on culture plates.

Chlorophyll fluorescence

Chlorophyll fluorescence refers to the measurement by fluorometer whereby a calibrated wavelength of light specific to chlorophyll is directed into a given sample. If chlorophyll is contacted by the test beam, then the emitted fluorescent light of reaction is detected by the fluorometer and reported as a positive test result. Modern fluorometers are typically able to detect Chlorophyll as biological indicators in extremely low concentrations, often orders of magnitude lower than other test.

Dead stain

Stains are often utilized to enhance visualization during microscopy analysis. Dead staining agents such as Green SYTO further enhance this process due to the inability to strongly stain living cells due to their intact cell membranes. As a result dead stains allow for direct measure of deceased cells in a given sample, which is often compared to a live stain for a ratio of living to deceased cells.



Direct count

Direct counting refers to any test where organisms are visually counted in a given sample as a means to statistically derive their concentration in a larger population area (such as a ballast tank). Examples include counting of cell colonies on culture plates, or direct cell counts on microscope slides.

DNA/RNA

Deoxy- or Ribo- Nucleic Acids (DNA/RNA) refers to genetic material found in all forms of life. Some general test such as nucleic acid dyes can assist in direct microscopy analysis, while more advanced laboratory analysis, match specific DNA sequences to identify contaminants to a high degree of certainty. Typically the results of these tests are used as final verification of other testing protocols.

Enzymatic activity

Enzymatic activity testing refers to a technique used in microbe analysis as an identifying characteristic filter. In its most basic test forms, small samples are directly exposed to an enzymatic substrate capable of reaction with cells of interest. If there is a visible reaction (i.e. bubbling), then the test is recorded positive for the presence of this group of applicable organisms. More modern forms of this test combine use of a fluorometer with this test, in order to quantify the chemical energy released from the chemical reaction as a direct measure of the quantity of cells in solution. Depending on the specific enzyme reaction selected for testing, the protocol can be targeted at broad or specific ranges of microbial species.

General stain

General stain refers to a broad category of test utilizing chemical stains/dyes for microscopy analysis to enhance visualization of transparent microbes. While there are broad catalogues of available staining agents, each one typically is good for a spectrum of organisms and test should further designed with staining agents relevant to the contaminating species of interest.

Live stain

In microscopy analysis live stains is a division within general cell stains, wherein the test is designed to show the number of living cells separate from deceased cells. Live stain techniques often utilize a broad scope stain such as SYTO red, to visualize all cells living or dead while a secondary dead stain such as SYTO green is used to differentiate dead cells. As a result the cells positive for SYTO red, but negative for SYTO green are inferred to be living cells.

Live + dead stain direct count

Live and dead stains are commonly done simultaneously on the same sample in order to calculate the amount of viable living cells versus total biomass. Each stain only provides additional information, but also provides a counter stain to the other, which is enhances the visualization of each stain.

Membrane potential

Tests utilizing membrane potential rely on a nearly universal biological principle that living cells require a proton gradient across a membrane in order to facilitate biological activity. As such variable fluorescent dye kits have been designed in order to visibly color living and dead cells differently. Once treated with these membrane potential reagent kits, the samples can be analyzed using either microscopy or high through-put flow cytometry.



Metabolic activity, enzyme

Enzymatic activity test are often used to quantify present living biomass via metabolic activity. FDA kits coupled with measure by a flow cytometer are effective means to detect metabolic activity via enzyme activity.

Movement

Movement is a detectible indicator of living organisms in test samples. Detection methods include manual microscopy, or computerized analysis of video from microscopy. Accurate counting is often difficult when done manually, due to the nature of constantly moving cells. Computerized optical recognition can be programmed for applicable characteristic patterns of microbial movement and used to take living cell counts versus total particulate background. It should be noted though, that while movement is an affirmative indicator, the absence of movement does not conclude the absence of living organisms due to the nature of many microbes lacking the capability of active movement. Algae, for example, rely on buoyancy for its position in the environment, but cannot control its own horizontal movement.

Nucleic acid stains

Virtually all forms of life current known contain a nucleic acid, either in the form of DNA or RNA. Nucleic acid stains are specifically designed to bind to these molecules. An example of a common nucleic acid stain is SYTOX-green, which is also a dead stain.

Respiration rates

Respiration rate refers to rate at which a given living biomass consumes a predictable amount oxygen. Tests measuring changes in oxygen concentration over a set time can be used to quantify the presence of biological activity in a closed ballast water tank.

Selective substrate most probable number

Selective substrates such as FDA are used to measure enzymatic activity in living organisms. Measured enzyme activity is then adjusted for variance between different sized cells, before finally estimating the most probable number of living cells.

Swimming organisms in small volume

Swimming organisms readily stand out to visual analysis under microscopy. For microorganisms, the space between the microscope slide and the cover slip often provides a small volume of water with a height of less than a millimeter, but plenty of room for microbial movement.

Variable fluorescence

Techniques utilizing variable fluorescence involve fluorescent agents capable of producing more than one color depending on exposed conditions. An example would be with membrane potential reagent where the reagent molecule fluoresces different colors depending on its location in the cell where different pH zones appear.



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APPENDIX E. INSTRUMENT OR ANALYSIS OF MEASUREMENT

3,3'-dihexyloxacarbocyanine (DiOC6(3))

DiOC6(3) is a lipophilic stain specific to mitochondrial cell components within eukaryotic cells (non-bacteria). Membrane potential test kits often utilize DiOC6(3) as a fluorescent stain because its emitted spectrum is ideal for reading by flow cytometers.

Automated video analysis

Optic recognition programs can be combined with video of visual analysis in order to automate visual analysis processes such as microscopy. Software packages can be programmed to look for general morphological profiles, movement profiles, or specific test fluorescence.

Biochemical oxygen demand/dissolved oxygen (DO) sensor, Electron Transport Systems

Living biomass is commonly detected by aerobic respiration and consumption of oxygen. Cellular consumption of oxygen by their electron transport can be detected either by dissolved oxygen monitoring of a closed system or by specialized electron transport chain fluorescent stains. In the case of electron transport chain fluorescent stains, the consumption of oxygen excites the electron transport chain complex, releasing energy which causes uniquely detectable fluorescence of the respiring cells. This provides real-time quantification of living biomass in a sample.

Chlorophyll fluorometer

Chlorophyll fluorometers are utilized in detection of plants, algae and cyanobacteria via the presence their green pigment, chlorophyll. The fluorometer technology takes advantage from chlorophyll's chemical property to react to the presence of light, and can read the excitation energy released by the pigment. As such the benefits are greatly increased detection limits by orders of magnitude.

Colorimetric immunoassay kit

Immunoassay kits refer to any test where antibodies are used to screen for and attach markers to specific molecules of interest. In the past such test were limited to lab testing, however modern commercial suppliers have incorporated this technology into effective field test strips. Colorimetric markers are often utilized by field test because they lack specialized equipment requirements in order to be read.

Coulter counter

A Coulter counter is a device used to count cells in a given solution, often times with the addition capability to differentiate between cell sizes. The primary technology relies on its ability to draw the solution through micro channels and measure the electrical resistance across these microchannels. As cells are drawn through each microchannel, they increase the electrical resistance in a predictable way relative to their cell size.

CTC

CTC refers to a bacterial live stain reagent also known as 5-cyano-2,3-ditolyl tetrazolium chloride. This reagent reacts within cells undergoing aerobic respiration and fluoresces Red in their presence.

Electron microscopy

Electron microscopy is a form of microscopy utilizing electrons in place of visible light. The advantage of this substitution is electrons have a drastically shorter wavelength, allowing for high resolution, up to 5,000 times greater than a light microscope. This is particularly useful for the smaller organisms like certain bacteria and viruses.



Epifluorescent microscope

An epifluorescent microscope is one that controls levels of background light, in order to maximize visualization of fluorescent light emitted by fluorescent dyes. This microscope is used in quantitative and qualitative lab test.

FDA fluorometer kit

A Fluorescein diacetate (FDA) kit is a pack of necessary reagents for performing an enzymatic activity test on a given sample. The test is an indirect measure of biological activity, which in combination with statistical models can derive quantities of living biomass versus dead biomass.

FDA/CMFDA

Fluorescein diacetate (FDA) and Chloromethylfluorescein diacetate (CMFDA) are similar variable fluorescent live stains used to differentiate viable living cells from dead cells via intracellular esterase activity. In living cells, naturally present enzymes will chemically convert these dyes changing the spectrum of fluorescent light emitted.

Flow cytometry

Flow cytometry is a high through-put version of automated microscopy whereby the machine analyzes a stream of cells in solution, and automatically counts cells satisfying programmable optic or conductive criteria. In many cases a flow cytometer can be programmed in combination to a fluorescent live or dead stain in order to differentiate living cells versus total biomass in solution.

Flow cytometers (imaging)

Flow Cytometers are designed to automate various evaluation task previously examined manually via microscopy. Flow Cytometers measure the difference in electrical resistance of a solution as a test sample is drawn through micro-channels. The process is sensitive enough to detect individual cellular bodies as they flow through the channel gates, allowing for automated counting of cells in a given sample. Additional advances in technology can further filter the counted results, by size or programmed spectrum of fluorescence given off by a stain administered prior to the flow cytometer.

Green nucleic acid stain (Sytox)

Green sytox is a common dead stain, which specifically binds to nucleic acids within the cell. Due to the internal nature of its binding activity, green sytox is only able to bind with dead cells with a permeated cell membrane.

Lab analysis

Lab analysis is any analysis that cannot be conducted in the field. Typically tests that require lab analysis will require specialized personnel and will have lower results.

Lab fluorescent antibodies

Lab fluorescent antibodies are biochemical substances with uniquely targeted binding properties for a targeted substance (substrate) of interest. Once bound, the attached fluorescent molecules allow the substrates to be detected and quantified. The lab designation separates this class of reagents from some newer age test strips able to be utilized in the field.



Laser optical plankton counter (LOPC)

Laser optical plankton counters track the flow of plankton cell bodies through a test stream, and provide quantitative analysis of plankton concentrations. Advanced instruments are able to further filter counted results via basic morphological shape readings specific to species of interest.

Live stain and flow cytometry

Fluorescent live stains when coupled with flow cytometry provide an automated means to quickly count the number of live organisms in a sample. Sample concentrations can but utilized to extrapolate the concentration within a larger system such as a ballast water tank.

Luminometer reagent kit

Luminometers are used to measure the intensity of fluorescent test, and as such their associated reagent kits typically contain cell fluorescent agents along with necessary reaction buffers and cell permeability enhancing chemicals.

Microscope

Microscopes are devices often utilizing lenses to enhance and magnify visualization of tiny objects normally too small for the human eye to see. Advanced microscope technologies such as electron microscopes may utilize backscatter reading technology similar to airport security scanners, in order to further magnify beyond the physical limits of lens technology.

m-TEC

Modified membrane-Thermotolerant Escherichia coli (m-TEC) agar is a specialized culture medium, used to enhance plate counts of E. coli, a common biological contaminant species in water samples. m-TEC agar includes an extra selective enzyme substrate, 5-bromo-6-chloro-3-indolyl- β -D-glucuronide, which is broken down by enzymes in E. Coli. The resulting chemical product produces a bright magenta color for each applicable colony on the test plate. The test increases visualization of colonies, and lowers the required expertise needed by the test technician.

Neutral red

Neutral red is a general stain, which non-discriminately attempts to dye all particles in solution, red. This general stain is commonly used in combination with dead stains to calculate the ratio of live to dead biomass.

Nucleic acid stains and epifluorescence microscope

Fluorescent acid stains are often used in combination with epifluorescence microscopy to enhance visualization in the display of the microscope. With fluorescence, special microscopes such as epifluorescence types are needed in order to block out background light that would overwhelm the weak fluorescent signals. Nucleic acid stains and epifluorescence microscopy can be used to quantify dead biomass in a sample.

Nucleic acid stains and flow cytometry

Flow cytometry can be utilized in junction with nucleic acid stains in order to automate manual processes such as microscopy which quantify dead biomass in sample solutions.

PAM fluorometer

PAM fluorometers are used in chlorophyll quantification test. The test laser is pulsed quickly on the sample chlorophyll in order to increase signal strength of detection.



Plate counts

When measuring microbes, culture plates are commonly used to grow and magnify bacteria within samples up to detectable limits. Colony forming microbes are particularly convenient to measure using plate counting techniques because the number of visible colonies can be easily counted without aided visual enhancement, and the be used to derive the original bacterial concentration in a given sample.

qPCR

Quantitative Polymerase Chain Reaction (qPCR) is an amplified detection method for measurement of low concentrations of DNA. PCR is normally a method for replicating DNA in order increase a sample size. The rate of replication is controlled closely by limiting reagent and controlled reaction temperatures. In qPCR the initial quantity of DNA before replication is calculated using the known rate of replication and the measured duration. qPCR is a laboratory technique requiring specialized personnel.

Selective substrate most probable number kit S

Selective substrates are test molecules designed to interact with specific target enzymes as a measure of living biomass. Due to variance in individual cells containing the target enzymes, statistical models have been designed to predict the most probable number (MPN) of cells in a given sample. These MPN models are calibrated to unique sample compositions using MPN kits.

Video

Video recordings of visual test are often created for repeatability, or for automated video analysis techniques.

Visual

Visual counting techniques generally refer to any technique where the test facilitator looks for visual indicators of biological contaminants.



APPENDIX F. SUBJECT MATTER EXPERT WORKSHOP SPEAKERS

The following is a list of speakers followed by their respective presentations as part of the SME workshop. This list is included to assist researchers and analysts for any follow-on projects.

1. Everett, Richard. U.S. Coast Guard: Review of the USCG proposed ballast water discharge regulations
2. Albert, Ryan. Environmental Protection Agency: Mandatory Reporting: Review of EPA General Vessel Permit in relation to ballast water discharge
3. Allain, Ryan and Fluke, Chuck. U.S. Coast Guard: Onboard Inspections: Regulatory case study – USCG compliance and enforcement of oily water discharge and separators
4. Allain, Ryan and Fluke, Chuck. U.S. Coast Guard: Onboard Inspections: Regulatory case study – USCG compliance and enforcement of Marine Sanitation Devices
5. Tamburri, Mario. University of Maryland, Center for Environmental Science: Review of currently available ballast water treatment systems
6. Drake, Lisa. Naval Research Laboratory Key West: Overview of the ETV Generic Protocols for the Verification of Ballast Water Treatment Technology
7. Veldhuis, Marcel. Royal Netherlands Institute for Sea Research: Review of International Testing Efforts
8. Danesi, Robin. Environmental Protection Agency: Overview of EPA water quality testing with regards to the Clean Water and Safe Drinking Water Acts and related system verification
9. Welschmeyer, Nick. Moss Landing Marine Laboratories: Indirect measures of non-compliance



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APPENDIX G. SUBJECT MATTER EXPERT WORKSHOP PRESENTATIONS

G.1 Richard Everett: Review of the USCG proposed ballast water discharge regulations



USCG

Ballast Water Management
Program and Discharge
Standard Rulemaking

www.Regulations.gov

Docket Number: USCG-2001-10486

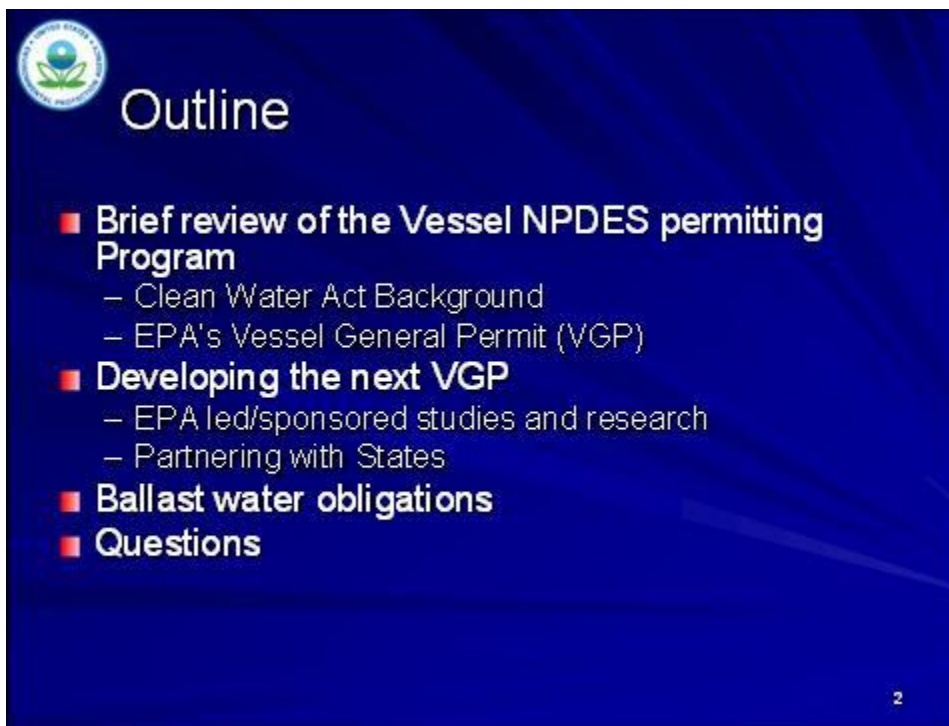
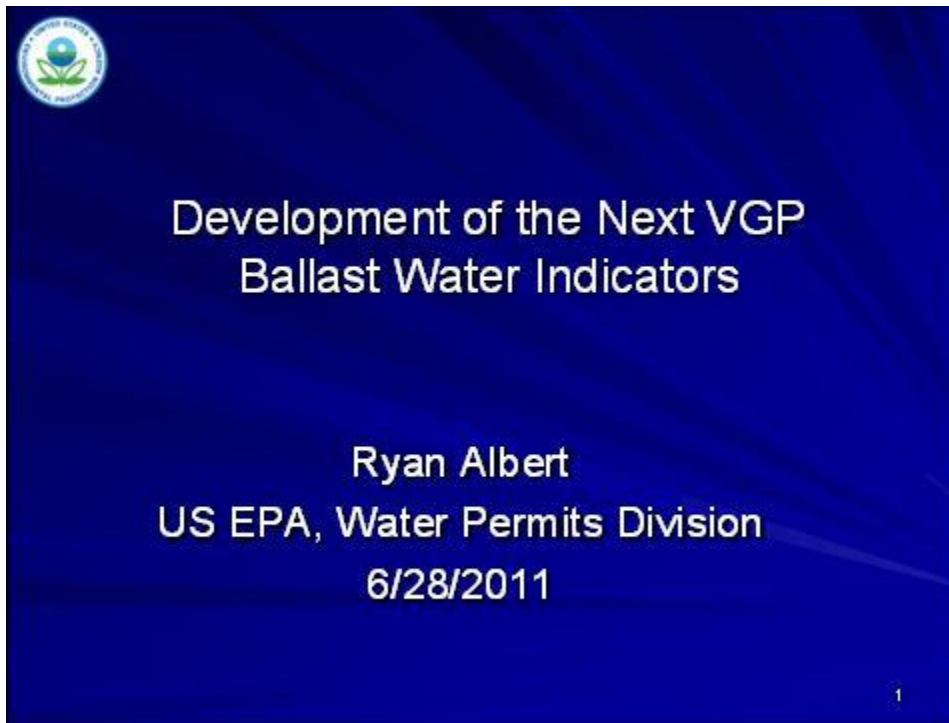


Table 1. Comparison Between Phase One and Phase Two Discharge Standards

Technical description	Large Organisms (> 50µm)	Small Organisms (≤ 10µ and ≤ 50 µm)	Very Small Organisms (≤ 10µm)	Bacteria		
				Toxicogenic <i>Vibrio cholerae</i> (01 & 0139)	<i>Escherichia coli</i>	Intestinal enterococci
Phase One	< 10 per m ³	< 10 per ml	N/A	<1 cfu per 100 ml	<250 cfu per 100 ml	<100 cfu per 100 ml
Phase Two	< 1 per 100 m ³	< 1 per 100 ml	< 1000 bacterial cells AND < 10,000 viruses per 100ml	<1 cfu per 100 ml	<126 cfu per 100 ml	<33 cfu per 100 ml



G.2 Ryan Albert: Mandatory Reporting: Review of EPA General Vessel Permit in relation to ballast water discharge





CLEAN WATER ACT (CWA) PERMIT BASICS

For more info visit <http://cfpub.epa.gov/npdes/>

- **"Discharge of a pollutant" generally prohibited without a permit [CWA § 301(a)]**
- **National Pollutant Discharge Elimination System (NPDES) Permits [CWA § 402]**
 - Individual permits
 - General permits
 - Permit term not to exceed 5 years
 - For EPA-issued permits, State 401 certification and CZMA concurrences required

3



CLEAN WATER ACT (CWA) PERMIT BASICS (cont.)

