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2009 ASSESSMENT OF THE EFFICACY, AVAILABILITY AND ENVIRONMENTAL IMPACTS OF BALLAST WATER TREATMENT SYSTEMS FOR USE IN CALIFORNIA WATERS

PRODUCED FOR THE
CALIFORNIA STATE LEGISLATURE

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EXECUTIVE SUMMARY

The Coastal Ecosystems Protection Act (Act) of 2006 expanded the Marine Invasive Species Act of 2003 to more effectively address the threat of nonindigenous species introduction through ballast water discharge. The Act charged the California State Lands Commission (Commission) to implement performance standards for the discharge of ballast water and to prepare a report assessing the efficacy, availability, and environmental impacts, including water quality, of currently available ballast water treatment technologies. The performance standards regulations were adopted in October 2007, and the first technology assessment report was approved by the Commission in December 2007 (see Dobroski et al. 2007). In response to the recommendations in the 2007 report, the California Legislature passed Senate Bill (SB) 1781 (Chapter 696, Statutes of 2008) which delayed the initial implementation of the performance standards from January 1, 2009 to January 1, 2010. Additionally, SB 1781 required an update of the technology assessment report by January 1, 2009. This report summarizes the Commission's conclusions on the advancement of ballast water treatment technology development and evaluation during 2008, discusses future plans of the Commission's Marine Invasive Species Program regarding the implementation of California's performance standards for the discharge of ballast water, and makes recommendations to the Legislature.

Significant progress has been made in the development of treatment systems since the previous technology assessment report (see Dobroski et al. 2007). Both the quantity and the quality of the recently received data on system performance attest to this fact. The field of treatment technology performance evaluation, however, has not kept pace with the rapidly evolving ballast water treatment industry. Scientific methods to assess the concentration of viable organisms present in ballast water discharge still must be developed so that Commission staff may rapidly assess vessel compliance with the ballast water performance standards.

California's standards for bacteria and viruses pose a significant challenge, as no widely accepted methods exist to both quantify and assess the viability of all bacteria and viruses in a sample of ballast water discharge. The best available technique for bacterial assessment involves the use of a subset or proxy group of organisms to represent treatment of bacteria as a whole. While this technique is not without some debate, it is scientifically supported by many experts in microbiology and technology assessment (see Appendix A). The viruses pose a greater challenge. Without strong evidence for the selection of proxy organisms in this size class, Commission staff believes that there are no acceptable methods for verification of compliance with the total viral standard at this time, and that the Commission should proceed with assessment of technologies for the remaining organism size classes in the standards.

Based on the available information and using best assessment techniques, Commission staff reviewed 30 ballast water treatment systems for this report. Staff believes that at least two treatment systems have demonstrated the potential to comply with California's performance standards. Many additional systems are close to completing system performance verification testing and will soon have data available for review. Commission staff expects that before 2010 several systems will be ready to meet California standards.

Over 20 systems are anticipated to be commercially available by the end of 2009 (Lloyd's Register 2008). Systems cannot clearly be deemed "available" for use, however, unless they have demonstrated the ability to meet California's performance standards. The treatment systems that met California's standards under the review for this report are commercially available at this time, and the several additional systems that are close to meeting all of California's standards are also commercially available.

Treatment vendors and vessel operators will also need to assess potential water quality impacts from treatment system usage in California waters. Commission staff, in consultation with the State Water Resources Control Board, has recently distributed to technology vendors a set of "Ballast Water Treatment Technology Testing Guidelines" that provides guidance on relevant water quality control plans and objectives for vessels intending to discharge treated effluent in State waters. Further guidance will be provided by the U.S. Environmental Protection Agency's National Pollution Discharge Elimination System (NPDES) Vessel General Permit for Discharges Incidental to the Normal Operation of Vessels, and the California-specific provisions added to the Vessel General Permit through the Clean Water Act Section 401 certification process. As of the writing of this report, however, those provisions are not available. Based on the available data it is clear that not all treatment systems will meet California's water quality objectives, particularly for chlorine residuals. Vessel owners and operators will need to consult with the Water Board to better assess the potential for water quality impacts from treatment system usage in California waters.

The Commission is preparing to implement the performance standards for new vessels with a ballast water capacity of less than 5000 MT in 2010. This review indicates that systems are or will soon be available to meet California's performance standards, particularly in light of the small number of new vessels that will likely need to meet the standards beginning in 2010. Commission staff is working closely with the shipping industry and treatment vendors to ensure a smooth transition to the new standards.

Commission staff is currently undertaking several projects to develop a comprehensive program for the implementation of California's performance standards including: 1) Developing protocols to verify vessel compliance with the performance standards; 2) Amending the performance standards regulations to bring the regulations inline with recent changes in statute and to specify requirements for ballast water sample collection and analysis; 3) Revising the Ballast Water Treatment Technology Testing Guidelines, as necessary; and 4) Supporting the development of performance standards and a technology assessment program at the federal level.

Staff will conduct another assessment of available treatment technologies by July 1, 2010 in anticipation of the 2012 implementation date for new vessels with a ballast water capacity greater than 5000 MT.

At this time, the Commission recommends that legislation be adopted to:

1. Authorize the Commission to amend the ballast water reporting requirements via regulations.

In 2007, the Commission recommended that the Legislature provide the Commission with the authority to change the ballast water reporting requirements to include information on the timing of, and requirements for, treatment system use, deviations from suggested system operation, and certifications for operation from vessel classification societies and other organizations/agencies. The statute currently limits the Commission's ability to amend the existing ballast water reporting form or develop a new form to collect necessary information about treatment system usage. To address this challenge, the Legislature proposed and passed Assembly Bill 169 in 2008, which was later vetoed by the Governor along with hundreds of other bills, due to the late passage of the budget. Nonetheless, the need for more information about treatment system installation and usage remains. The Commission should be authorized to amend the ballast water reporting requirements to meet these needs.

2. Support continued research promoting technology development and performance evaluation.

Ballast water treatment is an emerging industry that will continue to develop as California's Performance Standards are progressively implemented and as new vessel types are built. The scientific evaluation of treatment technology performance is also in its infancy, and new methods and techniques will be necessary to assess discharge compliance. The research and development needed to meet and assess compliance with these standards will require substantial financial resources. Funds necessary to support these research needs could be obtained through three mechanisms: general funds, grants, or through the existing fees assessed on ships. The Commission and the Legislature should support future budget change proposals or other fiscal actions to ensure that the development of evaluation methods may keep pace with the advancement of treatment technologies and with the performance standards implementation.

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ABBREVIATIONS AND TERMS

AB	Assembly Bill
Act	Coastal Ecosystems Protection Act
CCR	California Code of Regulations
CFR	Code of Federal Regulations
CSLC/Commission	California State Lands Commission
CTR	California Toxics Rule
Convention	International Convention for the Control and Management of Ships' Ballast Water and Sediments
CWA	Clean Water Act
EEZ	Exclusive Economic Zone
EPA	United States Environmental Protection Agency
ETV	Environmental Technology Verification Program
FIFRA	Federal Insecticide, Fungicide, and Rodenticide Act
GESAMP-BWWG	Joint Group of Experts on the Scientific Aspects of Marine Environmental Protection – Ballast Water Working Group
IMO	International Maritime Organization
MEPC	Marine Environment Protection Committee
Michigan DEQ	Michigan Department of Environmental Quality
MPCA	Minnesota Pollution Control Agency
MT	Metric Ton
NEPA	National Environmental Policy Act
NIS	Nonindigenous Species
nm	Nautical Mile
NPDES	National Pollution Discharge Elimination System
NRL	Naval Research Laboratory
PRC	Public Resources Code
SB	Senate Bill
Staff	Commission staff
STEP	Shipboard Technology Evaluation Program
TRC	Total Residual Chlorine
TRO	Total Residual Oxidant
USCG	United States Coast Guard
UV	Ultraviolet Irradiation
Vessel General Permit	Vessel General Permit for Discharges Incidental to the Normal Operation of Commercial Vessels and Large Recreational Vessels
Water Board	California State Water Resources Control Board
WDFW	Washington Department of Fish and Wildlife
WET	Whole Effluent Toxicity

I. PURPOSE

This report was prepared for the California Legislature pursuant to the Coastal Ecosystems Protection Act of 2006 (Act). Among its provisions, the Act added Section 71205.3 to the Public Resources Code (PRC) which required the California State Lands Commission (Commission) to prepare and submit to the Legislature, "a review of the efficacy, availability, and environmental impacts, including the effect on water quality, of currently available technologies for ballast water treatment systems." The initial technology assessment report, "Assessment of the Efficacy, Availability and Environmental Impacts of Ballast Water Treatment Systems for Use in California Waters," was approved by the Commission in December 2007 and submitted to the California Legislature (see Dobroski et al. 2007). In response to the recommendations in that report, the Legislature passed Senate Bill (SB) 1781 in 2008 (Chapter 696, Statutes of 2008) which amended PRC Section 71205.3 to delay the initial implementation of California's performance standards for the discharge of ballast water from January 1, 2009 to January 1, 2010. Additionally, the bill required an update of the initial technology assessment report by January 1, 2009 in anticipation of the implementation of the performance standards in 2010. This report summarizes Commission conclusions on the advancement of ballast water treatment technology development and assessment during 2008, discusses plans developed by Commission staff to implement California's performance standards for the discharge of ballast water, and makes recommendations to the Legislature.

II. INTRODUCTION

Nonindigenous Species and their Impacts

Also known as "introduced", "invasive", "exotic", "alien", or "aquatic nuisance species", nonindigenous species (NIS) are organisms that have been transported by human activities to a region where they did not occur historically, and have established reproducing populations in the wild (Carlton 2001). Once established, NIS can have serious human health, economic and environmental impacts in their new environment.

One of the most infamous examples is the zebra mussel (*Dreissena polymorpha*), which was introduced from the Black Sea to the Great Lakes in the mid-1980s (Carlton 2008) and was discovered in California in 2008 (California Department of Fish and Game 2008). This tiny striped mussel attaches to hard surfaces in dense populations that clog municipal water systems and electric generating plants, costing approximately $1 billion a year in damage and control for the Great Lakes alone (Pimentel et al. 2005). In San Francisco Bay, the overbite clam (*Corbula amurensis*) is thought to have contributed to declines of fish populations in the Sacramento-San Joaquin River Delta by reducing the availability of the plankton food base of the ecosystem (Feyrer et al. 2003). The Chinese mitten crab (*Eriocheir sinensis*), first sighted in San Francisco Bay in 1992, clogged water pumping stations and riddled levies with burrows costing approximately $1 million in 2000-2001 for control and research (Carlton 2001). In addition, the microorganisms that cause human cholera (Ruiz et al. 2000) and paralytic shellfish poisoning (Hallegraeff 1998) have been found in the ballast tanks of ships.

In marine, estuarine and freshwater environments, NIS may be transported to new regions through various human activities including aquaculture, the aquarium and pet trade, and bait shipments (Cohen and Carlton 1995, Weigle et al. 2005). In coastal habitats commercial shipping is an important transport mechanism, or “vector,” for invasion. In one study, shipping was responsible for or contributed to approximately 80% of invertebrate and algae introductions to North America (Fofonoff et al. 2003, see also Cohen and Carlton 1995 for San Francisco Bay). Ballast water was a possible vector for 69% of those shipping introductions, making it a significant ship-based introduction vector (Fofonoff et al. 2003).

Ballast water is necessary for many functions related to the trim, stability, maneuverability, and propulsion of large oceangoing vessels (National Research Council 1996). Vessels take on, discharge, or redistribute water during cargo loading and unloading, as they take on and burn fuel, as they encounter rough seas, or as they transit through shallow coastal waterways. Typically, a vessel takes on ballast water after its cargo is unloaded in one port to compensate for the weight imbalance, and will later discharge that water when cargo is loaded in another port. This transfer of ballast water from “source” to “destination” ports results in the movement of many organisms from one region to the next. In this fashion, it is estimated that more than 7000 species are moved around the world on a daily basis (Carlton 1999).

Ballast Water Management

Attempts to eradicate NIS after they have become widely distributed are often costly and unsuccessful (Carlton 2001). Between 2000 and 2006, over $7 million was spent to eradicate the Mediterranean green seaweed (*Caulerpa taxifolia*) from two embayments in southern California (Woodfield 2006). Approximately $10 million is spent annually to control the sea lamprey (*Petromyzon marinus*) in the Great Lakes (Lovell and Stone 2005). By 2010, over $12 million will have been spent in San Francisco Bay to control the Atlantic cordgrass (*Spartina alterniflora*) (Spellman, M., pers. comm. 2008). These costs reflect only a fraction of the cumulative expense over time as species control is an unending process. Prevention is therefore considered the most desirable way to address the NIS issue.

For the vast majority of commercial vessels, ballast water exchange is the primary management technique to prevent or minimize the transfer of coastal (including bay/estuarine) organisms. During exchange, the biologically rich water that is loaded while a vessel is in port or near the coast is exchanged with the comparatively species- and nutrient-poor waters of the mid-ocean (Zhang and Dickman 1999). Coastal organisms adapted to the conditions of bays, estuaries and shallow coasts are not expected to survive and/or be able to reproduce in the mid-ocean due to the differences in biology (competition, predation, food availability) and oceanography (temperature, salinity, turbidity, nutrient levels) between the two regions (Cohen 1998). Mid-ocean organisms are likewise not expected to survive in coastal waters (Cohen 1998).

Performance Standards for the Discharge of Ballast Water

Ballast water exchange is generally considered an interim tool because of its variable efficacy and operational limitations. Studies indicate that the effectiveness of ballast water exchange at eliminating organisms in tanks ranges widely from 50-99% (Cohen 1998, Parsons 1998, Zhang and Dickman 1999, U.S. Coast Guard 2001, Wonham et al. 2001, MacIsaac et al. 2002). When performed properly, exchange is considered an effective tool to reduce the risk of coastal species invasions (Ruiz and Reid 2007). However, new research demonstrates that the percentage of ballast water exchanged does not necessarily correlate with a proportional decrease in organism abundance (Choi et al. 2005, Ruiz and Reid 2007). Some vessels are regularly routed on short voyages or voyages that remain within 50 nautical miles (nm) of shore, and in such cases, the exchange process may create a delay or require a vessel to deviate from the most direct route. Such deviations can extend travel distances, increasing vessel costs for personnel time and fuel consumption.

In some circumstances, ballast water exchange may not be possible without compromising vessel or crew safety. For example, vessels that encounter adverse weather or experience equipment failure may be unable to conduct ballast water exchange safely. Unmanned barges are incapable of conducting exchange without transferring personnel onboard, a procedure that can present unacceptable danger if attempted in the exposed conditions of the open ocean. In recognition of these challenges, state and federal ballast water regulations allow vessels to forego exchange should the master or person in charge determine that it would place the vessel, its crew, or its passengers at risk. Though the provision is rarely invoked in California, the handful of vessels that use it may subsequently discharge un-exchanged ballast into State waters, presenting a risk of NIS introduction.

Regulatory agencies and the commercial shipping industry have therefore looked toward the development of effective ballast water treatment technologies as a promising management option. For regulators, such systems would provide NIS prevention including in situations where exchange may be unsafe or impossible. Technologies that eliminate organisms more effectively than mid-ocean exchange could provide a consistently higher level of protection to coastal ecosystems from NIS. For the shipping industry, the use of effective ballast water treatment systems might allow voyages to proceed along the shortest routes, in all operational scenarios, thereby saving time and money.

Despite these incentives, until recently, financial investment in the research and development of ballast water treatment systems has been limited and the advancement of ballast water treatment technologies slow. Many barriers have hindered the development of technologies, including equipment design limitations, the cost of technology development, and the lack of guidelines for testing and evaluating performance. However, some shipping industry representatives, technology developers and investors considered the absence of a specific set of ballast water performance standards as a primary deterrent to progress. Performance standards would set benchmark levels for organism discharge that a technology would be required to achieve for it to be deemed acceptable for use in eliminating the threat of species introductions. Developers requested these targets so they could design technologies to meet the standards (MEPC 2003). Without standards, investors were reluctant to devote financial resources towards conceptual or prototype systems because they had no indication that their investments might ultimately meet future regulations. For the same reason, vessel owners were hesitant to allow installation and testing of prototype systems onboard operational vessels. It was argued that the adoption of performance standards would address these fears, and accelerate the advancement of ballast treatment technologies. Thus in response to the slow progress of ballast water treatment technology development and the need for effective ballast water treatment options, state, federal and international regulatory agencies have adopted or are in the process of developing performance standards for ballast water discharges.

III. REGULATORY AND PROGRAMMATIC OVERVIEW

A thorough evaluation of the status of ballast water treatment technologies requires not only an understanding of the regulatory framework associated with the development and implementation of performance standards for the discharge of ballast water, but also knowledge of mechanisms for the testing and evaluation of treatment systems to meet those standards. Currently, no comprehensive international, federal or state program exists that includes performance standards, guidelines and/or protocols to verify the performance of treatment technologies, and methods to sample and analyze discharged ballast water for compliance purposes. California, other U.S. states, the federal government, and the international community are working toward the development of a standardized approach to the management of discharged ballast water, however, at this time existing legislation, standards and guidelines vary by jurisdiction. The following is a summary of the status of performance standards regulations, treatment system evaluation and discharge compliance verification as of the writing of this report.

International Maritime Organization

In February 2004, after several years of development and negotiation, International Maritime Organization (IMO) member countries adopted the International Convention for the Control and Management of Ships' Ballast Water and Sediments (Convention) (see IMO 2005). Among its requirements, the Convention imposes performance standards for the discharge of ballast water (Regulation D-2) with an associated implementation schedule based on vessel ballast water capacity and status as a new or existing vessel (Tables III-1 and III-2).

The Convention will enter into force 12 months after ratification by 30 countries representing 35% of the world's commercial shipping tonnage (IMO 2005). As of September 30, 2008, 16 countries representing 14.24% of the world's shipping tonnage have signed the convention (IMO 2008). The Convention cannot be enforced upon any ship until it is ratified (IMO 2007). Because insufficient time remains to ratify the Convention and have it enter into force before the first performance standards implementation date in 2009, the IMO General Assembly adopted Resolution A.1005(25), on November 29, 2007. The Resolution delays the date by which new vessels built in 2009 with a ballast water capacity of less than 5000 metric tons (MT) must comply with Regulation D-2 from 2009 until the vessel's second annual survey, but no later than December 31, 2011 (IMO 2007). For now, the implementation dates for all other vessel size classes remain the same as originally proposed (Table III-2).

Table III-1. Ballast Water Treatment Performance Standards

Organism Size Class	IMO Regulation D-2[1]	California[1,2]
Organisms greater than 50 µm[3] in minimum dimension	< 10 viable organisms per cubic meter	No detectable living organisms
Organisms 10 – 50 µm in minimum dimension	< 10 viable organisms per ml[4]	< 0.01 living organisms per ml
Living organisms less than 10 µm in minimum dimension		< 10^3 bacteria/100 ml < 10^4 viruses/100 ml
Escherichia coli	< 250 cfu[5]/100 ml	< 126 cfu/100 ml
Intestinal enterococci	< 100 cfu/100 ml	< 33 cfu/100 ml
Toxicogenic *Vibrio cholerae* (O1 & O139)	< 1 cfu/100 ml or < 1 cfu/gram wet weight zooplankton samples	< 1 cfu/100 ml or < 1 cfu/gram wet weight zoological samples

[1] See Implementation Schedule (below) for dates by which vessels must meet California Interim Performance Standards and IMO Ballast Water Performance Standards.
[2] Final discharge standard for California, beginning January 1, 2020, is zero detectable living organisms for all organism size classes.
[3] Micrometer – one-millionth of a meter
[4] Milliliter – one-thousandth of a liter
[5] Colony-forming unit – a measure of viable bacterial numbers

Table III-2. Implementation Schedule for Performance Standards

Ballast Water Capacity of Vessel	Standards apply to new vessels in this size class constructed on or after	Standards apply to all other vessels in this size class beginning in[1]
< 1500 metric tons	2009 (IMO)[2] /2010 (CA)[3]	2016
1500 – 5000 metric tons	2009 (IMO)[2] /2010 (CA) [3]	2014
> 5000 metric tons	2012	2016

[1] In California the standard applies to vessels in this size class as of January 1 of the year of compliance. The IMO Convention applies to vessels in this size class not later than the first intermediate or renewal survey, whichever occurs first, after the anniversary date of delivery of the ship in the year of compliance (IMO 2005).
[2] IMO has pushed back the initial implementation of the performance standards for vessels constructed in 2009 in this size class until the vessel's second annual survey, but no later than December 31, 2011 (IMO 2007).
[3] California Senate Bill 1781 (Chapter 696, Statutes of 2008) delayed the initial implementation of performance standards for vessels in this size class from January 1, 2009 to January 1, 2010.

In order to ensure global and uniform application of the relevant requirements of the Convention, the IMO Marine Environment Protection Committee (MEPC) has adopted 12 implementation guidelines (one additional guideline remains in draft form, see below for details) (Everett, R., pers. comm. 2008). Relevant to this report, the guidelines for the evaluation and approval of ballast water treatment systems were adopted at the 53rd session of the MEPC in July, 2005. Guideline G8, “Guidelines for Approval of Ballast Water Management Systems” (MEPC 2005a), and Guideline G9, “Procedure for Approval of Ballast Water Management Systems That Make Use of Active Substances” (MEPC 2005b), work together to create a framework for the evaluation of treatment systems by the MEPC and Flag State Administrations (i.e. the country or flag under which a vessel operates) (Figure III-3). Flag States (not the IMO) may grant approval (also known as “Type Approval”) to systems that are in compliance with the Convention’s Regulation D-2 performance standards based upon recommended procedures (as detailed in Guideline G8) for full-scale land-based and shipboard testing of the treatment system. A treatment system may not be used by a vessel party to the Convention to meet the D-2 standards in the Convention unless that system is Type Approved.

In addition to receiving Type Approval from the Flag State Administration, ballast water treatment systems using “active substances” must be approved by the IMO MEPC based upon procedures developed by the organization (IMO 2005). An active substance is defined by IMO as, “…a substance or organism, including a virus or a fungus that has a general or specific action on or against Harmful Aquatic Organisms and Pathogens” (IMO 2005). For all intents and purposes, an active substance is a chemical or reagent (e.g. chlorine, ozone) that kills or inactivates organisms in ballast water. The IMO approval pathway for treatment systems that use active substances is more rigorous than the evaluation process for technologies that do not. As required by Guideline G9, technologies utilizing active substances must go through a two-step “Basic” and “Final” approval process. Active substance systems that apply for Basic and Final Approval are reviewed for environmental, ship, and personnel safety by the IMO Joint Group of Experts on the Scientific Aspects of Marine Environmental Protection (GESAMP) –

Ballast Water Working Group (BWWG) in accordance with the procedures detailed in Guideline G9. The MEPC may grant Basic or Final Approval based upon the GESAMP-BWWG recommendation. Systems that do not use active substances (i.e. a system only using filtration) do not need Basic or Final Approval, and need only acquire Type Approval (Figure III-3).

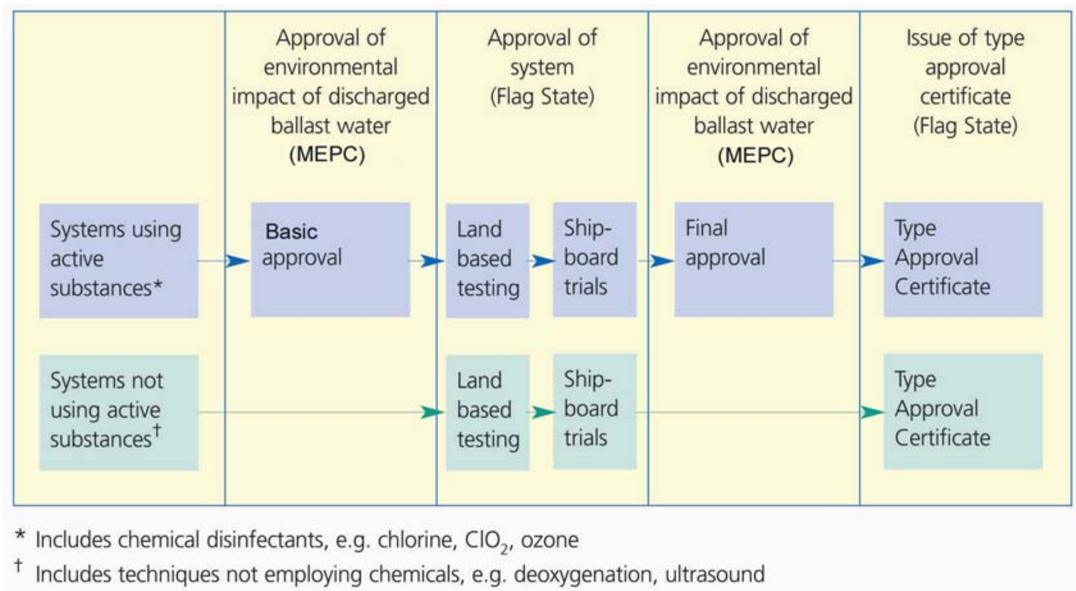

Figure III-3. Summary of IMO approval pathway for ballast water treatment systems. (Modified from Lloyd's Register (2007))

The entire IMO evaluation process (including approval for systems using active substances) has been estimated to take between six months and two years to complete (Everett, R., pers. comm. 2007, Lloyd's Register 2007). Once a ballast water treatment system has acquired Type Approval (and the Convention is ratified and in force), the system is deemed acceptable by parties to the Convention for use in international waters in compliance with Regulation D-2.

The U.S. has neither reviewed nor submitted applications to IMO on behalf of any U.S. treatment technology vendors thus far. Until the Convention is both signed by the U.S. and enters into force through international ratification, no U.S. federal agency has the authority (unless authorized by Congress) to manage a program to review treatment technologies and submit applications on their behalf to IMO. United States treatment vendors may approach IMO through association with other IMO Member States, and several have or are in the process of doing so. However, because the Convention has not yet been ratified, it does not have the force of international law, which draws into question the legality of MEPC approvals of treatment systems. While the U.S. is actively involved in developing and negotiating the various requirements of the Convention, until the U.S. signs on to the Convention, and it is ratified by enough member states to go into force, the U.S. is not party to the Convention requirements. Hence, vessels calling on U.S. ports have no authority to use systems approved through the IMO Type Approval process to meet U.S. ballast water management requirements.

One additional guideline related to the implementation of the IMO Convention bears mention here for its relevance to California's ballast water management program. Guideline G2, the "Guidelines for Ballast Water Sampling," provides valuable information, in the absence of U.S. federal guidance, on the location and equipment necessary to collect ballast water samples to assess compliance with the performance standards. Guideline G2 defines the preferred sampling point (i.e. the place in the ballast water piping where the sample is taken) and sampling facilities (i.e. the equipment installed to take the sample) for sample collection (BLG 2008). As California gets closer to the implementation of its own performance standards for the discharge of ballast water, these sampling guidelines will help direct the development of new California regulations and compliance verification procedures (see Section on California Legislation and Implementation of Performance Standards for details).

U.S. Federal Legislation and Programs

The authority to regulate ballast water discharges in the United States has recently shifted to include the U.S. Environmental Protection Agency (EPA) in addition to the U.S. Coast Guard (USCG). Beginning December 19, 2008, the EPA must regulate ballast water, and other discharges incidental to normal vessel operations, under the Clean Water Act (CWA). This requirement stems from the 2003 lawsuit filed by Northwest Environmental Advocates et al. against the EPA in U.S. District Court, Northern District of California, challenging a regulation originally promulgated under the CWA (Nw. Envtl. Advocates v. U.S. EPA, No. C 03-05760 SI, 2006 U.S. Dist. LEXIS 69476 (N.D. Cal. Sept. 18, 2006)). The regulation at issue, Title 40 of the Code of Federal Regulations (CFR) Section 122.3(a), exempted effluent discharges “incidental to the normal operations of a vessel,” including ballast water, from regulation under the National Pollution Discharge Elimination System (NPDES). The plaintiffs sought to have the regulation declared *ultra vires,* or beyond the authority of the EPA under the CWA. On March 31, 2005, the District Court granted judgment in favor of Northwest Environmental Advocates et al., and on September 18, 2006 the Court issued an order revoking the exemptive regulation (40 CFR. Section 122.3(a)) as of September 30, 2008. EPA filed an appeal with the Ninth Circuit U.S. Court of Appeals but was denied in July 2008 (Nw. Envtl. Advocates v. U.S. EPA, No. 03-74795, 2008 U.S. App. LEXIS 15576 (9th Cir. Cal. July 23, 2008)). In June 2008, EPA released for public comment the draft NPDES “Vessel General Permit for Discharges Incidental to the Normal Operation of Commercial Vessels and Large Recreation Vessels” (Vessel General Permit). All vessels greater than 300 gross registered tons, or with a ballast water capacity greater than 8 cubic meters, must submit a Notice of Intent with EPA in order to receive coverage under the permit. Vessels greater than 79 feet but less than 300 tons receive automatic permit coverage. In September 2008, the District Court granted a motion to delay the vacature of the 122.3(a) regulation from September 30 to December 19, 2008.

In large part, the draft NPDES Vessel General Permit maintains the regulation of ballast water discharges by the USCG through regulations found in 33 CFR Part 151. The USCG regulations, developed under authority of the Nonindigenous Aquatic Nuisance Prevention and Control Act of 1990, which was revised and reauthorized as the National Invasive Species Act of 1996, require ballast water management (i.e. ballast water exchange) for vessels entering U.S. waters from outside of the 200 nautical mile (nm) Exclusive Economic Zone (EEZ) of the U.S. Vessels may use onboard treatment systems to meet the current ballast water management requirements if that system is approved by the Commandant of the USCG, however, as of October 2008 no approval process is in place.

The draft NPDES Vessel General Permit does not include performance standards for the discharge of ballast water. Performance standards may be included in the next iteration of the permit (in 2013) based on the outcome of an anticipated USCG rulemaking on ballast water treatment standards, and if treatment technologies are determined to be commercially available and economically achievable to meet those standards. The lack of a federal discharge standard precludes the approval of any treatment system at the national level.

The EPA's draft NPDES Vessel General Permit and the USCG regulations do not relieve vessel owners/operators (permittees) of the responsibility of complying with applicable state laws or regulations. Additionally, states with authority to implement the CWA may add specific provisions, including performance standards, for vessel discharges in state waters to the EPA's general permit through the CWA Section 401 certification process. Thus we do not expect to see any impact from the implementation of the NPDES permit on individual states' ability to implement performance standards for the discharge of ballast water in state waters, including California. Vessels will, however, have to comply with both state and federal regulations for ballast water management under the NPDES permit and the USCG regulations. This may result in vessels having to exchange ballast water to comply with federal management requirements under the CWA and the USCG regulations and treat ballast water to comply with state regulations. Legislation may be required to clarify this potentially confusing situation.

Several bills have been introduced in the U.S. House of Representatives and Senate in recent years to legislatively establish a national discharge standard. In 2007 the following bills were introduced:

- The Ballast Water Management Act of 2007 (H.R. 2423, S. 1578)
- Prevention of Aquatic Invasive Species Act of 2007 (H.R. 889)
- National Aquatic Invasive Species Act of 2007 (S. 725)
- Great Lakes Invasive Species Control Act (H.R. 801)

- Coast Guard Authorization Act of 2007 (H.R. 2830)
- Great Lakes Collaboration Implementation Act (S. 791, H.R. 1350)
- Aquatic Invasive Species Research Act (H.R. 260).

These bills seek to clarify the goals and role of the federal government in ballast water management. Several of the bills introduce performance standards that would be less stringent than California's standards. More importantly, however, many of these bills also introduce language that would preempt state laws and set back California's efforts to better manage ballast water discharges and other ship-mediated vectors of NIS introductions.

As of October 2008, only H.R. 2830 (the Coast Guard Authorization Act) has cleared its house of origin. Recently, the Senate Committee on Commerce, Science and Technology has been working with the House and states to draft ballast water legislation that would establish a federal discharge standard while allowing states, such as California, to retain authority over their ballast water management programs. This new language could either be inserted into the Senate USCG authorization bill (S. 1892), the House USCG bill currently in the Senate (H.R. 2830), a separate bill or could be addressed in conference committee. Commission staff (staff) will continue to follow and assess the potential impacts of any new federal legislation on ballast water management and California's program.

While the federal implementation of performance standards for the discharge of ballast water remains uncertain in the near future, two promising federal programs are currently working proactively to support the development of treatment technologies and facilitate the testing and evaluation of those systems: 1) The USCG Shipboard Technology Evaluation Program (STEP), and 2) The EPA's Environmental Technology Verification (ETV) program.

The USCG STEP is intended to facilitate the development of ballast water treatment technologies. Vessel owners and operators accepted into STEP may install and operate specific experimental ballast water treatment systems on their vessels for use in U.S. waters. In order to be accepted, treatment technology developers must assess the efficacy of systems for removing biological organisms, residual concentrations of treatment chemicals, and water quality parameters of the discharged ballast water (USCG 2004). STEP provides incentives for vessel operators and treatment developers to test promising new technologies. Vessels accepted into the program may operate the system to meet the USCG ballast water management requirements and will be grandfathered for operation under future ballast water discharge standards for the life of the vessel or the treatment system. During the summer of 2008, the draft environmental assessments for three vessels that applied to the program were released for comment. Those three vessels were accepted into STEP in the fall of 2008. One more vessel has applied to the program and is currently undergoing review. The USCG has plans to streamline the review process for future applicants (USCG 2008).

The EPA ETV program is an effort to accelerate the development and marketing of environmental technologies, including ballast water treatment technologies. The USCG and the EPA established a formal agreement to implement an ETV program focused on ballast water management. Under this agreement, the ETV program developed a draft protocol in 2004 for verification of the performance of ballast water treatment technologies. Subsequently, the USCG established an agreement with the Naval Research Laboratory (NRL) to evaluate, refine, and validate this protocol and the test facility design required for its use. This validation project resulted in the construction of a model ETV Ballast Water Treatment System Test Facility at the NRL Corrosion Science and Engineering facility in Key West, Florida. The innovative research conducted at the NRL facility is intended by the USCG to result in technical procedures for testing ballast water treatment systems for the purpose of approval and certification. Based on the information collected during the evaluation of the 2004 draft protocol, the ETV program, in consultation with an advisory panel (of which CSLC staff is a member), is currently developing a revised final treatment technology verification protocol which is expected to be released in late-2009 or early 2010 (Stevens, T., pers. comm. 2008).

U.S. State Legislation and Programs

Washington

The Washington Department of Fish and Wildlife (WDFW), in consultation with a Ballast Water Work Group, is working on a comprehensive rewrite of the state's ballast water management regulations in response to Washington state legislation passed in 2007 (see E2SSB 5923, the Aquatic Invasive Species Enforcement and Control Act). The new regulations are anticipated to replace the interim percent reduction-based performance standards with permanent concentration-based standards that are in-line with California regulations. These changes would help bring the U.S. Pacific coast states into greater management consistency. Additional revisions are also being made to Washington's treatment technology approval process. The WDFW will no longer independently approve treatment systems for use in state water and will instead rely on regional, national or international approvals. Systems previously approved under the interim regulations will remain approved for their original period of use. WDFW staff expects the new regulations to be adopted in early 2009 (Pleus, A., pers. comm. 2008).