- **Effluent limits [CWA § 301(b)]**
 - **Technology-based [CWA § 304(b)]**
 - BAT/BPT (can be Best Management Practices – BMP)
 - BPL
 - **Water quality-based [CWA § 303]**

4





2003 Lawsuit

Northwest Environmental Advocates

- For 35 years, regulations excluded from NPDES permitting “discharges incidental to the normal operation of a vessel” while operating as a means of transportation (40 CFR 122.3(a))
- 2006 Court decision vacated this regulation
- As a result, EPA issued the Vessel General Permit (VGP) in December 2008

5



The 2008 Vessel General Permit

- The current VGP expires on December 19, 2013
 - The current VGP covers
 - All discharges incidental to normal operation of non-recreational vessels 79 feet or longer, except commercial fishing vessels
 - For ballast water discharges, permit covers all non-recreational vessels (including commercial fishing vessels and vessels less than 79 feet)



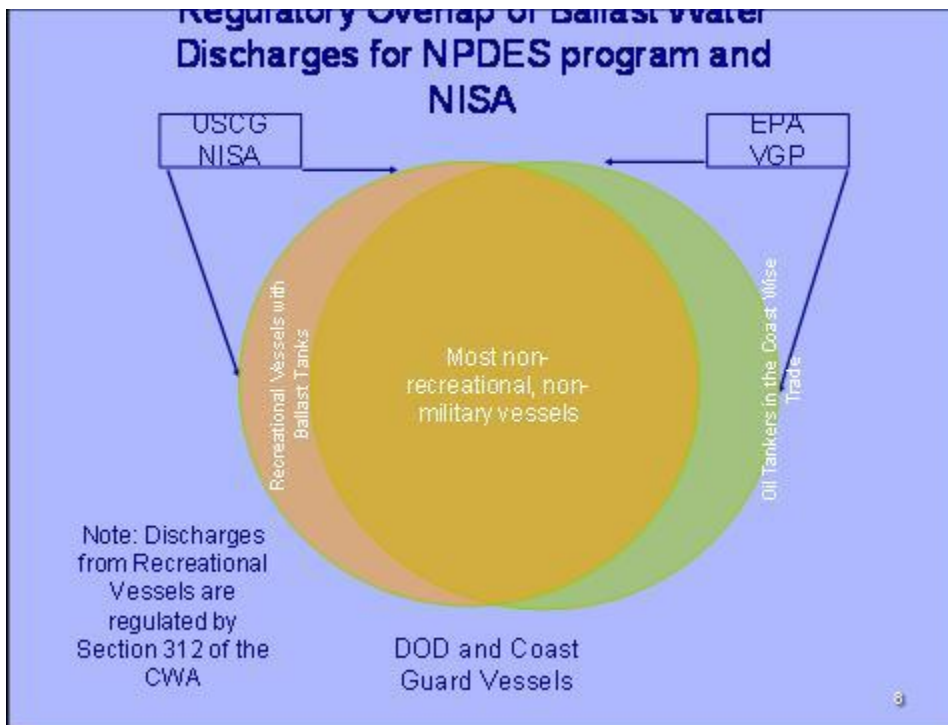


Vessel Universe

■ From a Clean Water Act regulatory perspective, there are four distinct classes of vessels




Class	Coverage	Estimated Universe
VGP Vessels - includes vessels >79 feet and all ballast water discharges	VGP	70,000
Moratorium Vessels - Non military, non-recreational vessels less than 79 feet and commercial fishing vessels regardless of size	Currently exempt except for ballast water discharges	120,000-140,000
Recreational Vessels	Clean Boating Act (CWA 312)	18 million
Vessels of the Armed Forces	Uniform National Discharge Standards (CWA 312)	~7,000





Developing the Next VGP



9



Three Primary Goals of VGP reissuance

- Improve environmental protection in key areas
- Clarifying edits for lower priority discharge types and requirements
- Improve efficiency



10





New vessel information since 2008

- EPA Vessels Report to Congress (2010)
- Science Advisory Board Shipboard Technology Ballast Water Study (In Progress)
- National Academy of Science Risk Assessment
- VGP NOI Analysis (In Progress)
- EPA One-time report information (available 2012)
- Other technical research on certain discharges, e.g., graywater, bilgewater
- Information received from Listening Session and 60+ day public comment period
 - Listening session held December 15, 2010
 - Written comments received from over 4,500 commenters, with ~250 unique comments



11



Developing VGP Ballast Water Limits: EPA's Science Advisory Board

EPA's Science Advisory Panel (SAB) on Ballast Water Treatment System Technological Availability

- SAB's charge was to evaluate the status of existing and potential shipboard ballast water treatment technologies and their ability to meet different discharge standards
- SAB draft report stated existing systems are available

12





Developing VGP Ballast Water Limits: National Academy of Sciences

- **National Academy of Science National Research Council (NAS) Study on Methods for Evaluating Risk**

- The NAS study assessed methods to evaluate the risk of invasive species introductions associated with ballast water discharges
- Released June 2, 2011
- Found that our ability to adequately quantify risk suffers from a profound lack of data
- Available via EPA's website at:
<http://cfpub.epa.gov/npdes/vessels/programdevelopment.cfm>

13



Partnering with the States

- **Several States have added conditions to the VGP**
- **For example, ballast water limits range from IMO (Minnesota) to 1000 times IMO (New York) to prescribing certain treatment approaches (Michigan)**
- **Regional inconsistency has caused confusion in the industry**

14





 **State Level Communication**

- In order to assist in regional/national consistency of standards, EPA plans to:
 - build tools to facilitate communication between states
 - will sponsor regional calls and/or a national co-regulator meeting to allow communication between states
 - provide information about the current state of ballast water science

16



EPA Ballast Water Obligations

- Propose numeric concentration based TBELs and/or WQBELs
 - If no numerics for WQBELs or TBELs, must include detailed analysis in the administrative record
- Take comment on “California Standards”
- “Commence consultation” on ESA
- Include self monitoring of discharges

17

14. EPA will include in the Draft Next VGP monitoring requirements for ballast water treatment systems onboard vessels specifying the parameter(s) to be monitored (*e.g.*, treatment unit flow, treatment unit pressure, active substance concentration, residual active substances, suspended solids, and/or indicator organisms) as EPA determines necessary to assess compliance with the ballast water effluent limits, consistent with 40 C.F.R. § 122.44(i)(1)(i)&(ii) and 122.45(e), with at least one of these parameters to be monitored on a monthly or more frequent basis. EPA will include in the administrative record for the Next Draft VGP an explanation of how it determined the frequency of reporting of monitoring results by assessing the nature and effect of the discharges, as required by 40 CFR 122.44(i). EPA will propose to make any ballast water monitoring information transmitted to the Agency in electronic form available to the public in electronic form.





VGP Schedule

- November 2011: Propose VGP
- November 2012: Finalize VGP
- December 2013: VGP comes into effect
 - Extra year planned to give industry an opportunity to adapt to the new permit.

19

G.3 Ryan Allain and Chuck Fluke: Onboard Inspections: Regulatory case study – USCG compliance and enforcement of oily water discharge and separators

Conducting Port State Control and MARPOL Annex I Examinations

Ballast Water Discharge Standard Workshop
June 2011



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Overview

- History and Philosophy of Port State Control
- USCG PSC Procedures
- USCG Policy
- Conducting MARPOL Annex I Examinations
- Case Studies



2



Philosophy of Port State Control

- Global effort to identify and eliminate substandard shipping
- Part of overall safety net – IMO, owners, operators, Administrations, Recognized Organizations, Port States
- International Standards provide minimum requirements for ships
- Use International protocol to prevent substandard ships from sailing until they meet minimum standards
- PSCOs verify substantial compliance.
- Avoid undue delay; facilitate commerce for ships that meet standards
- Engage and communicate with International partnerships to improve shipping worldwide



International PSC Authority/Responsibility

IMO Instrument	Purpose	Premise/Vessel Example
International Convention for the Safety of Life at Sea (SOLAS), 1912 – 2004	Life Saving and Fire Fighting	1912 - RMS Titanic Sinking – 1503 lives lost
International Convention for the Prevention of Pollution from Ships (MARPOL 73/78)	Prevent Maritime Pollution	1967 – Torrey Canyon – English Channel 1976 – Argo Merchant – Nantucket 1978 – Amoco Cadiz – Portsall, France
International Convention on Load Lines (LL 66)	Stability and Watertight integrity	Shipping casualties in UK in 1860s doubled in 30 years, raising insurance rates.
International Convention on Standards of Training, Certification and Watchkeeping for Seafarers (STCW 95)	Crew competency and safe manning	1992 - Aegean Sea – Spain – 21.5 million gallons
International Ship and Port Facility Security Code (ISPS 2002)	Prevention of terrorism	2000 & 2001 – USS Cole Attack/ Sept 11 Terrorist attacks on Pentagon and World Trade Center

How PSC got started in the U.S.

In 1994, Congress recognized that within the greater influx of non-U.S. ships, there undoubtedly existed a number of substandard vessels that posed an unacceptable risk to the safety of ports, waterways, and the marine environment of the country.

Accordingly, Congress directed the Coast Guard to develop a program to eliminate substandard vessels from the nation's waters, and to submit annual reports on the status of this newly mandated program.



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Goals of Port State Control in the U.S.

- Identify and eliminate foreign merchant ships found to be substandard.
- Encourage those committed to trading with the U.S. to adopt management philosophies that ensure compliance with accepted standards.
- Every foreign ship is examined at least annually.
- Vessels found to be substandard are detained.



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U.S. Coast Guard and Port State Control

- The U.S. Coast Guard verifies that foreign vessels operating in the U.S. comply with applicable International Conventions, U.S. Laws and U.S. Regulations.
- Foreign vessel examinations may be initiated by the Coast Guard, requested by another flag State administration on the basis of information regarding a potential substandard ship or based on information regarding a substandard ship provided by members of a ship's crew, a professional body, an association, a trade union or any other involved individual.



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Port State Control Policy in the U.S.

- Navigation and Vessel Inspection Circulars (NVICs)
- Marine Safety Manual
- Policy Letters
- Procedures for Port State Control, IMO Resolution A.787(19), as amended by IMO Resolution A.882(21)
- Other Non-mandatory IMO Resolutions and Circulars



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Navigation and Inspection Circular (NVIC) 06-03

- Comprehensive Policy for Coast Guard Port State Control Program
- Outlines Procedures for:
 - Risk Based Targeting of Vessels
 - Vessel Examination
 - Control of Substandard Vessels
 - Reporting and Documentation of Port State Control Exams



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Targeting Vessels/Risk Factors

- Different reasons for utilizing targeting process
 - Finite PSC resources
 - Foster compliance by targeting ships with risk factors
- Different factors could be used for targeting of vessels
 - Owners, Operators, Charterers
 - Flags, ROs, RSOs
 - Vessel Types, Vessel Ages
- Possible area of harmonization between MOUs



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Vessel Targeting

Three targeting matrices...two are unclassified

PSC Safety Matrix



•Legacy Mission

High Interest Vessel
Matrix



~~SECRET~~
•Intelligence driven

ISPS Matrix



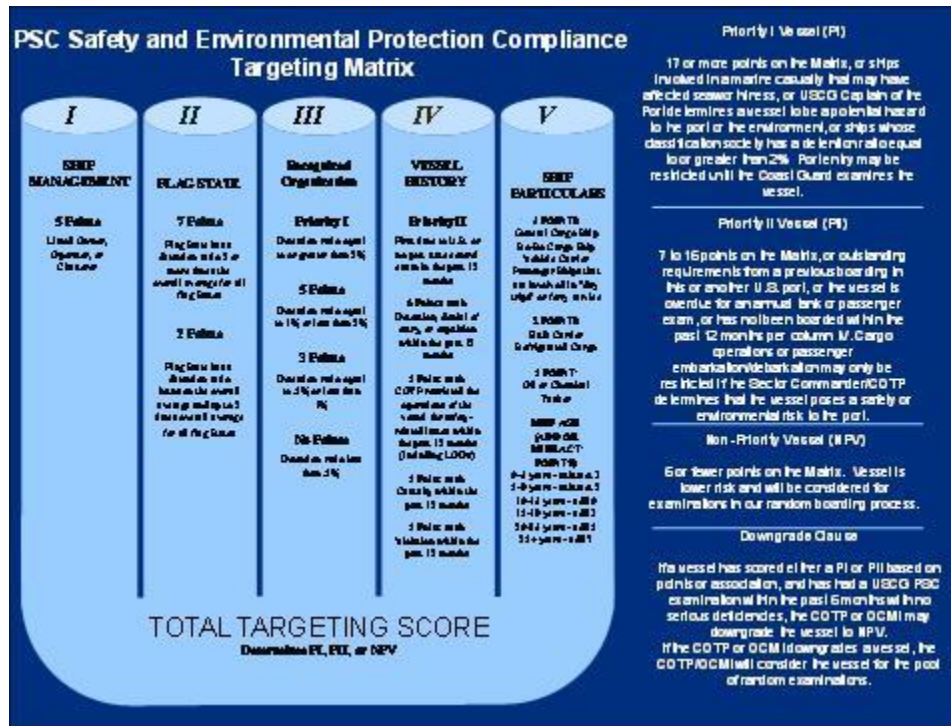
•Security
•SOLAS based/PSC

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11





Port State Control Exams Minimum Effort of Examination

- ✓ Evaluate the Vessel's Security System
- ✓ Evaluate the Safety Management System
- ✓ Conduct Navigation Safety Checks
- ✓ Conduct a Deck Walk and Evaluate the Vessel's Structure
- ✓ Conduct Steering Gear Tests
- ✓ Test the Oily Water Separator and Bilge Monitor
- ✓ Test the Fire Detection System
- ✓ Test the Fixed Deck Foam System (Oil Tankers Only, use water)
- ✓ Operate the Main and Emergency Fire Pump
- ✓ Examine Emergency Lighting
- ✓ Test the Emergency Generator
- ✓ Witness Fire & Boat Drills
- ✓ Evaluate ILO Conditions That May Be Unacceptable



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Research & Development Center

COAST GUARD PSC EXAMINATION BOOK

- CG 840 Book

United States Coast Guard

1-800-441-4622

COMMERCIAL VESSEL

Name of Vessel		Flag
HSA Number		Accident Number
Exam Completed	Priority	Notes
Priority	Priority	Priority
Priority	Priority	Priority
Location		
Vessel Call Sign		
Port State Control Reference		
1.	2.	
3.	4.	
Vessel Description:		
Master/Owner	Reg-Ship	Ship
Master/Owner	Reg-Ship	Ship



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ISM Code Enforcement

- The U.S. Coast Guard began enforcement of the ISM Code in July 1998 on passenger vessels and tankers, bulk carriers, gas carriers and high speed craft of 500 gross tons or more. In July 2002 our enforcement expanded to other cargo ships of 500 gross tons or more.
- In August of 2005, we refined guidance via Navigation and Vessel Inspection Circular (NVIC) 04-05.
- The NVIC can be found on our website:
<http://homeport.uscg.mil>
- We fully support the promotion of a "safety culture" through the use of Safety Management System implementation at the administrative company level and on the vessel.
- When we find non-compliance, we will use international and domestic authorities to correct the deficiencies.
- We carefully review all detention cases involving ISM deficiencies.



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Key Definitions of ISM Code Terms

- **Non-conformity:** ISM Code 1.1.9 ... **Non-conformity** means an observed situation where objective evidence indicates the non-fulfillment of a specified requirement. WHAT DOES SPECIFIED REQUIREMENT MEAN?
- **Major Non-conformity:** ISM Code 1.1.10.... means an identifiable deviation that poses a serious threat to the safety of personnel or the ship or a serious risk to the environment that requires immediate corrective action **and** includes the lack of effective and systematic implementation of a requirement of this Code. WHAT DOES "LACK OF EFFECTIVE AND SYSTEMATIC IMPLEMENTATION OF A REQUIREMENT OF THIS CODE MEAN?



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ISM Checklist

ISM Compliance Assessment Tool			
ISM Code Reference	Description of Non-Conformity (NC)	Problem Apparent	
		Yes	No
ISM Guidelines 1.1.11	Are deficiencies present that pose a serious threat to personnel, the ship, or serious risk to the environment, and that require immediate corrective action?	O	O
ISM Guidelines 1.1.11	Is there an apparent lack of effective and systematic implementation of a requirement of the ISM Code? If so, does such apparent failure apply to:	O	O
ISM Code 1.4	• The adherence to the ship's approved, self-developed Safety Management System?	O	O
ISM Code 4	• The responsiveness of the Company's Designated Person(s) to the ship's non-conformity reporting and requests for assistance/corrective action?	O	O
ISM Code 5	• The Master's defined responsibilities, authority, and his performance of those responsibilities?	O	O
ISM Code 6	• Critical crewmember's knowledge and familiarity with the ship-specific SMS?	O	O



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ISM Checklist (con't)

ISM Compliance Assessment Tool			
ISM Code Reference	Description of Non-Conformity (NC)	Problem Apparent	
		Yes	No
ISM Code 1.4, 7, 8, 9, 10, 11, 12	Are the SMS-specified reporting and documentation procedures being followed? <i>(Note: This is a broad, catchall topic. Use judiciously when hard documentation of NCs posing serious threat are apparent due to the lack of adherence to the reporting and documentation requirements throughout the Code. Cite the specific Code ref. for the evidence discovered.)</i>	O	O
ISM Code 10	- Do the maintenance procedures for the ship systems identified in the SMS; include frequency, scope, and documentation for the maintenance and necessary corrective action(s)? - Does the overall performance of the crew indicate that they have been trained to perform their assigned tasks in accordance with the training needs identified in the SMS?	O	O
ISM Code 6		O	O
Familiarity with the Code is critical to the identification of specific SMS breakdowns. The PSCO must document on the boarding report (Form B) those items suspected to be ISM-related deficiencies, citing the ISM Code element(s) associated with the non-conformity (deficiency). The thoroughness of the explained non-conformity will play an essential role in the external audit, enabling the Auditor for the RO to focus on the non-conformities identified by the PSCO as the impetus for the external audit.			



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MARPOL Annex I Enforcement

- U.S. committed to environmental protection of U.S. waters, coasts, and natural resources
- U.S. diligently enforces U.S. & MARPOL Annex I requirements
- MARPOL Annex I Applicability:
 - Tank ships – 150 GT
 - Other ships – 400 GT
 - Attention to GT necessary for thresholds for certain Annex I requirements
- MARPOL Annex I Examination Procedures
- Expanded Examinations
- Referrals for criminal prosecution



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MARPOL Annex I Examination

■ Documentation Review

- IOPP Certificate
 - Validity, ship arrangements, exemption, equivalencies
- Oil Record Book
 - Signatures
 - Irregularities: missing pages, incorrect entries, repetitive entries, information does not agree with conditions onboard
 - Required entries made



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MARPOL Annex I Examination

■ Documentation Review (Continued)

- Oil Discharge Monitoring & Control System Records
- Shipboard Oil Pollution Emergency Plan
 - Onboard and approved
 - Pollution prevention equipment onboard
- Safety Management System
 - Spot check environmental compliance policy



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MARPOL Annex I Examination

▪ Vessel Examination

- Walk-through examination (On deck & in machinery spaces)
- Oily water and overboard piping; indications of leaks and tampering
- Unused Pipe nipples and blanked flanges in piping
- No Installed bypasses
- Unused bypasses stowed below deck plates
- Sludge tank/dirty oil tank levels and capacities
- Oil containment
- Standard discharge connection



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MARPOL Annex I Examination

▪ Witness Tests/Examine Equipment

- Oily Water Separator
 - Approved equipment
 - Condition
 - Operational, no bypass, Crew Competence in its operation
- Bilge Alarm
 - Approved equipment
 - Condition
 - Operational, no bypass, Crew Competence in its operation

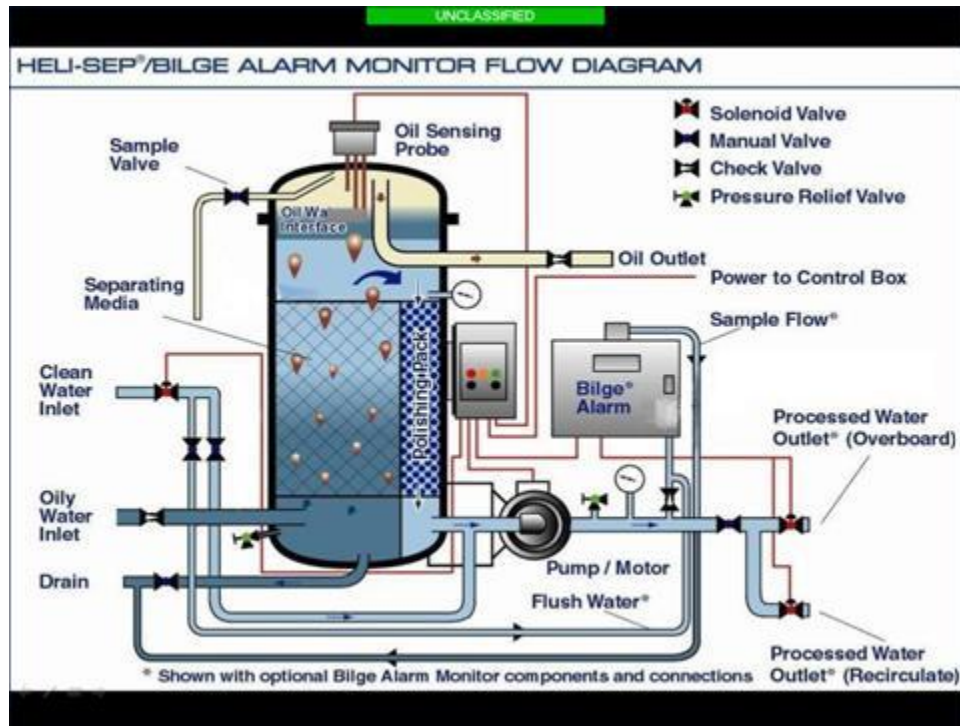


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MARPOL Annex I Examination

▪ Witness Tests/Examine Equipment (Continued)

- Oil Content Meter (Tank Ships)
 - Approved equipment
 - Condition
 - Operational, no bypass, Crew Competence in its operation
- Incinerator
 - Condition
 - Operational (and Crew Competence in its operation)
- Equipment installed, but not required



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Clear Grounds for Expanded Examination under MARPOL

- Absence of principal equipment or arrangements
- Evidence that certificates are clearly invalid
- Documents required by convention not on board, incomplete are not maintained, or falsely maintained



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Clear Grounds for Expanded Examination under MARPOL (Continued)

- Evidence from the PSCO's impressions or observations that serious deficiencies exist in pollution prevention equipment
- Evidence that the master or the crew are not familiar with essential shipboard procedures relating to the prevention of pollution
- Receipt of a report or complaint that the ship is substandard with respect to pollution prevention



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MARPOL Annex I Enforcement

- Our enforcement of MARPOL Annex I has been enhanced by issuance of Policy Letter 06-01.
 - This letter is available on line; see <http://homeport.uscg.mil>
 - The Coast Guard refer pollution cases involving potential criminal violations of U.S. law to the Department of Justice for criminal investigation when MARPOL Annex I violations show:
 - Vessel/crew concealment of pollution discharge caused by poorly maintained or faulty equipment.
- U.S. Coast Guard continues to partner with IMO and other Port State Control regimes to ensure consistent enforcement of MARPOL Annex I requirements.

Crew Competency Standards

- **SOLAS 74-06**, Chapter XI-1, Reg 4 "... clear grounds for believing that the master or the crew are not familiar with essential shipboard procedures relating to the safety of the ships."
- **MARPOL**
 - Annex I, Reg II "...relating to the prevention of pollution by oil."
 - Annex II, Reg 16.9.1 "...prevention of pollution by NLS."
 - Annex III, Reg 8 "...by harmful substances."
 - Annex V, Reg 8 "...by garbage."
 - Annex VI, Reg 10 "...air pollution from ships."
- **STCW** Reg I/4.1.3.4 "the ship is otherwise being operated in such a manner as to pose a danger to persons, property or the environment."

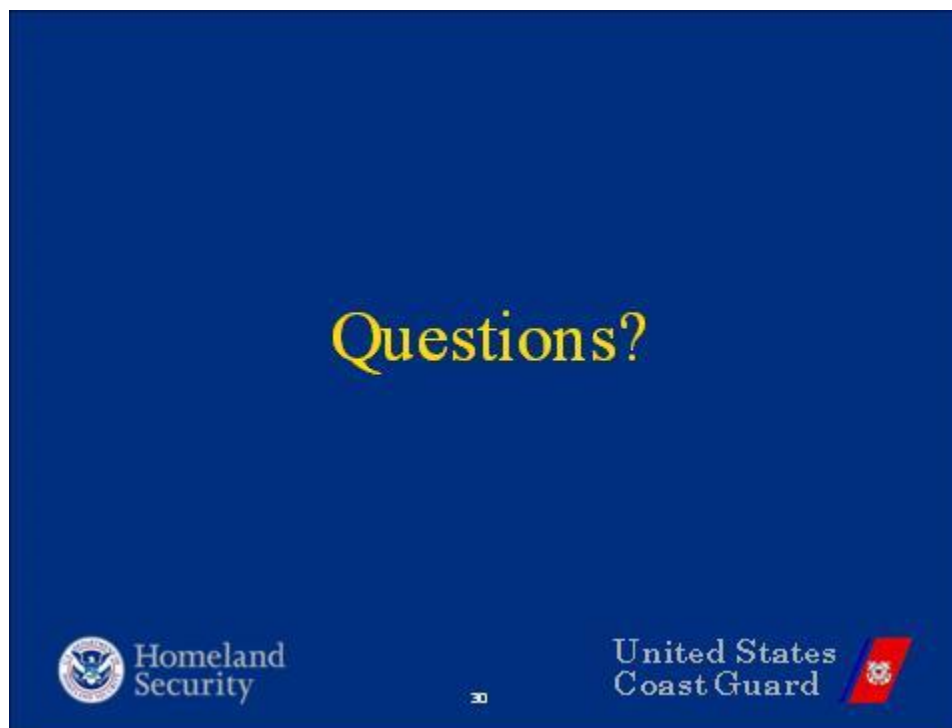


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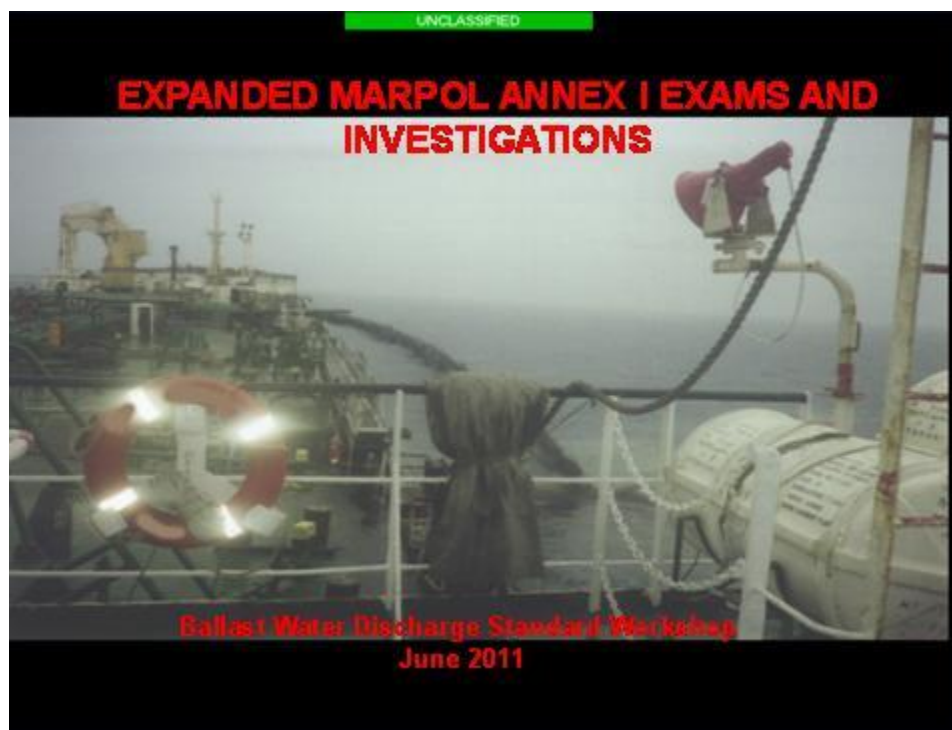
29

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G.4 Ryan Allain and Chuck Fluke: Onboard Inspections: Regulatory case study – USCG compliance and enforcement of marine sanitation devices.



EXPANDED MARPOL ANNEX I EXAMS AND INVESTIGATIONS

- Expanded or More Detailed Exams
- Technical Aspects to Consider
- Investigations



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PROCEDURES FOR CONDUCTING AN "EXPANDED" MARPOL ANNEX I EXAM

- Ensure **clear grounds** exist to justify a more in-depth examination;
 - crew inexperience in Oily Water Separator (OWS) operation
 - use of flush water to demonstrate Oil Content Monitor (OCM)
 - recently painted parts on OWS piping
 - wrench marks in overboard piping
 - Inoperable OWS or OCM
 - Inconsistencies in ORB
 - Evidence of possible use of Standard Discharge Connection with no ORB entries
 - Etc.
- If clear grounds exist, then the PSCO should expand the scope of examination to gather further information
 - Don't immediately assume a criminal violation has taken place



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PROCEDURES FOR CONDUCTING AN "EXPANDED" MARPOL ANNEX I EXAM

Conducting a more detailed expanded examination:

- Verify no electrical bypass, jumpers, or extra switches configured within OCM
- Verify bilge piping matches approved OWS piping diagram to ensure no unauthorized modifications
- OWS discharging may be opened if bypass suspected
 - A bypass does not always directly involve the OWS overboard skin valve
- Compare SMS requirements for OWS preventative maintenance against actual maintenance conducted
 - Spare parts (filters), receipts of any service, documentation of maintenance completed, tech reports, etc.



These are some of the important technical aspects of vessel waste management:

- Are the wastes being properly managed?
- Do records support proper activities and processes?
- Do records conflict with each other?
- Can shipboard personnel confirm or deny certain activities?
- What does the other evidence indicate?
- Who runs the engine room?
- What are the roles and activities of shore side personnel?
- What is their impact on vessel maintenance, operating, and environmental budgets?





PROCEDURES FOR CONDUCTING AN "EXPANDED" MARPOL ANNEX I EXAM

Conducting a more detailed expanded examination (cont):

- Review OCM calibration records if available
 - Crew is not required to know how to calibrate an OCM
 - OCMs built to comply with MEPC.107 (49) requires seal on bilge alarm
- Take note of any cleaning products in the engine room.
 - Can emulsify the oil in bilges
 - Can render OWS inoperative
 - OWS manual contains approved cleaning products



8



PROCEDURES FOR CONDUCTING AN "EXPANDED" MARPOL ANNEX I EXAM

Conducting a more detailed expanded examination (cont):

Incinerator –

- Review ORB for quantities of sludge incineration.
- Conduct operational test of the incinerator.
- Consult manufacturer's operations manual before test.
- Sound and note level and verify content of source tank. – i.e. ensure there is actually sludge in the tank.
- Examine firebox refractory – if routinely used it should not be too clean
- Let incinerator run for 15 to 20 minutes
- Check for drops in source tank consistent to ORB entries
- Verify vessel has spare parts as per manufacture's manual.



9



CONDUCTING AN INVESTIGATION FOR SUSPECTED MARPOL ANNEX I DISCHARGE VIOLATION

When to initiate an *investigation* into a possible discharge violation:

- Upon discovery of some evidence that the vessel is possibly illegally discharging oil overboard. (e.g. find black oil in the overboard discharge pipe, find evidence of tampering/bypassing, etc) or if during expanded examination, things just aren't adding up.
- The investigation may result in an IMO reportable detention, referral for criminal prosecution or both.
- Use guidance from Appendix 2 of Procedures for Port State Control, Resolution A.787(19), as amended by resolution A.882(21)



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CONDUCTING AN INVESTIGATION FOR SUSPECTED MARPOL ANNEX I DISCHARGE VIOLATION

Investigations reveal the difference between:

What is said to be done onboard.

Policy, procedures, standing orders, etc...

What is actually done.

Efforts to cut costs, conform, independently manage vessel problems, etc...



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CONDUCTING AN INVESTIGATION FOR SUSPECTED MARPOL ANNEX I DISCHARGE VIOLATION

The key to any successful investigation into a possible criminal violation is:

- Preparation
- Coordination
- Communication



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CONDUCTING AN INVESTIGATION FOR SUSPECTED MARPOL ANNEX I DISCHARGE VIOLATION

Guidelines in conducting an investigation:

- Conduct interviews with all engineering department personnel and/ or require written statements.
- Take pictures of evidence that cannot be seized
- Collect oil samples from bilge, OWS, slop tanks, bilge hold tank, etc
- SEIZURE OF EVIDENCE
 - USCG can seize evidence if we have probable cause to believe it is evidence of a crime. 14 U.S.C. § 89(a).



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CONDUCTING AN INVESTIGATION FOR SUSPECTED MARPOL ANNEX I DISCHARGE VIOLATION

Guidelines in conducting an investigation (cont):

- What kind of evidence do we to seize?
 - Piping, valves, hoses
 - Oil record books
 - Sounding tables & logs
 - Engineering logs
 - Deck logs
 - Maintenance logs
 - Automation printouts
 - Disposal receipts
 - Crew rosters
 - Ship to shore Communications
 - Transfer procedures
 - Equipment manuals
 - Piping diagrams
 - SOLAS certificates
 - "Work Done" logs
 - Engineers' diaries
 - ISM documents
 - Alarm print outs
 - Computer hard drives
 - "Overtime" records



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Logs and Documents:

Cast a wide net

- Oil Record Books
- Waste disposal receipts
- Sounding logs (and tank capacity table)
- Deck/engine/incinerator logs
- Ship prints (diagrams)
- Alarm print outs
- Automated machinery control tapes
- Oily Water Separator and incinerator maintenance logs
- Incoming/outgoing comms w/shore office



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“Tell-Tale Signs”

- Evidence that suggests the component is removed on a frequent basis (different colors of paint on piping, paint chips on piping, flanges, and bolts, greasy hand prints on piping, oil splatter or staining on bulkheads) or these items that have been very recently painted.