Michigan

Michigan passed legislation in June 2005 (Act 33, Public Acts of 2005) requiring a permit for the discharge of any ballast water from oceangoing vessels into the waters of the state beginning January 2007. Through the general permit (Permit No. MIG140000) developed by Michigan Department of Environmental Quality (DEQ), any ballast water discharged must first be treated by one of four methods (hypochlorite, chlorine dioxide, ultraviolet radiation preceded by suspended solids removal, or deoxygenation) that have been deemed environmentally sound and effective in preventing the discharge of NIS. Vessels must use treatment technologies in compliance with applicable requirements and conditions of use as specified by Michigan DEQ for use in state waters. Vessels using technologies not listed under the Michigan general permit may apply for individual permits if the treatment technology used is, "environmentally sound and its treatment effectiveness is equal to or better at preventing the discharge of aquatic nuisance species as the ballast water treatment methods contained in [the general] permit," (Michigan DEQ 2006).

Minnesota

Effective July 1, 2008, Minnesota state law (S.F. 3056) requires vessels operating in state waters to have both a ballast water record book and a ballast water management plan onboard that has been approved by the Minnesota Pollution Control Agency (MPCA) (MPCA 2008). Additionally, based on the authority in Minn. Stat. 115.07, Minn. R. 7001.0020, subp. D, and Minn. R. 7001.0210, and to implement the recently enacted legislation, the MPCA approved a State Disposal System general permit for ballast water discharges into Lake Superior and associated waterways in September 2008 (MPCA 2008). Under the permit, vessels that wish to discharge into Minnesota waters must comply immediately with approved best management practices. No later than January 1, 2012, new vessels will be required to comply with the IMO D-2 performance standards for the discharge of ballast water (see Table III-1), and existing vessels will be required to comply with those standards no later than January 1, 2016 (MPCA 2008).

California Legislation and the Implementation of Performance Standards

Review of Legislation

California's Marine Invasive Species Act of 2003 directed the Commission to recommend performance standards for the discharge of ballast water to the State Legislature in consultation with the State Water Resources Control Board (Water Board), the USCG and a technical advisory panel (see PRC Section 71204.9). The legislation directed that standards should be selected based on the best available technology economically achievable, and should be designed to protect the beneficial uses of the waters of the State.

In 2005, Commission staff convened a cross-interest, multi-disciplinary panel consisting of regulators, research scientists, industry representatives and environmental organizations and facilitated discussions over the selection of performance standards. Many sources of information were used to guide the performance standards selection including: biological data on organism concentrations in exchanged and un-exchanged ballast water, theories on coastal invasion rates, standards considered or adopted by other regulatory bodies, and available information on the efficacy and costs of experimental treatment technologies. Though all sources and panel members provided some level of insight, none could provide solid guidance for the selection of a specific set of standards that would reduce or eliminate the introduction and establishment of NIS. At a minimum, it was determined that reductions achieved by the selected performance standards should improve upon the status quo and decrease the discharge of viable ballast organisms to a level below quantities observed following legal ballast water exchange. Additionally, the technologies used to achieve these standards should function without introducing chemical or physical constituents to the treated ballast water that may result in adverse impacts to receiving waters. Beyond these general criteria, however, there was no concrete support for the selection of a specific set of standards. This stems from the key knowledge gap that invasion risk cannot be predicted for a particular quantity of organisms discharged in ballast water (MEPC 2003), with the exception that zero organism discharge equates to zero risk.

The Commission ultimately put forward performance standards recommended by the majority of the Panel because they encompassed several desirable characteristics: 1) A significant improvement upon ballast water exchange; 2) In-line with the best professional judgment of scientific experts that participated in the development of the IMO Convention; and 3) Approached a protective zero discharge standard. The proposed interim standards were based on organism size classes (Table III-1). The standards for the two largest size classes of organisms (>50 micrometers (µm; one-millionth of a meter) in minimum dimension and 10 – 50 µm in minimum dimension) were significantly more protective than those proposed by the IMO Convention. The majority of the Panel also recommended standards for organisms less than 10 µm including human health indicator species and total counts of living bacteria and viruses. The recommended bacterial standards for human health indicator species, *Escherichia coli* and intestinal enterococci, are identical to those adopted by the EPA in 1986 for recreational use and human health safety (EPA 1986). The standards for total living bacteria and viruses have not been adopted by any other state, federal or international administration or agency. The implementation schedule proposed for the interim standards was similar to the IMO Convention (Table III-2). A final discharge standard of zero detectable organisms was recommended by the majority of the Panel. The Commission included an implementation deadline of 2020 for this final discharge standard.

The Commission submitted the recommended standards and information on the rationale behind its selection in a report to the State Legislature in January of 2006 (see Falkner et al. 2006). By the fall of that same year, the Legislature passed the Coastal Ecosystems Protection Act (Chapter 292, Statutes of 2006) directing the Commission to adopt the recommended standards and implementation schedule through the California rulemaking process by January 1, 2008. The Commission completed that rulemaking in October, 2007 (see 2 CCR § 2291 et seq.).

In anticipation of the implementation of the interim performance standards, the Coastal Ecosystems Protection Act also directed the Commission to review the efficacy, availability and environmental impacts of currently available ballast water treatment systems by January 1, 2008. The review and resultant report was approved by the Commission in December, 2007 (see Dobroski et al. 2007). Additional reviews must be completed 18 months prior to the implementation dates for all other vessel classes and 18 months before the implementation of the final discharge standard on January 1, 2020 (see Table III-2 for full implementation schedule). During any of these reviews, if it is determined that existing technologies are unable to meet the discharge standards, the report must describe why they are not available.

In response to the recommendations in the initial technology assessment report (Dobroski et al. 2007), the Legislature passed SB 1781 in 2008 (Chapter 696, Statutes of 2008). SB 1781 amends PRC Section 71205.3(a)(2), delaying the implementation of the interim performance standards for new vessels with a ballast water capacity of less than 5000 MT from January 1, 2009 to January 1, 2010. Commission staff is currently preparing a rulemaking package to bring the performance standards regulations (2 CCR § 2291 et seq.) in-line with statute. SB 1781 also requires an additional assessment of available ballast water treatment technologies by January 1, 2009 (this report) in anticipation of the standards implementation in 2010.

Implementing California's Performance Standards

Commission staff is in the process of instituting a comprehensive plan for the implementation of California's performance standards. The delay of initial implementation of the standards from January 1, 2009 to January 1, 2010 has provided technology developers with the necessary time to prepare systems for sale and installation on vessels in order to meet California's performance standards. The delay also provided Commission staff with additional time to compile information about treatment system operation and safety, develop procedures for treatment system evaluation, and begin development of vessel compliance verification protocols.

As discussed in Dobroski et al. (2007), the Commission will not be approving ballast water treatment systems for use in California waters. Instead, the Commission will focus on dockside inspection of vessels for verification of compliance with the performance standards (in accordance with PRC Section 71206). Nonetheless, Commission staff believes that before systems enter the commercial marketplace, it is in the best interest of the State and concerned stakeholders that systems undergo a thorough performance, safety and environmental impact evaluation. Therefore, Commission staff has developed ballast water treatment technology testing guidelines to bridge the gap between treatment system development and operation in California waters.

The "Ballast Water Treatment Technology Testing Guidelines" (Appendix A) provide technology vendors with a standardized approach to evaluating treatment system performance relative to California's discharge standards and water quality objectives. Commission staff developed these protocols in consultation with the Water Board, USCG, ETV program staff and an expert panel of scientists (Appendix A1). System verification testing according to these guidelines is not required by the Commission, however staff strongly encourages technology vendors to conduct verification testing according to these guidelines to ensure a uniform, cost-effective, scientifically-rigorous, independent assessment of system performance and environmental safety. The results generated from system evaluation according to these guidelines will provide Commission staff and potential treatment technology customers with a valuable upfront assessment of the ability of systems to meet California's performance standards and water quality objectives. The guidelines and an associated information sheet were completed and distributed in October, 2008. Initial response from industry has been positive, although it is still too early to determine whether or not these guidelines have influenced the testing methods and verification protocols used by vendors and testing organizations.

While the testing guidelines will provide useful information about the potential of treatment system to meet California's performance standards, they are not a substitute for in-the-field sampling and discharge compliance verification. Commission staff is currently in the process of developing procedures for use by the Commission's Marine Safety Personnel to verify vessel compliance with the performance standards. The compliance verification procedures, to be developed in consultation with technical experts, will make use of the best available techniques to assess organism concentration for each of the standards.

It is expected that the best available techniques to assess vessel compliance with the performance standards will change over time as technology advances. The Commission will need to clarify the manner by which it holds vessels accountable for meeting the standards to ensure that vessels compliant under the current set of verification protocols will not fail compliance in the future simply because the sensitivity of assessment techniques improves. This may be accomplished by grandfathering installed treatment systems under a specific set of compliance verification techniques. Further discussion will be necessary to determine how such a grandfathering system might work while remaining protective of California's waters and consistent with the law.

Commission staff is also developing regulations regarding the selection of sampling points (i.e. location) and sampling facilities (i.e. equipment) on vessels for compliance verification purposes. According to PRC Section 71206 Commission staff is mandated to "take samples of ballast water and sediment from at least 25 percent of the arriving vessels…and make other appropriate inquiries to assess the compliance of any vessel subject to this division." The new regulations will specify that ballast water samples must be taken during ballast water discharge (per 2 CCR § 2291 et seq.). Additionally, the regulations will offer guidance on the selection of sampling facilities so as to reduce or eliminate the possibility of artificially-induced organism mortality (that may skew compliance assessment) associated with passage through the sampling apparatus. Commission staff expects to complete this rulemaking in 2009.

Finally, the effective implementation of California's performance standards will require regular monitoring of the treatment technologies as performance standards are implemented. Commission staff will continue to gather information about treatment system development, installation, and use on board vessels. This information will guide the development of new regulations which take into account development within the rapidly advancing ballast water treatment technology industry.

IV. TREATMENT TECHNOLOGY ASSESSMENT PROCESS

Public Resources Code (PRC) Section 71205.3 directs the Commission to prepare, "a review of the efficacy, availability, and environmental impacts, including the effect on water quality, of currently available technologies for ballast water treatment systems." In accordance with the law, the Commission has consulted with, "the State Water Resources Control Board, the United States Coast Guard, and the stakeholder advisory panel described in subdivision (b) of [PRC] Section 71204.9." This stakeholder panel also provided guidance in the development of the performance standards report to the California Legislature (Falkner et al. 2006).

During the preparation of the initial technology assessment report (Dobroski et al. 2007), Commission staff received input from a small technical workgroup prior to consulting with the stakeholder advisory panel. The workgroup met in May 2007 to assess the current availability of treatment systems, the efficacy of those systems, and any potential environmental and water quality impacts. This group included individuals with expertise in ballast water treatment technology development, water quality and biological monitoring and evaluation, naval architecture and engineering, and technology efficacy analysis (see Dobroski et al. 2007 for workshop participants and summary). The conclusions drawn during the workshop in 2007 continued to provide valuable guidance and direction in the preparation of the current report.

As with the initial technology assessment report, Commission staff conducted an exhaustive literature search to prepare this report. Staff focused its review on recently available scientific papers and performance verification reports from independent testing organizations. Staff also contacted treatment technology vendors in order to gather the most up-to-date information about system development, testing and approvals. On several occasions, staff held meetings in person with technology vendors. These face-to-face gatherings proved to be extremely valuable opportunities to inform vendors about California's performance standards requirements and engage in dialogue about performance verification testing and the Commission's technology assessment report.

Commission staff compiled the available data to develop a treatment system matrix (see Tables V-1, VI-1, VI-3, VI-4, and Appendix B). Upon completion of the data analysis, Commission staff drafted a preliminary report for review by the Commission's stakeholder advisory panel (see Appendix C for list of panel members), the Water Board and USCG. The advisory panel met in October, 2007 to review the initial technology assessment report (see Dobroski et al. 2007), and met in October, 2008 to review the current updated report (Appendix C). Advisory panel discussions were considered by staff to help guide the development of this final report.

V. TREATMENT TECHNOLOGIES

The goal of ballast water treatment is to remove or inactivate organisms entrained in ballast water. Given societal experience with wastewater treatment technologies, the design and production of ballast water treatment systems may seem simple in concept, but has instead proved to be difficult and complex in practice. A system must be effective under a wide range of challenging environmental conditions including variable temperature, salinity, nutrients and suspended solids. It must also function under difficult operational constraints including high flow-rates of ballast water pumps, large water volumes, and variable retention times (time ballast water is held in tanks). Treatment systems must be capable of eradicating a wide variety of different organisms ranging from viruses and microscopic bacteria to free-swimming plankton, and must operate so as to minimize or prevent impairment of the water quality conditions of the receiving waters. The development of effective treatment systems is further complicated by the variability of vessel types, shipping routes and port geography.

Two general platform types have been explored for the development of ballast water treatment technologies. Shoreside ballast water treatment occurs at a facility following transfer from a vessel. Shipboard treatment occurs onboard vessels through the use of technologies that are integrated into the ballasting system. Shipboard treatment systems are attractive because they allow flexibility to manage ballast water during normal operations, while shoreside treatment may be a good option for vessels with small ballast water capacity and/or dedicated port calls.

The shoreside treatment of ballast water is an appealing option because of the potential similarity in design to waste water treatment systems, however, shoreside treatment poses several challenges. Current shoreside wastewater treatment plants are not equipped to treat saline water (Water Board 2002, Moore, S., pers. comm. 2005). If existing municipal facilities are to be used for the purposes of ballast water treatment, they will need to be modified, and a new extensive network of piping and associated pumps will be required to distribute ballast water from vessels at berth to the treatment plants. The establishment of new piping and facilities dedicated to ballast water treatment, while technically feasible, would be complex and costly in California port areas. Shoreside treatment is not feasible for vessels that must take on or discharge ballast water while underway, for example, if the vessel must adjust its draft to navigate through a shallow channel or under a bridge. The cost of retrofitting of vessels to discharge ballast to shoreside facilities at a rate that prevents vessel delays in port might also be prohibitive (CAPA 2000).

On the other hand, shoreside treatment does provide options for treatment technologies and/or methods that are not feasible onboard vessels due to space and/or energy constraints, such as reverse osmosis. Additionally, shoreside treatment facilities could be staffed by trained wastewater engineers instead of ships' crew who may not be specifically trained in the operation and maintenance of water treatment facilities. To date, however, only limited feasibility studies have been conducted on shoreside treatment (see references in Falkner et al. 2006). Shoreside treatment has been generally considered a good option for unique terminals such as those with limited but dedicated vessel calls (e.g. cruise ships). Nonetheless, one study specific to cruise ships indicated that due to the operational practices of cruise ships and the current regulatory requirements in California and the Port of San Francisco there is little demand at this time for shoreside treatment except in emergency situations (Bluewater Network 2006). Additional studies will be necessary to determine feasibility of and demand for shoreside treatment for other vessel types and across the State as a whole. This may include assessments by those involved in the wastewater treatment sector on whether existing technologies could meet California's performance standards. Because the majority of time, money, and effort in the development of ballast water treatment technologies during recent years has been focused on shipboard treatment systems, we will focus on shipboard systems for the remainder of this report.

Shipboard systems allow for greater flexibility during vessel operations. Vessels may treat and discharge ballast while in transit, and thus will not need to coordinate vessel port arrival time with available space and time at shoreside treatment facilities. As with shoreside treatment, however, shipboard treatment systems face their own set of challenges. They must be engineered to conform to a vessel's structure, ensure crew safety, and withstand the vibrations and movements induced by the vessel's engine and rough seas. Additionally, shipboard systems must be effective under transit times that range from less than 24 hours to several weeks, and must treat ballast water in compliance with water quality requirements in recipient regions.

The timing and location of shipboard ballast water treatment can be varied according to the needs of the treatment system and the length of vessel transit. Ballast water may be treated in the pipe during uptake or discharge (in line) or in the ballast tanks during the voyage (in tank). While mechanical separation (such as filtration) generally occurs during ballast uptake in order to remove large organisms and sediment particles before they enter the ballast tanks, other forms of treatment may occur at any point during the voyage. Some treatment systems treat ballast water at multiple points during the voyage, such as during uptake and discharge.

Because of this wide range of variables associated with shipboard ballast water treatment, the identification of a single treatment technology for all NIS, ships, and port conditions is unlikely. Each technology may meet the objective of killing or inactivating NIS in a slightly different manner and each could potentially impact the water quality of the receiving environment through the release of chemical residuals or alterations to water temperature, salinity, and/or turbidity. Thus a suite of treatment technologies will undoubtedly need to be developed to treat ballast water industry-wide and across all ports and environments.

Treatment Methods

The development of ballast water treatment systems that are effective, environmentally friendly and safe has been a complex, costly and time consuming process. At the root of many treatment systems are methods that are already in use to some degree by the wastewater treatment industry. A preliminary understanding of these treatment methods forms the basis for more detailed analysis and discussion of ballast water treatment systems. The diverse array of water treatment methods currently under development for use in ballast water treatment can be broken down into five major categories: mechanical, chemical, physical, biological and combination.

Mechanical Treatment

Mechanical treatment traps and removes mid-size and large particles from ballast water. Mechanical treatment typically takes place upon ballast water uptake in order to limit the number of organisms and amount of sediment that may enter ballast tanks. Common options for mechanical treatment include filtration and hydrocyclonic separation.

Filtration works by capturing organisms and particles as water passes through a porous screen or filtration medium, such as sand or gravel. The size of organisms trapped by the filter depends on the mesh size in the case of screen or disk filters, and on the size of the interstitial space for filtration media. In ballast water treatment, screen and disk filtration is more commonly used over filter media, however, there has been some interest in the use of crumb rubber as a filtration medium in recent studies (Tang et al. 2006). Typical mesh size for ballast water filters ranges from 25 to 100 µm (Parsons and Harkins 2002, Parsons 2003).   Most filtration-based technologies also use a backwash process that removes organisms and sediment that become trapped on the filter, and can discharge them at the port of origin before the vessel gets underway. Filter efficacy is a function not only of initial mesh size, but also of water flow rate and backwashing frequency.

Hydrocyclonic separation, also known as centrifugation, relies on density differences to separate organisms and sediment from ballast water. Hydrocyclones create a vortex that cause heavier particles to move toward the outer edges of the cyclonic flow where they are trapped in a weir-like device and can be discharged before entering the ballast tanks (Parsons and Harkins 2002). Hydrocyclones in use in ballast water treatment trap particles in the 50 to 100 µm size range (Parsons and Harkins 2002). One challenge associated with hydrocylone use, however, is that many small aquatic organisms have a density similar to sea water and are thus difficult to separate using centrifugation.

Chemical Treatment

A variety of chemicals (i.e. active substances) are available to kill or inactivate organisms in ballast water. While the vast majority of chemicals are biocides, some chemicals may be used to clump or coagulate organisms in order to assist with their mechanical removal. Chemical treatment may take place during ballast uptake, vessel transit, or discharge. Chemicals may be stored onboard in liquid or gas form, or they may be generated on demand through electrolytic or electrochemical processes.

Chemical biocides can be classified into two major categories: oxidizing and non-oxidizing. Oxidizing agents (e.g. chlorine, chlorine dioxide, bromine, hydrogen peroxide, peroxyacetic acid, ozone) are commonly used in the wastewater treatment sector and work by destroying cell membranes and other organic structures (NRC 1996, Faimali et al. 2006). Electrochemical oxidation combines electrical currents with naturally occurring reactants in seawater and/or air (e.g. salt, oxygen) to produce killing agents. For example, electrochemical oxidation can produce reactants such as hydroxyl radicals, ozone or sodium hypochlorite (chlorine) that are capable of damaging cell membranes. Non-oxidizing biocides, including Acrolein®, gluteraldehyde, and menadione (Vitamin K3), are reported to work like pesticides by interfering with an organism's neural, reproductive or metabolic processes (NRC 1996, Faimali et al. 2006).

The ultimate goal of chemical biocides is to maximize organism inactivation or mortality while minimizing environmental impact. Environmental concerns surrounding chemical use in ballast water focus on the impacts of residuals or byproducts in treated discharge on receiving waters. The effective use of chemical biocides in ballast water treatment requires a balance between the amount of time required to achieve inactivation of organisms, with the time needed for those chemicals and residuals to degrade or be treated to environmentally acceptable levels. Both of these times vary as a function of ballast water temperature, organic content and sediment load. As a result, certain chemicals may be more effective than others based on ballast volume, voyage length, and water quality conditions. Additional concerns about chemical use specific to shipboard operation include corrosion, safety (personnel and ship safety), and vessel design limitations that impact the availability of space onboard for both chemical storage and equipment for dosing.

Physical Treatment

Physical treatment methods include a wide range of non-chemical means to kill or inactivate organisms present in ballast water. Like chemical treatment, physical treatment may occur on ballast uptake, during vessel transit or during discharge. Examples of physical treatment of ballast water include heat treatment, ultraviolet irradiation, ultrasonic energy and some forms of deoxygenation.

Rigby et al. (1999, 2004) discuss the use of waste heat from the ship's main engine as a mechanism to heat ballast water and kill or inactivate unwanted organisms during vessel transit. However, it would be difficult to heat ballast water to a sufficient temperature to kill all species of bacteria due to lack of sufficient surplus energy/heat on a vessel (Rigby et al. 1999, Rigby et al. 2004). An alternative approach to heat treatment involves the use of microwaves. Currently such a treatment technology would be prohibitively expensive (up to \$2.55/m^3), but additional research and development may reduce costs to acceptable levels (Boldor et al. 2008).

Ultrasound (ultrasonic treatment) kills through high frequency vibration that creates microscopic bubbles that rupture cell membranes (Viitasalo et al. 2005). The efficacy of ultrasound varies based on the intensity of vibration and length of exposure. Ultraviolet (UV) irradiation is another method of sterilization that is commonly used in waste water treatment. UV damages genetic material and proteins which disrupts reproductive and physiological processes. UV irradiation can be highly effective against pathogens (Wright et al. 2006).

Deoxygenation involves the displacement or stripping of oxygen with another inert gas such as nitrogen or carbon dioxide. This process is primarily physical in nature, although the addition of carbon dioxide may trigger a chemical response and result in a reduction in ballast water pH (Tamburri et al. 2006).

Biological Treatment

By far, the least common method of ballast water treatment involves the use of biological organisms to directly kill or produce conditions that will kill or inactivate organisms present in ballast water. These treatment organisms are considered an “active substance” according to the IMO definition (IMO 2005). One example of biological treatment is the use of yeast to produce low-oxygen (hypoxic) conditions in ballast tanks. In this instance, yeast cells extract the available oxygen in the ballast water tank during cell replication (Bilkovski, R., pers. comm. 2008). The resultant hypoxic environment is toxic to the remaining organisms in the ballast tank. Vendors of biological treatment systems will likely need to address how systems will meet the performance standards, as the organisms responsible for producing the desired killing effect on NIS may trigger non-compliance if detected in the discharged ballast.

Combination Treatment

Several treatment technologies inactivate organisms by combining mechanical, chemical, physical and/or biological treatment processes, and are referred to as “combination treatment” in this report. In combination treatment, any single treatment method may not be sufficient to treat the ballast water to required standards, but in combination the methods produce the desired result. For example, while filtration is rarely sufficient to remove organisms of all size classes from ballast water, and UV irradiation may be insufficient to deactivate dense clusters of organisms, paired together they may be an effective method of ballast water treatment. The most common combined treatment methods pair mechanical removal with a physical or chemical process.

Treatment Systems

Twenty-eight treatment technologies were reviewed in the first technology assessment report for the California Legislature (see Dobroski et al. 2007). As of the writing of this report, one treatment vendor (L. Meyer Gmbh) appears to no longer be active in the international market, and was therefore removed from the list of reviewed systems

(Table V-1). Two manufacturers - Hamann and Evonik Degussa - were condensed into one listing because their treatment system is a combined effort. The Japan Association of Marine Safety was renamed as Mitsui Engineering, and four vendors - ATG Willand, EcologiQ, Panasia, and the Toagosei Group - were added to the list based on new information (Table V-1). Thus for this report, Commission staff compiled and reviewed information on 30 shipboard ballast water treatment systems developed in 10 countries (Table V-1).

Twenty-one of the treatment systems reviewed here utilize combination treatment methods, 18 of which pair mechanical treatment with another treatment method(s). Aside from mechanical separation, the most common method used in ballast water treatment systems is chemical. Of the 30 systems reviewed, 18 use a chemical in the treatment process (Table V-1). Specifically, six systems use chlorine or the electrolytic generation of sodium hypochlorite, one uses chlorine dioxide to treat ballast water, four systems use ozone, one uses ozone and electrolytic chlorination, one uses ferrate, one uses a proprietary mixture of peracetic acid, hydrogen peroxide and acetic acid (Peraclean Ocean) and three use advanced oxidation or electrolytic processes that can generate an array of oxidants including bromine, chlorine, and/or hydroxyl radicals (Table V-1).

The next most commonly used method of ballast water treatment amongst the 30 systems reviewed is UV irradiation. Six treatment systems use UV as the primary means to kill or deactivate organisms found in ballast water. All of these systems pair UV treatment with either filtration or hydrocyclonic mechanical separation methods.

Only three systems used deoxygenation as the major form of treatment. Technology treatment categorized as “other” include systems that used various methods including a non-oxidizing biocide (menadione), a heat treatment technology, and one technology using a combination of coagulation and magnetic separation (Table V-1).

Table V-1. Ballast Water Treatment Systems Reviewed by Commission Staff

Manufacturer	***Country***	***System Name***	***Technology Type***	***Technology Description***	***Approvals***
Alfa Laval	Sweden	PureBallast	combination	filtration + advanced oxidation technology (hydroxyl radicals)	IMO Basic and Final Type Approval (Norway)
ATG Willand	United Kingdom		combination	hydrocyclone + UV	
Ecochlor	USA	Ecochlor™ BW Treatment System	combination	filtration + biocide (chlorine dioxide)	IMO Basic
EcologiQ	USA/Canada	BallaClean	biological	deoxygenation	
Electrichlor	USA	Model EL 1-3 B	chemical	biocide (electrolytic generation of sodium hypochlorite)	
Environmental Technologies Inc.	USA	BWDTS	combination	ozone + sonic energy	
Ferrate Treatment Technologies LLC	USA	Ferrator	chemical	biocide (ferrate)	
Greenship Ltd	Netherlands	Sedinox	combination	hydrocyclone + electrolytic chlorination	IMO Basic
Hamann Evonik Degussa	Germany	SEDNA System	combination	hydrocyclone + filtration + biocide (Peraclean Ocean)	IMO Basic and Final, Type Approval (Ger.)
Hi Tech Marine	Australia	SeaSafe-3	physical	heat treatment	Queensland EPA
Hitachi	Japan	ClearBallast	combination	coagulation + magnetic separation + filtration	IMO Basic
Hyde Marine	USA	Hyde Guardian	combination	filtration + UV	WA Conditional
JFE Engineering Corp.	Japan	JFE BWMS	combination	filtration + biocide (sodium chlorine) + cavitation	
MARENCO	USA		combination	filtration + UV	WA General Approval
Maritime Solutions Inc.	USA		combination	filtration + UV	

Table V-1 (Continued). Ballast Water Treatment Systems Reviewed by Commission Staff

Manufacturer	***Country***	***System Name***	***Technology Type***	***Technology Description***	***Approvals***
MH Systems	USA	BW treatment system	combination	deoxygenation + carbonation	
Mitsubishi Heavy Industries	Japan	Hybrid System	combination	filtration + electrolytic chlorination	
Mitsui Engineering	Japan	Special Pipe	combination	mechanical treatment + ozone	IMO Basic
NEI	USA	Venturi Oxygen Stripping (VOS)	combination	deoxygenation + cavitation	Type Approval (Liberia)
NK-O3	Korea	BlueBallast	chemical	ozone	IMO Basic
Nutech 03 Inc.	USA	SCX 2000, Mark III	chemical	ozone	
OceanSaver	Norway	OceanSaver BWMS	combination	filtration + cavitation + nitrogen supersaturation + electrodialysis	IMO Basic and Final
OptiMarin	Norway	OptiMarin Ballast System	combination	filtration + UV	
Panasia Co. Ltd	Korea	GloEn-Patrol	combination	filtration + UV	IMO Basic
Resource Ballast Technologies	South Africa	RBT Reactor	combination	cavitation + ozone + sodium hypochlorite + filtration	IMO Basic
RWO Marine Water Technology	Germany	CleanBallast	combination	filtration + advanced electrolysis	IMO Basic
SeaKleen (Hyde)	USA	SeaKleen	chemical	biocide (menadione)	
Severn Trent DeNora	USA	BalPure	chemical	electrolytic generation of sodium hypochlorite + neutralizing agent (sodium bisulfite)	WA Conditional
Techcross Inc.	Korea	Electro-Cleen	chemical	electrochemical oxidation + neutralizing agent (sodium thiosulfate)	IMO Basic and Final
Toagosei Group	Japan	TG BallastCleaner TG Environmentalguard	combination	filtration + biocides (sodium hypochlorite) and neutralizing agent (sodium sulfite)	IMO Basic

VI. ASSESSMENT OF TREATMENT SYSTEMS

The Coastal Ecosystems Protection Act required the adoption of regulations to implement performance standards for the discharge of ballast water. Over 80% of voyages to California ports report that they do not discharge ballast into California waters (Falkner et al. 2007). These vessels will comply with the performance standards simply by retaining all ballast onboard. Vessels that do discharge but use nontraditional sources for ballast water (such as freshwater from a municipal source or treated grey water) will likely meet the discharge standards without the need for onboard ballast water treatment systems. Vessels that utilize coastal or ocean water as ballast, however, will require ballast treatment prior to discharge. For these vessels, the assessment of treatment system efficacy, availability, and environmental impacts (as required by PRC Section 71205.3(b)) is an important step towards understanding if systems will be available prior to the implementation of the interim performance standards for newly built vessels with a ballast water capacity of less than 5000 MT beginning in 2010.

Efficacy

During the preparation of the initial technology assessment report, the evaluation of ballast water treatment system performance (i.e. efficacy) was a challenge for a number of reasons. First and foremost, the lack of available data precluded any form of assessment for many systems. For those systems with data for review, the inconsistency in testing methodologies among systems and occasionally between tests of a single system made comparison of data impossible. Results often varied in scale (pilot vs. full-scale) and location (laboratory vs. dockside vs. shipboard), and were frequently presented in metrics incompatible with California's standards (i.e. as percent reduction instead of concentration of organisms). Perhaps most importantly, the majority of the available information was not subject to rigorous evaluation by independent, third-party scientific testing organizations.

While some of these challenges remain, most notably the lack of information for some systems, Commission staff has seen a significant improvement in the quantity and particularly the quality of available data. Many treatment vendors and developers are in the midst of full-scale land-based and shipboard evaluations of treatment system performance in order to receive Type Approval before the ratification and implementation of the IMO Convention. As a result, there is more data available for Commission staff to review than for the previous report. Much of this new research is being conducted by independent, third-party testing organizations and is presented using methods and in metrics directly comparable to California's standards. Therefore the quality of the new information is substantially higher than seen previously, and the data directly lends itself to comparison against California's performance standards.

Commission staff compiled and reviewed all available literature and performance data in order to assess system potential to meet California's performance standards (see Table III-1 for performance standards). While all of the new data was presented according to organism size class, some of the data that has not been updated since the previous report is presented by organism type (i.e. zooplankton, phytoplankton). In an effort to standardize results, staff evaluated any data on zooplankton abundance as representative of the largest size class of organisms (greater than 50 µm in size), and phytoplankton abundance was evaluated on par with organisms in the 10 – 50 µm size class. These substitutions were solely for the purpose of this report and will not be applicable to future compliance verifications. Ultimately, Commission staff gathered efficacy data on 20 of the 30 technologies reviewed in this report (Table VI-1, Appendix B).

Staff evaluated the data in light of the best available methods and techniques for assessing organism concentration and viability for each of the size classes in California's performance standards. The technical advisory panel that guided the development of the "Ballast Water Treatment Technology Testing Guidelines" assisted Commission staff with the production of a table (see Table 5-1 in Appendix A) listing commonly accepted methods of organism enumeration and viability determination for each of the organism size classes. While this list is not all-inclusive, it provides a framework that guided staff's critical evaluation of data on treatment system efficacy.

As a whole, the field of treatment performance assessment, like that of treatment technology development, is still emerging. Scientists are striving to find rapid, innovative techniques that can be used by both scientists and regulatory agencies to assess vessel discharge compliance with the relevant performance standards. Because California's performance standards for organisms less than 10 micrometers in size (bacteria and viruses, but not including protists) have not been adopted by any other regulatory entity in the world, there is not a worldwide push to develop assessment techniques for these size classes of organisms. Currently, there are no available techniques to both quantify and assess the viability of all bacteria and viruses in a sample of ballast water (see Appendix A1 for discussions on this topic).

To assess compliance with the bacterial standard, Commission staff used a proxy group of organisms (culturable, aerobic, heterotrophic bacteria – hereafter culturable heterotrophic bacteria) to represent the larger group of all bacteria. Culturable heterotrophic bacteria were selected as a proxy for total bacteria because, unlike total bacteria, there are reliable, well-accepted standard methods to both enumerate and assess viability of these organisms. Culturable heterotrophic bacteria are a well-studied group of bacteria, and research is being conducted to examine the relationship between their populations and the larger pool of bacterial species (see Appendix A1, Dobbs, F., pers. comm. 2008). Staff examined the data on treatment system performance at reducing culturable heterotrophic bacteria to levels within the California standard of 1000 bacteria (in this case expressed as colony-forming units) per 100 ml of ballast water. At a subsequent advisory panel meeting, members debated whether such a proxy group of organisms should be held to a different standard than that written in the law (see Appendix C for discussion). For instance, because heterotrophic bacteria are a subset of all bacteria they should be held to a standard in proportion to their relative abundance in nature (for example if heterotrophic bacteria represent 10% of the total population of bacteria, the standard for assessment using this proxy group might be more appropriate if set at 10% of 1000/100 ml or 100 CFU/100 ml). However, until such a debate is settled, Commission staff will continue to analyze all data using best available techniques and the numerical standard found in the law.

Analysis of viral species is challenging at this time. While several representative organisms exists for viruses (see discussions in Appendix A1), their relationship to the greater population of all viral species is more tenuous than for bacteria (cf. Culley and Suttle 2007). One option for future analyses involves the use of a subset of viruses known as bacteriophages (viruses that infect bacteria) (see Appendix A), but further discussion will be necessary to determine how this proxy organism could be analyzed to broadly represent treatment of viral species. For the purposes of this analysis, Commission staff believes that no widely accepted technique or proxy is available, and thus systems were not evaluated for compliance with the viral standard. Staff will continue to monitor the development of new assessment techniques for all organism size classes and incorporate them into future technology assessment reports.

Staff summarized the potential for all reviewed treatment systems to meet both the IMO and California performance standards (as assessed using best available methods) in Table VI-1. A positive compliance assessment for the purpose of this report, however, does not relieve the vessel owner/operator of the responsibility of complying with California's performance standards for the discharge of ballast water. Potential treatment system customers should consult extensively with vendors to ensure that thorough system verification work has been conducted and that the system is appropriate for the type of vessel under normal ballasting conditions.

Table VI-1. Summary of systems with available results for assessment of efficacy

Those systems with at least one replicate in compliance with the performance standards are denoted by a “Y” in the appropriate column in Table VI-1. Non-compliance is denoted by an “N,” and those systems with data in metrics not directly comparable to the performance standards were designated as “unknown.” A blank cell or hashing indicates that no data was available.