Examples of “Tell-Tale” signs



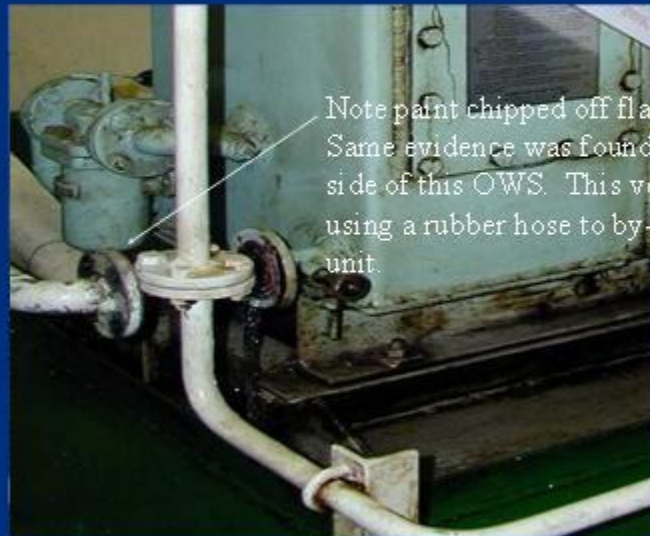
New bolts installed

Different colored piping

Paint chipped off pipe
and flanges from frequent
handling

Oil staining on bulkhead
and oil splatter &
accumulation found below
this area.

OWS Tell-Tale Sign

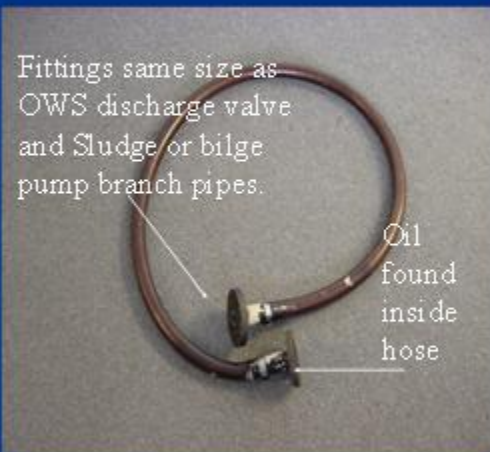


Inspection of OWS & associated equipment (cont.)

- Potential By-Pass equipment
 - If you find evidence of frequent removal of piping or branch pipes on the discharge sides of the bilge pump, sludge pump or the OWS outlet line, look for a hose or pipe in the vicinity that could be used as a by-pass.

Fittings same size as OWS discharge valve and Sludge or bilge pump branch pipes.

Oil found inside hose



Oil inside OWS discharge valve

Oil inside valve



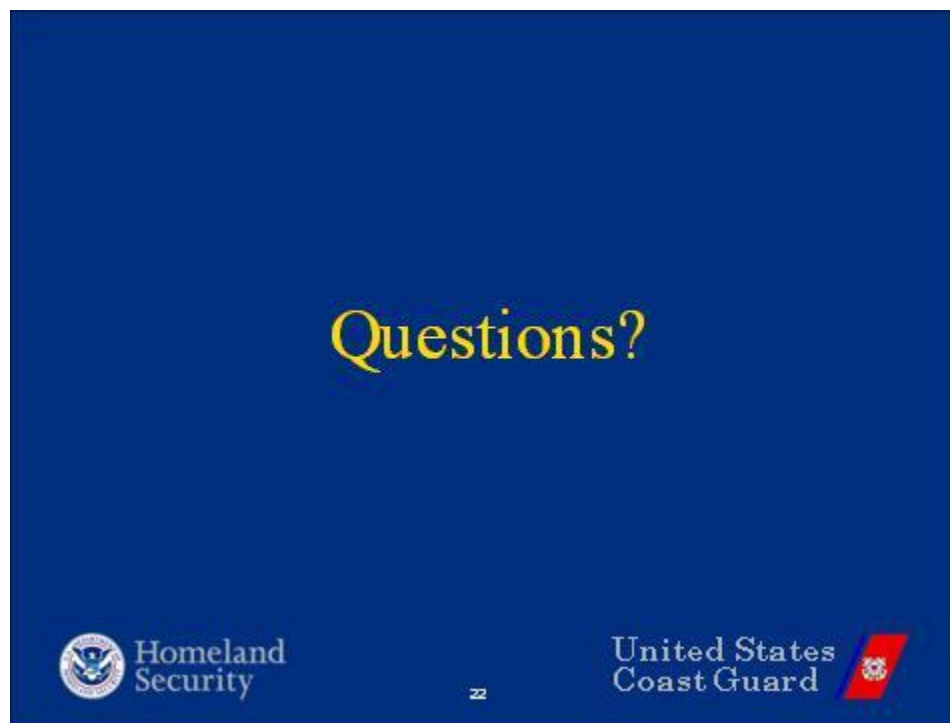
EXPANDED MARPOL ANNEX I EXAMS

- Discharges of oil contaminated bilge water and sludge is a direct contravention of MARPOL requirements.
- Port State Control data in the U.S. indicates an increasing frequency of non-compliance with MARPOL Annex I requirements.
- Data indicates that ship owners and vessel crews conceal accidental or deliberate discharges of oily waste and sludge, usually caused by malfunctioning equipment, poorly managed maintenance programs or as an effort to reduce operational costs.

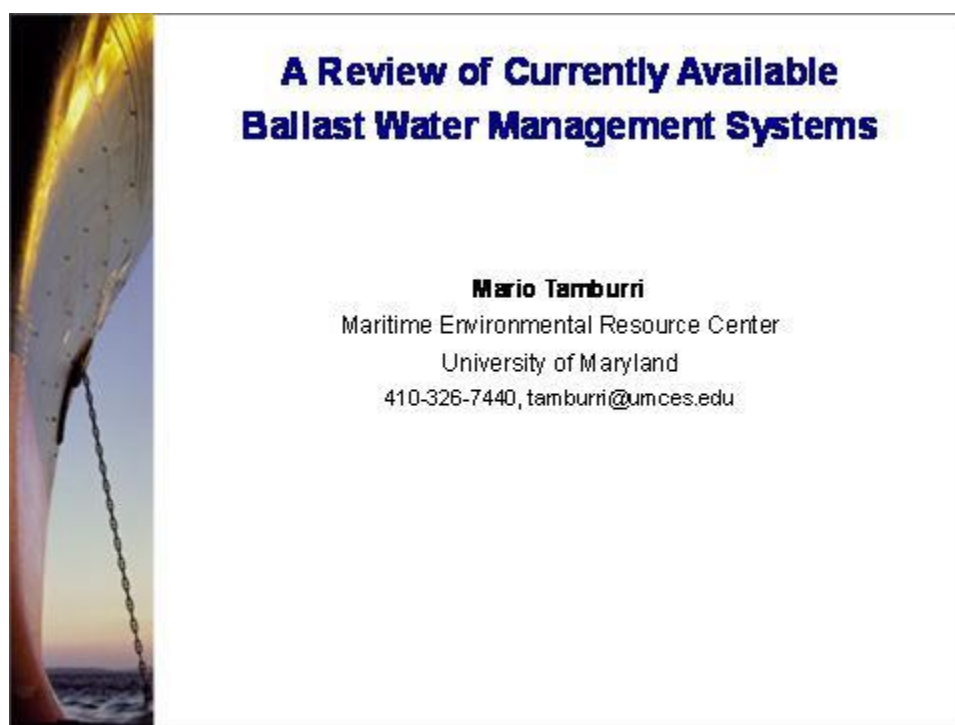


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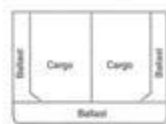




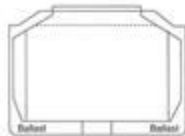
G.5 Mario Tamburri: Review of currently available ballast water treatment systems



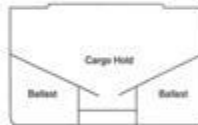
Ship Ballast Water



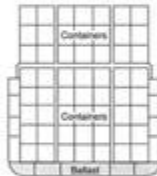
Tanker



Panamax size oil bulk ore carrier



Great Lakes bulk vessel,
intermediate class

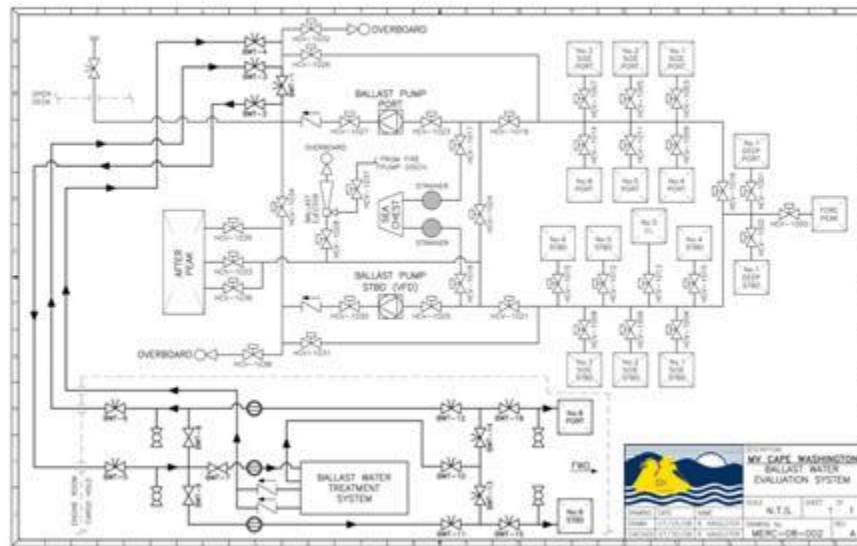


Container ship

- Adjust trim and draft
- Rates 100 - 12,000 m³/hr
- Volumes 5,000 - 100,000 m³
- System capacity depends on rate of draft or trim adjustment
- Tankers and Containerships required rate depends on cargo loading/unloading times, min/max drafts and equipment tolerances

- Total Capacity of tanker ballast pumps typically sufficient to fill/discharge in 15 hours
- Common goal is to load with full cargo to carry no ballast

Typical Ballast System



Treating Ballast Water

- Effective at killing/removing organisms and reliably meet discharge standards
- Safe for vessel and crew
- Environmentally benign
- Feasible and affordable



Ballast Water Management Systems

Physical Separation

- Filtration (screen or disk)
- Hydrocyclone

Chemical Treatment (Biocides)

- Oxidizing – sodium hypochlorite, chlorine dioxide, ozone (bromine/bromate), peracetic acid
- Non-Oxidizing – SeaKleen (medadione), Mexel (alkylamines)
- Most "neutralize" prior to discharge

Physical/Chemical Treatment

- Cavitation
- Deoxygenation
- Flocculation
- Heat
- Ultrasound
- Ultraviolet Radiation

Most are Combinations

- e.g., Separation + Chemical
- Two to five phases
- Uptake and discharge



Recent Review of BWMS

- **Lloyd's Register Review of Ballast Water Treatment Technology (2010)**
- **CSLC's Assessment of the Efficacy, Availability, and Environmental Impacts of Ballast Water Treatment Systems for Use in California Waters (2010)**
- **American Bureau of Shipping Ballast Water Treatment Advisory (2010)**
- **Albert et al., Availability and Efficacy of Ballast Water Treatment Technology: Background and Issue Paper (2010)**
- **Efficacy of Ballast Water Treatment Systems; a report by the EPA Science Advisory Board (2011)**

Maturity of BWMS

- **> 50 existing or developing individual BWMSs**
- **17 BWMSs with Type Approval Certification (and growing)**
- **5 categories of BWMS had been demonstrated to meet IMO D-2 and USCG Phase 1 (Draft EPA SAB 2011):**
 - Cavitation + deoxygenation (inert gas)**
 - Filtration + chlorine dioxide**
 - Filtration + electrochlorination (NaOCl)**
 - Filtration + UV**
 - Filtration + UV + TiO₂**



Category of BWMS

Type or Category of BWMS	Type or Category of BWMS
Deoxygenation	Filtration + cavitation + electrochlorination
Deoxygenation + cavitation	Filtration + electrochlorination + ultrasound
Deoxygenation + bioactive agent	Filtration + cavitation + ozone + electrochlorination
Electrochlorination	Filtration + plasma + UV
Electric pulse	Filtration + cavitation + nitrogen + electrochlorination
Filtration	Filtration + hydrocyclone + electrochlorination
Filtration + chlorine	Heat
Filtration + chlorine dioxide	Hydrocyclone + filtration + peracetic acid
Filtration + coagulation	Hydrocyclone + electrochlorination
Filtration + UV	Hydrodynamic shear + cavitation + ozone
Filtration + UV + TiO ₂	Hydrocyclone + filtration + UV
Filtration + ultrasound	Menadione
Filtration + ozone + ultrasound	Mexel
Filtration + UV + ozone	Ozone
Filtration + electrochlorination	Ozone + cavitation
Filtration + UV + ozone + electrochlorination	Shear + cavitation + ozone
Filtration + electrochlorination + advanced oxidation	Shear + cavitation + peracetic acid

From Draft EPA SAB 2011

Type Approved BWMS

Manufacturer	Country	System Name	Technology	Technology Description	Approvals
Alfa Laval	Sweden	PureBallast 2.0/2.0 Ex	combination	filtration + advanced oxidation technology (UV + TiO ₂)	IMO Basic and Final, Type Approval (Norway)
China Ocean Shipping Company (COSCO)	China	Blue Ocean Shield	combination	hydrocyclone + filtration + UV	IMO Basic, Type Approval (China)
Hitachi	Japan	ClearBallast	combination	coagulation + magnetic separation + filtration	IMO Basic and Final, Type Approval (Japan)
Hyde Marine	USA	Hyde Guardian	combination	filtration + UV	Type Approval (U.K.), STEP ¹
JFE Engineering Corp.	Japan	JFE BallastAce	combination	filtration + biocide (sodium hypochlorite) ² + cavitation + neutralizing agent (sodium sulfite)	IMO Basic and Final, Type Approval (Japan)
MAHLE Industrial Filtration GmbH	Germany	Ocean Protection System (OPS)	combination	filtration + UV	Type Approval (Germany)
NEI	USA	Venturi Oxygen Stripping (VOS)	combination	deoxygenation + cavitation	Type Approval (Liberia, Malta, Marshall Islands, Panama), STEP ¹
NK-03	Korea	BlueBallast	chemical	ozone	IMO Basic and Final, Type Approval (Korea)
OceanSever	Norway	OS BWMS	combination	filtration + electrolysis	IMO Basic and Final, Type Approval (Nor.)
OptiMarin	Norway	OptiMarin Ballast System	combination	filtration + UV	Type Approval (Norway)
Panasia Co. Ltd.	Korea	GloEon-Patrol™	combination	filtration + UV	IMO Basic and Final, Type Approval (Korea)
Qingdao Headway Technology Co. Ltd.	China	OceanGuard™ BWMS	combination	filtration + electrolysis + ultrasound	IMO Basic and Final, Type Approval (China)
RWO Marine Water Technology	Germany	CleanBallast	combination	filtration + electrolysis + neutralization	IMO Basic and Final, Type Approval (Ger.)
Sunui CFCC	China	BalClor™ BWMS	combination	filtration + electrochlorination + neutralizing agent (sodium thiosulfate)	IMO Basic and Final, Type Approval (China)
Techcross Co. Ltd.	Korea	Electro-Clean™ System	chemical	electrolysis + neutralizing agent (sodium thiosulfate)	IMO Basic and Final, Type Approval (Korea)
Wilhelmsen/Resource Ballast Technologies	Norway/ South Africa	Unitor BWTS	combination	cavitation + ozone + sodium hypochlorite + filtration	IMO Basic and Final, Type Approval (South Africa)
Wuxi Brightsky Electronic Co. Ltd.	China	BSKY™ BWMS	combination	filtration + UV	Type Approval (China)

From Draft CSLO 2011



Compliance Monitoring for Ballast Water Regulations

- A. How can we monitor BWMS performance?**
- B. How can we detect gross non-compliance with discharge regulations?**
- C. How can we quantify directly non-compliance with discharge regulations?**



G.6 Lisa Drake: Overview of the ETV Generic Protocols for the Verification of Ballast Water Treatment Technology



Overview of the Environmental Technology Verification (ETV) Generic Protocol for the Verification of Ballast Water Treatment Technology

Lisa A. Drake, Edward J. Lemieux,
Timothy P. Wier, and Jonathan F. Grant



U.S. Coast Guard Research and Development Center

28 – 30 JUN 2011



Outline



- Background
- Considerations for biological sampling in compliance testing:
 - Statistics of sampling rare populations
 - Representative sample collection
 - Current viability methods
 - Organisms $\geq 50 \mu\text{m}$ (nominally zooplankton)
 - Organisms $\geq 10 \mu\text{m}$ and $< 50 \mu\text{m}$ (nominally protists)
 - Organisms $< 10 \mu\text{m}$ (bacteria)
 - QA/QC

2

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Environmental Technology Verification (ETV) protocol



- The USCG and the EPA, under the ETV Program, collaboratively developed a protocol for verifying the performance of ballast water treatment systems at land-based test facilities
 - Developed with the input of stakeholders and panels of technical experts
 - Provides guidance on methods for engineering design, statistical rigor, and biological testing
 - Tested the draft Protocol at the Naval Research Laboratory (Key West) in 2006-2007; changes were incorporated
- Final version (5.1) published September 2010
 - A test of 5.1 is underway at two test facilities – USCG RDC's Intercalibration Project

3

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Statistics: Poisson Distribution



- Used to determine the probability of a rare occurrence, e.g., 10 zooplankters m^{-3} or 10 protists mL^{-1}
- The distribution starts at 0, since there is a chance of no occurrences
 - At larger means (> 20), it can be approximated by a normal distribution (and the left side of the distribution curve—previously very close to zero—would be filled out)
- Mean = Variance

4

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Poisson Distribution - Assumptions



- Samples must be representative of the entire volume of interest (e.g., an entire tank or a 10 m^3 volume of the discharge)
- Subsamples must be well mixed
- The cumulative or total count is the key test statistic
- A chi-square transformation can be used to approximate the confidence intervals
- The volume that must be filtered is dependent on
 - Concentration factor (e.g., concentrating $5 \text{ m}^3 \rightarrow 1 \text{ L}$)
 - Number of subsamples analyzed
 - Requisite detection limit

5

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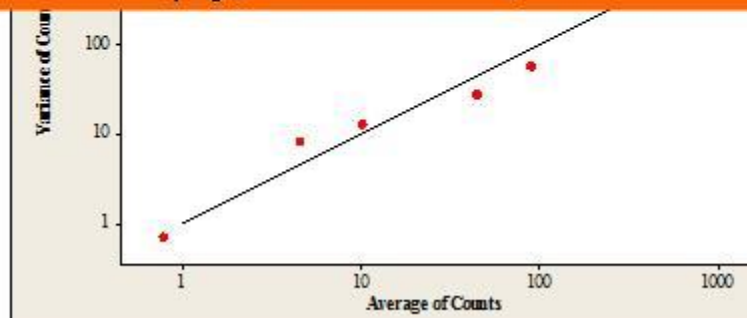
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Poisson Distribution - Validation

At low (IMO and USCG proposed discharge standard) values,
variance $\approx$ mean

The ballast water community shows wide support for this
distribution (e.g., Miller et al. 2011; Nelson et al. 2008)



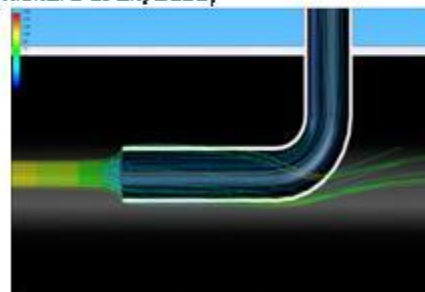
Lemieux et al., 2008

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Representative Sampling

- An 'isokinetic' sampling port is recommended
 - To ensure the sample collected is representative of all constituents (i.e., organisms) in the flow stream
 - Computational fluid dynamics modeling shows that an L-shaped sample wand with a diameter 1.5 – 2.0x the isokinetic diameter yields the optimum flow transitions from the main flow stream (Richard et al., 2008)
- The opening of the sampling pipe should be chamfered to provide a smooth transition into the pipe
- For flow control, diaphragm or similar valve types are recommended



Richard et al., 2008

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Zooplankton Viability



- Samples are analyzed immediately and held at ambient temperature
- The maximum sample hold time should be validated so mortality $\leq 5\%$
- Samples are analyzed microscopically at 10-40x magnification
 - If a fixative is used, validation is advised
 - Zooplankton are tallied as 'live' if they move
 - Unmoving but intact zooplankton are gently touched with a probe and evaluated for up to 10s for movement



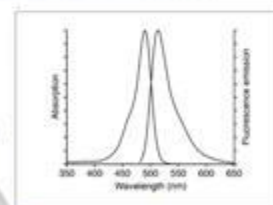
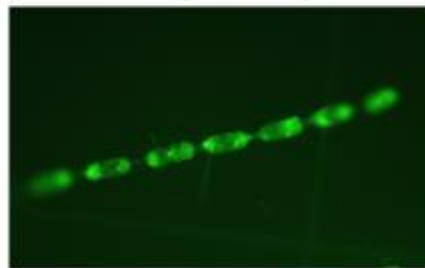
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Protist Viability

Vital Stains + Direct Counting = Viability + # Organisms

Fluorescein Diacetate (FDA) +
5-chloromethylfluorescein diacetate
(CMFDA)



Fluorescein spectra

www.jm-lab.com
www.Moran.com



Protist Viability

- Samples are incubated in the dark for 10 min
- Samples are evaluated for up to 20 min using epifluorescence microscopy
 - Protists are categorized as 'live' if they are stained, moving, or both
- It is recommended to take a photomicrograph of living cells

Protist Viability



- Multi-site validation:
 - False positives (Type I error): 3% to ~40%
 - False negatives (Type II errors): 0% to 1%
- These results highlight the importance of validating any viability method
- To address the Type I errors, killed organisms (i.e., negative controls) can be analyzed



12

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Bacteria Viability



- All methods require grow-out techniques from unconcentrated samples
- Culturable, aerobic, heterotrophs:
 - 10-fold dilution series in Phosphate Buffered Saline or sterile, ambient water
 - Plate onto a minimum of two general-purpose media
 - Incubate for 25 °C for 5 days (3-5 days if colony overgrowth appears imminent on all plates)

13

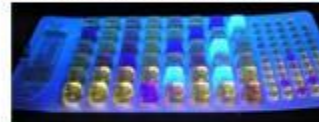
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Bacteria Viability



- Methods are described for three indicators and pathogens, although there are no requirements for concentrations in the Protocol (e.g., treated water)
 - *Escherichia coli*:
 - ▶ EPA Method 1603 (mTEC agar plates) or IDEXX Collert kit
 - *Enterococci*:
 - ▶ Modified EPA method 1106.1 (mEA and BEA agar plates) or IDEXX Enterolert kit
 - *Vibrio cholerae*:
 - ▶ DNA colony blot hybridization (TCBS agar and antibodies)



<http://www.uscg.mil/uscg/rdc/bwds/compliance/Workshop/Agenda>

14

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QA/QC



- **A.5.1 Data Quality Indicators**
- Statistical analyses shall be carried out on data obtained for all performance measurements. As part of the assessment of data quality, six data quality indicators (DQIs) can be used to interpret the degree of acceptability or utility of the data. At a minimum, the QAPP shall include a protocol for assessing the following DQIs, and acceptable limits and criteria for each of these indicators: **representativeness, accuracy, precision, bias, comparability, and completeness.**
- DQIs will be relevant in compliance testing

15

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Acknowledgements



U.S. Coast Guard Environmental Standards Division
(CG-5224)

U.S. Coast Guard Acquisition Directorate – Research
and Development Center



16

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Challenge Water



- The following table has required values for land-based testing

17

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Reference	DOC, POC, TSS (Salt to Fresh Water)		
US ETV v. 5.1 "Challenge water" minimum values For land-based test facilities A minimum of three tests at each of two salinities	S = 28-36 PSU (Marine) DOM*: 6 mg L ⁻¹ POM*: 4 mg L ⁻¹ MM: 20 mg L ⁻¹ TSS*: 24 mg L ⁻¹ T = 4 - 35 °C	S = 10-20 PSU (Brackish) DOM*: 6 mg L ⁻¹ POM*: 4 mg L ⁻¹ MM: 20 mg L ⁻¹ TSS*: 24 mg L ⁻¹ T = 4 - 35 °C	< 1 PSU (Fresh) DOM*: 6 mg L ⁻¹ POM*: 4 mg L ⁻¹ MM: 20 mg L ⁻¹ TSS*: 24 mg L ⁻¹ T = 4 - 35 °C
IMO G8 "Influent water" For land-based testing ⁺ At least 2 cycles (= 5 replicate tests) each with a different salinity, separated by at least 10 PSU	S > 32 PSU DOC: > 1 mg L ⁻¹ POC: > 1 mg L ⁻¹ TSS: > 1 mg L ⁻¹	3-32 PSU DOC: > 5 mg L ⁻¹ POC: > 5 mg L ⁻¹ TSS: > 50 mg L ⁻¹	< 3 PSU DOC: > 5 mg L ⁻¹ POC: > 5 mg L ⁻¹ TSS: > 50 mg L ⁻¹

*DOM as DOC; POM as POC
 **TSS = POM + MM

⁺Not addressed for shipboard testing other than the following parameters must be measured in source water: T, S, POC, TSS



Draft Environmental Technology Verification Protocol, v. 3.3

Count Data

Scaled Densities and Confidence Intervals
(Assuming the entire sample volume is concentrated to 1L and 20 mL is sub-sampled from the concentrate for analysis)

SAMPLE VOLUME=			V = 1 m ³		V = 2 m ³		V = 10 m ³		V = 30 m ³		V = 80 m ³	
Count	95% Upper Bound	Upper Bound / Count	Mean Density (m ⁻³)	95% Upper Bound (m ⁻³)	Mean Density (m ⁻³)	95% Upper Bound (m ⁻³)	Mean Density (m ⁻³)	95% Upper Bound (m ⁻³)	Mean Density (m ⁻³)	95% Upper Bound (m ⁻³)	Mean Density (m ⁻³)	95% Upper Bound (m ⁻³)
0	3.00		0	150	0	50	0	15	0	5	0	2.5
1	4.74	4.74	50	257	17	79	5	24	2	8	1	4.8
2	8.33	3.15	100	315	33	105	10	31	3	10	2	5.2
3	7.76	2.68	150	386	50	129	15	39	5	13	3	6.5
4	9.16	2.29	200	458	67	153	20	46	7	15	3	7.6
5	10.61	2.10	250	529	83	175	25	53	8	18	4	8.8
6	11.84	1.97	300	592	100	197	30	59	10	20	5	9.9
7	13.16	1.88	350	657	117	219	35	66	12	22	6	11.1
8	14.43	1.80	400	722	133	241	40	72	13	24	7	12.0
9	16.71	1.76	450	785	150	262	45	79	15	26	8	13.1
10	18.98	1.70	500	848	167	283	50	85	17	28	8	14.1
11	18.21	1.68	550	911	183	303	55	91	18	30	9	15.2
12	19.44	1.62	600	972	200	324	60	97	20	32	10	16.2
13	20.87	1.60	650	1033	217	344	65	103	22	34	11	17.2
14	21.89	1.60	700	1094	233	365	70	109	23	36	12	18.2
15	23.10	1.54	750	1155	250	385	75	115	25	38	13	19.2
16	24.30	1.52	800	1215	267	405	80	122	27	41	13	20.3
17	26.60	1.60	850	1275	283	425	85	127	28	42	14	21.2
18	28.89	1.48	900	1335	300	445	90	133	30	44	15	22.2
19	27.38	1.47	950	1396	317	465	95	139	32	46	16	23.2
20	29.08	1.46	1000	1453	333	484	100	145	33	48	17	24.2

Lemieux et al., 2009

Poisson Distribution: Zooplankton



- Assume (for organisms $\geq 50 \mu\text{m}$) the desired minimum precision at the IMO discharge standard is:
 - The upper bound of the chi square statistic should not exceed 2x the observed mean (= a CV of 40%), then
 - A count of 6 organisms is required
 - If more than 6 organisms are counted, the water exceeds the discharge standard



Poisson Distribution: Protists

Since the protist volumes are relatively small, the issue of filtering large volumes of water applies only to zooplankton

n is the number of 1 ml subsamples analyzed
(ETV Protocol v. 3.3)

n=	2	3	4
Standard Limit (individuals ml ⁻¹)	SAMPLE VOLUME REQUIRED (l)		
0.1	600	400	300
1	60	40	30
10	6	4	3

G.7 Marcel Veldhuis: Review of International Testing Efforts



NIOZ Royal Netherlands Institute for Sea Research

*Testing Ballast Water Technologies,
Compliance Enforcement and Research*

Marcel Veldhuis
Department of Biological Oceanography
Royal Netherlands Institute for Sea Research
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1790 AB, Den Burg, TEXEL
The Netherlands
Marcel.Veldhuis@nioz.nl

NIOZ is part of the Netherlands Organisation for Scientific Research (NWO)





outline

- Introduction of *Royal*-NIOZ and history BWT activities
- Why is NIOZ so unique for land-based testing
- Mission of academic research institute in BWT testing
- The North Sea Ballast Water Opportunity project (NSBWO)
- Standard-D2, Phase-2 and the "NIOZ approach"
- Compliance Enforcement and Port State Inspection
- Future plans

- Representing view of NIOZ and not necessary of NA's



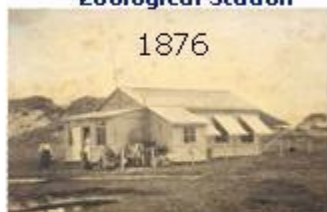
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Royal Netherlands Institute for Sea Research

Zoological Station

1876



Texel - NIOZ



Den Helder - Zoological Station/NIOZ

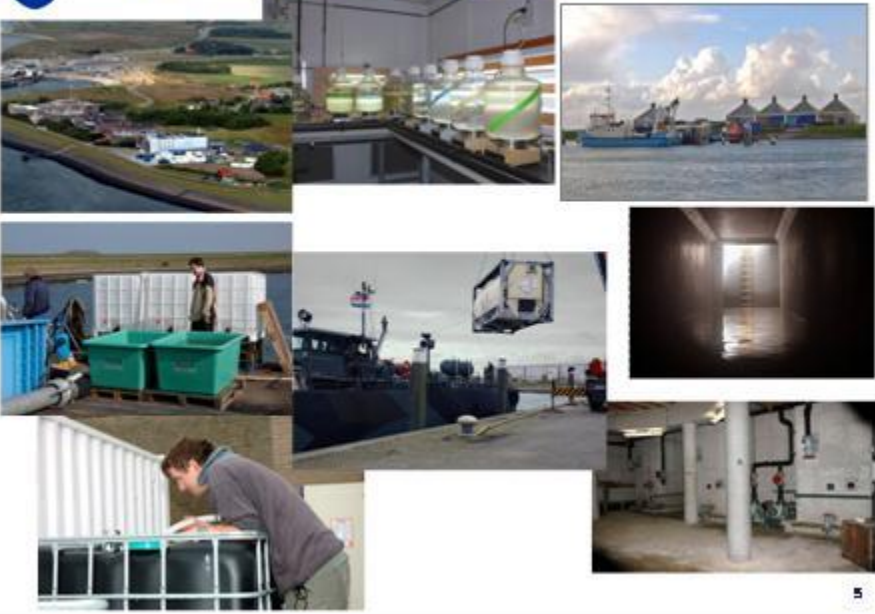
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3



 Why is NIOZ so unique for land-based testing



5

 Why is NIOZ so unique for land-based testing

Be Nice to us

With a minimum of effort we can make things very difficult



6



Mission academic research institute in BWT testing

- Academic (oceanographic) institute; publish all research in international peer reviewed journals
- Developing generic and specific tools and technologies
- Viruses, bacteria, phytoplankton and zooplankton (life stages)
- Holistic approach multiple tools to asses numbers and viability, applying state of the art tools and technologies
- Critical review of G8/G9 and legal aspects,
- Input for future legislation based on acquired data
- Specialized in innovative research ('silver bullets')

7



North Sea Ballast Water Opportunity Project

An Interreg IVB program (www.NorthSeaBallast.eu)

Goal: facilitate ratification of IMO Ballast Water Management Convention
2009 - 2013

- Overcome barriers:
- Boost technological innovation
- Stimulate maritime industry (ca 40% BWT systems)
 - !! Market 8 billion euro !!
 - 40% of industry in NS region
- Common standards / uniform implementation
- Involvement of stakeholders (public & private)
- Knowledge exchange and dedicated research
- Develop expertise and knowledge center

Where policy meets science and industry





Harmonization

- National Administration (7 NS countries, chaired by Germany; BSH)



EU-EMSA (Brian Elliot)
- Ballast water exchange NS and Baltic area
- PSC and exemptions
- Within the NSBWO project (DHI, NIVA, Wageningen-Imares, GoConsult, NIOZ); including NA

> < ((((*>

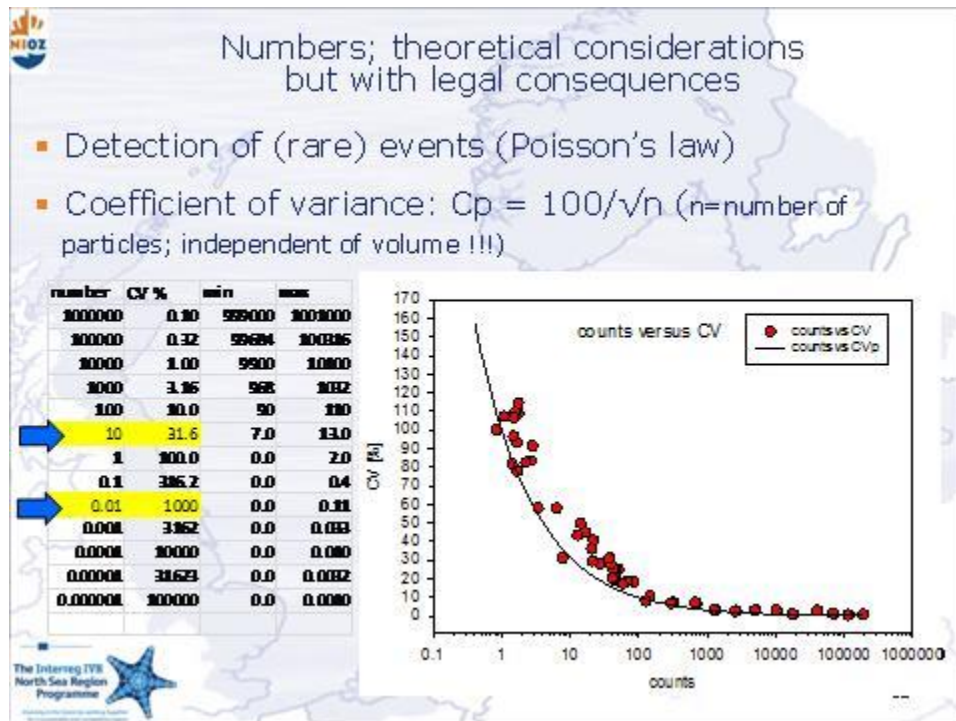
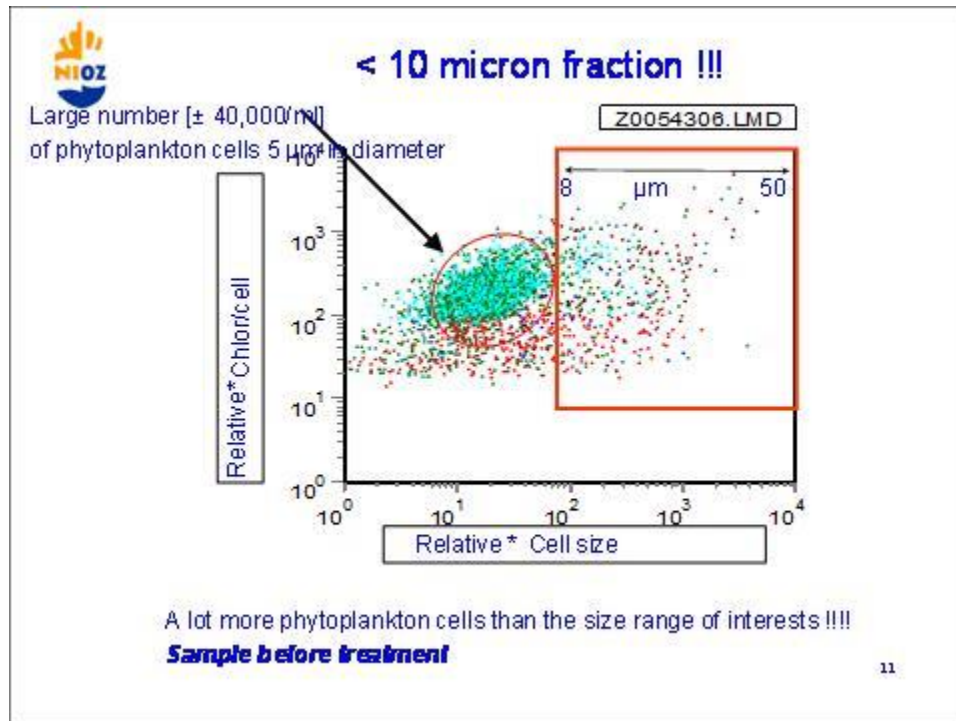
- 2010: Extension NSBWO, including MERC, GSI, Golden Bear
- IMO/Globallast Test Facilities Forum (global, but excludes NA)