Manufacturer	> 50 µm		10 - 50 µm		< 10 µm (bacteria)		*E. coli*		Enterococci		*V. cholerae*		Reference[3]
	IMO	CA	IMO	CA	IMO	CA[1,2]	IMO	CA	IMO	CA	IMO	CA	
Alfa Laval	Y	Y	Y	Y	N/A	N	Y	Y	Y	Y	Y[4]	Y[4]	1,68,93,94
ATG Willand					N/A								
Ecochlor	Y	Y	Y	Y	N/A	Y	Y	Y	Unknown		Y	Y	63,99
EcologiQ					N/A								
Electrichlor					N/A								
ETI			Y	N	N/A	N							59,60,61,62
Ferrate Treatment Tech.	Unknown				N/A		Y	N	Y	N	Y	Y	22
Greenship	Y	Y	Y	Y	N/A	Y	Y	Y	Y	Y			25,114
Hamann Evonik Degussa	Y	Y	Y	Y	N/A	Y	Y	Y	Y	Y			38,91,129
Hi Tech Marine	Unknown		Unknown		N/A								41
Hitachi	Y	Y	Y	Y	N/A		Y	Y					75
Hyde Marine	Y	Y	Unknown		N/A	N	Y	Y	Y[4]	Y[4]			56,57,138
JFE Engineering Corp.					N/A								
MARENCO	Y	Y	Y	N	N/A	Y							51,52,135
Maritime Solutions Inc.					N/A								
MH Systems	Unknown				N/A						Unknown		42
Mitsubishi Heavy Ind.					N/A								
Mitsui Engineering	Y	N	Y	Unknown	N/A	Unknown	Unknown		Unknown		Unknown		46,48,49
NEI	Y	Y	Y	Unknown	N/A	N	Y	Y	Y	Y	Y	Y	118,119,120
NK-03					N/A								
Nutech 03 Inc.	Y	Y	Y	N	N/A	Y	Y[4]	Y[4]	Y[4]	Y[4]	Y[4]	Y[4]	40,104,140
OceanSaver	Y	Y	Y	Y	N/A	Y	Y	Y	Y	Y	Y[4]	Y[4]	6,95,123
OptiMarin	Y	Y	Y	Y	N/A	Y	Y	Y	Y	Y	Y[4]	Y[4]	11,47,92,134
Panasia Co.					N/A								
Resource Ballast Tech.					N/A								
RWO Marine Water Tech.	Y[5]	Y[5]	Y[6]	Y[6]	N/A		Y	Y	Y	Y			71
SeaKleen (Hyde)	Y	Y	Y	Y	N/A	Unknown	Y	Y	Y[4]	Y[4]			8,21,36,57
Severn Trent DeNora	Y	Y	Y	Y	N/A	Y							39
Techcross Inc.	Y	Y	Y	Y	N/A		Y	Y	Y	Y	Y[4]	Y[4]	50,69
Toagosei Group					N/A								

[1] Bacteria were assessed through examination of culturable heterotrophic bacteria (expressed as colony forming units).

[2] No methods exist quantify and assess viability of viruses at this time.

[3] Numbered references can be found in Literature Cited section

[4] Concentration at intake was zero or non-detectable

[5] *Artemia* cysts only

[6] *Tetraselmis suecica* only

In the largest organism size class (organisms greater than 50 µm in size), 19 systems provided data and 15 demonstrated potential, in at least one testing replicate, to meet the required standard of no detectable living organisms per cubic meter of discharged ballast water (Table VI-2, Appendix B1). Similar results were seen in the 2007 report (Dobroski et al. 2007) when 14 systems were designated as compliant for this largest size class of organisms. In the 10 – 50 µm size class, 18 systems were reviewed and 11 systems had at least one test replicate that indicated compliance with the requirement of less than 0.01 living organisms per milliliter (ml) (Table VI-2, Appendix B2). This is an increase of 3 systems since 2007.

The results of analyses for human health indicator species (*Escherichia coli*, intestinal enterococci and *Vibrio cholerae*) and organisms less than 10 µm (bacteria and viruses) are varied. Several more vendors have completed assessment of system performance at eliminating human health indicator species since the 2007 report. Fifteen systems provided results of *E. coli* concentration in treated ballast water (Appendix B3). Fourteen of those were comparable to the California standard, and 13 demonstrated compliance – up from 10 in the 2007 report. Fourteen systems tested for the presence of intestinal enterococci, and eleven systems demonstrated potential compliance compared to three in 2007 (Appendix B4). Finally, ten systems examined treated ballast water for toxicogenic *Vibrio cholerae* and eight systems demonstrated potential compliance with the California performance standard (Appendix B5). Although this is an increase in both the number of systems testing for the presence of *Vibrio cholerae* and the number in compliance since the 2007 report, the low, and sometimes non-detectable, concentration of *Vibrio cholerae* in coastal waters makes it difficult to adequately assess system performance at eliminating the species.

As previously mentioned, the assessment bacteria and viruses relative to California's standards in treated ballast water has been problematic due to a lack of techniques to both enumerate and evaluate the viability of all bacterial and viruses in ballast water. A technical panel of experts that have assisted Commission staff with the development of the treatment technology testing guidelines (see Appendix A) recommend that the bacterial standard be assessed using culturable heterotrophic bacteria as a proxy group of bacterial organisms. Available data was analyzed for compliance with the bacterial standard of 1000 bacteria or CFU per 100 ml (Table VI-1). Fourteen systems analyzed system performance at treating culturable heterotrophic bacteria and twelve presented results in a metric comparable to the standard (CFU/100 ml) (Appendix B6). Eight demonstrated compliance with the standard. Many additional treatment vendors have conducted analyses of treated ballast water for culturable heterotrophic bacteria, but have not yet provided those data to Commission staff.

Results for the number (counts) of viruses or virus-like particles in ballast water samples either pre- or post-treatment were only available for two systems and only using bacteriophages (Appendix B7). Further discussion is required before staff can assess how phage concentrations relate to the total pool of viral species in ballast water and compliance with California’s standards. These numbers are simply recorded for now. On a theoretical note, experts variously refer to “viruses” as “virus-like particles”, and “virus-sized particles,” but no term is fully agreed upon within the scientific community (Dobbs, F., pers. comm. 2008). None of the systems that have provided information on virus assessment have defined what they referred to as a virus. Furthermore, some scientists contend that infectivity must be proved before calling an object a virus—and we cannot know simply by looking at one in a ballast water sample that it is infective. Staff will continue to follow advances in the field of viral science and will assess how future technology developments will impact our ability to assess compliance with the viral standard.

Table VI-2. Summary of Potential Treatment System Performance with Respect to California Performance Standards

	Organisms Greater than 50	Organisms 10 – 50	Organisms less than 10 (bacteria)[1]	*Escherichia coli*	Intestinal enterococci	*Vibrio cholerae*
Total Systems that provided Results[2]	19	18	14	15	14	10
Number Systems that Meet Standard[3]	15	11	8	13	11	8

[1] Bacteria examined using culturable heterotrophic bacteria (1000 CFU/100 ml)
[2] Of out of the 30 total systems assessed in this report, only 20 had testing results available for review. Not all 20 covered testing under each of the organism size classes. The total number of systems with results in a given size class is indicated in this category.
[3] This category reflects the number of systems with at least one replicate of system testing in compliance with the California performance standards (see Table III-1 for standards).

As seen in Table VI-1, 20 treatment systems have results available for analysis of system efficacy; the potential for the remaining ten systems to meet the California standards is not clear at this time. For those systems with results, sixteen systems demonstrated the potential to meet at least 1 out of seven performance standards organism size classes, fifteen systems met at least 2 size classes, fourteen systems met at least 3 size classes, eleven systems met at least 4 size classes, eight systems met at least 5 size classes, and two systems met 6 size classes (Table VI-1, Appendix B). Systems cannot be assessed for compliance with the viral standard at this time. Thus at least two systems, OceanSaver and OptiMarin, are capable of meeting all standards that can be assessed using the best available techniques and methods at this time. Overall, this is a marked improvement since 2007.

Commission staff expects several additional systems will meet California's standards in the near future. Many systems utilize similar treatment methods (i.e. chlorination/de-chlorination) and may likely produce similar types of results. Therefore a specific treatment method which has been shown to be effective for one system may likely be effective for a similar system for which data is lacking but which uses the same treatment method. While Commission staff did not assess system compliance in Table

VI-1 based on this assumption (i.e. in the absence of specific data from a particular system), the number of systems potentially capable of meeting California's performance standards is likely greater than directly evident based on currently available data.

Availability

An assessment of the availability of ballast water treatment systems requires an understanding of the relationship among many elements including the number of vessels that will be impacted by the performance standards (i.e. industry demand), commercial availability, and the relationship between government approval of systems and overall market demand for treatment technologies. Commercial availability is not simply a function of whether or not a system is available for purchase; it is also dependent on sufficient production of systems to meet demand and the availability of customer support. System availability is also influenced by the presence of an available market (i.e. demand) to purchase treatment systems. This market, in turn, will depend upon the development of mechanisms for systems approval, particularly at the federal and international levels, as vessel operators may be hesitant to purchase systems without government assurance that such systems will meet applicable standards. For the purposes of this report, however, treatment system availability is ultimately linked to system performance - the ability of a system to treat ballast water to a level in compliance with California's performance standards.

Industry Demand

The California performance standards have a phased implementation schedule similar to that of the IMO Convention (see Table III-2). The phased implementation provides greater time for large and/or existing vessels to execute plans for system installation including possible retrofits of vessel structures and machinery. The first implementation date for California will affect only new vessels built on or after January 1, 2010 with a ballast water capacity of less than 5000 MT. The number of new vessels that must meet the performance standards beginning in 2010 will greatly influence how quickly treatment vendors must have their systems available for sale. Lloyd's Register (2008) estimates that in 2009, worldwide construction will commence on 540 new vessels with a ballast capacity of less than 5000 MT. Presumably, a similar number of vessels will be constructed (i.e. as defined by keel laid date or commencement of major conversion) beginning in 2010, although no specific estimates are currently available. Exactly how many vessels will ultimately operate and discharge ballast in California waters is difficult to determine, however the numbers are expected to be relatively small.

Examination of the number of vessels that have previously arrived in California provides some insight into, and a very conservative estimate of, the number that must be prepared to meet the performance standards in 2010. Between January 2000 and August 2008, 908 unique vessels with a ballast water capacity less than 5000 MT arrived at California ports (Figure VI-1). Presuming a 20-year vessel replacement cycle, approximately 5% (45) of these 908 vessels may be replaced by new vessels and be required to meet the performance standards in 2010 (Reynolds, K., pers. comm. 2007). As only 20% of vessels, on average, discharge ballast in California waters (Falkner et al. 2007), an even smaller number of vessels (~ 9) will likely discharge in California waters and require treatment system usage. In the class of vessels with a ballast water capacity greater than 5000 MT, 5682 unique vessels arrived at California ports between January, 2000 and August, 2008 (Figure VI-1). Again, assuming a 5% yearly replacement rate, 284 vessels will likely be replaced with new vessels and be required to meet the performance standards beginning in 2012. Clearly, a much smaller number of new vessels will be required to meet the standards beginning in 2010 than in 2012, however, the precise number is less clear.

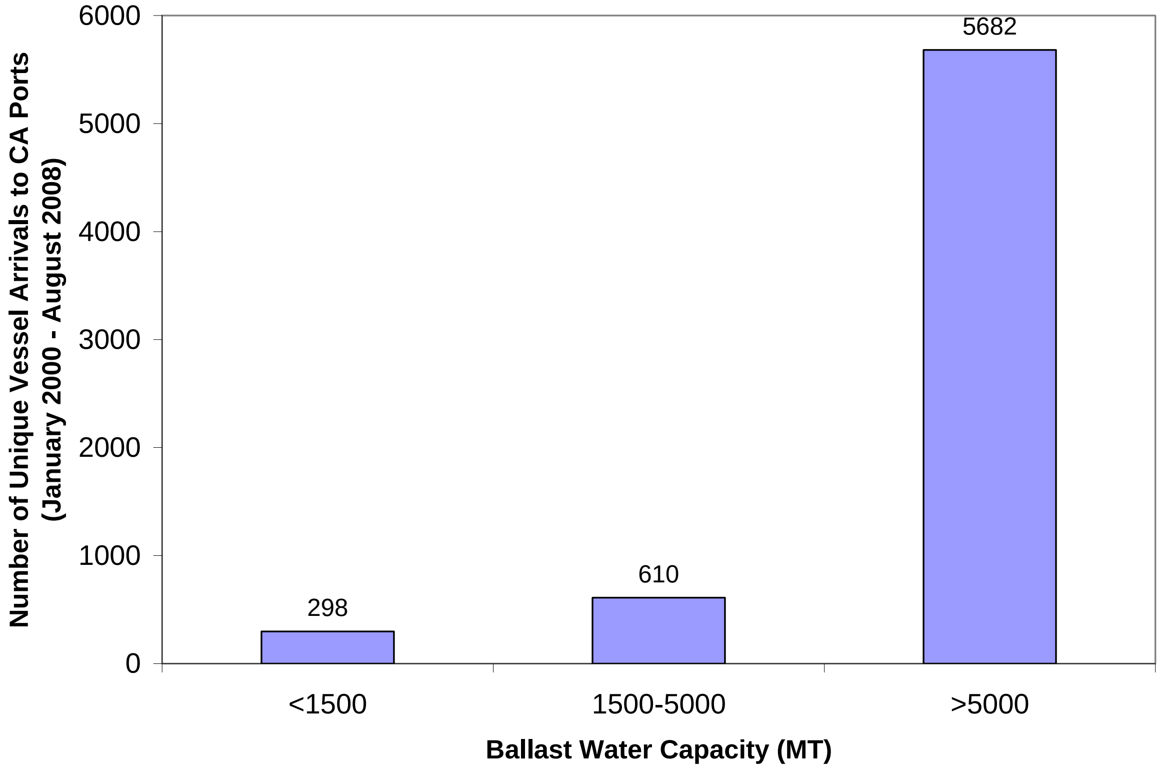

Figure VI-1. Number of unique vessels that arrived to California ports between January, 2000 and August, 2008 as a function of ballast water capacity (MT).

Assessing industry demand at any given time is further complicated by factors such as the timing of when a vessel owner chooses to purchase a treatment system. Vessel owners, particularly of existing vessels with later implementation dates, may choose to purchase a system earlier than required by the standards implementation date so that installation dovetails with drydock and repair schedules. In this case, estimates of demand based solely on the standards implementation dates are likely inaccurate. Commission staff will continue to follow trends in vessel visits to California and treatment system purchase and installation, particularly as the performance standards are implemented for newly built vessels, and will assess system availability for existing vessels in future reports.

Commercial Availability

System vendors will need to have systems commercially available by the time the initial interim performance standards take effect in 2010. The definition of commercial availability differs depending on who you talk to. Many treatment vendors consider commercial availability to be the time when systems are available for sale and purchase. Vessel owners, however, may not consider systems to be commercially available until all required approvals (IMO or otherwise) are in place (see Market Availability below for further explanation). In 2008, 23 treatment technology vendors provided Lloyd's Register with an actual or anticipated date of commercial availability. Ten companies reported that their systems were commercial availability by 2007, four expected to be commercial availability in 2008, seven anticipated availability in 2009 and two in 2010 (Lloyd's Register 2008). Similar data collected by Commission staff indicate that as of October 2008 at least 12 technology vendors consider their systems to be commercially available. On the other hand, only three systems have currently received Type Approval as required by IMO (Table V-1).

In addition to having systems ready for purchase, treatment vendors will also need to produce sufficient quantities of those systems to meet market demand. Several of the large, multinational technology vendors already produce many other products for the maritime industry and have a pre-existing infrastructure in place that may be modified to globally produce and support ballast water treatment systems (Reynolds, K., pers. comm. 2007). However, it is more difficult to gauge the ability of small technology vendors to meet projected production and support needs of the shipping fleet. Treatment vendors may be able to space out delivery of systems for new vessels with a ballast capacity less than 5000 MT over a couple of years while infrastructure and production are brought up to speed, as even the largest marine corporations require significant lead time for existing marine product lines (Reynolds, K., pers. comm. 2007). While vessels in this size class are subject to the standards as of 2010, the construction of large commercial vessels can take several years, and many of those vessels may not actually be ready for treatment system installation and operation until 2011 or later.

System support is equally important as commercial availability. Following installation, system developers will need to have personnel and infrastructure in place to troubleshoot and fix problems that arise during system operation. Maritime trade is a global industry, and vessel operators will need to have global support for onboard machinery. The Lloyd's Register (2008) report does not address the issue of after-purchase support of systems. The initial influx of systems into the marketplace will no doubt challenge developers to provide adequate service. Larger companies entrenched in the maritime logistics or equipment industries may already be prepared to respond to technological challenges and emergencies as they arise, but smaller ballast water treatment vendors may face an initial period to ramp up service and access to replacement parts. It is currently unclear if system support service will be adequate as the first of California's performance standards is implemented in 2010, and if a lack of service could impact commercial availability.

Market Availability

The availability of ballast water treatment systems is not only a function of commercial availability but also of market demand to purchase those technologies. Previous discussions addressed one aspect of demand - the number of vessels that will be required to meet the performance standards beginning in 2010. However, demand may also be influenced by the availability of systems that have received government approval to operate in a given water body.

In the U.S., the lack of a regulatory framework for the approval of ballast water treatment systems at the federal level is a major hindrance to the demand for systems. While California law requires initial compliance with the interim performance standards beginning in 2010, shipping companies may be hesitant to purchase treatment systems with little or no assurance that the system will be permitted to operate in federal waters. As of October 2008, neither the EPA nor the USCG has a ballast water treatment approval program in place. Vessels cannot use treatment systems to comply with the federal ballast water management requirements unless they are approved. Therefore, unless these federal agencies begin to approve systems before 2010, a vessel intent on discharging ballast in California after arriving from outside of the 200 nm Exclusive Economic Zone will need to conduct a mid-ocean exchange to comply with federal ballast water management requirements and will additionally be required to treat that water to meet California requirements. This conflict in ballast management regulation between federal and state governments will no doubt cause confusion and may even temper demand to install treatment systems onboard vessels. While it is extremely unlikely that all vessels that visit California can refrain from discharging all ballast, the implementation of the performance standards regulations in California may spur renewed interest in developing ballast water management plans that will limit ballast water discharges in the state.

Availability for Use in California

Commercial availability should not, however, at any time be confused with a system's capability to meet California's performance standards. Systems that may be deemed commercially available and ready for sale by technology vendors must demonstrate system efficacy to vessel owners/operators who will purchase those systems and to regulatory agencies. Systems that have received IMO approval for active substances and Type Approval may be available for purchase in compliance with the IMO D-2 standards, but for the purposes of this report, those systems are not deemed "available" for use in California until they demonstrate system efficacy and environmental safety in compliance with California's performance and water quality standards. Based on the information reviewed for this report, at least two systems, OceanSaver and OptiMarin, are both commercially available and have demonstrated the potential to comply with California's performance standards that can be currently quantified using best available assessment techniques (see previous discussion in Efficacy section). Several additional systems are close to completing performance verification testing and/or receiving Type Approval, and Commission staff believes that these systems will be available for use in California prior to the initial implementation of the performance standards in 2010.

Environmental Regulation and Impact Assessment

An effective ballast water treatment system must comply with both performance standards for the discharge of ballast water and applicable environmental safety and water quality laws and regulations. The discharge of treated ballast should not impair water quality so as to impact the designated beneficial uses of the State's receiving waters (e.g. recreation, fisheries, fish/wildlife habitat). The IMO, federal government and individual states have developed specific limits for discharge constituents and/or whole effluent toxicity evaluation procedures in order to protect the beneficial uses of waterways from harmful contaminants. Commission staff has drawn on the environmental review of ballast water treatment systems and active substance constituents from all levels of government (international, federal, state) in the assessment of environmental risk from the 30 treatment systems reviewed here.

International Maritime Organization Regulation

As discussed in Section III (Regulatory Overview), the IMO has established an approval process through Guideline G9 for treatment technologies using active substances (i.e. chemicals) to ensure systems are safe for the environment, ship, and personnel. The two-step process is comprised of an initial “Basic Approval” utilizing laboratory test results to demonstrate basic environmental safety followed by “Final Approval” upon evaluation of the environmental integrity of the full-scale system.

Guideline G9 of the Convention requires applicants to provide information identifying: 1) Chemical structure and description of the active substance and relevant chemicals (byproducts); 2) Results of testing for persistence (environmental half-life), bioaccumulation, and acute and chronic aquatic toxicity effects of the active substance on aquatic plants, invertebrates, fish, and mammals; and 3) An assessment report that addresses the quality of the tests results and a characterization of risk (MEPC 2005b). Systems that apply for Basic and Final Approval are reviewed by the IMO Joint Group of Experts on the Scientific Aspects of Marine Environmental Protection (GESAMP) – Ballast Water Working Group (BWWG) in accordance with the procedures detailed in Guideline G9. The Guideline does not address system efficacy, only environmental safety (MEPC 2005b).

Federal Regulation

Outside of USCG’s Shipboard Technology Evaluation Program (STEP), ballast water treatment systems are not currently approved for use in compliance with federal ballast water management requirements. Consequently, there is no formal environmental assessment approval program (like that of IMO) for ballast water treatment systems at the federal level. EPA, however, recognizes that ballast water treatment systems will be used both experimentally at the federal level and in compliance with state ballast water management requirements, and has therefore included provisions in the draft NPDES Vessel General Permit for discharges from vessels employing ballast water treatment systems.

The effluent limits and best management practices described in the draft NPDES Vessel General Permit are specific to those treatment systems that make use of biocides. Under the permit, all biocides that meet the definition of a "pesticide" under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA; 7 U.S.C. § 136 *et seq*.) must be registered for use with the EPA. Biocides generated onboard a vessel solely through the use of a "device" (as defined under FIFRA) do not require registration. Additionally, the permit sets a limit for Total Residual Chlorine (100 µg/l) in ballast water discharge, and states that discharges of other biocides or residuals must not "exceed acute water quality criteria as listed in EPA's 1986 Quality Criteria for Water [the Gold Book], or any subsequent revisions" (EPA 2008). Furthermore, EPA requested public comment on whether it was appropriate to include Whole Effluent Toxicity (WET) standards in the permit to complement or to serve in lieu of complying with chemical monitoring. Though the permit has not been released at the time of this report, it is possible that EPA will include WET monitoring options. In lieu of complying with the aforementioned conditions, vessels that discharge ballast containing biocides or chemical residuals may apply for an individual NPDES permit.

Vessels participating in the STEP must comply with the NPDES Vessel General Permit and additionally conform to the environmental compliance requirements associated with STEP participation including: 1) Compliance with the National Environmental Policy Act (NEPA) process; 2) Due diligence by the applicant in providing requested biological and ecological information and obtaining necessary permits from regulatory agencies; and 3) A provision that systems found to have an adverse impact on the environment or presenting a risk to the vessel or human health will be withdrawn from the program (USCG 2006).

State of Washington Regulation

The Washington State Department of Ecology developed a framework for "Establishing the Environmental Safety of Ballast Water Biocides" in 2003 and revised it in 2005 to be included as Appendix H in the *Laboratory Guidance and Whole Effluent Toxicity Test Review Criteria* manual (Washington State Department of Ecology 2005). Thus far, three systems have completed toxicity testing in accordance with Washington requirements (Table VI-3).

The tests used in the Washington State framework for evaluating ballast water biocides include EPA-approved acute, chronic and sensitive life stage toxicity tests on invertebrate, fish and algal species. If treated ballast water might be discharged more than once in the same location during a week or in sensitive marine areas in the state, then additional tests are also required to determine the biocide environmental safety. The results of the toxicity testing are used to set system discharge conditions such as maximum concentration or minimum degradation time (Marshall, R., pers. comm. 2007).

California Regulation

California does not have a formal environmental evaluation process for ballast water treatment systems. Vessels that discharge in California waters will need to comply with the applicable provisions of the EPA's NPDES Vessel General Permit including all California-specific conditions added by the State Water Resources Control Board through the Section 401 certification process. As of the writing of this report, the permit conditions included in the 401 certification were not available.

All vessels using treatment technologies that make use of biocides should also ensure that any residuals or reaction by-products in treated ballast water discharges meet applicable water quality objectives as outlined in the California Ocean Plan (Water Board 2005), Regional Water Quality Control Board Basin Plans, and the EPA's

California Toxics Rule (CTR) and associated State Implementation Policy for the CTR. Vessel owners/operators will need to consult with Water Board staff regarding the development and implementation of monitoring programs for all relevant discharge constituents. The "Ballast Water Treatment Technology Testing Guidelines" that were distributed to treatment vendors in October 2008 were developed in consultation with the Water Board, and summarize the water quality objectives and acute and chronic water toxicity criteria that systems will need to comply with when discharging in California waters (see Appendix A).

Environmental Assessment of Treatment Systems

Staff has compiled environmental assessment reports and water quality data reported to the IMO and the State of Washington, as well as information made available to Commission staff, to assess the treatment systems for potential environmental impacts to California waters. The IMO active substance approval documents, in particular, have proved to be a valuable resource to assess a treatment system's broad-scale environmental safety prior to comparison of specific system effluent constituents with California's water quality objectives.

Of the 30 treatment systems reviewed for this report, 19 use a biocide or chemical additive in the treatment process (Table VI-3), and will therefore require monitoring of discharges for chemical residuals under the EPA's NPDES Vessel General Permit and the State's Ocean Plan. As discussed in Section V (Treatment Technologies), eighteen of the 19 systems that use chemicals employ a chemical oxidant or oxidative technology as the active substance to kill or inactivate organisms in ballast water (Table VI-3). An assessment of the potential impacts from the wide variety of chemical residuals associated with the use of oxidants by each technology cannot be adequately addressed in this report and is the purview of the Water Board and EPA. Instead, Commission staff has focused this environmental assessment on Total Residual Chlorine (TRC) concentrations in discharged ballast water because both EPA (through the draft NPDES Vessel General Permit) and the Water Board (through the California Ocean Plan) have identified TRC as a particular concern due to its widespread toxicity to all organisms. Vendors and vessel owners/operators will need to consult with the Water Board and EPA to ensure that vessel discharges comply with all other applicable effluent requirements.

Table VI-3 lists the active substances and summarizes the status of environmental approvals/assessments for each of the technologies reviewed in this report. Where applicable, the available data has been analyzed to determine whether or not treated ballast would comply with California's water quality objective for chlorine in ocean waters (= instantaneous maximum of 60 µg/l in discharged waters).

Many systems have initiated toxicity testing of treated discharges and have applied to IMO for Basic and Final Approval. The IMO Basic Approval application, however, may include data from general literature review or laboratory analysis of system toxicity. Until such time that a system submits a full dossier of whole effluent toxicity data as required for IMO Final Approval, it will be difficult to anticipate the potential environmental impacts to California waters from the discharge of treated ballast from a fully functioning treatment system. Currently only four treatment systems have received Final Approval from IMO (Table VI-3).

The "pesticide" registration requirement under FIFRA is one mechanism to regulate and assess the impacts to U.S. federal waters from biocide use in treatment systems. The thorough chemical safety analysis and registration process required under FIFRA has been completed by one system (Hamann Evonik Degussa), and a few others are in the process of completing the process. FIFRA has a loophole, however, for chemicals that are generated onsite and used in place (e.g. generated and used by a vessel). Most treatment systems using biocides generate that chemical through onboard electrochemical processes, and thus will not be subject to FIFRA registration. This FIFRA loophole provides significant room for systems to operate in U.S. waters without any kind of biocide regulation except as provided by the NPDES Vessel General Permit, and at this time, it is uncertain how EPA will enforce the permit's provisions.

Table VI-3. Summary of environmental assessment and approval of treatment systems

Note: Table does not address whether or not toxicity testing was performed in accordance with California Ocean Plan

Manufacturer	Active Substance	Toxicity Testing	Environmental Related Approvals	CA TRC Compliant[1]	Source
Alfa Laval	free radicals	X	IMO Basic and Final	Y	73,93
ATG Willand	n/a (UV)				
Ecochlor	chlorine dioxide	X	IMO Basic, Rec WA Cond.[2]	Y	84
EcologiQ	yeast	X			3
Electrichlor	sodium hypochlorite				
ETI	ozone	X			62
Ferrate Treatment Tech.	ferrate				
Greenship	free active chlorine, total residual chlorine	X	IMO Basic		80,86
Hamann Evonik Degussa	Peraclean Ocean (peracetic acid, hydrogen peroxide, acetic acid)	X	IMO Basic & Final, EPA Reg., Rec. WA Conditional[2]		77,91
Hi Tech Marine	n/a (heat)		Queensland EPA		122
Hitachi	triiron tetraoxide, poly aluminum chloride, poly acrylamide sodium acrylate	X	IMO Basic		75
Hyde Marine	n/a (UV)				
JFE Engineering Corp.	sodium chlorine				
MARENCO	n/a (UV)				
Maritime Solutions Inc.	n/a (UV)				
MH Systems	n/a (deoxygenation)				
Mitsubishi Heavy Ind.	free active chlorine				72
Mitsui Engineering	ozone	X	IMO Basic		69
NEI	n/a (deoxygenation)	X			10
NK-03	ozone, total residual oxidant	X	IMO Basic	Y	85
Nutech 03 Inc.	ozone	X		N	140
OceanSaver	free and total residual oxidant	X	IMO Basic and Final	Y	2,82,87
OptiMarin	n/a (UV)	X		Y	92
Panasia Co.	photon		IMO Basic		78,81
Resource Ballast Tech.	ozone, hydroxyl radicals	X	IMO Basic	N	74
RWO Marine Water Tech.	hydroxyl radicals, free active chlorine	X	IMO Basic	N	76,79
SeaKleen	menadione (Vitamin K3)	X			8
Severn Trent DeNora	sodium hypochlorite, sodium bisulfite	X	Rec. WA Conditional[2]	Y	39
Techcross Inc.	hypochlorite, hypobromite, ozone, hydroxyl radicals, hydrogen peroxide	X	IMO Basic and Final	Y	83,86
Toagosei Group	sodium hypochlorite, sodium sulfite	X	IMO Basic		86

Blank cells indicate that data was not available

[1] CA Ocean Plan instantaneous maximum for Total Residual Chlorine = 60 micrograms/liter (µg/l)

[2] WA Dept. of Ecology Water Quality Program has recommended Conditional Approval of the system to WA Dept. Fish and Wildlife. As of the writing of this report, approval has not been granted.

A system's feasibility for operation in California is inherently based on its ability to meet all of California's requirements regarding discharges, not simply the performance standards. While it is the purview of the Water Board to review and regulate the effluent from treatment systems, Commission staff is working to educate technology vendors, particularly those from foreign countries, about California's water quality objectives. The "Ballast Water Treatment Technology Testing Guidelines" were recently distributed and summarize the pertinent information for vendors. Staff will also work closely with the Water Board to ensure that vendors are made aware of California's Section 401 provisions in the NPDES Vessel General Permit, once released. In the meantime, staff has attempted to compile data on Total Residual Chlorine (TRC) in treated effluent because of its broad-scale toxicity, and because so many systems use chlorine and related byproducts in the treatment process. Of the 30 systems reviewed, thirteen use chlorine in the treatment process or may have chlorinated residuals in treated effluent. Based on the available data, seven appear to meet California's objective of 60 µg/L of residual chlorine (Table VI-3). Clearly, not all treatment systems will meet California's stringent water quality standards. However, it is difficult to assess at this time whether systems are simply not able to meet the standards or whether additional water quality data must be gathered from operation of full-scale systems under real world scenarios. Commission staff will continue to work with the Water Board, vessel owners/operators and technology vendors to ensure that systems are tested with California's water quality objectives in mind and that the information is made available to interested parties.

Economic Impacts

An assessment of the economic impacts associated with the implementation of performance standards and the use of treatment technologies requires consideration not only of costs connected with the purchase, installation and operation of treatment systems, but also the impacts related to the control and/or eradication of NIS if performance standards are not met. As discussed in the Introduction (Section II), the U.S. has suffered major economic losses as a result of attempts to control and eradicate NIS (aquatic and terrestrial; Carlton 2001, Lovell and Stone 2005, Pimentel et al. 2005). The rate of new introductions is increasing (Cohen & Carlton 1998, Ruiz & Carlton 2003) which suggests that economic impacts will likely increase as well.

California had the largest ocean-based economy in the U.S. in 2004, ranking number one for employment, wages and gross state product (NOEP 2007). California's natural resources contribute significantly to the coastal economy. For example, in 2007 total landings of fish were over 380 million pounds, bringing in more than $120 million (NOEP 2008). Squid, the top revenue-generating species in 2007, brought in almost $30 million (NOEP 2008). The health of coastal natural resources is also closely tied to the tourism and recreation industries which accounted for almost $12 billion of California's gross state product in 2004 (NOEP 2007). NIS pose a threat to these and other components of California's ocean economy including commercial fisheries, aquaculture, sport and recreational fisheries, tourism and recreation, and education.

The use of ballast water treatment technologies to combat NIS introductions will involve economic investment on the part of ship owners. This investment in treatment systems reflects not only initial capital costs for the equipment and installation, but also the continuing operating costs for replacement parts, equipment service and shipboard energy usage. Cost estimates are strongly linked to vessel-specific characteristics including ballast water capacity, ballast pump rates and available space. Additionally, the retrofit of vessels already in operation (existing vessels) with ballast water treatment technologies may cost significantly more than installation costs for newly built vessels due to: 1) The necessity to rework existing installations (plumbing, electric circuitry); 2) Non-optimal arrangement of equipment that may require equipment be broken into pieces and mounted individually; 3) Relocation of displaced equipment; and 4) The time associated with lay-up (Reynolds, K., pers. comm. 2007). Nonetheless, the use of these treatment technologies will help minimize or prevent future introductions of NIS and relieve some of the future economic impacts associated with new introductions.

Many treatment technology vendors are hesitant to release costs at this point because system prices represent research and development costs and do not reflect the presumably lower costs that would apply once systems are mass produced. In the 2008 Lloyd's Register report, only 16 of 29 technologies profiled provided estimates of system capital expenditures (equipment and installation) and half (14) provided estimates of system operating expenditures (parts, service, and energy usage; Table VI-4). Commission staff has also acquired some data on capital and operating costs. Capital expenditure costs are dependent on system size. A 200 cubic meters per hour (m^3/h) capacity system may require an initial capital expenditure between $145,000 and $780,000 with an average cost of $387,500 (Lloyd's Register 2007, Lloyd's Register 2008, Commission data from technology vendors 2007-2008). A 2000 m^3/h capacity system ranges from $175,000 to $2,300,000 with an average cost of $894,600 per system (Lloyd's Register 2007, Lloyd's Register 2008, Commission data from technology vendors 2007-2008). Operating costs range from negligible, assuming waste heat is utilized, to $1.50 per m^3 with an average of $0.13 per m^3 (Lloyd's Register 2007, Lloyd's Register 2008, Commission data from technology vendors 2007-2008).

Treatment systems will likely increase the cost of a new vessel by 1-2%. For example, a new 8500 TEU (twenty-foot equivalent unit) container ship built by Seaspan Corporation costs approximately $132.5 million per vessel (Seaspan Corporation 2007). Installation of the most expensive treatment system currently available at $2.3 million (as indicated in Table VI-4) would increase the cost of that vessel by 1.7%. Many treatment technology developers claim that their systems will last the life of the vessel, so the capital costs for treatment systems should be a one-time investment per vessel.