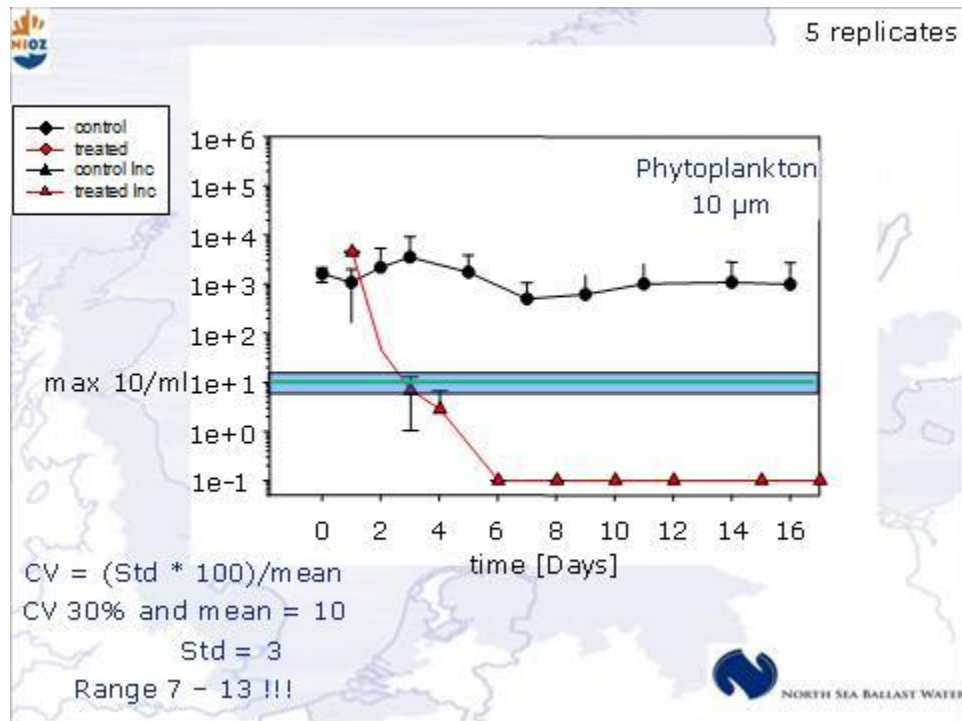
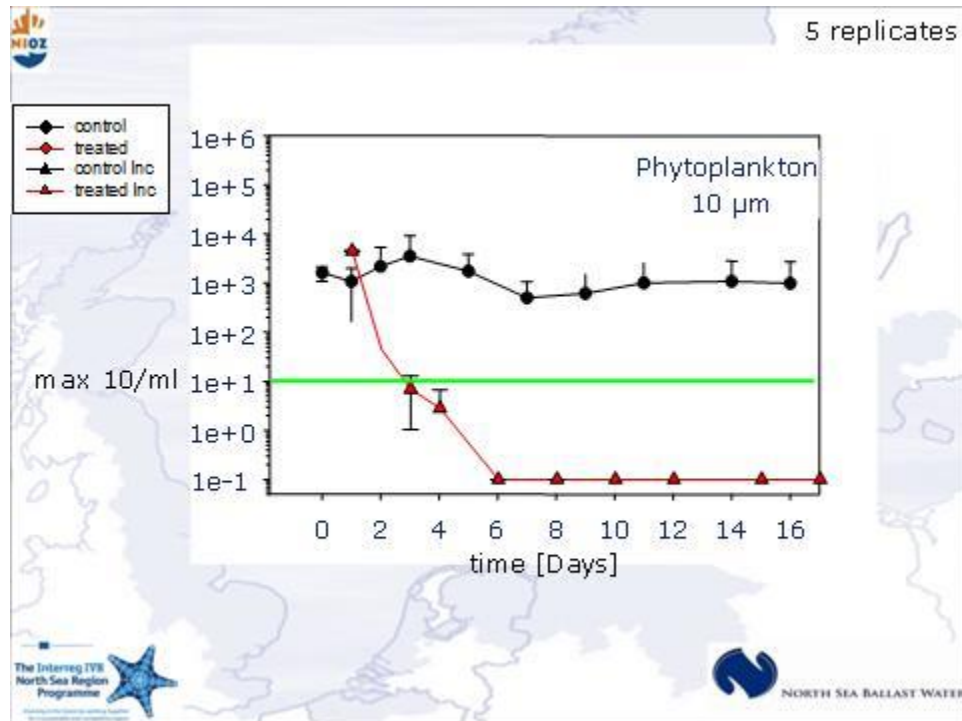


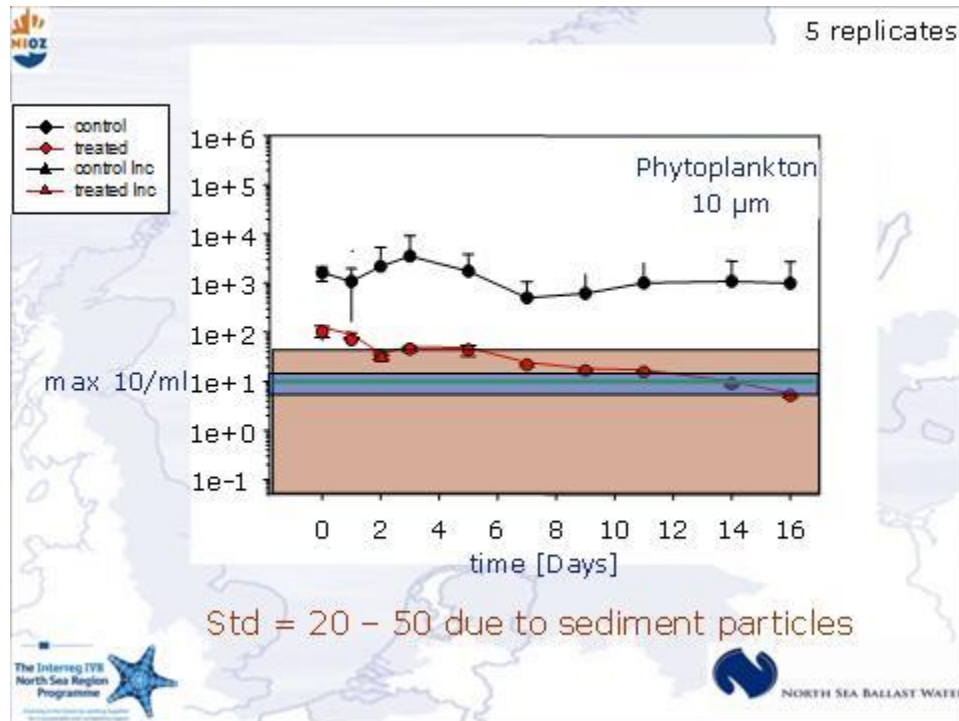
 - Comparison/intercalibration of test protocols (3 workshops)
 - > 50 micron (Texel, autumn 2010)
 - 10 – 50 micron (Moss Landing, CA, spring 2011)
 - < 10 micron (?)

IMO D2-Standard, USCG Phase-1 and USCG Phase-2 , and NIOZ recommendation

Management approach	IMO Standard -D2	USCG Phase 2	NIOZ	nature	Log reduction
Org. > 50 µm	< 10 /m ³	<0.01 /m ³	n.d.	10 ¹ -10 ⁵ /m ³	1 – 4 3 – 7
Org. 10-50µm	<10 /mL	< 0.01 /mL	n.d.	10-10 ⁴ /mL	1 – 3 3 – 6
Org. ≤ 10 µm Phytoplankton	N/S	N/S	n.d.	10-10 ⁶ /mL	N/S
bacteria	-	< 10 /mL	-	10 ⁵ -10 ⁸ /mL	N/S 4 – 7
viruses	-	< 100/mL	-	10 ⁴ -10 ⁹ /mL	N/S 2 – 7
n.d.= non-detectable	N/S= no standard				







Compliance Enforcement The Pragmatic approach

- aspects to be considered (practical constraints)
 - Dead volume, piping, tanks, BWTS (technical solution)
 - Average sample, representative volume/time of deballasting
- Biological versus statistical robustness
- Sampling: fixed volume, replicates
- Analyzing: fixed volume (exact 1 ml, concentration steps)
- (semi) automated analysis, data archiving
- regrowth of organisms in ballast water tank over time
- Indicative versus full scale sampling

The Interreg IVB North Sea Region Programme

European Union

The European Regional Development Fund



Sample size (theory) E. Meshabi (BWO meeting Malmö 2010).

The formula

$$n = \frac{N z_{\alpha/2}^2 P(1-P)}{(N-1)e^2 + z_{\alpha/2}^2 P(1-P)}$$

n = Sample size or the number of 1m³ ballast water samples that needs to be taken in order to have a true representation of the ballast water discharged.
 N = "population" size or total amount of ballast water discharged, in m³.
 P = probability of success or the estimated proportion of an attribute that is present in the "population". In this context it refers to the probability of having ≥10 viable organisms/m³ of ballast water sampled.
 $(1-P)$ = is the probability of failure (Q) that represents the probability of having < 10 viable organisms/m³ of ballast water sampled.
 $z_{\alpha/2}$ = confidence coefficient for a given confidence interval (for a confidence level of 95%; $z_{\alpha/2}=1.96$)
 e = standard sampling error: error that is assumed while the sampling is carried out. A value of 1-5% is considered as normal.

 Newcastle University
 The Interreg IVB North Sea Region Programme
 NORTH SEA BALLAST WATER

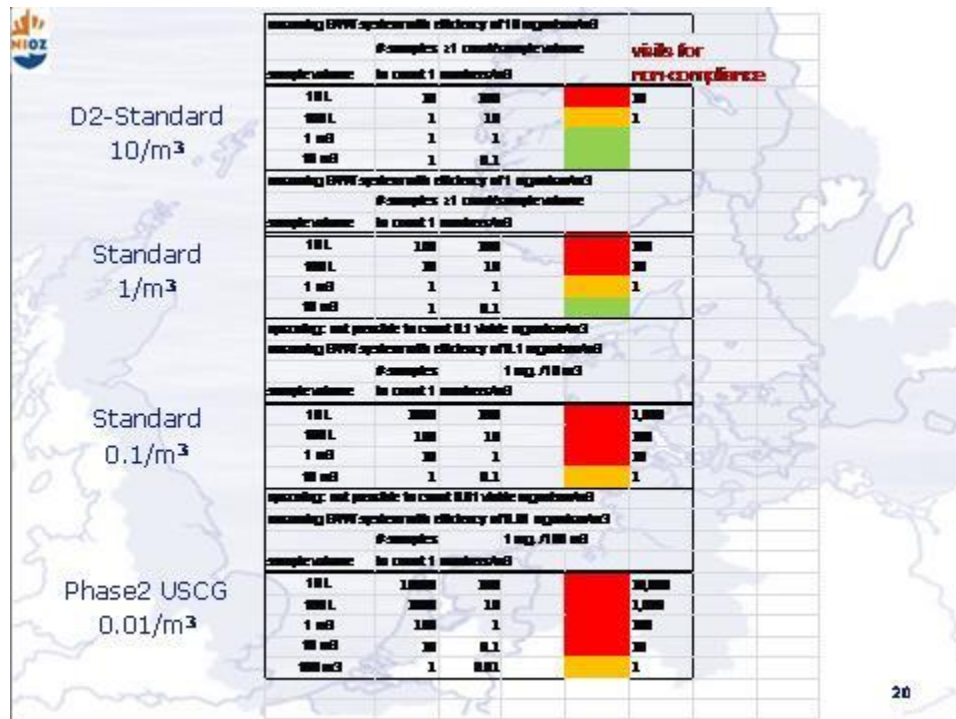
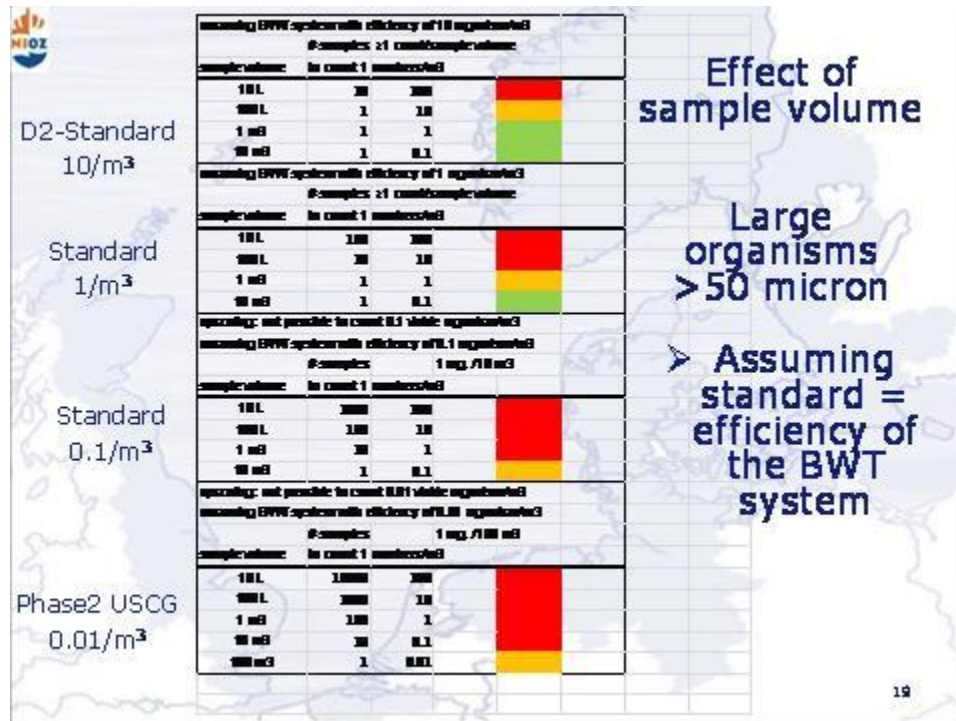
Experiences NIOZ

- Main guidance G8-guidelines (must strictly obeyed)
 - sample volume and replicates based on
 - 3 replicates of 1 m³ for >50 micron
 - 3 replicates of 1 ml for < 10 micron





 The Interreg IVB North Sea Region Programme
 NORTH SEA BALLAST WATER





G.8 Robin Danesi: Overview of EPA water quality testing with regards to the Clean Water and Safe Drinking Water Acts and related system verification

Developing, Validating and Approving Clean Water Act Analytical Methods

Robin Danesi
EPA Office of Wastewater Management
June 28, 2011



Office of Water



Developing, Validating and Approving Clean Water Act Analytical Methods

Robin Danesi
EPA Office of Wastewater Management
June 28, 2011



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Who Uses Methods and How?