While the economic investment by the shipping industry in ballast water treatment technologies will be significant, when compared to the major costs to control and/or eradicate NIS, the costs to treat ballast water may be negligible. Treating ballast water with treatment technologies will help to prevent further introductions and lower future costs for control and eradication. Additional studies will be necessary to obtain actual economic impacts associated with treating ballast water.

Table VI-4. Summary of capital and operating cost data for select treatment systems. Unless otherwise denoted, source of data was Lloyd's Register (2008).

Manufacturer	Capital Expenditure *(Equipment & Installation)*			Operating Expenditure
	200 m3/h *($ in thousands)*	2000 m3/h *($ in thousands)*	Other *($ in thousands)*	*($ per m3, unless otherwise noted)*
Alfa Laval				0.015[1]
ATG Willand				
Ecochlor	500	800		0.08
EcologiQ			<50[1]	1-1.50[1]
Electrichlor	350			0.019
ETI		500		0.005
Ferrate Treatment Tech.				
Greenship	300	2300		
Hamann Evonik Degussa				0.2
Hi Tech Marine	780	1600	16.5 – 300[1] *(equipment only)*	nil[2]
Hitachi		400		
Hyde			174 – 503[1]	0.01
JFE Engineering				0.04
MARENCO	145	175		0.0006-0.001
Maritime Solutions Inc.				
MH Systems	650	950		0.06
Mitsubishi				
Mitsui			100[1] *(installation only)*	0.15[3]
NEI	360	690		0.15
Nutech 03	288	150		0.32
OceanSaver		1600		0.06[3]
OptiMarin	430	1800		
Panasia				
Resource Ballast Technologies	200	500		
RWO Marine				
SeaKleen (Hyde)				
Severn Trent	350	500		0.013
Techcross	297	559		0.003
Toagosei Group				

[1] Source: Communications with technology vendors (2007-2008).
[2] Assumes waste heat utilized
[3] Source: Lloyd's Register (2007)

VII. CONCLUSIONS

Ballast water treatment remains an emerging industry. New technologies continue to be developed and existing ones refined in search of the most effective methods to reduce and/or eliminate the spread of nonindigenous species via ballast water release. While some hurdles remain to the full implementation of all of California's performance standards, significant progress has been made in the development of treatment systems since the previous technology assessment report (see Dobroski et al. 2007). Both the quantity and the quality of the recently received data on system performance attest to this fact.

Like the ballast water treatment industry, the fields of treatment technology assessment and compliance verification are still evolving. Commission staff has been working closely with a panel of technical experts in order to develop a set of ballast water treatment technology testing guidelines. The guidelines provide vendors with a summary of California's performance standards and relevant water quality objectives and toxicity criteria. Moreover, they provide some initial guidance on the selection of methods and techniques to assess system compliance with California's discharge standards.

The selection of best available assessment techniques for the guidelines has also informed staff's evaluation of system efficacy data for the purposes of this report. Challenges remain in assessing system compliance with the standards for organisms that are less than 10 micrometers in size – the bacteria and viruses. The best available technique for bacterial viability assessment involves the use of a subset or proxy group of organisms to represent treatment of bacteria as a whole. While this technique isn't without some debate, it is scientifically supported by many experts in microbiology and technology assessment (see Appendix A). The viruses pose a greater challenge. Without strong evidence for the selection of proxy or representative organisms in this size class, Commission staff believes that there are no acceptable methods for verification of compliance with the standard and that the Commission should proceed with assessment of technologies for the remaining six standards.

Based on the available information and using best assessment techniques, Commission staff believes that at least two treatment systems have demonstrated the potential to comply with the Commission's performance standards. Many additional systems are close to completing system performance verification testing and will soon have data available for review. Commission staff expects that before 2010 several systems will be ready to meet California standards.

The treatment systems that meet California's standards under the review for this report are commercially available at this time. The additional systems that are close to meeting California's standards are also commercially available. Many of these systems also expect to receive Type Approval in 2009, and thus these systems will be considered available for use both in California waters and in compliance with the IMO Convention, upon ratification.

The IMO approval pathway for systems utilizing active substances has been a resource for information about the potential environmental impacts from the discharge of treated ballast water. The number of systems that have received IMO Final approval remains small at this time, however, and thus environmental impact analysis of whole effluent toxicity remains hampered by a lack of data. The data available on Total Residual Chlorine concentration in treated ballast effluent makes it clear that not all systems will comply with California's water quality standards but also that additional information is necessary. The recently distributed treatment technology testing guidelines will inform vendors about California's water quality objectives and toxicity criteria and should influence environmental assessment of system discharges in the near future. Commission staff is also working closely with the Water Board to track the implementation of the NPDES Vessel General Permit in California and assess the acceptability of discharges under this new regulatory program. Ultimately, treatment vendors and vessel operators will need to consult with the Water Board to better assess the potential for water quality impacts from treatment system usage in California waters.

In conclusion, the Commission is preparing to implement the performance standards for new vessels with a ballast water capacity of less than 5000 MT in 2010. This review indicates that systems are or will soon be available to meet California's performance standards, particularly in light of the small number of new vessels that will likely need to meet the standards beginning in 2010. Commission staff is developing verification procedures to assess vessel compliance with the performance standards, and is working closely with the shipping industry and treatment vendors to ensure a smooth transition to the new standards. Staff will conduct another assessment of available treatment technologies by July 1, 2010 in anticipation of the 2012 implementation date for new vessels with a ballast water capacity greater than 5000 MT.

VIII. LOOKING FORWARD

Staff is currently engaged in the following activities to establish a comprehensive program for the implementation and enforcement of California's performance standards for the discharge of ballast water.

1. Develop protocols to assess vessel compliance with the performance standards

Staff must develop protocols for use by the Commission's Marine Safety personnel to verify vessel compliance with the performance standards. Commission staff will consult with a technical advisory panel in order to select the best available methods for organism enumeration and viability assessment taking into account ease of use, cost effectiveness, accuracy, precision and acceptance by the scientific community. The compliance verification protocols will describe on-site sampling, the handling of samples between vessel and testing laboratory (chain of custody), mechanisms for the identification and approval of independent laboratories to conduct the sample analysis, and requirements for reporting of compliance by laboratories to the Commission. The protocols are expected to be complete in mid-2009.

2. Amend performance standards regulations

Staff must make several amendments to the performance standards regulations during the next year. The passage of SB 1781 delayed the initial implementation of the standards from 2009 to 2010. The regulations must now be amended to maintain consistency with the statute. The proposed rulemaking will be presented to the Commission for approval in December, 2008.

Additionally, the advisory panel has brought to light potential issues with the use of best available assessment techniques to determine vessel compliance with the performance standards. It is possible that vessels may install systems compliant with the standards using today's assessment techniques, only to discover that the system is out of compliance at a future date when new and potentially more accurate assessment techniques are developed. The cost and burden to the industry is too great to risk having systems, operating at the same level as when they were installed, become out of compliance shortly after installation. The Commission must address how to account for changing compliance verification techniques without requiring vessels to frequently update costly ballast water treatment systems. One option would be to grandfather treatment systems under the compliance verification protocols in use during system installation. The specifics of this process have yet to be determined, but will likely need to be clarified via regulation in 2009 prior to the implementation of the performance standards in 2010.

Finally, Commission staff must amend the regulations to guide the selection of specific locations and sampling devices needed for onboard sample collection to verify compliance with California's performance standards. According to PRC Section 71206, Commission staff is mandated to inspect and take samples of ballast water and sediment from at least 25% of the arriving vessels to California ports in order to assess compliance with the law. The proposed changes are necessary because not all vessels are currently designed to include sampling "ports" for in-line collection of ballast water discharge. Staff has made efforts in the development of the proposed regulations to maintain consistency with proposed language for ballast water sampling in the IMO

Convention. Preliminary response from industry has been positive. Commission staff expects to complete the rulemaking process in 2009.

3. Revise ballast water treatment technology testing guidelines, as necessary

Commission staff developed the "Ballast Water Treatment Technology Testing Guidelines" in 2008 to promote a uniform, cost-effective, scientifically-rigorous, independent assessment of treatment system performance and environmental safety. In an effort to standardize ballast water treatment evaluation, the testing guidelines draw on the EPA's draft ETV protocols for ballast water performance verification. EPA is currently in the process of revising the draft ETV protocols and expects to release the next version in late-2009 or 2010. As the ETV protocols are updated, Commission staff will revise the testing guidelines in order to eliminate variability between the proposed federal technology evaluation program and California's recommended guidelines. Staff is also working with the Water Board staff to stay informed about the proposed provisions in the State's 401 certification of the NPDES Vessel General Permit and any changes to the California Ocean Plan or relevant monitoring programs associated with vessel discharges. Commission staff will update the testing guidelines as new information becomes available.

4. Support the development of performance standards and ballast water treatment technology performance verification protocols at the federal level

Commercial shipping is an international industry; any single ship may operate throughout several regions of the world. Ideally, performance standards should align both at the federal and international level and is preferable to a patchwork of standards adopted by individual states. Commission staff continues to work with the federal government, including the U.S. Congress, USCG and EPA, on the development of federal performance standards and treatment technology performance verification protocols. Commission staff has consulted with congressional staffers about proposing California's performance standards as the national standards. Additionally, staff participates on both the EPA ETV program Ballast Water Technical Panel and Stakeholder Advisory Panel. These panels are working with ETV program staff and the

USCG to finalize the technology verification protocols for ballast water treatment systems.

IX. RECOMMENDATIONS TO THE LEGISLATURE

The Commission recommends that the Legislature take the following actions to enhance the Commission's ability to effectively implement California's performance standards and to continue to prevent or minimize the introduction of NIS in California waters.

1. Authorize the Commission to amend the ballast water reporting requirements via regulations

In the previous treatment technology assessment report (see Dobroski et al. 2007), Commission staff recommended that the Legislature provide the Commission with the authority to develop a form via regulations to acquire information about ballast water treatment system installation and use onboard vessels. Currently, PRC Section 71205 specifies that voyage information must be submitted to the Commission on a form developed by the United States Coast Guard. However, PRC Section 71205 does not accommodate the Commission's need to develop a form to collect additional information about the use of ballast water treatment systems on board vessels, specifically: the timing of and requirements for treatment system use, deviations from suggested system operation, certifications for operation from vessel classification societies and other organizations/agencies, or additional information as deemed necessary by Commission staff in consultation with an advisory panel. Assembly Bill 169 was introduced in 2008 and passed by lawmakers to address the recommendation that the Commission be granted the authority to develop a form on ballast water treatment system use via the rulemaking process. The bill was not opposed by any organization, but was vetoed by the Governor, along with hundreds of other bills due to the delay in passage of the budget. Nonetheless, as the performance standards are implemented, the need for more information about treatment system installation and usage remains. The Commission should be authorized to amend ballast water reporting requirements to gather additional information about treatment system operation.

2. Continue to support research promoting technology development and performance evaluation.

Ballast water treatment remains a burgeoning industry that will undergo significant development as the IMO and California's performance standards are progressively implemented and as new vessel types are built. In 2012, the standards will go into effect for new vessels with the largest ballast water capacity (over 5000 MT), and technologies will need to be able to effectively inactivate organisms under high volume and pump rate conditions. Existing vessels built before 2010 will need to be retrofitted for approved treatment systems by 2014 or 2016 (depending on ballast water capacity). Those technologies must be installable under limited space conditions, and must be able to integrate with the existing engineering of ships (piping, electrical, computer, etc.). While several of the systems evaluated in this report meet or come close to meeting California's Standards, many must still be evaluated on vessels. Additionally, scientific research is needed to develop new and refine existing scientific methods to assess treatment system performance and verify vessel compliance with California's performance standards. Funds necessary to support these research needs could be obtained through three mechanisms: general funds, grants, or through the existing fees assessed on ships. The Commission and the Legislature should support future budget change proposals or other fiscal actions as necessary to fund this important research.

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XI. APPENDICES

APPENDIX A

BALLAST WATER TREATMENT TECHNOLOGY TESTING GUIDELINES

FOREWORD/DISCLAIMER

The staff of the California State Lands Commission (Commission) Marine Invasive Species Program (MISP) has developed the “Ballast Water Treatment Technology Testing Guidelines” to provide treatment technology vendors with a standardized protocol to verify treatment system compliance with California’s ballast water performance standards and water quality objectives. Verification testing according to these guidelines is not required by Commission staff, nor will the Commission be approving ballast water treatment systems for use in California waters. Commission staff strongly recommends, however, that vendors utilize these protocols to ensure a uniform, cost-effective, scientifically-rigorous, independent assessment of system performance and environmental safety. The guidelines provide a mechanism for vendors to declare that their systems are compliant with California's ballast water discharge regulations. These testing guidelines also contain useful information for determining the likelihood of compliance with relevant aspects of California’s water quality control plans and policies under the federal Clean Water Act and the California Water Code. The guidelines will be updated as new information becomes available and relevant regulations and programs are implemented.

TABLE OF CONTENTS FOR APPENDIX A

ABBREVIATIONS AND ACRONYMS

CCR	California Code of Regulations
CFR	Code of Federal Regulations
CFU	Colony-Forming Unit
Commission	California State Lands Commission
CTR	California Toxics Rule
EPA	U.S. Environmental Protection Agency
ETV	Environmental Technology Verification Program
IMO	International Maritime Organization
LC50	Lethal Concentration 50%
MEPC	Marine Environment Protection Committee
MISP	Marine Invasive Species Program
NIS	Nonindigenous Species
NOEL	No Observed Effects Level
NPDES	National Pollution Discharge Elimination System
NTU	Nephelometric Turbidity Unit
PRC	Public Resources Code
State Water Board	State Water Resources Control Board
STEP	Shipboard Technology Evaluation Program
TUa	Acute Toxicity Units
TUc	Chronic Toxicity Units
USCG	U.S. Coast Guard

CHAPTER 1. INTRODUCTION

The California Coastal Ecosystems Protection Act of 2006 required the California State Lands Commission (Commission) to adopt performance standards for the discharge of ballast water (Public Resources Code (PRC) Section 71205.3(a)(1)). The "Performance Standards for the Discharge of Ballast Water for Vessels Operating in California Waters" (Title 2 California Code of Regulations (CCR) §2291 et seq.) were approved in October 2007, and set both interim and final performance standards that will be implemented on a graduated time schedule (Tables 1-1, 1-2). The interim performance standards set limits for organism concentration as a function of organism size class. The final performance standard of zero detectable living organisms for all organism size classes in ballast water discharge will be implemented on January 1, 2020.

Table 1-1. California's Interim Performance Standards

Organism Size Class	Performance Standards[1,2]
Organisms greater than 50 µm[3] in minimum dimension	No detectable living organisms
Organisms 10 – 50 µm[3] in minimum dimension	< 0.01 living organisms per ml[4]
Living Organisms less than 10 µm[3] in minimum dimension:	< 10^3 bacteria/100 ml[4] < 10^4 viruses/100 ml[4]
Escherichia coli	< 126 CFU[5]/100 ml[4]
Intestinal enterococci	< 33 CFU[5]/100 ml[4]
Toxicogenic *Vibrio cholerae* (O1 & O139)	< 1 CFU[5]/100 ml[4] or < 1 CFU[5]/gram wet weight zoological samples

[1] See Implementation Schedule (Table 1-2) for dates by which vessels must meet California Interim Performance Standards
[2] The Final Discharge Standard for California, beginning January 1, 2020, is zero detectable living organisms for all organism size classes.
[3] Micrometer
[4] Milliliter
[5] Colony-forming unit

Table 1-2. Performance Standards Implementation Schedule

Ballast Water Capacity of Vessel	Standards apply to new vessels in this size class constructed on or after	Standards apply to all other vessels in this size class beginning in
< 1500 metric tons	2010*	2016
1500 – 5000 metric tons	2010*	2014
> 5000 metric tons	2012	2016

* California Senate Bill 1781 (Chapter 696, Statutes of 2008) delayed the initial implementation of the interim performance standards from January 1, 2009 to January 1, 2010

Compliance with California's performance standards regulations can be achieved through the use of at least one of the following ballast water management practices: 1) Retain all ballast on board the vessel; 2) Discharge ballast to an approved reception facility (although currently no such facilities exist in California); or 3) Discharge ballast that meets or exceeds the performance standards. The majority of those vessels intent on discharging into California waters will need to treat their ballast with a ballast water treatment system in order to comply with the performance standards.

To better ascertain the availability of treatment systems to meet the performance standards, the California State Legislature required the Commission to prepare a report assessing the efficacy, availability and environmental impacts of ballast water treatment systems (PRC Section 71205.3(b)). The review and resultant report, "Assessment of the Efficacy, Availability and Environmental Impacts of Ballast Water Treatment Systems for Use in California Waters" was completed in 2007 (see Dobroski et al. 2007). Among the major findings of the report, Commission staff found that the methods used by vendors and testing organizations for the verification of system performance were inconsistent across treatment systems, and many of the methods used to evaluate treatment systems produced results in metrics incompatible with California's performance standards (e.g. results were presented as percent reduction instead of concentration of organisms). The lack of standardized methods for evaluating system efficacy and environmental impacts hindered staff's ability to determine if those systems were capable of meeting or exceeding California's performance standards and water quality objectives.

In response to the lack of consistency among testing methods and metrics as outlined in Dobroski et al. (2007), staff has developed these “Ballast Water Treatment Technology Testing Guidelines.” The testing guidelines will provide treatment vendors with a standardized protocol to assess treatment system compliance with California’s performance standards and water quality objectives. Verification reports produced as a result of testing according to the guidelines will not only provide potential customers with the information necessary to make informed purchases to suit the needs of their specific vessels, but will also provide managers with much needed detail about system operation, performance and environmental safety.

CHAPTER 2. RESPONSIBLE CALIFORNIA AGENCIES

California State Lands Commission

The California State Lands Commission’s Marine Invasive Species Program (MISP) is charged with moving the state, “expeditiously towards elimination of the discharge of nonindigenous species into the waters of the state” (PRC Section 71201(d)). To that end, Commission staff is responsible for monitoring and developing management strategies for vessel vectors of nonindigenous species (NIS), including ballast water and vessel fouling. Since the passage of the Coastal Ecosystems Protection Act in 2006 (Chapter 292, Statutes of 2006), Commission staff has focused its attention on the implementation and enforcement of California’s performance standards for the discharge of ballast water. These testing guidelines are part of a proactive, multi-pronged approach to provide information to industry and enable vendors to assess system compliance with California’s performance standards. The “Ballast Water Treatment Technology Testing Guidelines” were developed by Commission staff in consultation with a panel of technical experts in marine engineering, oceanography, microbiology and treatment system evaluation (see Appendix A for a list of panel members and notes from panel meetings). For more information about the Commission’s Marine Invasive Species Program go to http://www.slc.ca.gov.

State Water Resources Control Board

The California State Water Resources Control Board (State Water Board) and the Regional Water Quality Control Boards are responsible for regulating water quality to protect the beneficial uses of California's waters. The Commission consults with the State Water Board to ensure that the Commission's Marine Invasive Species Program develops vessel vector management strategies that are consistent with state water quality standards including, but not limited to, acute and chronic toxicity criteria. Pertinent to California's performance standards, all treatment technologies that make use of active substances (i.e. chemicals) should ensure that any residuals or reaction by-products in treated ballast water discharges meet applicable water quality objectives as outlined in the California Ocean Plan (State Water Board 2005), Regional Water Quality Control Board Basin Plans, the U.S. Environmental Protection Agency's (EPA) California Toxics Rule (CTR) and associated State Implementation Policy for the CTR, and the California-specific provisions in Section 401 certification of the U.S. federal National Pollution Discharge Elimination System (NPDES) Vessel General Permit for Discharges Incidental to the Normal Operation of Commercial Vessels and Large Recreational Vessels. For more information go to http://www.waterboards.ca.gov.

CHAPTER 3. TESTING GUIDELINES

The Commission will not be approving ballast water treatment systems for use in California waters. Instead, Commission staff will focus on dockside inspection of vessels (as specified in PRC Section 71206) for verification of compliance with the performance standards. The "Ballast Water Treatment Technology Testing Guidelines" are intended to bridge the gap between treatment system development and operation in California waters. Commission staff believes that before systems enter the commercial marketplace, it is in the best interest of the State and concerned stakeholders for vendors to ensure that systems undergo a thorough performance, safety and environmental impact evaluation. The results generated from system evaluation according to these guidelines will provide Commission staff and potential treatment technology customers with a valuable upfront assessment of the ability of systems to meet California's performance standards and water quality objectives.

Treatment system verification protocols are under development or have been developed by both the International Maritime Organization (IMO) and the U.S. federal government. The IMO "Guidelines for approval of ballast water management systems (G8)" (Marine Environment Protection Committee (MEPC) 2005) offer test and performance specifications for evaluating ballast water management systems relative to the IMO Regulation D-2 performance standards (see IMO (2005) for more details). The U.S. federal government has encouraged the development of ballast water treatment technologies through the U.S. Coast Guard's (USCG) Shipboard Technology Evaluation Program (STEP), and the development of ballast water treatment technology verification protocols through a partnership between the U.S. EPA's Environmental Technology Verification (ETV) Program and the USCG.

The Commission recognizes the importance of establishing a standardized system for verifying system performance, and therefore does not intend to develop a new California-specific verification protocol. Instead, Commission staff offers these "Ballast Water Treatment Technology Testing Guidelines" to augment the federal ETV protocols with specific issues relevant to California's performance standards. Specifically, the testing guidelines merges: 1) The ETV Program's "Draft generic protocol for verification of ballast water treatment technologies" (NSF International 2004); with 2) Specific guidance on verifying system compliance with California standards and objectives. Commission staff highly recommends that vendors adhere to both parts of the system verification process and consult with and submit verification reports to Commission staff, ETV and other relevant agencies and organizations.

Generic Protocol for System Verification – The ETV Program

The ETV Program, "verifies the performance of innovative technologies that have the potential to improve protection of human health and the environment" (EPA 2008). The objective of the ETV ballast water treatment technology protocol is to "verify the performance characteristics of commercial-ready treatment technologies with regard to specific verification factors, including biological treatment performance, system reliability, cost, environmental acceptability, and safety" (NSF International 2004). When finalized, the ETV protocol will offer a federally-approved, standardized approach to evaluating ballast water treatment system performance. The ETV protocol is being developed in concert with a wide array of experts and through a formal Memorandum of Agreement between the EPA and the USCG. Commission staff highly recommends that all ballast water treatment systems to be used in California participate in this program. For more information on the ETV program for ballast water treatment technologies go to http://www.epa.gov/etv/center-wqp.html.

The final ETV protocol is expected to be finalized in late-2009 or early 2010. Until the ETV program for ballast water treatment technologies is accepting applications for system verification, Commission staff recommends that vendors contract with an independent testing organization to conduct system verification according to the most recently available draft ETV protocol (see NSF International 2004). Copies of the most recent draft protocol may be found on the Commission website http://www.slc.ca.gov. As updated information about the ETV protocol is released, Commission staff will update California's "Ballast Water Treatment Technology Testing Guidelines", as necessary, to reflect changes in the ETV protocol.

Regardless of whether verification testing proceeds through the ETV program or in conjunction with an independent testing organization using the draft ETV protocol, vendors should consult with Commission staff and ETV representatives throughout the verification process in order to address both the state and federal needs and minimize duplicative testing at a later date.

Treatment System Evaluation for California Compliance

In addition to conducting generic system verification through the ETV program, vendors should evaluate system performance relative to California's performance standards and water quality objectives. For this purpose, vendors and testing organizations should proceed with all components of the ETV protocols, but additional samples should be collected to be analyzed according to Commission staff recommended methods (see

Chapter 5 for sampling and analysis methods). Use of these methods will help ensure that test results are presented in metrics consistent with California's standards. Vendors whose systems meet all of California's performance standards may choose to declare that their systems are California compliant. This vendor-certified compliance with California's performance standards does not relieve the vessel owner or operator of the responsibility of complying with California discharge standards, but this declaration and associated verification reporting may be a resource to potential customers seeking treatment systems that have been evaluated with California's standards in mind.

CHAPTER 4. TEST PLAN DEVELOPMENT

All ballast water treatment verification tests should be completed following a written Test Plan. The Test Plan should be developed by an independent testing organization in conjunction with the vendor. Elements of the test plan are described in Chapter 4 of the draft ETV protocol (see NSF International 2004). The California component of the verification process should be included in the Test Plan development. Vendors are advised to consult with Commission staff and ETV representatives during the development of the Test Plan.

In developing the test plan, Commission staff also advises vendors to be familiar with the guidance provided by the USCG for preparation of applications for acceptance to the STEP (for more information go to http://www.uscg.mil/hq/g-m/mso/step.htm). While vendors are not required to work through the USCG program, Commission staff considers the approach used in this program to be appropriate for the development of the types of test plans and performance verification procedures necessary to verify compliance with California's performance standards.

CHAPTER 5. EXPERIMENTAL METHODS

California's specific ballast water performance standards and water quality objectives necessitate additional verification testing above and beyond that described in the ETV protocols. The following protocols discuss relevant California parameters including biological performance, water quality and environmental toxicity that should be evaluated during system verification testing.

Biological Performance

Parameters

California's performance standards (Table 1-1) will be implemented on a graduated time schedule beginning January 1, 2010 (Table 1-2). The final discharge standard of zero detectable living organisms in all organism size classes will be implemented on January 1, 2020. Commission staff intends to enforce California's performance standards using similar logic to that found in MEPC (2005), which states that compliance with the IMO performance standards for the discharge of ballast water "should be interpreted to be an instantaneous standard rather than an average over whole discharge. If any of the discharge samples exceed any of the discharge standards, this is grounds for finding non-compliance with the standards. It is unnecessary to show non-compliance in multiple samples or in mean values."

Sampling

California's performance standards set allowable levels of organism concentration in discharged ballast water. Upon implementation of the performance standards, all vessels will be required to provide the Commission's Marine Safety Inspectors access to sample ballast water discharge. The location and method of sample collection for system verification analysis should closely approximate the method of sampling that will be used by Commission staff for compliance purposes.

Until the specific regulations governing ballast water sampling are implemented in California, Commission staff recommends that vendors follow the draft IMO "G2" Guidelines for Ballast Water Sampling (BLG 2008) to establish the location of sampling (i.e. sampling point) and the equipment necessary to take the sample (i.e. sampling facility). Whether the sampling point is integrated into a ballast water treatment system or into the vessel's ballast water system is at the discretion of the vessel owner/operator in consultation with the treatment vendor, so long as the access point is located downstream from the ballast tanks and allows for sampling immediately prior to or during discharge. Commission staff highly recommends that vendors include sampling facilities in the design of ballast water treatment systems because port state authorities will require ballast water samples from vessels in order to assess compliance with relevant performance standards.

California's performance standards are set as the number of living organisms (or analogues/proxies for living organisms [i.e. colony-forming units; CFU]) per unit volume of discharged ballast water. Samples collected for purposes of compliance verification should be analyzed or appropriately processed immediately to accurately assess the concentration of living organisms at the time of discharge, ensuring that results are attained and presented in appropriate metrics.

The volume of water collected and equipment for sample collection and transport should be appropriate for the method of analysis and specific performance standard being examined. Sample collection methods should be scientifically defensible upon review. Commission staff should be consulted about the selection of appropriate methods and equipment for sample collection (see Appendix B, General Sampling Considerations).

Analytical Methods

The analytical methods described in the 2004 draft ETV protocol do not sufficiently address sample analysis for purposes of determining compliance with California's performance standards (see Table 5-8 "Core Parameter Methods" in NSF International (2004)). Table 5-1 provides a list of recommended methods to assess viability and organism concentration in each of the organism size classes in California's performance standards. California has marine, brackish and freshwater ports, so vendors and testing organizations should consider methods appropriate for assessing organism viability and concentration under each of these salinity regimes. The list of recommended methods in Table 5-1 is not all-inclusive. Those methods listed are commonly accepted for widespread use by U.S. laboratories. However, any scientifically defensible method that produces results in metrics consistent with California's standards would be appropriate for the purpose of performance verification. Methods outside of those listed should be suggested and/or approved by the independent testing organization.

Table 5-1. Recommended Methods for Organism Enumeration and Viability Determination

Organism Size Class	Units	Method or Reference[1,2]
Greater than 50 µm in minimum dimension	No Detectable	Note: At this time, there is no universally accepted method for enumerating live organisms greater than 50 µm in minimum dimension. The following methods may be useful, but will require modification to be sufficiently sensitive to determine compliance with California's performance standards: • Microscopic evaluation – Observe and probe, MEPC 53/2/7 Annex (2005) • Freshwater (may be adapted for marine conditions): GSI/SOP/RDTE/SA/Z/1(GSI 2008)
10 – 50 µm in minimum dimension	individuals/ml	Note : At this time, there is no universally accepted method for enumerating live organisms between 10 – 50 µm in minimum dimension. The following methods may be useful, but will require modification to be sufficiently sensitive to determine compliance with California's performance standards: • Freshwater : GSI/SOP/RDTE/SC/P/1 and GSI/SOP/RDTE/SA/P/1 (GSI 2008) • Nelson et al. (In Review) • Tamburri et al. (2006) – see method for assessment of viable organisms
Less than 10 µm in minimum dimension:		Note: There are no universal methods for enumerating all viable bacteria and viruses in any given sample because of the inability to culture many microorganisms in a lab setting, yet many of these very diverse taxa are routinely present in virtually all environmental water samples. In addition, most viruses found in aquatic systems infect species other than humans. Some viruses may survive in seawater better than in freshwater (especially true of bacteriophages, viruses that infect bacteria). However there are some methods that you may consider:
Bacteria	CFU/100 ml	• Heterotrophic Bacteria: Standard Method 9215 (Clesceri et al. 1998) o For freshwater bacteria, recommend R2A Agar or NWRI Agar o For marine bacteria, recommend Difco Marine Agar 2216
Viruses	Viruses/100 ml	• Viruses: Many viruses are naturally present in freshwater and seawater. Staining methods are available to detect and enumerate the total number of viruses, but results are reported as "virus-like particles". No methods are available to measure the viability of all viruses in aquatic samples. Specific types of viruses can be quantified, but these represent only a small fraction of, and may not always correlate with, the total number of viruses present. As potential surrogates for viruses pathogenic to humans the following could be used to evaluate the efficacy of a treatment system: Somatic and Male-specific Phage use Modified EPA Method 1601[2]; Adenovirus 40 and 41 and Norwalk-like Virus use qPCR. For information on sample size and concentration of samples using PCR see Standard Method 9510 (Clesceri et al. 1998).
Escherichia coli	CFU/100 ml	• Standard Method 9222.G (Clesceri et al. 1998) • Noble et al. (2004) • EPA Method 1603[2] or EPA Method 1103.1[2] • Freshwater: GSI/SOP/RDTE/SA/M/3 (GSI 2008)
Intestinal enterococci	CFU/100 ml	• Standard Method 9230.C (Clesceri et al. 1998) • Noble et al. (2004) • EPA Method 1600[2] or EPA Method 1106.1[2] • Freshwater : GSI/SOP/RDTE/SA/M/1 (2008)
Toxicogenic *Vibrio cholerae* (O1 & O139)	CFU/100 ml	• Standard Method 9260.H (Clesceri et al. 1998) • Choopun et al. (2002) • Chun et al. (1999)

[1] Methods specific to freshwater or marine water will be indicated as such. All other techniques listed should be considered appropriate for all salinities.
[2] EPA methods in this table can be found at U.S. EPA Microbiology Home Page. Website: http://www.epa.gov/nerlcwww/index.html. Accessed October 10, 2008.

Water Quality Considerations and Analysis

Parameters

A detailed listing of water quality objectives for California's ocean waters can be found in the California Ocean Plan (State Water Board 2005). The water quality objectives are set forth to protect the beneficial uses of the ocean waters of the State, including "industrial water supply; water contact and non-contact recreation, including aesthetic enjoyment; navigation; commercial and sport fishing; mariculture; preservation and enhancement of designated Areas of Special Biological Significant; rare and endangered species; marine habitat; fish migration; fish spawning and shellfish harvesting." (State Water Board 2005). The State Water Board is currently in the process of developing amendments to the California Ocean Plan. Read about the proposed amendments in the "California Ocean Plan Triennial Review and Workplan" and in associated documents at: http://www.waterboards.ca.gov/water_issues/programs/ocean/.

The California Ocean Plan includes both narrative and numerical water quality objectives. Those objectives pertinent to discharges from ballast water treatment systems are listed below. However, this list is not all-inclusive, and thus vendors and independent testing organizations should consult with Commission and State Water Board staff during the verification process to gain an understanding of the applicable water quality laws and regulations that vessels must comply with when discharging treated ballast water.

Discharges of ballast from treatment systems should meet the following criteria, generally based on the California Ocean Plan's narrative objectives and implementation provisions (See Appendix C for definition of "*" select terms):

1. The discharge should be essentially free of floating materials that would be visible in the receiving water.
2. The discharge must not cause grease and oil to be visible in the receiving water.
3. The discharge must not cause aesthetically undesirable discoloration of the surface of the receiving water.

4. Natural light shall not be significantly* reduced in the receiving water as the result of the discharge.
5. The discharge must not contain settleable materials or organic substances that will degrade benthic communities.
6. The discharge must not contain toxic substances in toxic concentrations, and substances that could accumulate to toxic levels in the receiving water or sediments.
7. The discharge must not contain substances that bioaccumulate, in fish, shellfish, or other marine life used for human consumption, to levels that are harmful to human health.
8. The discharge must not contain substances that alter the taste, odor or color of fish, shellfish, or other marine life used for human consumption.
9. The discharge must not contain radioactive wastes or byproducts.
10. The discharge must not contain nutrient concentrations that would cause objectionable aquatic growths or degrade* indigenous biota in the receiving water.
11. The discharge must not cause dissolved oxygen concentrations in the receiving water to be depressed more than 10 percent from that which occurs naturally, as the result of the discharge of oxygen demanding wastes.
12. The discharge must not cause pH in the receiving water to be changed more than 0.2 units from that which occurs naturally.
13. The discharge must not cause dissolved sulfide concentrations in the receiving water to be increased above that present under natural conditions.

Furthermore, discharges from vessels utilizing treatment systems into State ocean waters should comply with the numerical water quality objectives and effluent limits in the California Ocean Plan (State Water Board 2005). Discharges from treatment systems into inland surface waters, enclosed bays, and estuaries should comply with the numerical water quality objectives in the California Toxics Rule (http://www.epa.gov/waterscience/standards/rules/ctr/index.html) and Regional Water Quality Control Board Basin Plans (http://www.waterboards.ca.gov/plans_policies/).

Based on the aforementioned water quality objectives, Table 5-2 contains some selected relevant numeric limits that should be met when testing treatment system discharges. Because of the episodic nature of ballast discharges many of the limits presented in Table 5-2 are based on California Ocean Plan instantaneous maximums, daily maximums or 30-day averages relevant to specific constituents. The ammonia nitrogen limit is based on the San Francisco Bay Regional Board's Basin Plan maximum level (http://www.waterboards.ca.gov/sanfranciscobay/basin_planning.shtml). For pH, the range is based on impacts to freshwater, which has less buffering capacity than seawater, using the Central Valley Regional Board's Basin Plan (http://www.waterboards.ca.gov/centralvalley/water_issues/basin_plans/).

All vendors of systems using active substances are encouraged to consult with Commission and State Water Board staff about specific system residuals and treatment by-products to ensure that discharges will comply with California's water quality objectives.

As discussed in the 2004 draft ETV protocol, vendors of treatment systems employing biocides (i.e. active substances) should conduct toxicity testing during the start-up phase of verification testing. "If the post treatment effluent passes the toxicity tests, then verification testing can proceed. If, however, the effluent fails the toxicity test, verification testing shall not be initiated and further toxicity tests shall be required (NSF International 2004). Vendors should comply with all methods of toxicological analysis as described in the ETV protocols.

In addition to the ETV protocol requirements, California has specific objectives for acute and chronic toxicity (see Table 5-2) as described in California's Ocean Plan (State Water Board 2005). Toxicity is measured in acute and chronic toxicity units (see Appendix B for specific definition according to the California Ocean Plan). Acute toxicity units (TUa) are the inverse of the laboratory endpoint "Lethal Concentration 50%" (LC50) - the percent of the effluent giving 50% survival of test organisms. Chronic toxicity units (TUc) are the inverse of the laboratory endpoint "No Observed Effects

Level" (NOEL) - the maximum percent of the effluent that causes no observed effect on test organisms.

Table 5-2. Selected Water Quality Constituent Limits Relevant to Treatment Technologies **(adapted from State Water Board 2005)**

Constituent	Units	Limit	Method
Arsenic[1]	µg/l	80	EPA 200.8[2], for freshwater and EPA 1640[3] for seawater
Cadmium[1]	µg/l	10	"
Chromium[1]	µg/l	20	"
Copper[1]	µg/l	30	"
Lead[1]	µg/l	20	"
Nickel[1]	µg/l	50	"
Zinc[1]	µg/l	200	"
Ammonia N	mg/l	0.16	Standard Method 4500-NH3-D[4] or EPA 350.1 (Rev 2.0)[2]
Tributyltin	µg/l	0.0014	Standard Method 6710[4]
Total Chlorine Residual[5]	µg/l	60	Standard Method 4500-Cl-E[4]
Halomethanes	µg/l	130	EPA 601[2] or 624[2]
Grease and Oil	mg/l	75	EPA 1664[2]
Turbidity	NTU	225	EPA 180.1[2] or Standard Method 2130 B[4]
pH	pH units	Between 6.5 and 8.5	EPA 150.2[2] or Standard Method 4500-H^+-B[4]
Suspended solids	mg/l	60	Standard Method 2540-D[4]
Settleable Solids	ml/l	3	Standard Method 2540-F[4]
Acute toxcity	TUa	0.3	See Table 5-3 below
Chronic toxicity	TUc	1.0	See Tables 5-4, 5-5 below

1. A single metals analysis will result in all of the listed inorganic metals.
2. EPA methods can be found at 40 CFR Part 136 or at EPA website (Approved General-Purpose Methods): http://www.epa.gov/waterscience/methods/method/ . Accessed October 10, 2008.
3. Go to http://www.epa.gov/waterscience/methods/method/files/1640.pdf . Accessed October 10, 2008.
4. Clesceri et al. 1998
5. Both total residual chlorine and chlorine produced oxidants refer to the sum of free and combined chlorine and bromine as measured by the methods for total residual chlorine. The term "chlorine produced oxidants" is sometimes used in seawater samples because of the many oxidative reactions that chlorine can undergo in salt water.

Sampling and Analysis

Ballast water should be sampled immediately prior to or during discharge, as discussed in Chapter 5, Biological Performance, Sampling. Some general sampling considerations including appropriate equipment and maximum holding times for analysis of water quality samples can be found in Appendix B.

Samples for chemical analysis should be collected, preserved, handled and transported in accordance with Standard Methods for the Examination of Water and Wastewater (Clesceri et al. 1998) and the Code of Federal Regulations (CFR) in 40 CFR Part 136. The CFR can be found at www.gpoaccess.gov/ECFR/. Analysis for chemical constituents should be performed in accordance with the methods and minimum levels (to the lowest detectable concentration) described in Appendix II, of the California Ocean Plan (State Water Board 2005), and according to 40 CFR Part 136 or Standard Methods (Clesceri et al. 1998) where appropriate (see Table 5-2).

Acute toxicity should be assessed in accordance with EPA approved protocols as provided in 40 CFR PART 136 (http://www.epa.gov/waterscience/methods/wet/). At least one marine species and one freshwater species should be tested. Table 5-3 provides species and test methods that may be used for marine acute toxicity tests.

Monitoring for chronic toxicity for seawater under the California Ocean Plan (State Water Board 2005) and the State Implementation Policy for the Toxics Standards in the CTR(http://www.waterboards.ca.gov/water_issues/programs/state_implementation_policy/docs/final.pdf) requires the use of critical life stage toxicity tests as specified in Table 5-4 (modified from Table III-1 in the California Ocean Plan). "A minimum of three marine test species with approved test protocols shall be used to measure compliance with the toxicity objective. If possible, the test species shall include a fish, an invertebrate, and an aquatic plant" (State Water Board 2005). Out of state vendors/testing organizations that do not have access to the California species listed in Table 5-4 should contract with a laboratory approved under the California Department of Public Health, Environmental

Laboratory Accreditation Program. Go to http://www.cdph.ca.gov/certlic/labs/Pages/ELAP.aspx for a list of certified labs.

Table 5-3. Methods for Assessing Marine Acute Toxicity

EPA Method	Common and Species Names	Water Type
2007.0	Mysid, *Mysidopsis bahia*	marine
2004.0	Sheepshead Minnow, *Cyprinodon variegatus*	marine
2006.0	Silverside, *Menidia beryllina, Menidia menidia,* and *Menidia peninsulae*	marine
2002.0	Water flea, *Ceriodaphnia dubia*	fresh
2021.0	Water flea, *Daphnia puplex* and *Daphnia magna*	fresh
2000.0	Fathead Minnow, *Pimephales promelas*, and Bannerfin shiner, *Cyprinella leedsi*	fresh
2019.0	Rainbow Trout, *Oncorhynchus mykiss*, and brook trout, *Salvelinus fontinalis*	fresh

Source: EPA. 2002.

Vendors are encouraged to consult with both Commission staff and staff from the State Water Board prior to initiating toxicological evaluation to ensure that testing will fulfill all applicable state requirements.

Table 5-4. State Water Board Approved Tests for Chronic Toxicity (TUc)
(Adapted from State Water Board 2005)

Common and Species Names	Effect	Tier	Reference
Giant kelp, *Macrocystis pyrifera*	Percent germination; germ tube length	1	Chapman et al. 1995 State Water Board 1996
Red abalone, *Haliotis rufescens*	Abnormal shell development	1	Chapman et al. 1995 State Water Board 1996
Oyster, *Crassostrea gigas*; mussels, *Mytilus* spp.	Abnormal shell development; percent survival	1	Chapman et al. 1995 State Water Board 1996
Urchin, *Strongylocentrotus purpuratus*; sand dollar, *Dendraster excentricus*	Percent normal development	1	Chapman et al. 1995 State Water Board 1996
Urchin, *Strongylocentrotus purpuratus*; sand dollar, *Dendraster excentricus*	Percent fertilization	1	Chapman et al. 1995 State Water Board 1996
mysid, *Holmesimysis costata*	Percent survival; growth	1	Chapman et al. 1995 State Water Board 1996
mysid, *Mysidopsis bahia*	Percent survival; growth; fecundity	2	Klemm et al. 1994 Weber et al. 1988
topsmelt, *Atherinops affinis*	Larval growth rate; percent survival	1	Chapman et al. 1995 State Water Board 1996
Silversides, *Menidia beryllina*	Larval growth rate; percent survival	2	Klemm et al. 1994 Weber et al. 1988

Table Note - The first tier test methods are the preferred toxicity tests for compliance monitoring. A second tier test method may be used if after contacting California certified laboratories first tier organisms are not available.

Testing for chronic toxicity in freshwater species should also be performed, since there are inland ports in California. According to the State Implementation Policy for the Toxics Standards at least one of the tests in Table 5-5 should be conducted.

Table 5-5. Short-term Methods for Estimating Chronic Toxicity--Fresh Water

EPA Method	Species	Effect	Test duration
1000.0	fathead minnow, *Pimephales promelas*	larval survival and growth	7 days
1002.0	water flea, *Ceriodaphnia dubia*	survival and reproduction	6 to 8 days
1003.0	Alga, *Selenastrum capricornutum*	growth	4 days

Source: EPA. 1994.

CHAPTER 6. VERIFICATION REPORTING

All results of system evaluation should be presented in the verification report. A copy of the report should be submitted to EPA as outlined in the ETV protocol once applications are accepted for that program. A copy of the report and associated data should also be submitted to the Commission for review by Marine Invasive Species Program staff.

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CHAPTER 8. REFERENCES

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- Procedure for Algae/Small Protozoan Sample Collection GSI/SOP/RDTE/SC/P/1 and Sample Analysis GSI/SOP/RDTE/SA/P/1
- Procedure for the Detection and Enumeration of Enterococci by Membrane Filtration GSI/SOP/RDTE/SA/M/1

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APPENDIX A1. ADVISORY PANEL MEMBERS AND MEETING NOTES

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California State Lands Commission
Technical Advisory Panel:
Testing Guidelines and Verification Protocols
February 6, 2008
Meeting Notes

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Notes

Nicole - Introduction/Background

- Marine Invasive Species Act (MISA) required Commission to recommend performance standards to the Legislature
- In 2005 a technical advisory panel met 5 five times, and a majority of the panel recommended standards that were included in the Commission's performance standards report to the Legislature.
- Legislature took the recommendations from the report and incorporated them into the Coastal Ecosystems Protection Act of 2006 (CEPA).
- Major provisions of CEPA include: 1) Removed sunset date from MISA, 2) Required implementation of performance standards in accordance with

performance standards report, and 3) Required a review of the efficacy, availability and environmental impacts of ballast water treatment systems by January 1, 2008 and 18 months prior to each subsequent compliance date.

- Performance regulations – Standards were prescribed by statute and implemented via regulations.
- We received input from industry. Comments focused on the standards themselves (desire for CA to have standard in-line with IMO or Feds) and not on other aspects of the regulations.
- Regulations were approved in October 2007. The initial implementation deadline is January 1, 2009.
- A copy of the regulations was emailed to you and is available on the CSLC website.
- Treatment technology report assessed systems relative to California's standards.
- Key components of the report: efficacy, availability, environmental impacts.
- If technologies are unavailable to meet the standards, why not.
- Approved by the Commission in December, 2007, and then provided to the Legislature.
- Compiled available scientific literature, grey papers, white papers, and promotional brochures. Held a workshop in Boston, and received input from a technical advisory panel in Sacramento. Ultimately reviewed 28 systems from 9 countries.
- Efficacy – only had system results for 20 of 28 systems. Lenient review of results by CSLC staff. Looked for demonstration of "potential for compliance" – at least one testing replicate in compliance with the standards. Evaluation was difficult due to variable testing methods and results in metrics inconsistent with CA standards.

John – Any additional technologies that we missed?

Nicole – No, we received some additional information on existing technologies, but no new systems.

Nicole – Continuing with Introduction/Background

- 11 systems had results of shipboard testing, but no technology has yet met more than 4 (of 7) of California's standards.
- Availability – function of system production, market demand, government approval, and efficacy. Many systems will be commercially ready by 2009. The lack of federal standards and approval mechanisms may be a hindrance to market demand. Ultimately, no systems meet California standards, thus none really available.
- Environmental impacts – 21 of 28 systems use biocides. Several systems are approved by IMO and have received positive recommendations from WA, but there are no evaluation procedures in CA yet. We will be working with the State Water Resources Control Board (SWRCB) to identify applicable water quality control plans and regulations.

Dominic – 21 that use biocide, how many use chlorine?
Nicole – Most use some kind of chemical oxidant – chlorine, ozone, peroxyacetic acid…

Nicole – Intro/Background continued

- Conclusions – systems require further development and testing, particularly at shipboard scale. The lack of standardized testing procedures makes evaluation difficult. Commission staff will continue to gather info on and support research addressing technology development and system evaluation, and we believe systems will meet CA standards in future.

John – Industry prefers to do any system testing in consort with state and federal agencies in order to provide sufficient credibility to any test results. Is there potential for additional partnerships between state or federal agencies and the shipping community beyond those already taken advantage of?

Dominic - There are protocols in order but that these were specific to discharges of chemicals, not ballast discharges.

Maurya - There is no big overriding program, other than STEP, but there are smaller programs like the funding available under California's Marine Invasive Species Control Fund.

Mario - There are plans for development of a testing facility in Baltimore Harbor, Maryland that may include shipboard platforms.

Andrea – For the facility in Washington, the conceptual drawings are complete but we are still a long way away from being ready to start testing technologies. The Great Ships Initiative facility is currently up and running but they are limited to freshwater tests only. The Naval Research Lab in Key West, Florida is also up and running but they will not be conducting commercial testing at that facility. The facility in Washington, which will be equipped to handle saltwater and freshwater tests, is next in line and then the facility in Maryland, but they are both far away. We hope for testing by mid-2009, both salt and freshwater. Allegra's group will be ready sooner but limited to freshwater. Port of Baltimore later still, mobile platform.

Kevin – IMO test guidelines already done by NEI. Federal are yet to get published, for CA the question is how to test and verify.

Nicole – Testing guidelines relevant to CA. IMO protocols not necessarily relevant, not a lot of focus on # critters/volume. Must also develop verification protocols. Testing guidelines will lead to verification protocols.

Dominic – How feasible to assess BW prior to discharge?

Kevin – Dip a tank. The question is how to accurately sample a tank.

Mario – Testing for efficacy of a system involves both: 1) Rigorous assessment, and 2) Compliance monitoring using indirect measures of treatment.

Nick – Some kind of applied test

Nicole - continued

- Recommendation to Legislature in technology assessment report – 1) Change initial implementation date for new vessels with ballast water capacity less than 5000 metric tons from 2009 to 2010 [Note: the Bill number was incorrect as provided during the meeting, will let you know when we know the correct bill number], 2) Authorize Commission to amend reporting requirements via regulations, 3) Support continued research promoting technology development

Next Steps -

- Change initial implementation date from 2009 to 2010 and change reporting requirements, will be introduced in omnibus bill
- Work with SWRCB to identify applicable water quality requirements
- Treatment system testing and evaluation guidelines – guidelines not system approval. CSLC won't approve systems but we don't want to take a complete hands off approach. Want to provide treatment developers with testing guidelines (hopefully for 3rd party/independent labs), so they can self-certify their systems as compliant with CA's standards. Guidelines will help inform us about results of system efficacy testing and will also provide valuable info to vessel owners/operators prior to system purchase.
- Protocols for verification of compliance with performance standards. A set of protocols that inspectors can take to a vessel to sample and bring to lab for analysis. Everything from how to get a sample to how to handle it to what labs can do it.
- Want to get guidelines out first because will provide developers with suggested methods for testing systems. Have more time to develop protocols. Even if implementation date remains 2009, most 2009 new builds won't hit the water until 2010 at the earliest. Verification protocols will follow from testing guidelines for sampling and sample analysis.

Chris – Panel will provide advice and expertise to fill in gaps. Guidelines will benefit all. Hope to get guidelines out by end of summer. Plan to hold 4 meetings: 1) Discuss overall framework, 2) Land based testing, 3) Ship based testing, and 4) Sampling/viability assessment. Approximately 4 meetings, one every 4 to 6 weeks. For today, discuss framework

Andrea – Don't move too far away from ETV protocols.

Tom – ETV protocols and issues of ambient vs. surrogate species. The updated ETV protocols may be available towards the end of the year, possibly sooner. Fred Dobbs is working on report on BW surrogates. Also Ted Lemieux's work in Key West will be useful.

Kevin – Three issues for discussion: 1) Reassure that our guidelines will be related to ETV/IMO protocols, 2) Self-certification, and 3) Verification.

Tom- Specific testing guidelines will need to change from system to system. The manufacturer and the testing lab should be compelled to “dream up” a plan. Build in flexibility to adapt to how a system functions.

Kevin - The focus of this TAP should be to start with the end of pipe testing methods and work backwards from there. These will be the methods that will be used to verify compliance so the suggested protocols should stem from them, not the other way around. Should also think about sticking to shipboard testing for the time being and how to enforce end of pipe discharge.

Dominic – SWRCB focus on end of pipe.

John – Concerned about ship operations.

Dominic – Bacteria testing procedures – simple.

John - How will vessels know these systems are working?

Mario – Once they understand how a system works they can develop indicators. Indirect measurement. Sensor testing – can make measurement ozone, chlorine, etc… Can be adapted to in-tank or upon-discharge. Engineering very do-able.

Kevin – Self-certification ties into end of pipe, self-certification needs to be linked to end of pipe.

Nicole - Moving on to testing guidelines and system documentation (operations, environmental assessment)

Nick – Where on ship to test for compliance? Will manufactures add specific ports for end of pipe testing for CA? How does a biologist measure and verify that CA standards are being met? Testing for WA, IMO and CA – appropriate test for each class. Test used needs to be specific for each size class. Not much quibbling over live/dead greater than 50 microns.

Mario – Work to quantify organisms. Zooplankton standard live/dead (no brainer), indicator pathogens utilize standard off the shelf MPN (no brainer) – 10-24 hours to get results etc…What to do about phytoplankton? Take whole water samples and 1) measure chlorophyll, then subsample and grow out and measure chlorophyll
Vs. 2) total cell counts. Conservative approach.

Mario - 10 – 50 microns, quantitative no. No assay that gives number.

Andrea – Need quick techniques. Ted's work will be key part of protocols, get agreement with our protocols and his work.

Nicole – For the sampling procedures and analysis, what are the basic components?

Mario – Recommend build framework but recognize that there are several way to treat, build in flexibility.

Kevin – Add end of pipe testing. ID measureable variables from end of pipe work. ID and measure. Vessel/treatment system needs automation, red light, green light to demonstrate that it's working. Self-certification involves lots of testing to ensure some real tracers to ease of end of pipe testing in certified lab.

John – Cost? E.coli/Enterococcus?

Dominic - $200 roughly

Nick – Message to industry, manufacturer must know that a self-test be developed. Machine – red light/green light control needs to be incorporated.

Nicole – Should we flip it around? Start with verification protocols.

Rian – Less emphasis on land based, this document should focus on application.

Kevin – Focus on items unique to CA. How to enforce end of pipe standard? Thrown around end of pipe test regimes. We need to discuss regimes.

1) Rapid assay – allow CSLC inspectors to quickly assess compliance
2) Routine inspections to test whether system is operating (e.g. are chemicals present in correct concentrations). Red light/green light controls with periodic biological testing.
3) Treatment developers – secondary indicators that based on past tests, meet standards, system being used accordingly.

Nicole – Can address items 2 and 3. We need to find answers to #1.

John – What happens when ship owner fined for non-compliance?

Maurya – Don't know yet.

Dominic – Rapid indicators works in 4 plus hours

Mario – Problem is diversity of organisms. Numbers generally small, very dilute.

Dominic – For bacteria, tests already available. Methods out there, but perhaps more expensive. Total bacteria/virus counts

Nick – Organism groups where are we? E.coli

Fred – Live bacteria? CFU, not applicable to marine systems.

Mario – Concerned

Nicole- Mario did you test to G8 guidelines for NEI?

Mario – Yes, sort of. Originally no then adapted as we went along. Independent 3rd party facilities – GSI, Batelle, Port of Baltimore.

Nicole - We can use the IMO guidelines and modify as appropriate. We need to standardize as much as possible while IDing unique CA component.

Dominic – Need to let everyone know about water quality requirements involved. Toxic assessment chlorine residuals requirements.

Nicole – Yes, we need to focus on this.

Nick - Want to hear from sewage treatment experts. Exactly what tests do they use? Freshwater/sewage background should have lists of appropriate tests, including precision estimates, for testing human pathogenic bacteria.

Dominic to put together a powerpoint presentation. 3 tests done: 1) Multiple tube fermentation, 2) membrane filtration, 3) IDEXX

Kevin – Really hard to do sophisticated testing on a vessel. No personnel dedicated to monitoring.

Mario – Sampling design side needs work. Need to understand appropriate sampling methodology. Need for a statistician to become involved. Given the volumes involved, there are many statistical considerations that may warrant the need for a statistician,

Dominic – Composite sampling may help ease concern over when to sample during ballast cycle. Sample container over time while discharging to achieve statistical rigor.

Tom – Meeting in Providence looked at stats about designs. But this document was general not specific to standards. Will send a copy to Nicole of report and participant list.

Mario – Will also look at document.

Kevin – Environmental assessment, 21 of 28 utilize biocide. GESAMP rejected TechCross electrochlorination system because they were uncomfortable with the robustness of the dechlorination system.

Nicole – IMO originally rejected NKO3

Dominic – Chlorine instantaneous max (from Ocean Plan) 60 ppb, but right now excludes vessel discharges. Plan to fix this in future.

Kevin – Treatment vendors submit to WA DEQ, follows WET test. DEQ would assess and DEQ can say they accept discharges.

Dominic – Broaden Ocean Plan, but performance standards responsibility of CSLC to include vessels standards.

Kevin – Need to include toxicity information

Allen – Federal legislation discusses reception facilities

Dominic - Sewage treatment facilities are unable to accept saltwater into their plants. Also, land-based ballast water reception facilities are unlikely because the land is too valuable/expensive to build shore side facilities. However, if that does happen, they could use the protocols that we develop.

Lucie- All the guidelines on sampling discharge are not that easy to do. May use more than one area from tanks for discharges. Retention of ballast water = compliance. Move ballast water from one tank to another, need to consider this. Not simple. Some vessels don't discharge their ballast and may store it for years.

Nicole – Our next steps will be to compile the notes. We'll work on a new framework and get that out to you. We'd like to hold another meeting somewhere around the 2nd or 3rd week of March. Will send email with proposed dates. Questions?

Adjourn

California State Lands Commission
Technical Advisory Panel:
Testing Guidelines and Verification Protocols
March 10, 2008
Meeting Notes

Participants:

Nicole Dobroski, CSLC	Dave Lawrence, University of Washington
Rich Everett, U.S. Coast Guard	Ted Lemieux, Key West Naval Research Lab
Maurya Falkner, CSLC	Lucie Maranda, University of Rhode Island
Daphne Gehringer, CSLC	Chris Scianni, CSLC
Dominic Gregorio, State Water Resources Control Board	Andrew Solow, Woods Hole Oceanographic Institute
Russ Herwig, University of Washington	Mario Tamburri, University of Maryland
Rian Hooff, Oregon Department of Environmental Quality	Nick Welschmeyer, Moss Landing Marine Lab

Meeting Summary:
Nicole welcomed everyone to the meeting. Participants introduced themselves, and Nicole discussed the purpose of the meeting - to consider methods of quantifying and assessing the viability of organisms greater than 10 micrometers (microns) in size (predominantly zooplankton and phytoplankton) for compliance with California's performance standards.

Nicole gave a brief overview of some considerations (cost, time, scientific acceptability…) the CSLC must keep in mind with respect to what assays may be appropriate in determining abundance and viability of zooplankton and phytoplankton. From there, the participants began a discussion of methods for organisms greater than 50 microns in size. Ted discussed his development of a video mobility tool that will examine a sample and then quantify the abundance of live organisms in the sample based on movement. He projects that the device will be ready for others by the end of the year. The device has not been used with vital stains yet. It will be automated, quick to operate (5-10 minutes per sample) and could be used by an untrained individual.

Russ pointed out that for filtration/concentration purposes the net mesh must be 50 microns on the diagonal (i.e. essentially a 33 micron mesh net) in order to capture the right size class of organisms.

The discussion then moved to the use of neutral red as a vital stain to assist with counting organisms in a sample. One method of determining the number of live organisms was to stain and then count all of the non-moving ones before preserving the entire sample and making a total count. The number of live organisms in the sample would be the difference. Ultimately the most common method of determining viability remains the “poke test.”

Rich argued for changing the focus of the discussion from specific techniques to a broader discussion of the hierarchical progression of determining whether or not a system is in compliance with the standard. He suggested that California will need a first-cut approach to verification testing that could be used by inspectors to broadly determine whether or not a system has been operational and meets the standard within an order of magnitude. This broad testing could then be followed by specific, intense testing if the vessel does not appear to have treated its ballast water in compliance with the standards.

Dominic mentioned that DHS has a relatively easy to use field microscope that is used in HAB determinations. A similar type field microscope could be used to determine the abundance of live/moving zooplankton and some phytoplankton species in a sample. Mario stated that chlorophyll fluorescence may serve as a similar first cut proxy for the relative abundance of phytoplankton cells in a sample. Although chlorophyll use may have more pitfalls because samples that include recently lysed cells may still have chlorophyll present in solution. This would lead to a false positive result.

For CSLC, this type of semi-quantitative first-cut assessment could then be used in conjunction with onboard paperwork demonstrating system operation over the appropriate time period. If any flags are raised during this process, the vessel could be identified for further inspection.

The group discussed the need for each treatment system to have some indicator or recording device that will demonstrate system operation over the appropriate time period. An inspector should be able to board the vessel and check this system or printout and determine that the system was operational. Russ and Mario stated that some systems already have such systems. Everyone agreed that the maritime industry should put pressure on technology developers to include these operational sensors/recording devices on their systems.

Nicole moved the discussion to the development of testing guidelines for technology developers. Rich urged CSLC to look at the ETV public draft [Version 2.6] because it is information-rich and will be standardized at the federal level. Maurya countered that the draft was out of date, but Rich commented that at least it is better than a set of unconnected test procedures, and the next ETV protocol draft should be available later this year.

The discussion moved back to verification protocols and most agree that for zooplankton, the poke test and neutral red staining (although not perfect) was the way to go for now.

Nicole then introduced Andrew to provide statistical advice on how much water to sample to determine compliance with the greater than 50 micron size class. Andrew wanted to know whether or not the sample could be assumed to be randomly distributed. Nick and Russ said patchy, but later Nick suggested that we ignore the patchiness prediction because it is impossible to know zooplankton behavior in a ballast tank or in the discharge stream. Nick then commented that the natural coastal environmental has 1-100 copepods per L, 100,000's animals per m^3. Andy suggested that CSLC must determine what the null vs. alternative hypothesis should be and then what level of confidence do we find appropriate for verification purposes. CSLC must also determine if the hypothesis involves wanting to know the mean density in the tank or the presence or absence of zooplankton in one sample. These are different questions and will require different methods.

As the meeting wrapped up, Nicole brought the discussion back to the 10 – 50 micron size class. Nick suggested MPN analysis is the most appropriate for this size class. Russ and Lucie both use a similar technique. Nick pointed out that the serial dilutions would have to be carried out to "nothingness" to be done correctly. The process also takes several weeks to grow out, and Lucie commented that the duration required for the culture based methods will depend on what species/concentration you are looking for.

Nicole said the notes would be compiled and distributed and that the next meeting would take place on March 17.

Detailed Meeting Notes:
Nicole began with an overview of considerations including cost, time, complexity, chemicals/equipments, applicability of techniques, scientific acceptability etc…
Question: What extent will we see phytoplankton in greater than 50 micron size class?

Lucie – Some species can create chains/colonies.

Ted - Chain formers are not a single organism. Address them by non-chain size. IMO says "in minimum dimension". Greater than 50 micron phytoplankton treat with "standard zooplankton technique." Take 1 ml aliquot, count non-moving (dissecting scope), hit with tonic water (?), count again, examine using video mobility tool (confident in technique), record for 10-30 seconds, note how many have moved, and how many haven't. It has been used for phytoplankton. Within this calendar year, we will develop method that can be used by an untrained person with repeatability.

Lucie - Similar method as Ted's. Look at control. Lots of live organisms, remove non-moving/look dead, treat with neutral red, then poke test. Treatment tank - remove dead, look at moving, poke them. Separate the critters by dead and alive, and pick out whichever group has less organisms using a stereomicroscope.

Nicole - Time consuming?

Lucie - Dominant species removed quickly. Nauplii take a lot of time.

Russ - Poke and prod test. Need to use a net less 50 µm to fractionate and keep 50um size (diagonal size of 50 µm). Concerned with sensitivity towards CA standards (need to collect $1m^3$ to look for the “rare” organism that may be still alive) because they are not collecting that much water. May collect many liters, then take 1 liter total from samples and examine under stereo microscope. Not a rapid throughput method. Could stain with neutral red. Try to target a few species, and not focus on all species.

Nicole - We use the live counts as more of a flag than a consequence. We’re looking at it with other regulators and statisticians: high volume or not. High level of precision is being discussed, but in reality is not going to be used for compliance in the field.

Russ- The smaller you go, the harder it becomes to assess live/dead and and it becomes nearly impossible to ID species.

Mario - For shipboard testing, if the system is working, it’s easy to tell. Use 1 m^3/tank, don’t count every single organism. It’s not hard to count 0-low individuals. Is there a problem or not- should be quick and easy to do. But won’t hold up in court.

Maurya- This is relative. Great than 50 rule is zero, that’s an easy criterion. If not met, we’ll allow more time to solve problems to allow developers to update technology.

Ted - The video allows us to have an automated stage, and first analysis takes 5-10 minutes. Goes fast. When deliberately testing for certain populations…need to look at surrogates with high sediment amounts. Employable by an untrained person.

Rian - Question to Ted: Do you use 1 ml aliquots (A: I don’t know)? Examine 1 field of view (A: Yes).

Ted - We can zoom in on the image digitally to look for smaller motion (flagella).

Rian - But the heat from lamp can cause convection.

Ted - We’re trying to make sure that alive is alive. It is not commercially available, and uses a MATLAB code.

Nicole - Have you used stains?

Ted - No, not now. We’re toying with that idea.

Nicole - What about Neutral red?

Ted - We're thinking about it.

Lucie - Neutral red and evan's blue, success varies between species and is quite variable. Most of the problems with the zooplankton is the abundance of sediment. It's difficult to filter and process sample. Could be a problem with the video camera method. Samples with 1" of sediment and ¼" of animals from discharge clog the nets, very difficult to see if alive.

Rian - Previous experience with poke test shipboard sampling had the same problems: time constraints, resource limitations. Are there advances in stains? Doesn't sound like it has changed much.

Nicole - Any other stains?

Rich - In looking for compliance methods, like ETV, consider hierarchical progression: look for things that can be done onboard that has simple-moderate technique and equipment. Second level, the sample can be taken back to a lab, to be examined with higher technology. Still semi-quantitative. Can be expanded to look at species composition. No preconceived ideas about what inspectors can use other than microscopes. You don't want to always have a microscope to do the test, because they won't always have time. Depends on what your lawyers say you can do. The result of the determination isn't a fine, but will advance to the next step. If your treatment is working, then it is pretty evident, determining concentration is easy. But if you can visually, with a minimum of microscopy, assess >10, 10-100 organisms or more, should be good enough. There is value there. Don't need to quantify further. Consider a probabilistic approach: probability table that tells you chance that the ballast water exceeds the discharge standard. Take more than one sample over a reasonable time (minutes), if there is anything swimming around, it would give you an idea if it is successful treatment. You could then investigate to take a more substantial sample, and maintain it until you can look at it more rigorously, and determine concentrations.

Dominic - For CSLC, do you examine before or while discharging.

Maurya - Upon discharge. What about a first crude estimate? Look at a glass of water in the light, do you see anything?

Rich - Yes, refraction of light off organisms, can maybe determine order of magnitude, not concentration.

Dominic - DPH's first cut for HAB: field microscope, sample in capillary tube. Any one can do it. Can distribute methods, should be online. I'll try to get that out.

Maurya - DPH has the same as us: simple microscope for quick analysis.

Dominic- Maybe need a bigger capillary, probably easy to make.

Rian - Gallon container with a flashlight. Garbage can of known volume run through sieve to condense.

Rich - Does it have to be a 1m^3? Or if it's a concentration, maybe you don't necessarily need that much. Maybe 3-5 1L samples?

Maurya - For the first phase, we want to evaluate the situation and find a solution. If things aren't working we need to work with developers. Is that what you do at Waterboard?

Dominic - Storm water is a new program, it's kind of like that.

Maurya - We're going to have to take an iterative approach, too, like storm water (Dominic)

Dominic - Give a little bit of slack.

Maurya - Try to keep the concentration within an order of magnitude.

Dominic - Maybe have a grace period.

Nicole - If there are larger guys (>50) are there always smaller guys (<50)?

Rich - Not necessarily. There could be differences in susceptibility. The HAB technique sounds like it's working, so you could switch back and forth between the two size groups and techniques. That covers 95% of the potential problem and is a huge improvement.

Nick - Does the State do any viability or proxy test: stain, oxygen detection test. What would be an okay first cut of is the system working, do you need a concentration?

Rich - For enforcement, yes. For first cut, the concentration gives you an idea about how to get to the next step.

Nicole - Do you flag that vessel, and look at them again later, then penalize if they fail again?

Rich - We agree with ramp approach for enforcement. If the ballast is teaming with animals, then stop the discharge. Are the ballast inspectors also examining water quality?

Maurya - Vendors need to provide documentation on their system that they have a working system (ETV, GSI or something about how the system works) so the inspectors have a reference sheet. If there is a chemical residual, we pull in Dominic for this conversation.

Dominic - Can you have enforcement without a number, yes. If there is oil floating in the water, you've violated the law. You can quantify it later, but if it is visible right there, that is a violation. There is a first cut, a notice, not a fine at the Waterboard. The inspector can do that on the spot. If you come back and do it again, then you are in trouble.

Maurya - Same here. Notice first and then target for top priority inspection.

Russ - Aliquot of discharge can be semi-quantitative: Collect 1 L and anything moving is an obvious violation. Increase volume on 10 fold scale (concentrate sample) and that should work.

Dominic - You could have a flat flask with a certain amount of water, and look at with magnifying glass to see zooplankton.

Russ - Would be nice to standardize with poke test.

Mario - Another simple approach for the next size class [10 – 50 microns] is chlorophyll fluorescence.

Dominic - Does that determine live/dead?

Russ - If chlorophyll is too low for detection, no cell is alive. But dead can have some chlorophyll.

Dave - Chlorophyll is pretty good indicator. However, If you use UV treatment (which does not lyse cells), they may still contain chlorophyll for some time after treatment. So chlorophyll would not be a good indicator of organism viability after UV treatment.

Mario - If they are treating upon uptake, then those cells should be pretty dead after a couple of days.

Dave - Yes.

Nicole - What about coastal voyages?

(???) - Depends on technology.

Lucie - After the first day, chlorophyll is gone.

Mario - Same idea that if you see chlorophyll you should test further. If no chlorophyll, then it could be okay.

Maurya - We do need to have some kind of red light green light, rapid assessment. We do need to come up with guidelines for how does a technology developer do testing to meet our standards because developers need more stringent test than what we will do in the field.

Nick - Agree with Mario, put it on the shoulder of the manufacturer. But things can get misinterpreted quickly. Does chlorophyll determines viability? Maybe too much of a blanket statement. Some people might not like it for one reason or another.

Rich - How does the regulator tell if the system is working correctly? How does the inspector tell if the system is working correctly? Look at some kind of law of operation. This kind of a treatment should have done X…

Nicole - Developers should give that out to each boat.

Maurya - Need an idiot proof system. Matson indicates need for integrated system: system recording, warning which requires a response from crew. A no effort, automated system.

Ted - Will the owner of the ship want to use a piece of equipment that they don't know if it works until examined by an inspector. Build in a testing capability.