- EPA and States to characterize pollutants to establish regulatory priorities, and develop limitations or standards
- States and POTWs to establish permit limits and monitor compliance
- Industry and municipalities to demonstrate compliance with permits
- Environmental testing laboratories
- Citizens groups and others to monitor water quality



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Pertinent Statutes & Regulations

- CWA Section 304(h) requires the Administrator to *"promulgate guidelines establishing test procedures for the analysis of pollutants ..."*
 - Approved CWA analytical methods - 40 CFR Part 136,
- Method Developers use the Alternate Test Procedure (ATP) program described at 40 CFR 136.4 – 136.5
- The National Technology Transfer and Advancement Act (NTTAA) covers standards published by voluntary consensus standards bodies (VCSBs), such as ASTM, International and the Standard Methods Committee



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Contents of an EPA Method

- EPA format has 18 sections that include
 - Scope: Analytes, Matrices
 - Equipment, Supplies, Reagents, Standards
 - Sample Collection, Shipment, Storage
 - Sample Prep
 - Analysis
 - Data Analysis and Reporting
 - Performance and Quality Control Specifications
 - Waste Disposal and Safe practices



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Life Cycle of an EPA-Developed CWA Method

1. Develop
2. *Publish, Peer Review and Web-Post Methods*
3. Multi-lab Study – several matrices
4. Revise, WebPost and Peer Review
5. Propose and Take Comment
6. Promulgate – Available for Compliance



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Method Validation

- Assess whether method instructions are clearly written and easy to follow
- Assess the quality of data produced by several labs
- Establish method performance characteristics (e.g., precision, accuracy, sensitivity, selectivity, calibration) in different labs
- Demonstrate whether the method “works” for different types of environmental samples



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Multi-lab Validation

- Laboratories test a number of appropriate sample types to characterize method performance
- We prepare study plans and conduct peer reviews prior to validating the method
- We use the study results to evaluate method performance and develop performance criteria



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Approval of CWA Methods at Part 136

- Requires rulemaking with notice and comment
 - Part 122 allows non-Part 136 methods in NPDES permits
- Drivers - when there are a sufficient number of new methods, new pollutants or modified Part 136 methods
 - *On the CWA Methods page we may describe potential actions*
- Publish description of proposed actions in *Federal Register* for public comment
 - Actions may include more than just methods
- Final rule (usually in a year) responds to public comment and announces final actions



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Proposed Amendments to Part 136

- Add new or revised method for chemicals and microbes
- Add more examples of CWA implementation of a performance approach that allows you to modify and use approved methods without prior EPA review
- Specify essential QC elements
- Revise sample collection, preservation & storage requirements
- Go Green – remove method text printed in Part 136 Appendices



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Differences in Method Approval Authority

- CWA Program
 - National ATPs require notice & comment rulemaking
 - EPA Regions may approve a method for Limited Use
 - Regulations explicitly allow you to modify and use methods without EPA review or approval – 40 CFR 136.6
 - Compliance monitoring: may use a non-Part 136 method
- SDWA Program
 - National ATPs only; Limited Use ATPs are not available
 - Statute allows Expedited Method Approval (CWA does not)
 - These are published in a special section of Part 141
 - Compliance monitoring generally requires Part 141 Methods



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CWA Methods Information

- **WWW.EPA.GOV/WATERSCIENCE/METHODS/**
 - Facts Sheets, FAQs, Rule Text, EPA Methods
- Email us at: **OSTCWAMETHODS @EPA.GOV**
 - For lab accreditation or permit questions consult your permitting or accrediting authority.
- **CWA Methods Team**
 - Dr. Maria Gomez-Taylor
 - Ms. Meghan Hessenauer
 - Ms. Marion Kelly
 - Dr. Robin Oshiro
 - Mr. Lemuel Walker



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Other Sources

-
- **CWA Methods Team**
 - Dr. Maria Gomez-Taylor
 - Ms. Meghan Hessenauer
 - Ms. Marion Kelly
 - Dr. Robin Oshiro
 - Mr. Lemuel Walker



Office of Water



G.9 Nick Welschmeyer: Indirect measures of non-compliance



CAL MARITIME
GOLDEN BEAR FACILITY



Moss Landing Marine Laboratories



THE GLOSTEN ASSOCIATES
Consulting Engineers Serving the Marine Community

***“Ballast Water Compliance Testing:
a) Prospects for a rapid, ‘gross exceedance’ test
b) Prospects for rapid, quantitative testing
at regulatory levels”***

Presented to:
USCG Ballast Water Compliance Workshop
June 27-30, 2011; Groton CT

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Moss Landing Marine Laboratories



Ballast Regulations

The *Big* Dilemma:

- Individual ‘living’ organisms are the propagules of biological invasion
- Therefore, numeric counts of ‘living’ organisms are the logical unit of measurement for ballast water regulations

Unfortunately, numeric ‘live’ counts are:

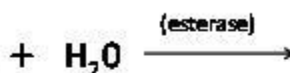
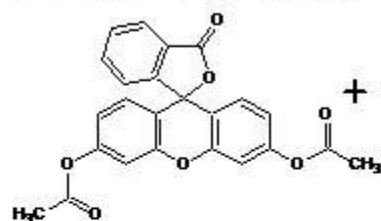
- Tedious (microscopy)
- Subjective (human error)
- Statistically-challenged (low counts)
- Logistically-challenged (organism die-off; huge volumes)
- Impossible for some plankton size classes (unicells)



What we need is an analytical technique for plankton viability that:

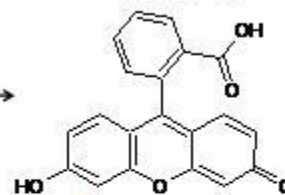
- Shows linear response to living biomass
- Has adequate sensitivity to detect <10 living organisms/mL (10-50 μ m)
- Relies on simple biochemical protocol
- Has rapid analysis time (<2 h)
- Has predictable temperature response
- Can be terminated and stored for later analysis
- Requires portable instrumentation for field technician or ship engineer (pocket-sized)
- Requires minimum technical training (pre-packaged 'test kit')
- ...and, has potential for online, automated implementation on ballast treatment system

Fluorescein diacetate (colorless)



(esterase)

Fluorescein



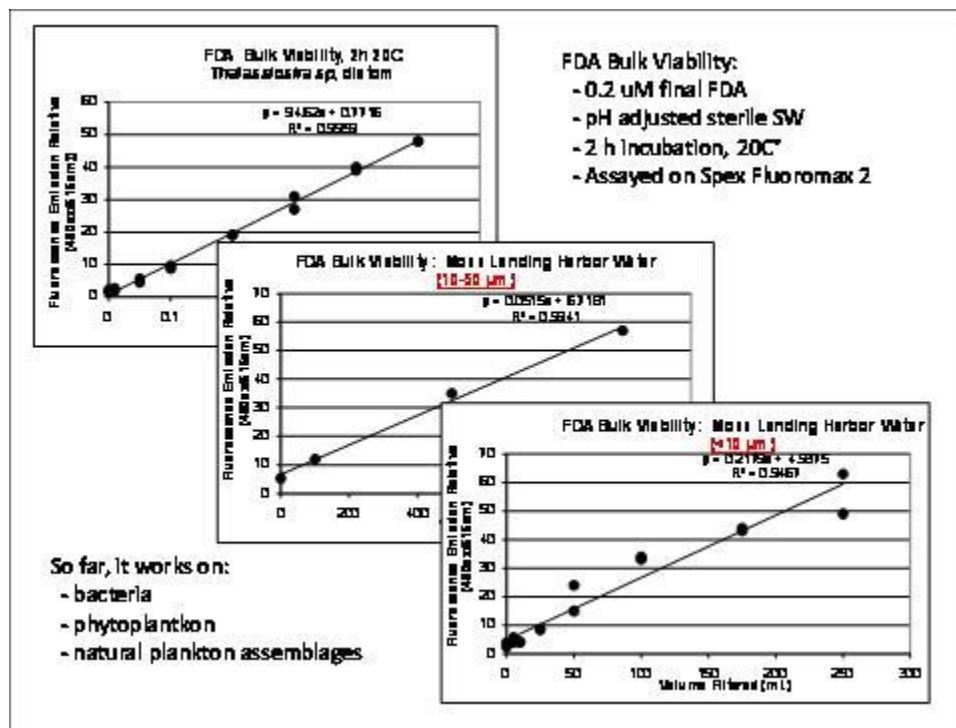
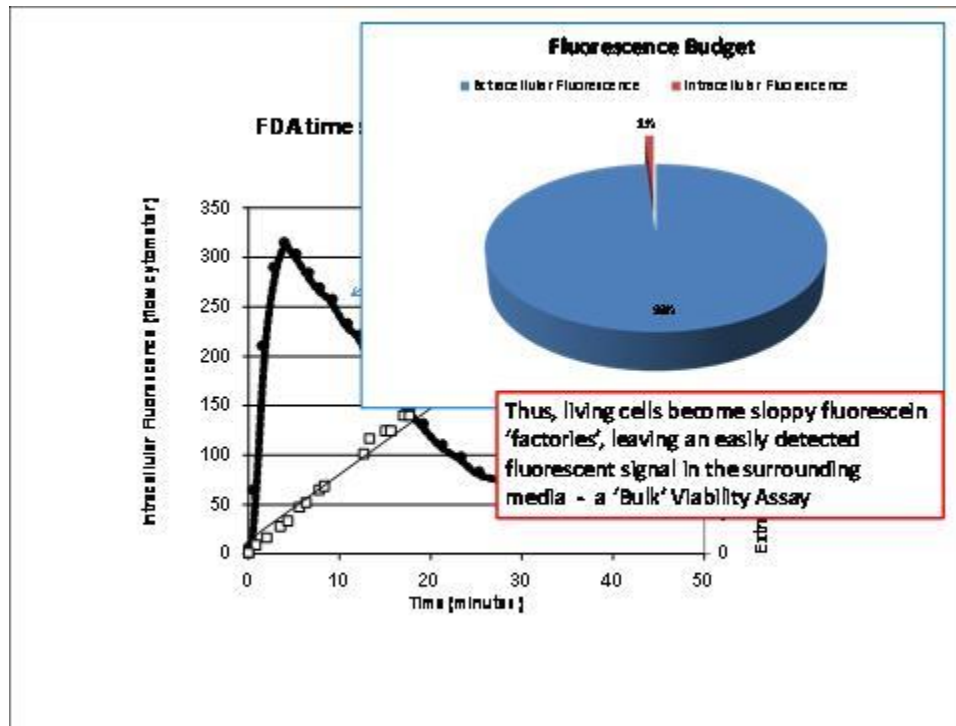
Good point:

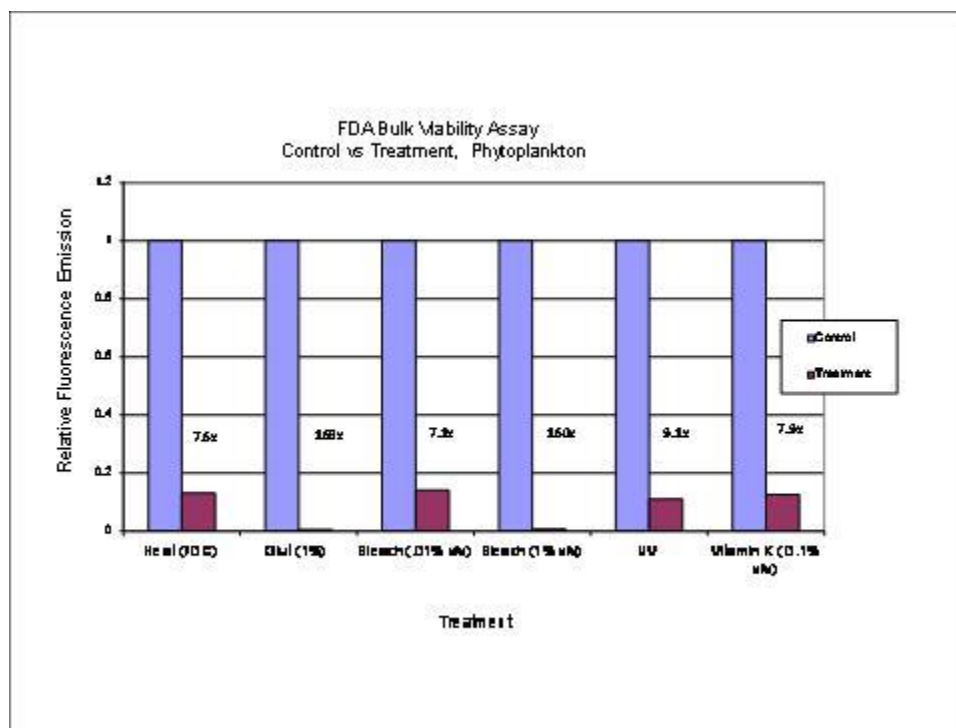
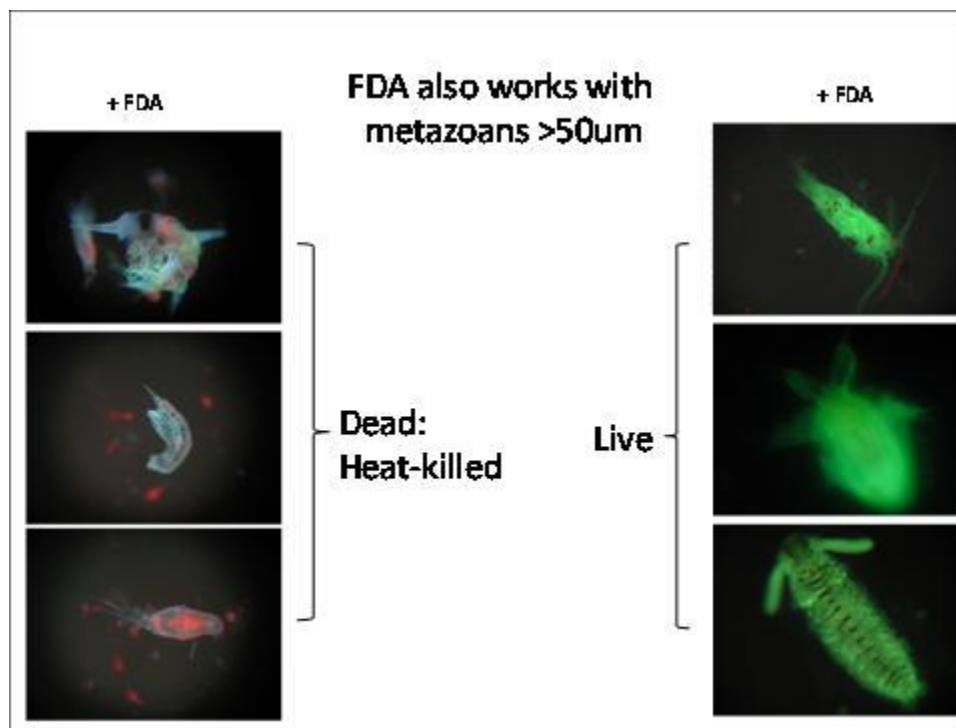
FDA tags live organisms, on the basis of viable esterase

Bad Point:

Fluorescein leaks out of cells







Strategy for defining compliance target

- well defined regulatory standards
- high numeric density
- minimum volume requirement
- ample 'viability' signal
- traceable lab calibration



{no standards} < 10 μm < {COMPLIANCE TARGET} < 50 μm < {too rare}

No regulations
(other than specific
pathogens)

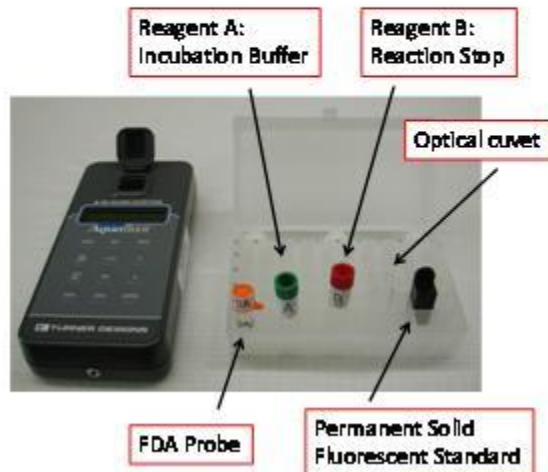
Excessive volume requirements

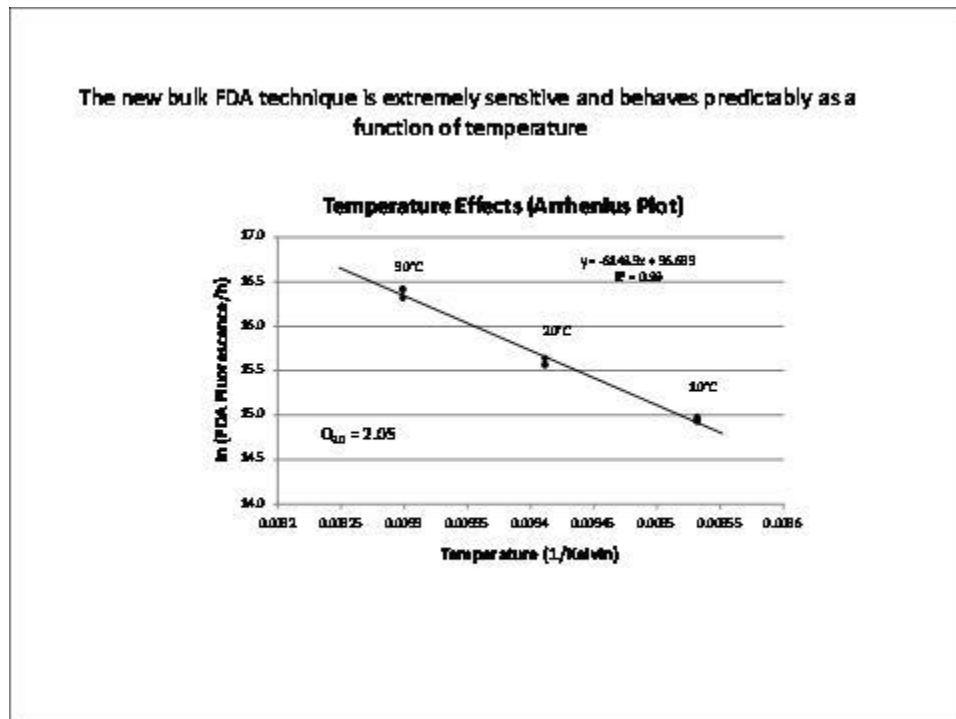
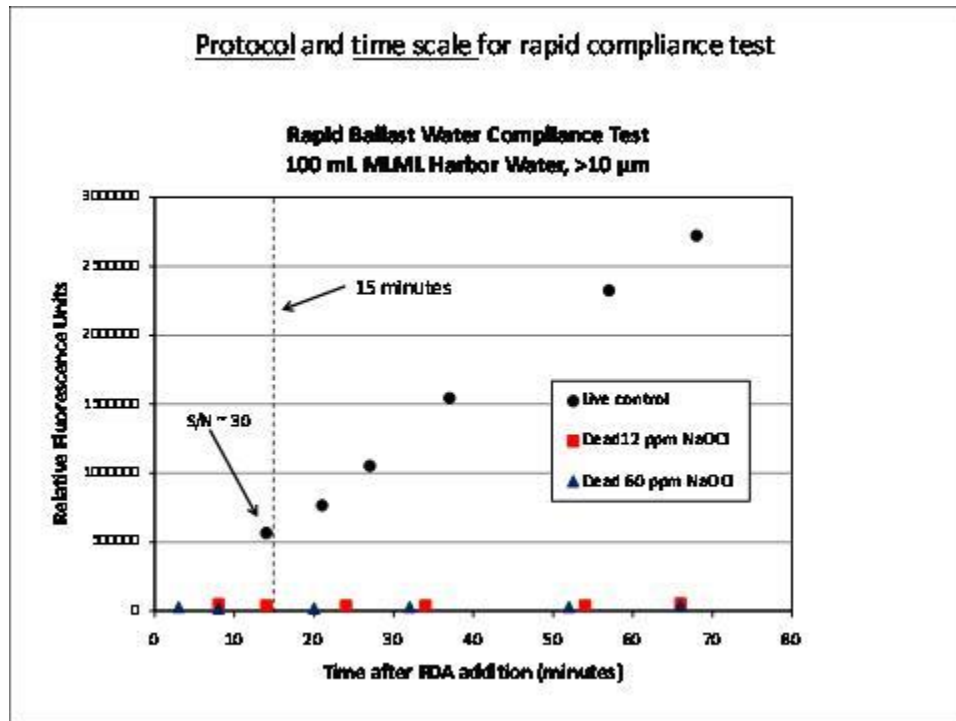
Protocol and time scale for rapid compliance test