Nicole - Are they building these in?

Nick - No. They wish they had it.

Mario - They [NEI] have a built-in indicator. Measured pretty straight forward, tests for oxygen concentration. For the biocides, they've worked out proper concentrations. They know when it will work and when it won't , and they know how to test for that.

Nick - Is there an oxygen sensor that gives you a number?

Mario – Measures temp, salinity, and oxygen. Then they know what the water is like (using a formula). Can measure indirectly.

Dominic - As sewage plants are discharging, the sensors continuously check the record.

Russ - Severn Trent putting inline sensors in their systems. [The inline systems can measure oxidant concentrations (called TRO, for total residual oxidant). A different sensor could measure Eh (oxidation reduction potential). In presence of oxidant, Eh is a large positive number.]

Nick - Counting on developers to have a perfectly running system that will tell you when it doesn't work is a big expectation for not wanting to prod anybody. Only test for CA is a proxy measurement.

Nicole - Vessels will want this because they will go after treatment developers if they fail inspection.

Nick - It is conceivable that their own monitoring device doesn't work, and there is no calibration. Maybe there needs to be prodding to match sensors with probability testing*.*

Maurya - There will be prodding. But tell maritime industry to go after technology developers. We are not going to approve systems. It has to meet this standard.

Dominic - SWRCB and EPA do not approve systems. There are unique situations. Generally, we set up limits, and they have to meet them.

Maurya - The ultimate goal is to provide guidance. Go to ETV, go to whoever, but you have to take this into consideration how to record specs on your system.

Rich - Question for Dominic: Maybe Nick's question has relevance for Waterboard. Water treatment plant uses chlorine, and has a probe, do you specify which probe to use?

Dominic - We do not approve certain products. Just standards, and manufacturers will have to meet them.

Rich - Right, probe vs. analytic measurements should be guided.

Maurya - Same with human health indicators. We can identify labs with in-house capabilities.

BREAK

Nicole - We have a good idea of what the inspectors could do (gross violations). When giving the developers guidelines, what techniques do we want to tell them about (ETV not ready) for testing the 50 µm size class?

Rich - ETV has a public draft. There are no ETV certified test facilities. Is there a problem if we tell developers to check out draft ETV? The general direction is clear. The updated draft will be available around summer, but do you need test facilities?

Nicole - We don't want to give them a test facility, we do need to give them direction.

Rich - That will open up a can of worms. They'll want guidance on how to go about testing. And we'll have to write something as information rich. Like, here are 1-2 tests you can do, but they'll all do them differently. Is there a sense that developers need the ETV, or are they moving just on the standards?

Nicole - They are starting to move on our standards (i.e. consistent units). If there are gross violations, we will need to get concentrations of critters.

Rich - You'll want to give something for the technology developers in a standardized fashion so that they verify the system is performing correctly. You are just recommending them the tests, so the guidelines are standardized. Results will be still be hodgepodge without being inconsistent with the guidelines. You will have to specify your tests, because that's what the developers will test their systems with. And they'll use that to sell their product. You have to direct them to develop a complete protocol.

Maurya - Which protocol?

Rich - Get the draft ETV protocol, get from Tom at NSF

Maurya - It hasn't changed since 2004.

Rich - Then recommend to them a set of unconnected test procedures (e.g. Nick, phyto; UW, zoop; X, bacteria). Every aspect of the tests will be so different, that the numbers will be irrelevant. So it doesn't help anyone.

Nicole - Maybe we should focus on what we are doing for compliance, and let them figure out how they are going to do it.

Dominic - Yep, send that out, and get comments.

Nicole - Vessel comes in and violates the "beaker and the flashlight" test. If we want to know what those numbers are, how much water do we need?

Nick - Is it time to say what our favorite methods are? My vision: things will change over time, scientists are still developing methods ourselves. Our group can only try to work on getting a protocol, it's not straightforward.

Nicole - Poke test isn't the ultimate test. We will have to update our protocols regularly. But we have to use what is around now for today's guidelines. Are there techniques are available?

Nick - Russ does the poke test, Mario isn't here. Will provide names of new tests.

Lucie - Zooplankton, poke and neutral red, sizeable volume that has been concentrated. There are problems with sediment. Poke method is something that is simple and seems to work.

Russ - I agree

Nick - I agree

(???) - NRL with video motion detection is a good direction, and sounds like a faster method. Sample size (1 M^3) sounds good. Split sample and look at representatives.

Nicole - To Andrew Solow. Intro to Andy.

Dominic - How many gallons of ballast water is going to be released?

Nicole - Andy says not relevant. It is about a concentration and if it's detectable per $1m^3$.

Andy - The question is: How powerful is the test? Given low density, what is the probability of finding none? If there are living organisms, then it failed the test.

Dominic: If you sample at the very beginning, then you should sample later on the discharge.

Andrew: That's the randomness assumption. If they are distributed patchy or non-random, then you have to do something differently.

Nick/Russ - Patchy

Russ - 1,2,3, 3rd of ballast discharge. 3x $1m^3$ samples. (per IMO).

Andy - If you can characterize the heterogeneity…if not, it is an ad hoc thing.

Lucie - IMO did this to get an idea of what is in the tank. The samples are combined statistically.

Rich - Is important to look at the IMO recommendation with caution…There is recommendation to sample the beginning, middle, end. The amount of water to be examined represents a periphery compromised solution and self interest of flag states and shipping industry, and makes sure that a port's ability to test those conditions is low. If you have a discharge standard that you have to have a concentration, and look at that in a hierarchical fashion: how are you going to do that …how extensive was the violation. You could take one sample and make your determination from that. If you want to repeat that, then it is an independent observation.

Nicole - If we take one sample, and know that it is a patchy distribution…?

Rich - You want to figure out when to sample to most likely get an animal. What's the likelihood that it is a minor or major violation.

Nick - Given a choice for the assumption of sampling, I'd go with Andrew and ignore the patchiness because it involves predicting zooplankton behavior, because we don't know how they act in a ballast tank. CA regulation is good because there is a set concentration, and you need to know the natural abundance level to determine the volume of water you need to analyze. Natural coastal environment has 1-100 copepods per L, or 100,000's animals per m^3. To show that we have zero copepods, how much water?

Andy - CA sets the standard, and to determine whether or not they meet the standard, do you test null hypothesis (there are no critters) or alternate hypothesis (there are critters), and that's up to you. How do you do this test? Assume non-zero standard and random distribution. Using the formula, you can get how much water you need. If the standard is zero, then if you find any then they failed. You can calculate the volume of water. In the case of the patchy distribution, there are standard statistical tests to make the same kind of calculations. All that matters is the total volume of the water. There is information in the variability between the samples. Need to sample separate quantities but same kind of test. You might be able just to take your separate sample and consolidate in the end if it is a depth patchiness.

Nick – This is about volume, not poke test, the more complicated you assume the vessel is, the more water you will have to sample? Andy?

Andy - The null is that standard is met, and the alternate that it is not…all a function of the true density in the tank. If the concentration is just above the standard, you will have to take a little water. The higher the concentration from than the standard, the less water you need. If you want to be 95% confident that the null is met, then you can …
If it's patchy, then you need separate samples. If patchy you need to know how patchy to develop the test (variability between samples). For the power, need to do more looking into.

Dominic - How long do they discharge?

Lucie - 45 minutes for the barge type vessels, or can take hours if they are loading.

Maurya - Do not discharge based on maximum ability. We are talking about inline sampling, it's pretty good (Ted), is better than dip net where the collection of animals is more patchy. Tanks are drained from bottom. How does the patchiness relate to inline sampling (we know that for dip nets, patchy). If the tank is draining from the bottom, is the patchiness still a problem?

(???): I have seen changes in turbidity/suspended material over a discharge. Brazil at IMO brought in data that you should not sample during the first 10% and the final 10% because they found greatly elevated sediments and would make it impossible to quantify plankton. BUT if plankton are just particles, that is the exact time that the plankton would be there. The temporal patchiness is hugely dependent on where plankton are in there. Can have affinity for water air interface….someone would have to do lots of work to determine when the most obvious point of the discharge. Focus on what is being discharged. What is the level of effort. Each moment is a violation of discharge.

Andy - I interpreted the mean density of animals in the tanks is X. It would be difficult if all zooplankton aggregate and you take that one sample, you have misconceived notion of the concentration in the tank. Strange.

Andy - Same thought: if that is your sampling rule, this is how well you will be able to detect things. There is a confusion of the goal and the sampling. Are you going to take 10 samples, and if you fail any one, then you fail. That's different from mean density. You need to work with the hypothesis, then develop method.

Nick - Better to filter 5m^3 per time, and get concentrations. You should get numbers of well above 100's, and there is no customization of rules. No matter how elegant the stats, the zooplankton counts have not been this regimented.

Nicole - We want to think about what volume of water

Nick - Need to know natural concentration, and base your stats on that to determine when you are confident.

Andy - You might need different volume based on origin, that is a different story

Nick - That is different and a moving target, but needed to know how well to study.

Andy - Natural density is not related to density needed to invade.

Nick - You don't want to be so conservative as to mention the entire tank. But need to be certainty in your zero. Too low of a volume would guarantee zero.
10 individuals/L is typical for coastal phyto. Chose the volume to get high power.

Andy - We can work on volume when Null =0

Nicole – Back to 10 -50 micron size class. Any techniques we should look up?

Nick - Not my favorite technique: Most probable number (MPN). Is it appropriate for this size class?

Russ - We use that [MPN] technique. It works. While we have not fractionated samples for MPN in the past, we plan to do so for future shipboard tests. We have a manifold system so you pour sample water through the top, it goes through the 35 µm mesh screen and then that water passes through the 10 µm filter. Done in triplicate. It works well. Capture 3 volumes (3 replicates) 100, 10, 1 mL. Set up with 3 funnel systems and for the first set, run the sample through each funnel. 25um filter goes onto a multiwall plate with media and F2 phytoplankton medium, and incubate 12 hours light /12 hours dark under ambient temperature. It's cultural technique, therefore you can only enumerate those who grow, so we use in conjunction of chlorophyll analysis of the same sample.

Lucie - MPN as well. We don't have manifold, do in tubes. Similar set up. Seems to work. There are limitations, so we use other methods at the same time (three dilutions, 6 replicates). There is growth in control or limited growth in treatment, in conjunction with our other measurements. Fairly conclusive.

Nick - There is a long history of MPN, but not common in terms of phytoplankton problems. When the treatment works, of course MPN works. Zero is the only good number from an MPN. You can dilute the original sample to “nothingness”, did it grow when there is only one cell? To do it properly…Diluting 1 order of magnitude is too much. If you had 0.1 animal/ml and used 1ml you will not get growth. MPN is not applicable to everything. You can test yourself by putting lots of cells in the MPN. It produces the result of …What contrives sample volume….Or as Russ is doing, take 100ml, concentrated it, then dilute it. Excellent chance that the one cell will not make it.

Russ - MPN has a stats table with 95% confidence intervals. The 100 ml subsample would not be diluted, but placed into a growth medium.

Nick - True, but the confidence is +/- an order of magnitude.

Russ - You only get presence/absence for a particular subsample, not direct enumeration. To reduce the confidence intervals, greater numbers of replicates are required for each sample volume.

Nick - Takes 3.5 weeks because you have one cell to get a detectable population. Inconvenient

Lucie - Culture based methods depend on what are you satisfied with. If it takes too long, look earlier.

Nick - If there is only one cell, you need all of that time.

Lucie - For compliance you could wait 6 months….

Nicole – Next meeting March 17, organisms less than 10 microns in size.

Adjourn

California State Lands Commission
Technical Advisory Panel:
Testing Guidelines and Verification Protocols
March 17, 2008
Meeting Notes

Participants:

Annie Cox , University of Rhode Island	Russ Herwig, University of Washington
Fred Dobbs, Old Dominion University	Lucie Maranda, University of Rhode Island
Nicole Dobroski, CSLC	Allen Pleus, WA Dept. Fish and Wildlife
Maurya Falkner, CSLC	Andrew Rogerson, soon to be Fresno State
Ray Frederick, ETV Program	Chris Scianni, CSLC
Daphne Gehringer, CSLC	Tom Stevens, NSF International
Dominic Gregorio, State Water Resources Control Board	Nick Welschmeyer, Moss Landing Marine Lab

Meeting Summary:
Nicole welcomed everyone to the meeting. Participants introduced themselves, and Nicole discussed the purpose of the meeting - to consider methods of quantifying and assessing the viability of organisms less than 10 microns in size (human health indicator species and total bacteria and virus counts) for compliance with California's performance standards.

Dominic presented an overview of California's water quality objectives for human health indicator species (coliform, fecal coliform, enterococci…) for coastal and ocean waters. He provided some specific examples for San Francisco Bay and the LA region. This information can be found in California's Ocean Plan or in specific regional Basin Plans. Dominic then proceeded to discuss the need for indicator/surrogate species in water quality analysis. He then focused on three common methods of determining indicator species presence and viability including: multiple tube fermentation, membrane filtration and enzyme substrate (IDEXX, Colilert, Enterolert)

There was some discussion about the need to dilute samples for the aforementioned tests and whether this could impact test sensitivity. Additionally, participants discussed how salt water or biocides could interfere with test processes and lead to false results.

Dominic went on to discuss *Vibrio cholerae*. *Vibrio* is not an indicator species. You can get interference from local waters when running the tests unless you know what serogroup you are looking for. Fred commented that serotypes O1 and O139 are associated with toxicogenicity, but these serotypes are not always toxicogenic. It would be incorrect to think that O1 and O139 = always toxic.

The discussion moved to rapid tests. Andrew discussed ATP test kits and that they might be promising, but these tests might experience interference from biocides if the enzymes are damaged. The test provides results as a range of numbers within an MPN type framework and not CFU, as in the California standards. Tests cost about $1.50/run. The luminometer costs approximately $2000

Nick was concerned that not all species under 10 microns in size are heterotrophic bacteria or viruses and that the discussion wasn't including smaller phytoplankton species. Methods such as FRR and PAM could pick up chlorophyll fluorescence and provide quick information on the presence of photosynthetic species in the sample. Nicole pointed out that the standards only consider bacteria and viruses in the under 10 micron size category. Regarding the cost of PAM, Nick said it costs roughly $10-15K right now.

Another rapid test using IDEXX trays was also discussed. This test creates a MPN result. They are widely used by the European community. This test may also lead to false positives, but was supported as probably the best approach for *E. coli* and intestinal enterococci by the meeting participants.

Fred provided some additional insight about *Vibrio* issues. He noted that the Germans are leaning towards assuming toxicogenicity if O1 or O139 are present, regardless of whether or not genes for toxicity are present. Using that assumption, then the methods are relatively quick using fluorescent antibodies. Each strain requires specific antibodies. Another rapid test, Cholera Smart, could give quick results to determine presence, but not abundance, of *Vibrio* in a sample. Methods to determine the presence of toxic *Vibrio* include infecting mice with a strain and see if they die or using PCR.

Fred mentioned that while the two *Vibrio* serotypes may be found together in the environment, he understands it is very unusual to find both during an epidemic. Maurya commented that if *Vibrio cholerae* was identified in any ballast water, DPH would be notified immediately.

The discussion moved on to living bacteria and viruses. The CA standard was determined to be inappropriate if expressed as individual bacteria and not colony forming units. We won't be able to tell dead from live bacteria without some kind of culture process. Also the performance standard as 10^3/100 ml is reasonable given that without treatment densities of colony forming bacteria should be 10^3/ml, so the treatment standard would be 1% of the normal population. State Lands will investigate changing the standard to read 10^3 culturable bacteria (same as CFU). This will have to be done in Legislation. It should be noted, that there are standard EPA plating methods to count culturable bacteria.

As for viruses, Fred noted that really the only way to count viruses is to look for virus-like particles that are stained and counted under an epifluorescent microscope, although there is no guarantee that each bit would then be an independent virus, could be a piece of fragmented DNA. The particles counted are referred to as virus like particles (VLP). While the procedure provides a number, it does not provide information about VLP viability. Another approach would be to look at a bacterial phage such as the coliphage MS2. If the phage is present on a plate with *E.coli*, the phage will kill the bacteria and leave a plaque where the colony was. This may be one way to use a surrogate virus to see if any viruses were killed. Of course, Russ pointed out that even if there are no coliphage, there may be many viruses left in the sample. Additionally, this technique would not work well for routine compliance monitoring, but could be useful for research and development.

Nicole wrapped up the meeting by asking for any other items. Fred suggested that CSLC further define several terms in the standards. Maurya will look into adding this language. CSLC will produce a rough draft protocol for distribution and comment by the panel members. Another meeting will likely also be required to finish up discussion of the 10 – 50 micron size class methods.

Detailed Meeting Notes:
Nicole: There are standards for 3 human health indicator species and two additional categories for under 10 um in size (bacteria and viruses). We need to develop 2 things: Technology guidance document (how to do the testing to meet CA standards) and verification protocols to determine vessel compliance. Last week we discussed the need for rapid techniques to quantify phytoplankton and zooplankton for the verification protocols. We also need more thorough/complete techniques for use in the testing guidelines. This was in the context of giving something [a protocol] to our inspectors, but we also need to take into consideration what developers need from us for testing purposes.

List of concerns for micro tests include cost, time, complexity, chemicals/equipments, applicability of techniques, scientific acceptability etc… But first I'll hand off to Dominic to tell us about CA methods for assessing microbes.

Dominic: Address issues about what methods are used to test microbes in CA water.
In terms of water quality standards for indicator bacteria, in general, Ocean Plan lists water quality standards for the open ocean, and also Basin Plans list standards for each watershed and bays/estuaries associated with the basin.

The Ocean Plan is standard under Clean Water Act, where limits are determined by beneficial uses and objectives/criteria [EPA criteria = CA objective]. The endpoints include the average concentration for multiple samples (30 day geometric means based on 5 samples collected within 30 days; units: CFU or most probable number), or can be a concentration for single sample. Geometric mean is 1000/100ml for total coliform, 200/100ml for fecal coliform, and 35/100ml for enterococci….single sample is allowed to have a higher result, as long as it doesn't get too high; there are limits on single sample that contributes to the mean. For example, Coliform can be up to 10,000/100ml. An epidemiological study in Santa Monica ~1995 resulted in state law and came up with new measure that incorporated the concern for human fecal matter from runoff. If the fecal: total coliform ratio is 0.1…If most of the total coliform is fecal, then 1000/100 ml is acceptable for a single sample standard. There are also standards for contact recreation (e.g. swimming), which has the same limits as Department of Public Health. Advisories are posted if beaches do not meet the standards.

The basin plan applies to beaches in bays/estuary, and there are different standards for shellfish. Median limits for total coliform is 70 cfu (or mpn)/100ml in water (not per grams shellfish tissue) and 10% of sample can't exceed 230/100ml. Terms are a little different than for recreation. We plan to amend the Ocean Plan to hopefully 14/100 ml concentration for a median.

Basin plan is very similar to Ocean Plan. Same kind of epidemiological background information. LA harbor- fecal coliform log mean, still has 30 day sample period but only 4 samples. Standards/criteria for contact recreation is a 200/100ml mean, and 10% can't exceed 400/100ml. They have the same limits but different sample number as Ocean Plan. For non-contact recreation (i.e. sailing): fecal coliform 2000/100ml mean, 4000/100ml single sample. 3 tubes are in multiple fermentation, so limit changes to 330/100ml, since there is less precision than in a 5 tube multiple fermentation (where limit is 400/100ml). Central Valley and San Francisco Bay are similar to other plans. In San Francisco Bay, contact recreation has a limit of 240/100ml on average, and 10,000/100ml for a single sample. I just wanted to point out the different regulatory levels.

Dominic Powerpoint presentation:
Why use indicator bacteria? They are surrogates for harmful species and it is easier to measure something abundant. Also, the tests are inexpensive and easy to perform, while pathogens are innumerable and expensive to test. There are drawbacks and pluses to using surrogates. There are known EPA-standardized methods, therefore it is easy to measure the surrogates. Ultimately, we do want to move away from only examining indicator bacteria. These surrogate bacteria include total and fecal coliforms, which are found in lots of different matrices: soil, wetlands, on algae, etc. A subgroup of the total coliforms are the fecal coliforms which originate from warm blooded animals (e.g. people) and birds. E. coli is a single species and is the largest subset of the fecal coliforms. A subgroup of fecal streptococcus is enterococcus.

Russ: There is a lot of history with these groups that were defined by the ability to behave or how they appear, operationally. They aren't really known to be different species.

Dominic: Methods for Coliform quantification:

1. Multiple tube fermentation - EPA approved. Units are most probable number, and the positive test is indicated by formation of acid (color change) or gases. It has 3 parts to the test and takes 96 hrs to get a result. It is a cumbersome test.
2. Membrane filtration: filter through membrane and use media to grow and incubate bacteria. Place filter onto surface of medium. Results are quicker than with multiple tube fermentation. Get CFU as a unit. The idea is to have a petri dish with a grid, and count the number of colonies ("blobs"). What if there is a hair or fiber, is there one blob or more? Use a dissecting scope to see them better. Bacteria like to cling to themselves so there could be multiple blobs, which is another source of error. It is hard to count how many colonies there are when the initial concentration of bacteria is high. But, you get a direct result. Each blob is assumed to come from about 1 bacterium and is referred to as a colony forming unit (CFU).
3. Enzyme substrate (IDEXX/Colilert). Enzyme substrate, pour sample in a multiple well tray. It is an 18 or 24 hr test. No one uses the 24 hr test. Sea water samples have to be diluted (1:10). Chromogenic/fluorometric test = color change. Testing for total coliform has a yellow product, and testing for E. coli appear blue under UV light. A survey in LA did a comparison between the multiple tube fermentation and the enzyme substrate tests, and the results came out the same. (examples: Colilert and Enterolert)

Nick: Do both multiple tube and enzyme substrate use sample dilution? Multiple separate sample into 5 tubes, IDEXX uses 96 wells, and must be diluted before going into the cells.

Fred: Need dilution

Andrew: EPA did lots of comparisons and found no correlation between the two tests. There were lots of false positives.

Dominic: Statistically the LA work showed that they performed the same. There were many false positives, mostly in freshwater and estuarine waters.

Andrew: This [false positives] could be an advantage for ballast water.

Russ: With membrane filtration, you can filter it down. It sounds like if you need to dilute it [for other tests], then you lose your sensitivity.

Nick: Add in replicates.

Dominic: Dilute sample in media and dilution distilled water (100ml) mix with dilution sample (1:10). Then pour into tray.

Nick: There is no serial dilution.

Andrew: You just want to lose some cells. Russ is right, you lose a lot of sensitivity.

Dominic: Just one step dilution.

Maurya: Is multiple tube a dilution?

Russ: Yes, you need 3 serial ten-fold dilutions. Dilute the sample to extinction so that you know where the cut off is. (There would be 9 tubes for a 3-tube MPN).

Dominic: So for enterococcus, you use the same 3 methods. You get a different color on the IDEXX method, The method is just tweaked for the different organisms. For enterococcus, the species you find is more of indication of whether or not you have sewage water, i.e., fecal contamination from humans. On 3rd slide of the powerpoint, there are four enterococcus species listed: to confirm sewage water, either S. faecilium or S. faecalis will be present…by knowing which of these species you have, you know if you have sewage. Others more common in storm water/non-point source pollution. However, sewage bacteria can grow on algae, and give you false idea of what is going on in the water.

Vibrio is a real pathogen, less of an indicator species. O1 serogroup [as is O139] is associated with cholera and it's hard to interpret results of a vibrio test unless you know what serotype you are testing for, can get interference from local waters. Standards for Vibrio are low because not very abundant.

Fred: Just to add, serotypes O1 and O139 are associated with cholera (epidemic serotypes). The confusing thing is these serotypes are not necessarily always toxicogenic. Looking at Vibrio in ballast water, O1 and O139 were present, but not all of the O1 and O139 individuals contained the genes to have toxic ability (tested using PCR). Incorrect to think serotype = toxicity, have to see if genes are present for toxicity.

Dominic: There is evidence that ballast contains Vibrio. We are paying SCCWRP [Southern California Coastal Water Research Project] to develop rapid methods, and they are making progress. One method is qPCR [Quantitative PCR], which takes about 3-4 hrs to complete. DNA technology. There are issues with false positives. Transcription Mediated Amplification (TMA): 2-3 hrs. and looks like it works well. This measures RNA, and there is no proof that Vibrio is alive. Must be calibrated with other methods for viability. Trying to work that out. Big advantage is the DPH can post beach safety notification pretty quickly, so it is a good system to protect human health.

Nicole: Let's focus on human health indicator species. Our standards are: E. coli 126 cfu/100 ml, intestinal enterococci 33 cfu/100 ml, and Vibrio 1 cfu/100 ml. Thinking about verification protocols, can we work with these methods that we just talked about?

Dominic: There is an immunological dipstick that did not work out very well, although they are very easy to use. Another is a variation of the Colilert test, using a colorimeter to identify bacteria. That idea kind of fell off. The last methods were the best.

Andrew: Live/dead is a problem with the highly technical tests. ATP rapid test kits might be promising. Can send details.

Fred: Is this a quick tool to see if you should take additional samples?

Andrew: Yes. Pretty idiot proof. The trouble is whether there is interference with biocides. Do different biocides inactivate the enzyme of the test. There needs to be more work. But you can't get faster than the color test. Tells you if something is living or not.

Fred: Very sensitive. Difficult to relate qPCR to CFU.

Nick: Agree with Andrew, ATP worth taking a look at – had good and bad results, very sensitive. You need to filter 10-100's of ml. It is very sensitive and might pick up dead organisms. Sometimes it is convincing, but if it works at ul level, that would be fabulous. Did you do that with natural seawater sample, unfiltered?

Andrew: Yes. We always did the rapid ATP test in unfiltered water. We tried to correlate to plate data. It was a reasonable relationship that was worth going forward with. Tells you if there is a need to examine the sample further.

Nick: Agree wholeheartedly. The other technique is FRR and PAM. Problem is we are only talking about bacteria. <10 um also include phytoplankton. There is an optical method of optical fluorescent. The FRR (fast repetition rate), PAM (variation in Canada, and England), the idea that you expose the sample to a fluorometer, and gives you a reading of low or high chlorophyll in a second or two. The disadvantage is that no one relates whether cells are alive or dead. However, the sample volume can be >1ml to 100ml and is pocket size. The answer is instant. If the tester has something to compare to (untreated water from elsewhere on the ship), you can see if there is a difference from the treatment. Huge practical appeal. Wishful, but will it pass mustard?

Nicole: We know there are phytoplankton in this size range, but we have no standard for phytoplankton in this size class.

Nick: PAM fluorometer tells you information based on chlorophyll. Okay for photosynthetic bacteria (cyanobacteria), not heterotrophic bacteria.

Dominic: How much does it cost? Could it measure larger organisms?

Nick: ~$10-15k and coming down in price. It measures everything with chlorophyll. You could filter the size class you want. Semiquantitatively, we have seen differences between treated and untreated water in 99.99% of the tests we've done. Doesn't necessarily mean the phytoplankton is dead, but there is a numerical difference.

Dominic: ATP dipstick sounds promising to me.

Andrew: It does need some work. If the biocide damages the enzyme it won't work, but it seems to work with chlorine.

Maurya: If the biocide does affect the ATP, could give you false negative?

Andrew: Yes.

Russ: If you could do a filtration and a wash, you could reduce the impact of the biocide.

Maurya: If the biocide does affect the test, then maybe there is too much residual biocide for disposal.

Andrew: You get a result within 18 hrs. It may not be absolutely related to organisms, but if you have zero count, you're looking good.

Dominic: Doesn't give you the units you want. You get MPN, not CFU.

Fred: But it gives you a range on numbers, are you at the low or high end of a range. It's okay along an order of magnitude.

Andrew: It shows that there was a treatment.

Dominic: For legislation, why was CFU used and not MPN?

Maurya: Because the majority of the advisory panel said so in the report. We could fix the bill to be MPN if we want to.

Andrew: Because everyone else is doing membrane filtration which uses CFU.

Dominic: Not really for CA regulation, use IDEXX.

Andrew: MEI agar is almost faultless.

Russ: How much do you need to dilute it?

Andrew: Not at all.

Dominic: You are talking about different species, media, and therefore units (?)

Andrew: False positives are okay, better than false negatives.

Fred: Detecting public health might be fundamentally different than detecting ballast treatment. We want a conservative approach, especially with regards to public health. But for treatment technology, there could be two aims. The vendor would be upset about false positive. But CSLC could be less concerned, trying to protect public from invasive species, so maybe false positives ok.

Maurya: Yep.

Fred: I just attended a meeting in Denmark that discussed these issues in an international crowd. These problems could end up in court, because what if vendor said we had 125 not 127 cfu?

Andrew: We need to confirm that the system will be working.

Nicole: That’s what we’re looking for.

Andrew: The only thing that works is plate counting, but it takes time. Perhaps combined with rapid method it’d be okay.

Dominic: We use both MPN and CFU in CA, and we issue fines based on both units.

Nick: Back to phytoplankton part, do the dynamic/pulse fluorometer which measures concentration and activity. My impression from the scientists that have tested other methods that I haven’t tested…when chlorination is used, the testing ability goes down, you can test for biomass indicator if there is a track record …there is a chlorophyll number …

Russ: IDEXX- Quanti-Tray creates MPN, 1-200 MPN / 100 ml, another model (Quanti-Tray/2000lets you test 1-2419 MPN/100 ml, has range for E. coli and coliforms. might be hard to penalize if still in development. Stuck with EPA approved samples. If you dilute sample, it should be okay with 2419 sample tray.

Fred: Recently been approved for wastewater treatment, and widely used by European colleagues.

Nicole: What about Vibrio?

Andrew: I like IDEXX, but if you want a yardstick…there are problems with using it as first step because you never know if they had those bacteria in the first place.

Dominic: If you had a record of treatment system operation, then you can tell if it worked. But Colilert will tell you if the system worked.

Nicole: Ultimately we’re interested in what is coming out the pipe. Whatever we do there should be an indication of paper work and how the system worked.

BREAK

Nick: Was the ATP test called Luminultra?

Andrew: I don't know. I'll send some info to Maurya. It's about ~$2000, or ~$1.50/run, and you do 5 replicates per test which can really tell you if it's an effective treatment. Also need the luminometer, ~$2k. The live/dead staining kits are pretty good, and can be related to photo counts

Fred: Live-dead tests work well on lab bacteria, but not as well with natural conditions. For technology testing, take some natural water, throw in glucose to amp up production and re-run the test, and see if there is an indication of the technology. Not a pure batch colony, but gives more ability to test. .

Nicole: Vibrio test seems sketchy, what is toxicogenic or not.

Fred: For toxicogenicity, the Germans are leaning toward O1 or O139 are present, assume toxicogenic. Might be easiest to say if you don't have those, then you have no toxicogenic problems. Methods are relatively quick: use fluorescent antibody. New Horizon Diagnostics (Rita Colwell) has one, but it is pretty expensive, $350 (with academic discount), about 100 tests. Each strain needs its own antibody. Take bacteria on slide, quick stain procedure (35 min) and epifluorescent microscope ($12-30k).

Maurya: Could you expect to find this in an EPA certified lab with human health?

Dominic: I don't know if it's EPA. It's in standard methods, which are usually one in the same but not always. Waterboard doesn't do it. It's an expensive test and it is not an indicator because it tells you less about the overall bacteria population.

Fred: CA picked it up [Vibrio standard] because of Brazil, who was concerned about an outbreak since an outbreak occurred in Peru. I understand that in some cases, Brazil just pours chlorox down in the tanks.

Maurya: No indication of how much [chlorine] went in.

Fred: No quick way to do this. The fluorescence doesn't really need cultures, although if you wanted to, you could. It is very specific.

Maurya: Are there any association between O1 and O139? Are they always together?

Fred: If there is an outbreak it's one or the other, if an environmental sample, one or the other or both. Our results show that the strains are overwhelming not toxicogenic. But if there is an outbreak from untreated sewage, there will likely be toxicogenic products.

Maurya: If you see the strain, we could just assume it's toxic to be safe

Fred: That is the mainstream approach. Alternatively, you could grow up a culture and infect mice, see if they die. That might be a bit much. There are PCR methods, therefore, there could be qPCR methods for each strain, but it gets really tricky because the region of the genome that you work with is co-regulated by a gene from a virus that has been inserted into the genome of the Vibrio.

Russ: Because Vibrio is a major killer internationally there is lots of work on creating quicker methods. Watch international public health agencies worldwide for techniques.

Maurya: If we did a rapid assessment for Vibrio and identify the two strains, the first response would to contact DPH, and get more minds involved. Since it is such a big issue, I would hope that people could address this. If we had a rapid test, we could pass it off to someone who has the better technology readily available.

Fred: CholeraSmart, is a rapid test, and is based on the same technology used for pregnancy testing and could be used by an unskilled person. Take same water, maybe concentrate it, and a blue line/pink line will give you an idea if it's in the water. It doesn't tell you the concentration, but tells you if it is there (presence/absence). Together with the ATP, now you have some good rapid methods.

Maurya: Is O1 more common than O139?

Fred: O1 (El Tor) was responsible for the 1^{st} 4 epidemics. Then O139 (Bengal) has shown up in the last 25 yrs. It is thought to be more prevalent world wide.

Russ: [Looking up test info online] O1 is 20 min assay, geared toward stool analysis.

Maurya: Do they work on O139?

Russ: New Horizon is an interesting company with a lot of these kinds of tests.

Dominic: Can the strains later mutate into a toxicogenic form?

Fred: They can be promiscuous with their genes. One individual that is toxicogenic can transfer genes to another individual.

Nicole: So the presence of either strain is not acceptable?

Fred: Yes, if you want to say that. Just think about the balance between legal pragmaticism and public health concerns…I don't know how the antigen changes the cell membrane. If the surface antigens have not deteriorated, the test could get a false positive.

Maurya: This would be an interesting note to point out in our document: if we want to look at rapid assessment, you can know that if you use biocide A, and it can get activated upon discharge (UV- intake and discharge), you might get a false positive because the cell membrane has not deteriorated yet.

Russ: There is a huge amount of work into creating the tests.

Maurya: That’s why we need assessments attached to the methods. The new tools, enzymes, PAM, we just have to jump through more hoops.

Nicole: Let’s move on to total bacterial and viral counts.

Fred: “Total” is wrought with peril. I think you mean total culturable bacteria. We need to think about what kind of agar to use, because you get different counts depending on what you use.

Nick: Was it in the conversation that it is a CFU from plate streaking method?

Andrew: Total number is not possible because you have to culture it, and you’ll go way over your standard with that. So your number is representative of total count. But you can’t tell dead from alive. After culturing bacteria, expect densities around 10^3/ ml

Maurya: Greg said this would be the most difficult; but it’s pretty important. While we would like to find some methods to make these measurements, we are not going to hold back about changing the standards. Total count doesn’t give live/dead, so do you just want to see a change from before/after?

Andrew: If you can culture 10^3/ ml, then aim for 95% reduction from that. That would be very reproducible, and is more or less the desired standard (10^3/100 ml).

Nick: 10 bacteria/ml would be CA standard, which is 1% of typical plate grow out. So we are talking about the same number. This is extremely difficult. Sea water is usually 10^6 bacterial/ml

Andrew: 10 cultural bacteria/ml, I think

Nick: What kind of reduction would we look for? This is 5x 10 fold reduction. Bacteria/ml vs CFU/ml.

Maurya: Culturable bacterial, I think.

Nick: That would be achievable.

Fred: Based on direct counts it would be difficult for technology to achieve that. For flow cytometry, very difficult to measure the number.

Nick: Yes, prone to technical mistake. The method should be everyone’s tried and true bacterial streak. Too many log orders below what could be done

Maurya: Should be 10 cfu/ml?

Nick: Yes.

Nicole: If it is CFU, should be easy: plate and count?

Nick: Yes, and is achievable in less than 4 hrs sometimes.

Andrew: You could do some serial dilutions, with replicates, grow the bacteria on agar plates, incubate them, and count colonies.

Russ: If treated ballast water, add 100-200 ul of ballast water to the plate, if you get colonies, that's a problem.

Fred: There is a 20 yr old technology (called the Spiral Plater and manufactured by Advanced Instruments company; see an image of the device at http://www.topac.com/spiralplater.html) that can plate bacteria quickly for you. It costs about $13k. [See another brand of spiral plater at http://www.neutecgroup.com/eddyjet.htm]

Russ: It plates the bacteria down the center, the amount of bacteria that is in the sample influences the distance from the center that the bacteria will grow.

Nicole: We'll investigate change the wording from total bacteria to total culturable bacteria.

Maurya: Is total culturable bacteria the same as CFU?

Russ: Yes. [The term culturable refers to the ability of an organism to grow on microbiological media. If it is culturable, then the organism must be alive. Samples can be placed onto agar medium resulting in the formation of colony forming units (CFU). If inoculated in broth medium then can count using the MPN (Most Probable Number) method.]

Fred and Andrew: and the bacteria have to be alive [to show up on plates]

Nick: You'll need to specifically state the test or it will be abused. These kinds of tests are always being taking for granted: the size of the squirt, the length of the incubation, the agar media, and there are big contamination problems to consider. Standard methods in EPA

Andrew: There are standard methods. It can be done. It must be written down.

Nicole: What do we want to say about viruses

Fred: How does CSLC define virus? Can define virus like particles with an epifluorescent microscope. Add stain, you can see them, but there is no guarantee that it is a virus.

Dominic: Could be a fragemented DNA from anything?

Fred: Using standard electron microscopy….there are specific bacteria, archaea, cyanobacteria for the viruses.

Andrew: Count total live and dead using Fred's methods. Will have to grow to see if a live, will have to rely on ?.if you kill the bacteria do you kill the viruses?? The only thing you can go for is coliphage. Take sample water + cultured E. coli. Viral infection occurs within 15 minutes. There will be a clear spot in the agar if there is a virus that killed the cells. Lets you know if it is a viable virus. Assume a clear spot represents a dead bacterial cell. You can go after older contamination. Looking for viruses is so difficult, we don't know that if you killed coliphage you kill other viruses

Lucie: Chlorine dioxide, MS2. Looking at different concentrations, the results were clear that above 1 ppm chlorine dioxide there is an efficiency of the treatment.

Russ: Difficult to culture many viruses. People are looking for surrogates [MS2 coliphage is used as a surrogate for viruses in disinfection tests and is widely accepted]. And it's hard to work with pathogenic human viruses.

Lucie: MS2 coliphage was used.

Russ: Fred's method totally accepted. If you have a treatment that looks the same as before ... Too bad you put viruses in the regulation.

Nicole: We should look into modification?

Maurya: As Andy Cohen said, just because we can't measure it doesn't mean we shouldn't have it in the regulation. It's like BWE, we still moved forward. It's something we are working on and towards and is a long lasting question. We should still have it as a standard.

Dominic: There are many similar instances in water quality. You need to have it there anyway.

Nick: The regulation reads 10^4/100ml viruses, not viable viruses. Just put that in perspective. In a VLP assay will be 10^7-10^8 Viral Like Particles. We've made those measurements. To get that low is absurd for viruses. This is the one test case that if you lost it from regulation, you'd have a shotgun approach (?), and I don't believe that any treatment would pass that test.

Maurya: I recognize this is problem. But why didn't anyone argue earlier?

Nick: I remember this discussion. You couldn't dilute them that efficiently in the lab to meet these standards.

Andrew: I thought we were going to leave it at removing the bacteria….

Russ: About coliphage: as compliance, what if you don't find it, there still could be huge numbers of other viruses there. [Coliphages can be used as indicators of fecal contamination since they are viruses that infect coliform bacteria. If coliform or fecal coliform bacteria are absent, then would be unlikely you would find coliphage. Using coliphage in testing and efficacy testing is another story. Here you could add coliphage to the system and examine the efficacy of the treatment system.]

Andrew: Make sure the testers check for coliphage.

Nick: It is out in the open that it is more of a disservice than an aid.

Maurya: I just want people to get some numbers. What does your system do?? You can't enforce something you can't measure. I would like the technology developers to at least keep the viruses in mind, and know how to get the numbers.

Andrew: No one tests for viruses. Keep it in mind.

Maurya: The only people that have thoughts of litigation are the people who can't test it. It is an area that should be looked at.

Russ: Some test will kill MS2 and coliphage, maybe some biocides will oxidize some VLP. Looking for pinpoints of light is pretty hard. Makes you wonder if you are imagining them.

Maurya: Next we need to work on our straw man document …Anything else?

Fred: Define terms. Have a glossary in the beginning.

Maurya: Legislation will extend the first standards. If we can change bacteria to CFU, and insert some definitions, I'd like some of your input so that it is logical.

Nick: Just to recap: the 10-50um discussion went quickly. We talked about the pros/cons of MPN, and the conclusion was that we didn't get anywhere…did you get anywhere?

Nicole: No. We need to talk again about that.

Maurya: Might do straw dog, and reconvene after that.

Nick: Counting viruses were the least cost effective technique out of all…

Nicole: Thanks to all for participating. Will send out notes for review and information on next meeting. Adjourn.

California State Lands Commission
Technical Advisory Panel:
Ballast Water Treatment Technology Testing Guidelines
July 16, 2008
Meeting Notes

Participants

Andrea Copping, Pacific Northwest National Laboratory	Rian Hooff, Oregon Department of Environmental Quality
Fred Dobbs, Old Dominion University	Dave Lawrence, University of Washington
Nicole Dobroski, CSLC	Lucie Maranda, University of Rhode Island
Rich Everett, U.S. Coast Guard	Kevin Reynolds, The Glosten Associates
Maurya Falkner, CSLC	Greg Ruiz, Smithsonian Environmental Research Center
Daphne Gehringer, CSLC	Chris Scianni, CSLC
Dominic Gregorio, State Water Resources Control Board	Mario Tamburri, University of Maryland
Russ Herwig, University of Washington	Nick Welschmeyer, Moss Landing Marine Lab

Summary

The advisory panel met to discuss the draft "Ballast Water Treatment Technology Testing Guidelines." After a brief introduction, Nicole began the discussion by asking for comments on the testing guidelines as a whole. Rich voiced concern about the self-certification process and its validity. While Nicole understood Rich's concerns, she pointed out that the guidelines are voluntary, and it will be up to vessel owners and operators to determine whether or not they have sufficient evidence that a system will be able to meet California's performance standards. The vendor self-certification does not relieve the vessel owner/operator of the responsibility of complying with the performance standards.

Rich also suggested removing USCG from the list of contact people available to review the test plans because USCG can only discuss items relevant to established regulations. Nicole agreed to remove USCG from that section.

The conversation moved on to discuss the methodologies in Table 5-1. Lucie, Russ and Nick commented that the freshwater methodologies from the Great Ships Initiative (GSI) were confusing because they were mixed in with tests for marine systems. Nicole agreed to separate out the freshwater and marine methods. Rich noted that based on the title of the table it appears that the methods are required. Nicole will clarify that the methods are recommended and that they may change with time as new methods are developed.

Nick brought up the topic of the less than 10 µm size class and whether or not the standard applied to living/culturable bacteria. Nicole said yes, although Maurya commented that it will be at least next year before the performance standards can be changed to reflect that. The discussion also covered the use of specific marine media for culturing the bacteria. Dominic pointed out that the IDEXX methods, which give a MPN endpoint, are comparable to the CFU methods.

As for the 10 – 50 µm size class, there are no standardized methods for assessing compliance. Fred commented that the GSI protocol is not sensitive enough to determine compliance with California's standards.

The discussion touched on water quality issues and the ability to apply the Ocean Plan limits to vessel discharges. Dominic discussed the need to think of the cumulative environmental impacts of these discharges. For the moment though, we have a poor idea of the metal concentrations in ballast discharges. Greg is undertaking work on this topic.

Nicole will work on a new draft of the testing guidelines and distribute it to the advisory panel and the technology vendors for their input. She thanked everyone for their participation.

Detailed Meeting Notes

Nicole welcomed everyone to the meeting. Participants introduced themselves, and Nicole discussed the purpose of the meeting - to discuss the draft ballast water treatment technology testing guidelines. Nicole reiterated that CSLC will not approve treatment technologies for use in California waters. Instead, CSLC will conduct inspections and enforce the ballast water performance standards. The Testing Guidelines should work in conjunction with the IMO Convention and pending federal treatment technology evaluation guidelines, while incorporating California's standards. The purpose of the Testing Guidelines is to provide technology developers with a mechanism to assess system compliance with California's performance standards. Systems that meet California's standards can be vendor certified as compliant with California's requirements. This certification may serve as a marketing tool to provide information to potential customers. The certification does nothing to relieve the responsibility of the vessel owner/operator to comply with California's performance standards.

Andrea pointed out that the Testing Guidelines are important as they refer to the IMO and proposed federal evaluation guidelines, and are written in a way that is comparable to those other documents.

Nicole and Maurya discussed that this document will continue to evolve, and, as stated on page 7 of the Testing Guidelines, that CSLC will update the guidelines as necessary. The Testing Guidelines, updates, and the CSLC contact information will be available on the CSLC website.

Greg suggested that if CSLC receives a high volume of questions and concerns from technology developers and vessel owners, we could consider creating a Frequently Asked Questions page on our website to streamline our work efforts. Nicole agreed that a FAQ page would be useful.

Rich brought up concerns about the self-certification process, and suggested incorporating text notifying the technology developers that certification from a third party, or independent testing center, could be another useful marketing tool and may be more credible than the self-certification alone. This could also help to streamline CSLC's review of verification reports generated from the verification process (i.e. the independent certification could reduce the amount of time CSLC staff would have to spend reviewing methodologies for applicability to California's standards). Rich noted that any system, even those that conduct limited evaluation, could self-certify as California compliant.

Nicole argued that competition between systems will require technology developers to conduct valid verification testing in conjunction with independent testing organizations.

Greg commented that if third party testing centers will be used, they could provide information regarding their reputation/experience and the quality of their data, and that this may help CSLC assess the quality of the system verification report.

Nicole commented that CSLC will not require the certification come from independent or third party labs. The guidelines are voluntary, and it is up to the system developer to accurately and honestly provide results about system performance. The market will demand such reporting.

Maurya stated that when it comes down to a treatment developer wanting to sell a system to the Maersks or the Matsons of the world, and if that shipping company plans to call on California, the developer will need to decide if they want to use these guidelines and/or go to an independent testing organization to conduct the evaluation. The treatment developer can then choose to self-certify compliance with California's standards. Vessels owners/operators will look to this self-certification. If vessels aren't meeting the standards and the system was certified as California compliant, the vessel owner/operator will look to the treatment developer as the responsible party. Most companies won't be willing to put a system on their vessel that they haven't heard much about, particularly since each compliance violation will cost $27,500/day. CSLC is working under a mandate to have strong performance standards without the ability to approve treatment systems to meet those standards. All we can do is require a sampling point/facility with which to draw a sample and determine compliance with the standards.

Nicole commented that it is ultimately up to the vessel owner to decide whether or not they have sufficient information about a system to warrant purchasing and installing that system on their vessel. So, while it is not necessary to require that the certification come from an independent source, CSLC can still strongly recommends that testing take place with an independent testing organization. We can request that those independent testing organization provide credentials, as Greg mentioned, to ease comparison between them.

Rich suggested CSLC remove USCG from the list of contact people available to review test plans because USCG can only discuss items relevant to regulations that are already established. USCG is not in a position to “consult extensively” on test plans, even when an approval program is in place. The consultation should be between the independent lab and the technology developers. USCG does not have staff to deal with that many people, and has to be careful with what they say to avoid “approving” a system test plan. Andrea commented that she can understand that USCG and NOAA could provide information regarding “gray areas”, but only once regulations are in place. Maurya and Nicole agreed to remove USCG from the recommendation for consultation on the test plans.

Nicole moved the discussion to Table 5.1 - the methodologies for testing ballast water to determine compliance with the performance standards. Lucie commented that the table was confusing because it included freshwater methodologies, and since most of CA ports are marine, you had to hunt through the table to find which tests were appropriate for marine systems. Andrea stated that in the past freshwater tests were ignored to some degree, but we now recognize that we will have to take these tests seriously given that many ports worldwide are freshwater or brackish. Russ suggested modifying the table to separate out freshwater and saltwater methodologies.

Nick agreed with Russ. He was also concerned that it is not clear that this table will be updated frequently, and that testers will do what the guidelines suggested. Nick commented that if he were new to testing technologies, he would not find this table very clear because there are no titles to the methods, and it appears that the resources required for the specific methodologies are set in stone without room for compromise.

Nicole pointed out that the table is intended as a guide and is not all inclusive.

Rich pointed out that that the title of the table is not clear because it suggests that following the methods in the table will ensure compliance. Perhaps if it said something along the lines of “recommended methodology” it would be more in line with CSLC’s intentions. As far as specifics of the contents of the table, Rich was concerned about the links for zooplankton, because they does not include information on how to quantify zooplankton, only how to sample them.

Rich and Russ discussed the importance of congruence for testing for compliance, but that IMO has not been able to agree on which specific tests are appropriate for a comprehensive evaluation. Nicole mentioned that CSLC will have to suggest methods that are currently being used, and that it cannot be all inclusive because new methods will continue to be developed. Russ thought that the technology developers will be interested in knowing how CSLC will enforce the performance standards, and that they will want to use those methods to test their systems. Nicole mentioned that we are still working through how CSLC will determine compliance with the performance standards, but if there are other methods we should know about, that this is the time to comment.

Nick had a question about the less than 10 µm size class in the CA regulation as specific in Table 1-1. Are the bacterial counts specifically meant to be live or total counts? It looks like there should be the word “live” written in the table. Table 5-1 lists standard methods involving growing out and plating. There are methods for live determination. Nicole answered that the 10^3 bacterial numbers do refer to live culturable bacteria.

Rich mentioned that Marcel Veldhuis (NIOZ) has pointed out that there are abundant phytoplankton in less than 10 µm size class, but they are not included in this table. Nicole clarified that California is concerned about the phytoplankton in that size class, but it has not been written into regulation thus far, and this could possibly change down the road.

Greg wanted to clarify the differences between live bacteria and those generated from MPN approaches, and that maybe we should define “live” bacteria in this document because of the issues about so many marine bacteria being unculturable. Fred also suggested to use the word culturable. Fred agreed with Russ’s suggestions that Table 5-1 should reference standard method 92-15 and should include the names of standard media for culture of heterotrophic bacteria.

Nick reiterated his concern about the table being vague about “live” or “culturable.” Maurya clarified that this would have to go through legislation to be corrected. We can make this comment in our Biennial Report, but it cannot be changed until next year - perhaps when we update the performance standards to have the earliest compliance date changed from 2009 to 2010. In the meantime, we can add “culturable” to the text.

In terms of bacteria media, Russ pointed out that marine media are not discussed in Method 9215, and of the four heterotrophic media, some are more preferable than others. Russ and Nick like the Difco Marine Agar 2216. Dominic commented that CSLC might want to investigate media that are used in culturing marine pathogens. Nicole agreed to add the marine agar to the table in addition to the media used in the standard method.

Regarding the 10-50 µm size class, Fred commented that the GSI protocols are appropriate, but to keep in mind that they are draft protocols and will change. Lucie commented that there is no indication of quantifying live phytoplankton in this method. Because there are no absolute methods for quantifying and determining viability available yet, Nicole stated that vendors and testing organizations will just have to use the best methods available that are scientifically defensible.

Fred made reference to the comments that he submitted to Nicole prior to the meeting regarding the detection limit of the GSI method for phytoplankton assessment. Fred discussed that the GSI method for quantifying phytoplankton has a higher detection limit than the CSLC performance standards. Therefore, technology developers might avoid that test because it would be too easy to fail because finding one organism would lead to non-compliance. Nick suggested that it might be appropriate to suggest that there is not a good test for the 10-50 size class because the test is not sensitive enough for CSLC performance standards, and suggesting the GSI test would send the researchers/developers down the wrong path. Nicole stated that we will think about this issue.

Nick and Fred pointed out a problem with the link to the GSI protocol page for the 10-50 µm methodology, and that GSI has since changed the link with regards to the direction of the slash marks.

Regarding Table 5-1, Fred can live with the bacteria section, but expressed concern about the virus section, in particular, using the words “live” and “virus-like particles,” as even experts can’t decide whether to use the term viruses or virus-like particles or virus-sized particles. He stated that these words should be explicitly defined, and Nicole agreed.

Regarding enterococci methods, Dominic pointed out that the IDEXX test results in an MPN endpoint, which is comparable to CFU, and he sent a report about this to CSLC. Russ mentioned that membrane filtration has better sensitivity than the plating method listed in Table 5.1, and is a good method. Fred did not have comments about the Vibrio standard method testing, but is looking into Chun’s method (using PCR) and thinks that it might be the best to use. As for the Vibrio methods, Fred speculated that everyone is going to go bust on Vibrio.

In a brief discussion, Nicole clarified that requirements for sampling points are going to be similar to the ETV guidelines and IMO G2 Guidelines. Andrea commented that GSI is doing a lot of work investigating sample port design and whether or not it kills organisms in the process of sampling. This information will be available soon.

Questions regarding testing for water quality were raised. Dominic stated that for dissolved oxygen, a ship cannot impact the port waters at any point, including directly at the outflow. He then clarified that settleable material only includes settleable material that degrades the port waters. This is important in thinking about cumulative effects, since ports can have lots of vessel arrivals, all with the same discharge practices. By including a statement such as this, developers can keep it in mind and be cognizant of downstream issues.

Rich commented that back flushing systems have filtrate that is much more concentrated than when it went in and has a lot of settleable material. The standard puts the onus on the developer or vessel owner to be aware of what they are discharging so that the amount is not harmful to communities. Of course, what is harmful is open to interpretation because it will vary between communities. Dominic pointed out that it is important to think about cumulative effects over time.

Dominic stated that Table 5-2 lists measurable/numeric (vs. narrative) effluent limits. Kevin wondered if Table 5-2 takes background levels into consideration. Dominic said that the Ocean Plan doesn't consider background, but other programs do. These levels are very high levels compared to what would be in port and ocean environments. If we had used the median levels, that would have been a problem. Kevin mentioned that Ukraine has a similar table as 5-2, and fines vessels for poor water quality. $20-60k per fine. This is a concern for ships.

Dominic discussed that the standards aren't capricious and are fair based on environmental impacts. As an example, he picked Zn. The median limit is 20 µg/l and the background ocean conditions are significantly less than that. The discharge limits in the Ocean Plan is an order of magnitude greater than these levels at 200 µg/l and is definitely toxic to marine life.

Kevin discussed that the best piping for vessels is Cu/Ni because it inhibits fouling. Dominic wants to know what the concentrations of some of these metals are under current conditions. What does come out of ballast? Greg states that he is getting some data on that. His intention is to sample a couple of locations and vessels to determine metal concentrations.

As far as risk assessment, Nicole stated that CSLC inspections will target all vessels eventually, but initially we might have to focus on vessels that have treatment technologies that are unknown to CSLC.

As far as sending out the testing guidelines, Nicole will send notes to the technology developers to let them know what we are working on, and some of them might provide comments. When the document is complete, it will be available on CSLC's website. Greg pointed out the value of a public release, so if vendors have questions first, they can have time to read it and ask.

Russ suggested also sending the draft document to Randy Marshall at Washington Dept. of Ecology. Nicole agreed, and thanked the panel for participating in the meeting and their contributions. A revised draft document will be sent out in a couple of weeks.

APPENDIX A2. GENERAL SAMPLING CONSIDERATIONS

Parameter	Sampling Equipment	Preferred/Maximum Holding Times
Conventional Parameters		
Temperature	Plastic or glass container or sample directly	immediately
Dissolved oxygen (D.O.)	Glass D.O bottle	Immediately/fix per protocol instructions, continue analysis within 8 hrs.
pH	Plastic or glass container	immediately
Conductivity	Plastic or glass container	Immediately/refrigerate up to 28 days
Turbidity	Plastic or glass container	Immediately/store in dark for up to 24 hrs.
Nutrients		
Ammonia	Van Dorn, LaMotte or plastic sampling bottle	immediately
Nitrates	Van Dorn, LaMotte or plastic sampling bottle	Immediately, refrigerate in dark for up to 48 hrs.
Phosphate	Van Dorn, LaMotte or plastic sampling bottle	immediately
Urban Pollutants – Field measurements		
Total Residual Chlorine	Van Dorn, LaMotte or plastic sampling bottle	immediately
Phenols	Van Dorn, LaMotte or plastic sampling bottle	immediately
Total Copper	Van Dorn, LaMotte or plastic sampling bottle	immediately
Detergents	Van Dorn, LaMotte or plastic sampling bottle	immediately
Laboratory Analysis of Chemical Parameters		
Total Organic Carbon	Acid and deionized water rinsed glass sampling bottle, Teflon liner in lid	Refrigerate to 4 degrees C, send to lab immediately
Metals	Plastic sampling bottle	Fix with Ultrapure (or comparable) nitric acid, send to lab immediately
Oil and Grease	Acid and deionized water rinsed glass sampling bottle, Teflon liner in lid	Refrigerate to 4 degrees C, send to lab immediately
PAH's	Acid and deionized water rinsed glass sampling bottle, Teflon liner in lid	Refrigerate to 4 degrees C, send to lab immediately
Pesticides and other synthetic organic compounds	Acid and deionized water rinsed glass sampling bottle, Teflon liner in lid	Refrigerate to 4 degrees C, send to lab immediately
Toxicity	Acid and deionized water rinsed glass sampling bottle, Teflon liner in lid	Refrigerate to 4 degrees C, send to lab immediately
Biological Parameters		
Organisms >50 µm	Flask, no preservation	Immediately
Organisms 10 -50 µm	Dark HDPE bottle, no preservation	Immediately
Bacteria	Sterile plastic, no preservation	Immediately

APPENDIX A3. SELECTED TERMS FROM THE CALIFORNIA OCEAN PLAN (State Water Board 2005) Appendix 1, Definition of Terms

ACUTE TOXICITY

a. Acute Toxicity (TUa)

Expressed in Toxic Units Acute (TUa)

$$TUa = \frac{100}{\text{96-hr LC 50\%}}$$

b. Lethal Concentration 50% (LC 50)

LC 50 (percent waste giving 50% survival of test organisms) shall be determined by static or continuous flow bioassay techniques using standard marine test species as specified in Appendix III, Chapter II. If specific identifiable substances in wastewater can be demonstrated by the discharger as being rapidly rendered harmless upon discharge to the marine environment, but not as a result of dilution, the LC 50 may be determined after the test samples are adjusted to remove the influence of those substances.

When it is not possible to measure the 96-hour LC 50 due to greater than 50 percent survival of the test species in 100 percent waste, the toxicity concentration shall be calculated by the expression:

$$TUa = (\log(100 - S))/1.7$$

where:

S = percentage survival in 100% waste. If $S > 99$, TUa shall be reported as zero.

CHRONIC TOXICITY: This parameter shall be used to measure the acceptability of waters for supporting a healthy marine biota until improved methods are developed to evaluate biological response.

a. Chronic Toxicity (TUc)

Expressed as Toxic Units Chronic (TUc)

$$TUc = 100/NOEL$$

b. No Observed Effect Level (NOEL)

The NOEL is expressed as the maximum percent effluent or receiving water that causes no observable effect on a test organism, as determined by the result of a critical life stage toxicity test listed in Table 5-3.

DEGRADE: Degradation shall be determined by comparison of the waste field and reference site(s) for characteristic species diversity, population density, contamination, growth anomalies, debility, or supplanting of normal species by undesirable plant and animal species. Degradation occurs if there are significant differences in any of three major biotic groups, namely, demersal fish, benthic invertebrates, or attached algae. Other groups may be evaluated where benthic species are not affected, or are not the only ones affected.

NATURAL LIGHT: Reduction of natural light may be determined by the Regional Board by measurement of light transmissivity or total irradiance, or both, according to the monitoring needs of the Regional Board.

OCEAN WATERS: Territorial marine waters of the State as defined by California law to the extent these waters are outside of enclosed bays, estuaries, and coastal lagoons. If a discharge outside the territorial waters of the State could affect the quality of the waters of the State, the discharge may be regulated to assure no violation of the Ocean Plan will occur in ocean waters.

SHELLFISH: Organisms identified by the California Department of Health Services as shellfish for public health purposes (i.e., mussels, clams and oysters).

WASTE: As used in this [Ocean] Plan, waste includes a discharger's total discharge, of whatever origin, i.e., gross, not net, discharge.

APPENDIX B

Ballast Water Treatment System
Efficacy Matrix

Thirty ballast water treatment systems were reviewed by Commission staff for compliance with the California performance standards. Twnety systems had data on system efficacy available for review. Commission staff was lenient in their assessment of system efficacy. Staff included data from shipboard, dockside and laboratory studies of system performance. In an effort to standardize results, staff evaluated any data on zooplankton abundance as representative of the largest size class of organisms (greater than 50 µm in size), and phytoplankton abundance was evaluated on par with organisms in the 10 – 50 µm size class. Results presented as percent reduction in organism abundance or as concentration of pigments or biological compounds associated with organism presence were noted, but these metrics were not comparable to the performance standards.

In the following tables, systems with at least one testing replicate in compliance with the performance standard are scored as meeting California standards. Testing results that had no testing replicates in compliance with the standard are scored as not meeting California standards. Systems that presented data for a given organism size class but presented the results in metrics not comparable to the standards are classified as "Unknown." For example, a system that presented results of system efficacy as percent reduction of zooplankton abundance could not be compared against the California standards, and thus ability of the system to comply with the standards is unknown. Open cells indicate lack of data for a given organism size class. Compliance with the bacteria standard was assessed using the concentration of culturable heterotrophic bacteria in discharged ballast water. Available data on ballast water treatment of viruses is included in this analysis, but due to the lack of available methods to both quantify and assess the viability of all viruses, compliance could not be assess for these samples at this time. The source(s) of the data for each system can be found in the Literature Cited section of the main report.

Appendix B1 Organisms > 50 µm

Manufacturer	Location	# Tests	# Tests Met Std	Replicates	Controls	# Organisms/cubic meter	Methods	Reference
Alfa Laval	Laboratory	1	0	-	-	Unknown (% Reduction)	Visual Assesment	68
	Land-Based	12	9	6	Y	0 - 26	Microscope/mobility	1,93
	Shipboard	5	2	12	Y	0 - 3	Microscope/mobility	68,94
ATG Willand	Laboratory	-	-	-	-	-	-	-
	Land-Based	-	-	-	-	-	-	-
	Shipboard	-	-	-			-	-
Ecochlor	Laboratory	2	2	2	Y	0 - 3.5×10^5	Visual, Neutral Red Stain	99
	Land-Based	-	-	-	-	-	-	-
	Shipboard	1	1	3	Y	0-5	Visual Assessment	63
EcologiQ	Laboratory	-	-	-	-	-	-	-
	Land-Based	-	-	-	-	-	-	-
	Shipboard	-	-	-			-	-
Electrichlor	Laboratory	-	-	-	-	-	-	-
	Land-Based	-	-	-	-	-	-	-
	Shipboard	-	-	-			-	-
ETI	Laboratory	-	-	-	-	-	-	-
	Land-Based	-	-	-	-	-	-	-
	Shipboard	-	-	-	-	-	-	-
Ferrate Treatment Tech.	Laboratory	-	-	-	-	-	-	-
	Land-Based	-	-	-	-	-	-	-
	Shipboard	-	-	-	-	-	-	-
Greenship	Laboratory	-	-	-	-	-	-	-
	Land-Based	5	5	Y	Y	0	Visual Assessment	114
	Shipboard	-	-	-	-	-	-	-
Hamann AG Evonik Degussa	Laboratory	2	2	Y	Y	0	Visual Assesment	35,129
	Land-Based	19	16	Y	Y	0-0.7	Visual Asses., Neutral Red	35,38,91,129
	Shipboard	5	4	3	Y	0-1.1	-	91
Hi Tech Marine	Laboratory	-	-	-	-	-	-	-
	Land-Based	-	-	-	-	-	-	-
	Shipboard	2	0	-	-	Unknown (% mortality)	-	41
Hitachi	Laboratory	-	-	-	-	-	-	-
	Land-Based	-	-	-	-	-	-	-
	Shipboard	-	-	-	-	-	-	-
Hyde Marine	Laboratory	1	0	Y	Y	-	Visual Assessment	56
	Land-Based	4	2	Y	Y	0 (100% Mortality)	Visual Assessment	57
	Shipboard	4	0	3	Y	3 - 161	Visual, Neutral Red Stain	138
JFE Engineering Corp	Laboratory	-	-	-	-	-	-	-
	Land-Based	-	-	-	-	-	-	-
	Shipboard	-	-	-	-	-	-	-
MARENCO	Laboratory	-	-	-	-	-	-	-
	Land-Based	3	2	Y	Y	0 - 1.57	Visual Assessment	51,52,135
	Shipboard	-	-	-	-	-	-	-
Maritime Solutions Inc.	Laboratory	-	-	-	-	-	-	-
	Land-Based	-	-	-	-	-	-	-
	Shipboard	-	-	-	-	-	-	-

Appendix B1 Organisms > 50 µm

Manufacturer	Location	# Tests	# Tests Met Std	Replicates	Controls	# Organisms/cubic meter	Methods	Reference
	Laboratory	1	0	3	N	Unk (No Units)	Visual Assessment	42
MH Systems	Land-Based	-	-	-	-	-	-	-
	Shipboard	-	-	-	-	-	-	-
	Laboratory	-	-	-	-	-	-	-
Mitsubishi Heavy Ind.	Land-Based	-	-	-	-	-	-	-
	Shipboard	-	-	-	-	-	-	-
	Laboratory	-	-	-	-	-	-	-
Mitsui Engineering	Land-Based	4	0	3-5	Y	BD, 2×10^5 - 1.4×10^6	Visual Assessment	48,49
	Shipboard	1	0	-	Y	8	Visual Assessment	46
	Laboratory	-	-	-	-	-	-	-
NEI	Land-Based	5	1	Y	Y	0, Unk (% Survival)	Visual Assessment	118,119
	Shipboard	4	1	Y	Y	0 - 7	Visual Assessment	120
	Laboratory	-	-	-	-	-	-	-
NK-O3	Land-Based	-	-	-	-	-	-	-
	Shipboard	-	-	-	-	-	-	-
	Laboratory	3	0	4	Y	1.2×10^2 - 1.2×10^4	Visual Assessment	104
Nutech O3 Inc.	Land-Based	3	1	Y	Y	Unk (% Live)	Visual Assessment	40
	Shipboard	3	2	12	Y	0 - 150	Visual Assessment	140
	Laboratory	-	-	-	-	-	-	-
OceanSaver	Land-Based	14	2*	3	Y	0*-135	Visual Assessment	2,95
	Shipboard	12	4	3	Y	0 - 9720	Visual Assessment	6,123
	Laboratory	1	0	-	Y	> 0	Visual Assessment	47
OptiMarin	Land-Based	13	8	3	Y	0-144	Microscope/Mobility	11,92
	Shipboard	7	0	Y	Y	1.4 - ~5500, Unk (% Reduction)	Visual Assessment	11,134
	Laboratory	-	-	-	-	-	-	-
Panasia Co.	Land-Based	-	-	-	-	-	-	-
	Shipboard	-	-	-	-	-	-	-
	Laboratory	-	-	-	-	-	-	-
Resource Ballast Tech	Land-Based	-	-	-	-	-	-	-
	Shipboard	-	-	-	-	-	-	-
RWO Marine Water Tech	Laboratory	1	1	-	-	0	Visual Assessment	71
	Land-Based	-	-	-	-	-	-	-
	Shipboard	-	-	-	-	-	-	-
	Laboratory	1	1	Y	Y	0	Visual Assessment	21,36
SeaKleen (Hyde)	Land-Based	2	2	3	Y	0	Visual Assessment	57
	Shipboard	1	1	3	Y	0	Visual Assessment	8
	Laboratory	-	-	-	-	-	-	-
Severn Trent	Land-Based	5	3	3-4	Y	0 - ~4×10^5	Visual Assessment	39
	Shipboard	-	-	-	-	-	-	-
	Laboratory	-	-	-	-	-	-	-
Techcross Inc.	Land-Based	11	8	3	Y	0-6	Visual Assessment	50
	Shipboard	3	3	3	Y	0	Visual Assessment	50
	Laboratory	-	-	-	-	-	-	-
Toagosei Group	Land-Based	-	-	-	-	-	-	-
	Shipboard	-	-	-	-	-	-	-

Unk = Unknown, BD = Below Detection Limits

Appendix B2 Organisms 10 - 50 µm

Manufacturer	Location	# Tests	# Tests Met Std	Replicates	Controls	# Organisms/ml	Methods	Reference
Alfa Laval	Laboratory	1	0	-	-	Unk (% Reduction)	Visual Assesment	68
	Land-Based	12	3	6	Y	0-92.5	Microscope/stain (CDFA_AM), MPN	1,93
	Shipboard	5	1	12	Y	0-1.7	Microscope/stain (CDFA_AM), MPN	68,94
ATG Willand	Laboratory	-	-	-	-	-	-	-
	Land-Based	-	-	-	-	-	-	-
	Shipboard	-	-	-	-	-	-	-
Ecochlor	Laboratory	2	0	2	Y	<0.1 - >60, Unk ([Chl a])	Visual Assessment, MPN, [Chl a]	99
	Land-Based	-	-	-	-	-	-	-
	Shipboard	1	1	3	Y	0-81	Visual Assessment, [Chl a]	63
EcologiQ	Laboratory	-	-	-	-	-	-	-
	Land-Based	-	-	-	-	-	-	-
	Shipboard	-	-	-			-	-
Electrichlor	Laboratory	-	-	-	-	-	-	-
	Land-Based	-	-	-	-	-	-	-
	Shipboard	-	-	-	-	-	-	-
ETI	Laboratory	-	-	-	-	-	-	-
	Land-Based	3	0	2-3	Y	1 - 1.5	Growout (+, -), Flowcam	60,61,62
	Shipboard	-	-	-	-	-	-	-
Ferrate Treatment Tech.	Laboratory	-	-	-	-	-	-	-
	Land-Based	-	-	-	-	-	-	-
	Shipboard	-	-	-	-	-	-	-
Greenship	Laboratory	-	-	-	-	-	-	-
	Land-Based	5	3	Y	Y	0 -7	Total Counts	114
	Shipboard	-	-	-	-	-	-	-
Hamann Evonik Degussa	Laboratory	3	3	Y	Y	0 (100% Mortality)	Visual Assessment, Sytox Green	35,36,129
	Land-Based	18	17	3	Y	0 - <0.01	Flow Cytometer, Sytox stain	38,91,129
	Shipboard	5	0	3	Y	<0.1	Flow Cytometer, Sytox stain, PAM fluorometry	91
Hi Tech Marine	Laboratory	-	-	-	-	-	-	-
	Land-Based	-	-	-	-	-	-	-
	Shipboard	2	0	-	-	Unk (% Mortality)	-	41
Hitachi	Laboratory	-	-	-	-	-	-	-
	Land-Based	-	-	-	-	-	-	-
	Shipboard	-	-	-	-	-	-	-
Hyde Marine	Laboratory	1	0	Y	Y	26 - 210	Visual Assessment, Coulter, MPN	56
	Land-Based	4	0	Y	Y	Unk ([Chl a])	[Chl a]	57
	Shipboard	5	0	3	Y	Unk (% of controls, [Chl a])	Visual Assessment, [Chl a]	138
JFE Engineering Corp	Laboratory	-	-	-	-	-	-	-
	Land-Based	-	-	-	-	-	-	-
	Shipboard	-	-	-	-	-	-	-
MARENCO	Laboratory	-	-	-	-	-	-	-
	Land-Based	3	0	Y	Y	0.05 - 0.186	MPN, [Chl a], ^{14}C, PAM	51,52,135
	Shipboard	-	-	-	-	-	-	-
Maritime Solutions Inc.	Laboratory	-	-	-	-	-	-	-
	Land-Based	-	-	-	-	-	-	-
	Shipboard	-	-	-	-	-	-	-