1. Gravity filter (<500 mL)
onto 10 μm 25mm filter:
(Killed sample, Live sample)

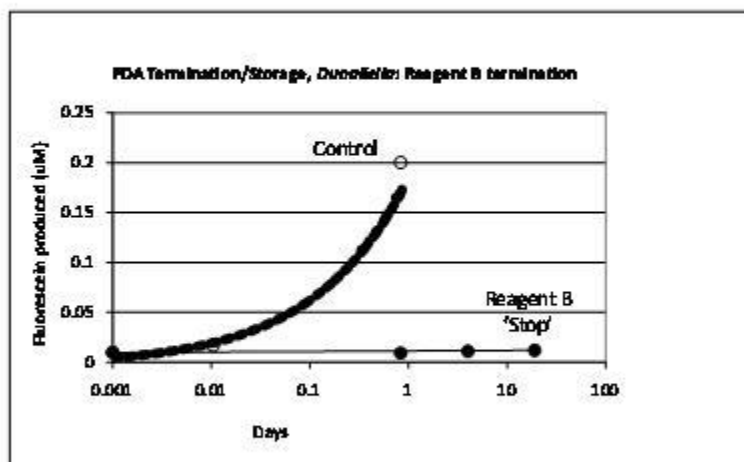
2. Add filter to optical cuvet
prefilled w/ Reagent 'A'
Incubation solution

3. Wait 10 minutes. Read
fluorescence on hand-held
fluorometer



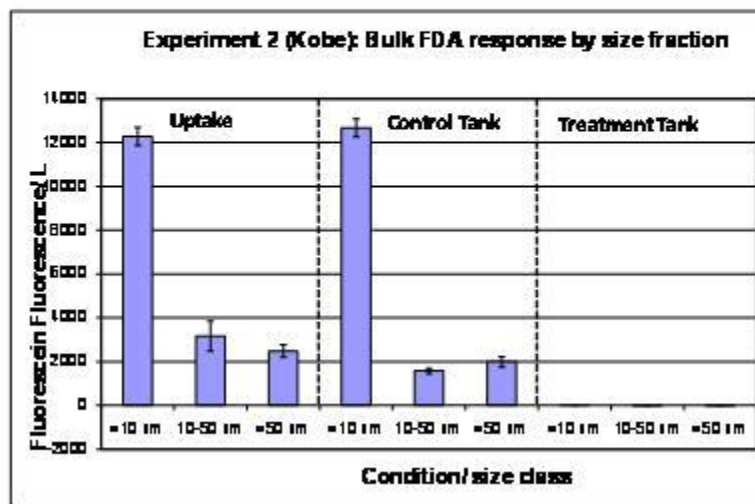


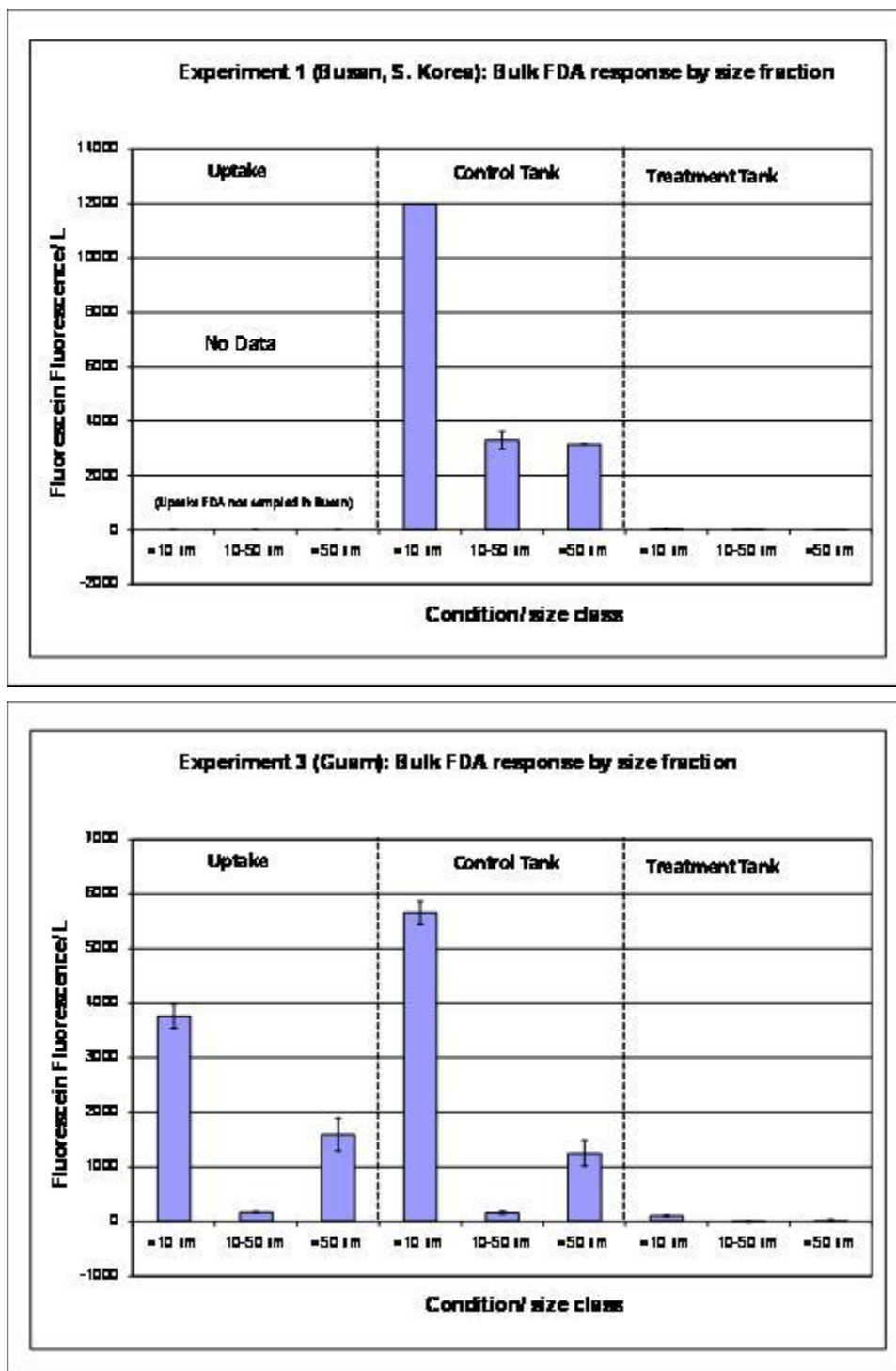
Bulk FDA viability assay can be stopped and stored for later readings



The bulk FDA viability assay provides a means of testing all required planktonic size categories with one method.

The full test can be completed in one hour.

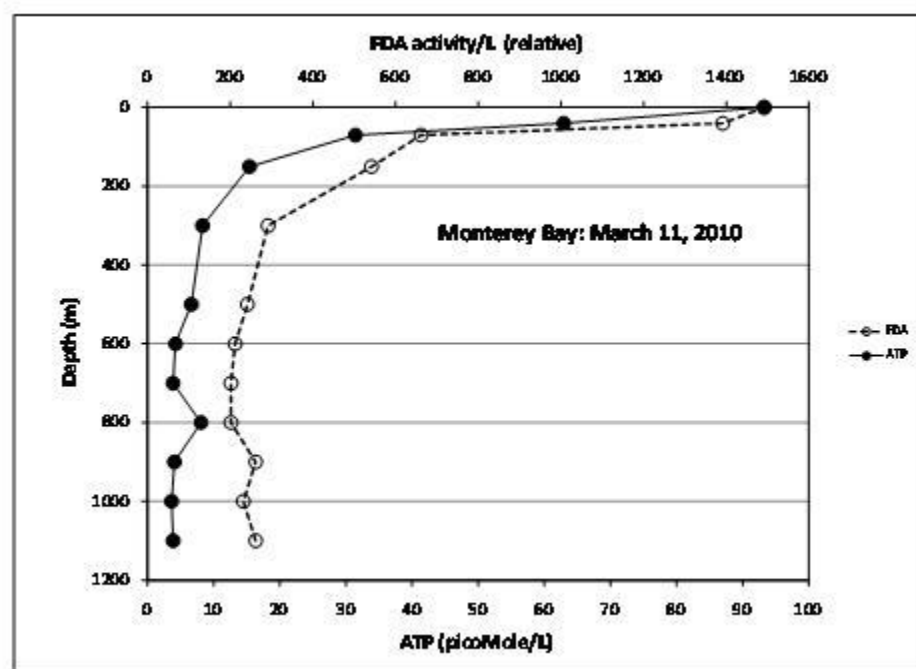


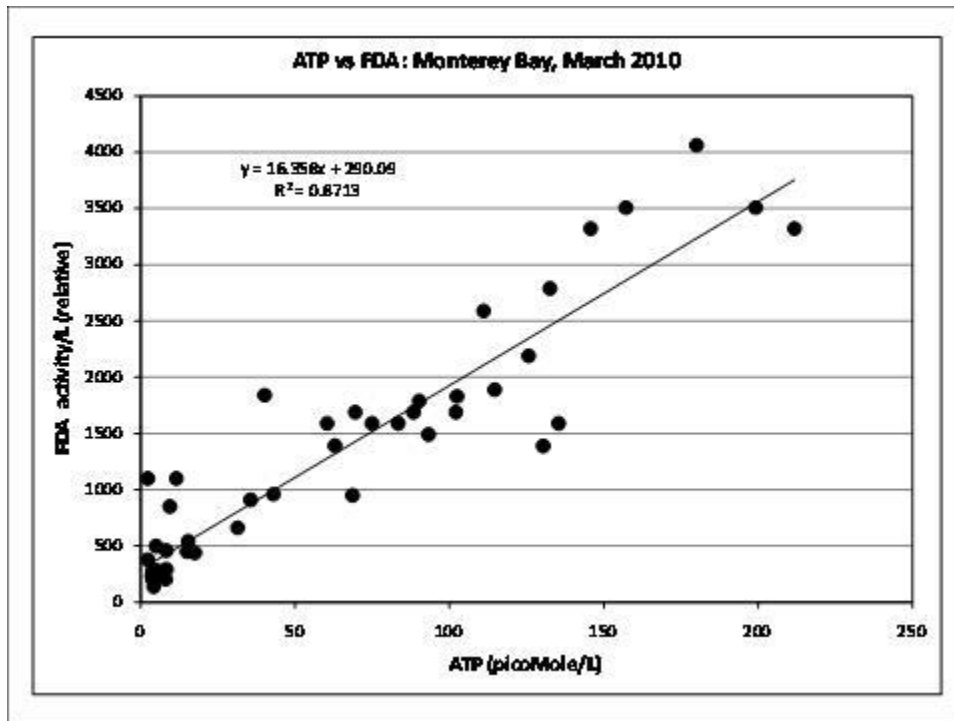


So...

Can we relate FDA-based fluorescein production to NUMERIC COUNTS??

Well... yes we can (*with some discussion*)





**ATP as a proxy for living biomass
(the concept of 'living carbon')**

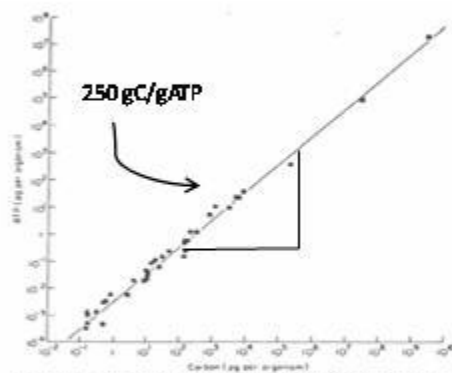


Fig. 2. Relationship of ATP (adenosine triphosphate) content of planktonic organisms to their carbon content. Units are picograms (10^{-12} g), for bacteria (▲), phytoplankton algae (●) and two zooplanktonic copepods (■). (From Hodin-Harmon, '97)

- ATP is present in all living organisms
- ATP rapidly degrades upon cell death
- Carbon/ATP ratio is relatively constant among unicellular and multicellular plankton
- POC/ATP ratio ~ 250 gC/gATP
- Therefore, ATP can be used to estimate 'living POC' concentrations



The Concept of 'Living Carbon'

(Holm-Hansen 1972; Karl 1993)

All living unicellular organisms (bacteria, phytoplankton, colorless protists) have a relatively constant POC/ATP ratio: ~250 gC/gATP

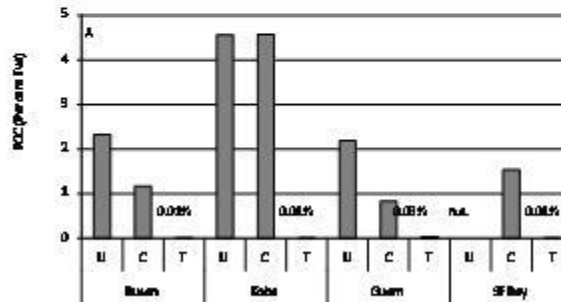
Thus, from measurements of:

- 1) total POC (live + dead) (CHN combustion analysis) and
- 2) ATP-derived 'living' POC (ATP luminescence assay),

we can calculate the Percent Living Carbon:

$$\text{Living Carbon \%} = [(gATP \times 250) / gPOC] \times 100$$

Figure 14: Living Carbon as percentage of total POC

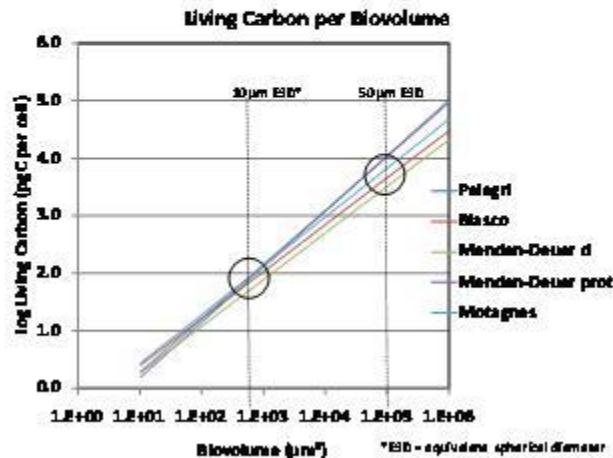


Can we relate biomass to numeric concentrations?

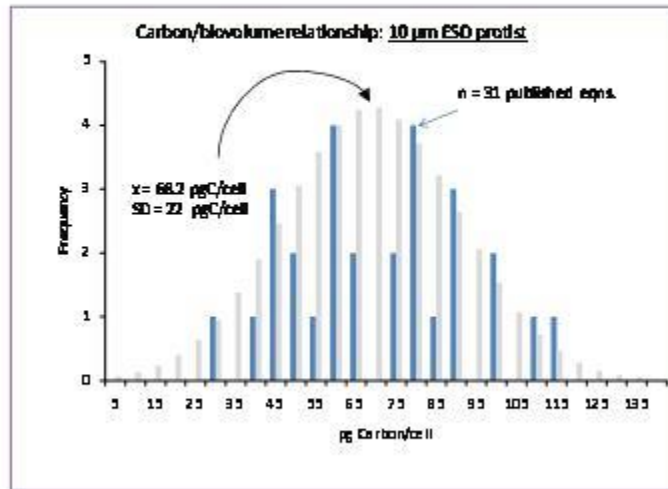
There are many examples of biovolume to living carbon (POC) conversions

Bretzke 1983
 Bonheim et al. 1987
 Reger et al. 1996
 Gundersen et al. 2002
 Herbert 1990
 Hildebrand et al. 1999
 Kuer 1994
 LaRocque et al. 1993
 Lee 1993
 Lee and Fuhrman 1987
 Menden-Deuer and Lessard 2000
 Montagne et al. 1994
 Montagne and Franklin 2001
 Mullin et al. 1966
 Nagata 1986
 Nagata and Watanabe 1990
 Norland 1993
 Pelagri et al. 1999
 Poth et al. 2001
 Putt and Stoeckel et al. 1989
 Roche and Duncan 1983
 Strathman 1967
 Theilacker and Sandegard 1993
 Thorne et al. 1997
 Verity et al. 1992
 Verity and Langdon 1984

Example: $\log \text{Carbon (pgC/cell)} = 0.94 \cdot \log \text{vol (}\mu\text{m}^3) - 0.6$ (Strathman 1967)



'Living carbon' for any cell size is reasonably predictable



ATP-derived numeric cell concentrations

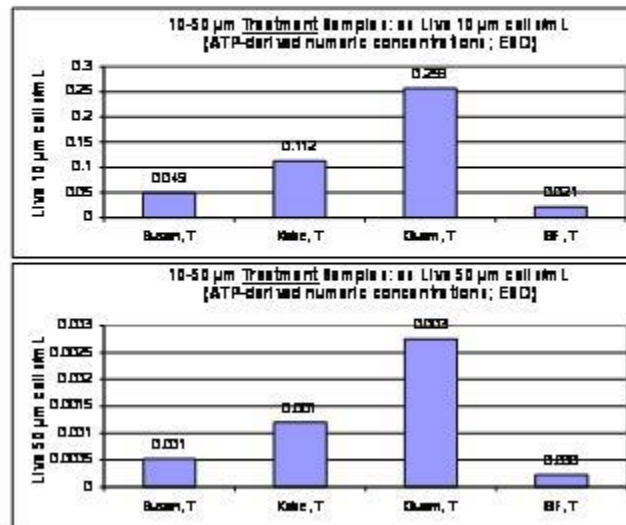
Assumptions:

10 μ m ESD (2768 cells/ng ATP)

50 μ m ESD (29.6 cells/ng ATP)

All ATP-based densities pass
IMO Standards for 10-50 μ m

Corroborated by:
- Flow Cytometry
- Epifluorescence Microscopy
- Chl-based MPN grow out





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