Unk = Unkown, MPN = Most Probable Number

Appendix B2 Organisms 10 - 50 µm

Manufacturer	Location	# Tests	# Tests Met Std	Replicates	Controls	# Organisms/ml	Methods	Reference
MH Systems	Laboratory	-	-	-	-	-	-	-
	Land-Based	-	-	-	-	-	-	-
	Shipboard	-	-	-	-	-	-	-
Mitsubishi Heavy Ind.	Laboratory	-	-	-	-	-	-	-
	Land-Based	-	-	-	-	-	-	-
	Shipboard	-	-	-	-	-	-	-
Mitsui Engineering	Laboratory	-	-	-	-	-	-	-
	Land-Based	4	0	3-5	Y	BD, 206.6 - 387.4, Unk	Visual Assessment (20 - 50um)	48,49
	Shipboard	1	0	-	Y	BD	Visual Assessment	46
NEI	Laboratory	-	-	-	-	-	-	-
	Land-Based	5	0	Y	Y	Unk	[Chl a]	118,119
	Shipboard	4	0	Y	Y	443 - 593	Total Counts (Preserved), [Chl a], Regrowth	120
NK-O3	Laboratory	-	-	-	-	-	-	-
	Land-Based	-	-	-	-	-	-	-
	Shipboard	-	-	-	-	-	-	-
Nutech O3 Inc.	Laboratory	3	0	4	Y	Unk	[Chl a]	104
	Land-Based	2	0	Y	Y	22 - 190	Total Counts (Preserved)	40
	Shipboard	3	0	5	Y	0.016 - 4	[Chl a], Grow Out, Counts	140
OceanSaver	Laboratory	-	-	-	-	-	-	-
	Land-Based	14	11	3	Y	0-8.7	dilution, microscopy (CFDA stain), plate counts	95
	Shipboard	3	3	3	Y	0-2.8	Microscope (CFDA stain), Photosynethic rates	123
OptiMarin	Laboratory	1	0	-	Y	26 - 210	MPN, Coulter	47
	Land-Based	13	6	3	Y	0-92	Microscope/stain, MPN, agar plates	11,92
	Shipboard	10	0	Y	Y	Unk ([Chl a], % Reduction)	[Chl a], HPLC, PAM, Counts, Growout	11,134
Panasia Co.	Laboratory	-	-	-	-	-	-	-
	Land-Based	-	-	-	-	-	-	-
	Shipboard	-	-	-	-	-	-	-
Resource Ballast Tech	Laboratory	-	-	-	-	-	-	-
	Land-Based	-	-	-	-	-	-	-
	Shipboard	-	-	-	-	-	-	-
RWO Marine Water Tech	Laboratory	1	1	-	-	0	Visual Assessment	71
	Land-Based	-	-	-	-	-	-	-
	Shipboard	-	-	-	-	-	-	-
SeaKleen (Hyde)	Laboratory	2	1	Y	Y	0, Unk (Unitless)	Epifluorescence, Hemacytometer, Sytox Green	21,36
	Land-Based	2	0	3	Y	Unk ([Chl a])	[Chl a]	57
	Shipboard	1	1	3	Y	0	Visual Assessment, [Chl a], Growout	8
Severn Trent	Laboratory	-	-	-	-	-	-	-
	Land-Based	5	2	3-4	Y	0.002 - 10, BD ([Chl a])	MPN, [Chl a]	39
	Shipboard	-	-	-	-	-	-	-
Techcross Inc.	Laboratory	-	-	-	-	-	-	-
	Land-Based	11	9	3	Y	0-4	Light micro., epifluor. and fluorometer (FDA stain)	50
	Shipboard	3	3	3	Y	0	Light micro., epifluor. and fluorometer (FDA stain)	50
Toagosei Group	Laboratory	-	-	-	-	-	-	-
	Land-Based	-	-	-	-	-	-	-
	Shipboard	-	-	-	-	-	-	-

Unk = Unknown, BD = Below Detection Limits, MPN = Most Probably Number

Appendix B3 ***E. Coli***

Manufacturer	Location	# Tests	# Tests Met Std	Replicates	Controls used	# CFU/100 ml	Methods	Reference
	Laboratory	1	0	-	-	Unk (% Reduction)	-	68
Alfa Laval	Land-Based	12	12	6	Y	0 - 800	Membrane filtration	1,93
	Shipboard	4	4*	9	Y	0*	Membrane filtration	94
	Laboratory	-	-	-	-	-	-	-
ATG Willand	Land-Based	-	-	-	-	-	-	-
	Shipboard	-	-	-			-	-
	Laboratory	-	-	-	-	-	-	-
Ecochlor	Land-Based	-	-	-	-	-	-	-
	Shipboard	1	1	3	Y	0 - ~21	Idexx Labs Colilert	63
	Laboratory	-	-	-	-	-	-	-
EcologiQ	Land-Based	-	-	-	-	-	-	-
	Shipboard	-	-	-			-	-
	Laboratory	-	-	-	-	-	-	-
Electrichlor	Land-Based	-	-	-	-	-	-	-
	Shipboard	-	-	-	-	-	-	-
	Laboratory	-	-	-	-	-	-	-
ETI	Land-Based	-	-	-	-	-	-	-
	Shipboard	-	-	-	-	-	-	-
	Laboratory	1	0	-	-	300	Idexx Labs QuantiTray MPN	22
Ferrate Treatment Tech.	Land-Based	-	-	-	-	-	-	-
	Shipboard	-	-	-	-	-	-	-
	Laboratory	1	1	-	Y	>1000 - 3000	Plate Counts	25
Greenship	Land-Based	5	5	Y	Y	0 - 1	Unk	114
	Shipboard	-	-	-	-	-	-	-
	Laboratory	1	1	Y	-	0	Plate Counts	36
Hamann Evonik Degussa	Land-Based	12	12	3	Y	<0.1/ml	Membrane filtration	91
	Shipboard	4	4*	3	Y	<0.1/ml	Membrane filtration	91
	Laboratory	-	-	-	-	-	-	-
Hi Tech Marine	Land-Based	-	-	-	-	-	-	-
	Shipboard	-	-	-	-	-	-	-
	Laboratory	-	-	-	-	-	-	-
Hitachi	Land-Based	-	-	-	-	-	-	-
	Shipboard	-	-	-	-	-	-	-
	Laboratory	-	-	-	-	-	-	-
Hyde Marine	Land-Based	-	-	-	-	-	-	-
	Shipboard	4	4	3	Y	0	Idexx Labs Colisure	138
	Laboratory	-	-	-	-	-	-	-
JFE Engineering Corp	Land-Based	-	-	-	-	-	-	-
	Shipboard	-	-	-	-	-	-	-
	Laboratory	-	-	-	-	-	-	-
MARENCO	Land-Based	-	-	-	-	-	-	-
	Shipboard	-	-	-	-	-	-	-
	Laboratory	-	-	-	-	-	-	-
Maritime Solutions Inc.	Land-Based	-	-	-	-	-	-	-
	Shipboard	-	-	-	-	-	-	-

Unk = Unknown, * = Initial concentration at intake was zero or non-detectable

Appendix B3 ***E. Coli***

Manufacturer	Location	# Tests	# Tests Met Std	Replicates	Controls used	# CFU/100 ml	Methods	Reference
	Laboratory	-	-	-	-	-	-	-
MH Systems	Land-Based	-	-	-	-	-	-	-
	Shipboard	-	-	-	-	-	-	-
	Laboratory	-	-	-	-	-	-	-
Mitsubishi Heavy Ind.	Land-Based	-	-	-	-	-	-	-
	Shipboard	-	-	-	-	-	-	-
	Laboratory	-	-	-	-	-	-	-
Mitsui Engineering	Land-Based	2	0	3	Y	BD, Unk	Plate Counts	48
	Shipboard	-	-	-	-	-	-	-
	Laboratory	-	-	-	-	-	-	-
NEI	Land-Based	5	1	Y	Y	10 - 160	Idexx Labs MPN Kit	118,119
	Shipboard	4	1	Y	Y	<100	Idexx Labs MPN Kit	120
	Laboratory	-	-	-	-	-	-	-
NK-O3	Land-Based	-	-	-	-	-	-	-
	Shipboard	-	-	-	-	-	-	-
	Laboratory	-	-	-	-	-	-	-
Nutech O3 Inc.	Land-Based	-	-	-	-	-	-	-
	Shipboard	3	3*	11-12	Y	0*	IDEXX Labs MPN Kit	-
	Laboratory	-	-	-	-	-	-	-
OceanSaver	Land-Based	14	14	3	Y	0-123	Membrane Filtration	95
	Shipboard	3	3*	3	Y	0*	Membrane Filtration	123
	Laboratory	-	-	-	-	-	-	-
OptiMarin	Land-Based	12	12	3	Y	0-2	Membrane Filtration	92
	Shipboard	-	-	-	-	-	-	-
	Laboratory	-	-	-	-	-	-	-
Panasia Co.	Land-Based	-	-	-	-	-	-	-
	Shipboard	-	-	-	-	-	-	-
	Laboratory	-	-	-	-	-	-	-
Resource Ballast Tech	Land-Based	-	-	-	-	-	-	-
	Shipboard	-	-	-	-	-	-	-
	Laboratory	-	-	-	-	-	-	-
RWO Marine Water Tech	Land-Based	-	-	-	-	-	-	-
	Shipboard	-	-	-	-	-	-	-
	Laboratory	1	1	Y	Y	0	Culture Growth	36
SeaKleen (Hyde)	Land-Based	-	-	-	-	-	-	-
	Shipboard	2	2*	3	Y	0*	Idexx Labs Colisure	8
	Laboratory	-	-	-	-	-	-	-
Severn Trent	Land-Based	-	-	-	-	-	-	-
	Shipboard	-	-	-	-	-	-	-
	Laboratory	-	-	-	-	-	-	-
Techcross Inc.	Land-Based	10	10	3	Y	0	Plate counts	50
	Shipboard	3	3	3	Y	0	Plate Counts	50
	Laboratory	-	-	-	-	-	-	-
Toagosei Group	Land-Based	-	-	-	-	-	-	-
	Shipboard	-	-	-	-	-	-	-

Unk = Unknown, BD = Below Detection Limits, * = Initial concentration at intake was zero or non-detectable

Appendix B4 Intestinal Enterococci

Manufacturer	Location	# Tests	# Tests Met Std	Replicates	Controls	# CFU/100 ml	Methods	Reference
	Laboratory	-	-	-	-	-	-	-
Alfa Laval	Land-Based	12	12	6	Y	0 - 4	Membrane filtration	1,93
	Shipboard	4	4*	10	Y	0	Membrane filtration	94
	Laboratory	-	-	-	-	-	-	-
ATG Willand	Land-Based	-	-	-	-	-	-	-
	Shipboard	-	-	-			-	-
	Laboratory	-	-	-	-	-	-	-
Ecochlor	Land-Based	-	-	-	-	-	-	-
	Shipboard	1	0	3	Y	Unk	Idexx Labs Enterolert	63
	Laboratory	-	-	-	-	-	-	-
EcologiQ	Land-Based	-	-	-	-	-	-	-
	Shipboard	-	-	-	-		-	-
	Laboratory	-	-	-	-	-	-	-
Electrichlor	Land-Based	-	-	-	-	-	-	-
	Shipboard	-	-	-	-	-	-	-
	Laboratory	-	-	-	-	-	-	-
ETI	Land-Based	-	-	-	-	-	-	-
	Shipboard	-	-	-	-	-	-	-
	Laboratory	1	0	-	-	80	Idexx Labs QuantiTray MPN	22
Ferrate Treatment Tech.	Land-Based	-	-	-	-	-	-	-
	Shipboard	-	-	-	-	-	-	-
	Laboratory	-	-	-	-	-	-	-
Greenship	Land-Based	5	5	Y	Y	0	Unk	114
	Shipboard	-	-	-	-	-	-	-
	Laboratory	-	-	-	-	-	-	-
Hamann Evonik Degussa	Land-Based	12	12	3	Y	<0.1/ml	Membrane filtration	91
	Shipboard	4	4*	3	Y	<0.1/ml	Membrane filtration	91
	Laboratory	-	-	-	-	-	-	-
Hi Tech Marine	Land-Based	-	-	-	-	-	-	-
	Shipboard	-	-	-	-	-	-	-
	Laboratory	-	-	-	-	-	-	-
Hitachi	Land-Based	-	-	-	-	-	-	-
	Shipboard	-	-	-	-	-	-	-
	Laboratory	-	-	-	-	-	-	-
Hyde Marine	Land-Based	-	-	-	-	-	-	-
	Shipboard	4	4*	3	Y	0	Idexx Labs Enterolert	138
	Laboratory	-	-	-	-	-	-	-
JFE Engineering Corp	Land-Based	-	-	-	-	-	-	-
	Shipboard	-	-	-	-	-	-	-
	Laboratory	-	-	-	-	-	-	-
MARENCO	Land-Based	-	-	-	-	-	-	-
	Shipboard	-	-	-	-	-	-	-
	Laboratory	-	-	-	-	-	-	-
Maritime Solutions Inc.	Land-Based	-	-	-	-	-	-	-
	Shipboard	-	-	-	-	-	-	-

Unk = Unknown, * = Initial concentration at intake was zero or non-detectable.

Appendix B4 Intestinal Enterococci

Manufacturer	Location	# Tests	# Tests Met Std	Replicates	Controls	# CFU/100 ml	Methods	Reference
MH Systems	Laboratory	-	-	-	-	-	-	-
	Land-Based	-	-	-	-	-	-	-
	Shipboard	-	-	-	-	-	-	-
Mitsubishi Heavy Ind.	Laboratory	-	-	-	-	-	-	-
	Land-Based	-	-	-	-	-	-	-
	Shipboard	-	-	-	-	-	-	-
Mitsui Engineering	Laboratory	-	-	-	-	-	-	-
	Land-Based	2	0	3	Y	BD, Unk	Plate counts	48
	Shipboard	-	-	-	-	-	-	-
NEI	Laboratory	-	-	-	-	-	-	-
	Land-Based	5	0	Y	Y	36	Idexx Labs MPN Kit	118,119
	Shipboard	4	2	Y	Y	<10	Idexx Labs MPN Kit	120
NK-O3	Laboratory	-	-	-	-	-	-	-
	Land-Based	-	-	-	-	-	-	-
	Shipboard	-	-	-	-	-	-	-
Nutech O3 Inc.	Laboratory	-	-	-	-	-	-	-
	Land-Based	-	-	-	-	-	-	-
	Shipboard	3	3*	11-12	Y	0*	Idexx Labs Enterolert	140
OceanSaver	Laboratory	-	-	-	-	-	-	-
	Land-Based	14	9	3	Y	0-133	Membrane Filtration	95
	Shipboard	3	3*	3	Y	0*-9	Membrane Filtration	123
OptiMarin	Laboratory	-	-	-	-	-	-	-
	Land-Based	-	-	-	-	-	-	-
	Shipboard	-	-	-	-	-	-	-
Panasia Co.	Laboratory	-	-	-	-	-	-	-
	Land-Based	-	-	-	-	-	-	-
	Shipboard	-	-	-	-	-	-	-
Resource Ballast Tech	Laboratory	-	-	-	-	-	-	-
	Land-Based	-	-	-	-	-	-	-
	Shipboard	-	-	-	-	-	-	-
RWO Marine Water Tech	Laboratory	-	-	-	-	-	-	-
	Land-Based	-	-	-	-	-	-	-
	Shipboard	-	-	-	-	-	-	-
SeaKleen (Hyde)	Laboratory	-	-	-	-	-	-	-
	Land-Based	-	-	-	-	-	-	-
	Shipboard	2	2*	3	Y	0*	Idexx Labs Enterolert	8
Severn Trent	Laboratory	-	-	-	-	-	-	-
	Land-Based	-	-	-	-	-	-	-
	Shipboard	-	-	-	-	-	-	-
Techcross Inc.	Laboratory	-	-	-	-	-	-	-
	Land-Based	11	11	3	Y	0-5	Plate counts	50
	Shipboard	2	2	3	Y	0	Plate counts	50
Toagosei Group	Laboratory	-	-	-	-	-	-	-
	Land-Based	-	-	-	-	-	-	-
	Shipboard	-	-	-	-	-	-	-

Unk = Unknown, BD = Below Detection Limits, * = Initial concentration at intake was zero or non-detectable

Appendix B5 ***Vibrio cholerae***

Manufacturer	Location	# Tests	# Tests Met Std	Replicates	Controls	# Organisms/100 ml	Methods	Reference
	Laboratory	-	-	-	-	-	-	-
Alfa Laval	Land-Based	10	10*	3	Y	<1*	Supplemented Agar Plates	93
	Shipboard	4	4*	9	Y	<1*	Supplemented Agar Plates	94
	Laboratory	-	-	-	-	-	-	-
ATG Willand	Land-Based	-	-	-	-	-	-	-
	Shipboard	-	-	-			-	-
	Laboratory	2	2	2	Y	0 (% cover)	Plate Counts	99
Ecochlor	Land-Based	-	-	-	-	-	-	-
	Shipboard	1	0	3	Y	BD - ~1000	Unk	63
	Laboratory	-	-	-	-	-	-	-
EcologiQ	Land-Based	-	-	-	-	-	-	-
	Shipboard	-	-	-	-		-	-
	Laboratory	-	-	-	-	-	-	-
Electrichlor	Land-Based	-	-	-	-	-	-	-
	Shipboard	-	-	-	-	-	-	-
	Laboratory	-	-	-	-	-	-	-
ETI	Land-Based	-	-	-	-	-	-	-
	Shipboard	-	-	-	-	-	-	-
	Laboratory	1	0	-	-	108	Indexx Labs QuantiTray MPN	22
Ferrate Treatment Tech.	Land-Based	-	-	-	-	-	-	-
	Shipboard	-	-	-	-	-	-	-
	Laboratory	-	-	-	-	-	-	-
Greenship	Land-Based	-	-	-	-	-	-	-
	Shipboard	-	-	-	-	-	-	-
	Laboratory	-	-	-	-	-	-	-
Hamann Evonik Degussa	Land-Based	-	-	-	-	-	-	-
	Shipboard	-	-	-	-	-	-	-
	Laboratory	-	-	-	-	-	-	-
Hi Tech Marine	Land-Based	-	-	-	-	-	-	-
	Shipboard	-	-	-	-	-	-	-
	Laboratory	-	-	-	-	-	-	-
Hitachi	Land-Based	-	-	-	-	-	-	-
	Shipboard	-	-	-	-	-	-	-
	Laboratory	-	-	-	-	-	-	-
Hyde Marine	Land-Based	-	-	-	-	-	-	-
	Shipboard	-	-	-	-	-	-	-
	Laboratory	-	-	-	-	-	-	-
JFE Engineering Corp	Land-Based	-	-	-	-	-	-	-
	Shipboard	-	-	-	-	-	-	-
	Laboratory	-	-	-	-	-	-	-
MARENCO	Land-Based	-	-	-	-	-	-	-
	Shipboard	-	-	-	-	-	-	-
	Laboratory	-	-	-	-	-	-	-
Maritime Solutions Inc.	Land-Based	-	-	-	-	-	-	-
	Shipboard	-	-	-	-	-	-	-

Unk = Unknown, * = Initial concentration at intake was zero or non-detectable

Appendix B5 ***Vibrio cholerae***

Manufacturer	Location	# Tests	# Tests Met Std	Replicates	Controls	# Organisms/100 ml	Methods	Reference
	Laboratory	1	0	3	N	Unk (% Reduction)	Plate Counts	42
MH Systems	Land-Based	-	-	-	-	-	-	-
	Shipboard	-	-	-	-	-	-	-
	Laboratory	-	-	-	-	-	-	-
Mitsubishi Heavy Ind.	Land-Based	-	-	-	-	-	-	-
	Shipboard	-	-	-	-	-	-	-
	Laboratory	-	-	-	-	-	-	-
Mitsui Engineering	Land-Based	2	0	3	Y	BD, Unk	Plate Counts	48
	Shipboard	-	-	-	-	-	-	-
	Laboratory	-	-	-	-	-	-	-
NEI	Land-Based	-	-	-	-	-	-	-
	Shipboard	4	1	Y	Y	0	DFA	120
	Laboratory	-	-	-	-	-	-	-
NK-O3	Land-Based	-	-	-	-	-	-	-
	Shipboard	-	-	-	-	-	-	-
	Laboratory	-	-	-	-	-	-	-
Nutech O3 Inc.	Land-Based	-	-	-	-	-	-	-
	Shipboard	3	3*	11-12	Y	0*	Unknown	140
	Laboratory	-	-	-	-	-	-	-
OceanSaver	Land-Based	14	14*	3	Y	<1*	Plate counts (TCBS agar)	95
	Shipboard	3	3*	3	Y	0*	Plate counts (TCBS agar)	123
	Laboratory	-	-	-	-	-	-	-
OptiMarin	Land-Based	12	12*	3	Y	<1	Supplemented Agar Plates	92
	Shipboard	-	-	-	-	-	-	-
	Laboratory	-	-	-	-	-	-	-
Panasia Co.	Land-Based	-	-	-	-	-	-	-
	Shipboard	-	-	-	-	-	-	-
	Laboratory	-	-	-	-	-	-	-
Resource Ballast Tech	Land-Based	-	-	-	-	-	-	-
	Shipboard	-	-	-	-	-	-	-
	Laboratory	-	-	-	-	-	-	-
RWO Marine Water Tech	Land-Based	-	-	-	-	-	-	-
	Shipboard	-	-	-	-	-	-	-
	Laboratory	-	-	-	-	-	-	-
SeaKleen (Hyde)	Land-Based	-	-	-	-	-	-	-
	Shipboard	-	-	-	-	-	-	-
	Laboratory	-	-	-	-	-	-	-
Severn Trent	Land-Based	-	-	-	-	-	-	-
	Shipboard	-	-	-	-	-	-	-
	Laboratory	-	-	-	-	-	-	-
Techcross Inc.	Land-Based	11	11*	3	Y	0*	Plate counts (TCBS agar)	50
	Shipboard	3	3*	3	Y	0*	Plate counts (TCBS agar)	50
	Laboratory	-	-	-	-	-	-	-
Toagosei Group	Land-Based	-	-	-	-	-	-	-
	Shipboard	-	-	-	-	-	-	-

Unk = Unknown, BD = Below Detection Limits, DFA = Direction Fluorescent Antibody, * = Initial concentration at intake was zero or non-detectable

Appendix B6 Organims < 10 μm (Bacteria)

Manufacturer	Location	# Tests	# Tests Met Std	Replicates	Controls	# Organisms/100 ml	Methods	Reference
Alfa Laval	Laboratory	1	0	-	-	Unk (% Reduction)	Visual Assesment	68
	Land-Based	10	0	6	Y	820/ml - $4x10^8$/ml	Agar Plate Counts	1,93
	Shipboard	-	-	-	-	-	-	-
ATG Willand	Laboratory	-	-	-	-	-	-	-
	Land-Based	-	-	-	-	-	-	-
	Shipboard	-	-	-			-	-
Ecochlor	Laboratory	2	2	2	Y	0,Unk (% of control, % Plate cover)	Plate Counts, ^{3}H-leucine	99
	Land-Based	-	-	-	-	-	-	-
	Shipboard	1	1	3	Y	BD	Plate Counts, ^{3}H-leucine	63
EcologiQ	Laboratory	-	-	-	-	-	-	-
	Land-Based	-	-	-	-	-	-	-
	Shipboard	-	-	-			-	-
Electrichlor	Laboratory	-	-	-	-	-	-	-
	Land-Based	-	-	-	-	-	-	-
	Shipboard	-	-	-	-	-	-	-
ETI	Laboratory	1	0	3	Y	-	Plate Counts, BacLight	59
	Land-Based	3	0	2-3	Y	$5x10^7$ - $1x10^9$	Growout (+, -), FCM/PicoGreen	60,61,62
	Shipboard	-	-	-	-	-	-	-
Ferrate Treatment Tech.	Laboratory	-	-	-	-	-	-	-
	Land-Based	-	-	-	-	-	-	-
	Shipboard	-	-	-	-	-	-	-
Greenship	Laboratory	-	-	-	-	-	-	-
	Land-Based	5	2	Y	Y	0 - 6000	Unk	114
	Shipboard	-	-	-	-	-	-	-
Hamann Evonik Degussa	Laboratory	2	0	Y	Y	$3.8x10^7$ - $4.6x10^7$	Plate Counts, PicoGreen	129
	Land-Based	13	1	3	Y	<10/ml - 4.6 x 10^7	PicoGreen, Agar Plate	91,129
	Shipboard	4	3	3	Y	5-15/ml	heterotrophic bacteria, plate	91
Hi Tech Marine	Laboratory	-	-	-	-	-	-	-
	Land-Based	-	-	-	-	-	-	-
	Shipboard	-	-	-	-	-	-	-
Hitachi	Laboratory	-	-	-	-	-	-	-
	Land-Based	-	-	-	-	-	-	-
	Shipboard	-	-	-	-	-	-	-
Hyde Marine	Laboratory	1	0	Y	Y	~5000 - 7000	Plate Counts	56
	Land-Based	2	0	Y	Y	Unk	Plate Counts, AODC	57
	Shipboard	4	0	3	Y	Unk	Plate Counts	138
JFE Engineering Corp	Laboratory	-	-	-	-	-	-	-
	Land-Based	-	-	-	-	-	-	-
	Shipboard	-	-	-	-	-	-	-
MARENCO	Laboratory		-	-	-	-	-	-
	Land-Based	3	2	Y	Y	0 - ~$5x10^8$	Plate Counts, Membrane Filtration	51,52,135
	Shipboard	-	-	-	-	-	-	-
Maritime Solutions Inc.	Laboratory	-	-	-	-	-	-	-
	Land-Based	-	-	-	-	-	-	-
	Shipboard	-	-	-	-	-	-	-

Unk = Unknown, BD = Below Detection Limits, FCM = Flow Cytometer

Appendix B6 Organims < 10 µm (Bacteria)

Manufacturer	Location	# Tests	# Tests Met Std	Replicates	Controls	# Organisms/100 ml	Methods	Reference
MH Systems	Laboratory	-	-	-	-	-	-	-
	Land-Based	-	-	-	-	-	-	-
	Shipboard	-	-	-	-	-	-	-
Mitsubishi Heavy Ind.	Laboratory	-	-	-	-	-	-	-
	Land-Based	-	-	-	-	-	-	-
	Shipboard	-	-	-	-	-	-	-
Mitsui Engineering	Laboratory	-	-	-	-	-	-	-
	Land-Based	2	0	3	Y	BD, Unk	Plate Counts	48
	Shipboard	1	0	-	Y	BD	Plate Counts	46
NEI	Laboratory	-	-	-	-	-	-	-
	Land-Based	5	0	Y	Y	> $1x10^8$	FCM	118,119
	Shipboard	4	0	Y	Y	$7.3x10^7$ - $7.9x10^7$	FCM	120
NK-O3	Laboratory	-	-	-	-	-	-	-
	Land-Based	-	-	-	-	-	-	-
	Shipboard	-	-	-	-	-	-	-
Nutech O3 Inc.	Laboratory	3	3	4	Y	$\leq 10^1$ - 10^8	Plate Counts, Membrane Filtration	104
	Land-Based	3	3	Y	Y	$3x10^{-1}$ - $3x10^2$	Plate Counts, Membrane Filtration	40
	Shipboard	2	2	9-12	Y	0	Plate Counts, Filtration	140
OceanSaver	Laboratory	-	-	-	-	-	-	-
	Land-Based	5	3	3	Y	0 - $8.2x10^5$/ml	Plate Counts	95
	Shipboard	-	-	-	-	-	-	-
OptiMarin	Laboratory	2	0	-	Y	~ $5x10^3$ - ~$7x10^3$	Plate Counts	47
	Land-Based	13	1	3	Y	9-220/ml	Agar Plate Counts	11, 92
	Shipboard	10	0	Y	Y	<10^3 - 10^4, Unk (% Reduction)	Plate Counts, SYBR Gold	11,134
Panasia Co.	Laboratory	-	-	-	-	-	-	-
	Land-Based	-	-	-	-	-	-	-
	Shipboard	-	-	-	-	-	-	-
Resource Ballast Tech	Laboratory	-	-	-	-	-	-	-
	Land-Based	-	-	-	-	-	-	-
	Shipboard	-	-	-	-	-	-	-
RWO Marine Water Tech	Laboratory	-	-	-	-	-	-	-
	Land-Based	-	-	-	-	-	-	-
	Shipboard	-	-	-	-	-	-	-
SeaKleen (Hyde)	Laboratory	-	-	-	-	-	-	-
	Land-Based	-	-	-	-	-	-	-
	Shipboard	1	0	3	Y	Unk (Unitless)	Plate Counts	8
Severn Trent	Laboratory	-	-	-	-	-	-	-
	Land-Based	4	4	3-4	Y	<1 - 10^{10}	Plate Counts, Membrane Filtration	39
	Shipboard	-	-	-	-	-	-	-
Techcross Inc.	Laboratory	-	-	-	-	-	-	-
	Land-Based	11	Unknown	3	Y	Unknown	Fluorescent microscopy (DAFI)	50
	Shipboard	3	Unknown	3	Y	Unknown	Fluorescent microscopy (DAFI)	50
Toagosei Group	Laboratory	-	-	-	-	-	-	-
	Land-Based	-	-	-	-	-	-	-
	Shipboard	-	-	-	-	-	-	-

Unk = Unknown, AODC = Acridine Orange Direct Counts, FCM = Flow Cytometer, BD = Below Detection Limits

Appendix B7 < 10 µm (Viruses)

Manufacturer	Location	# Tests	# Tests Met Std	Replicates	Controls	# Organisms/100 ml	Methods	Reference
	Laboratory	-	-	-	-	-	-	-
Alfa Laval	Land-Based	-	-	-	-	-	-	-
	Shipboard	-	-	-	-	-	-	-
	Laboratory	-	-	-	-	-	-	-
ATG Willand	Land-Based	-	-	-	-	-	-	-
	Shipboard	-	-	-			-	-
	Laboratory	2	Unknown	2	Y	0,Unk (% of Control)	Plaque Forming Units	99
Ecochlor	Land-Based	-	-	-	-	-	-	-
	Shipboard	-	-	-	-	-	-	-
	Laboratory	-	-	-	-	-	-	-
EcologiQ	Land-Based	-	-	-	-	-	-	-
	Shipboard	-	-	-	-		-	-
	Laboratory	-	-	-	-	-	-	-
Electrichlor	Land-Based	-	-	-	-	-	-	-
	Shipboard	-	-	-	-	-	-	-
	Laboratory	-	-	-	-	-	-	-
ETI	Land-Based	-	-	-	-	-	-	-
	Shipboard	-	-	-	-	-	-	-
	Laboratory	-	-	-	-	-	-	-
Ferrate Treatment Tech.	Land-Based	-	-	-	-	-	-	-
	Shipboard	-	-	-	-	-	-	-
	Laboratory	-	-	-	-	-	-	-
Greenship	Land-Based	-	-	-	-	-	-	-
	Shipboard	-	-	-	-	-	-	-
	Laboratory	-	-	-	-	-	-	-
Hamann Evonik Degussa	Land-Based	-	-	-	-	-	-	-
	Shipboard	-	-	-	-	-	-	-
	Laboratory	-	-	-	-	-	-	-
Hi Tech Marine	Land-Based	-	-	-	-	-	-	-
	Shipboard	-	-	-	-	-	-	-
	Laboratory	-	-	-	-	-	-	-
Hitachi	Land-Based	-	-	-	-	-	-	-
	Shipboard	-	-	-	-	-	-	-
	Laboratory	-	-	-	-	-	-	-
Hyde Marine	Land-Based	-	-	-	-	-	-	-
	Shipboard	-	-	-	-	-	-	-
	Laboratory	-	-	-	-	-	-	-
JFE Engineering Corp	Land-Based	-	-	-	-	-	-	-
	Shipboard	-	-	-	-	-	-	-
	Laboratory	-	-	-	-	-	-	-
MARENCO	Land-Based	-	-	-	-	-	-	-
	Shipboard	-	-	-	-	-	-	-
	Laboratory	-	-	-	-	-	-	-
Maritime Solutions Inc.	Land-Based	-	-	-	-	-	-	-
	Shipboard	-	-	-	-	-	-	-

Unk = Unknown

Appendix B7 < 10 µm (Viruses)

Manufacturer	Location	# Tests	# Tests Met Std	Replicates	Controls	# Organisms/100 ml	Methods	Reference
	Laboratory	-	-	-	-	-	-	-
MH Systems	Land-Based	-	-	-	-	-	-	-
	Shipboard	-	-	-	-	-	-	-
	Laboratory	-	-	-	-	-	-	-
Mitsubishi Heavy Ind.	Land-Based	-	-	-	-	-	-	-
	Shipboard	-	-	-	-	-	-	-
	Laboratory	-	-	-	-	-	-	-
Mitsui Engineering	Land-Based	-	-	-	-	-	-	-
	Shipboard	-	-	-	-	-	-	-
	Laboratory	-	-	-	-	-	-	-
NEI	Land-Based	-	-	-	-	-	-	-
	Shipboard	-	-	-	-	-	-	-
	Laboratory	-	-	-	-	-	-	-
NK-O3	Land-Based	-	-	-	-	-	-	-
	Shipboard	-	-	-	-	-	-	-
	Laboratory	-	-	-	-	-	-	-
Nutech O3 Inc.	Land-Based	-	-	-	-	-	-	-
	Shipboard	-	-	-	-	-	-	-
	Laboratory	-	-	-	-	-	-	-
OceanSaver	Land-Based	-	-	-	-	-	-	-
	Shipboard	-	-	-	-	-	-	-
	Laboratory	-	-	-	-	-	-	-
OptiMarin	Land-Based	1	Unknown	-	Y	Unk (% Reduction)	Spiked Coliphage MS2 Exp.	11
	Shipboard	5	Unknown	-	Y	Unk (% Reduction)	Spiked Coliphage, SYBR Gold	11,134
	Laboratory	-	-	-	-	-	-	-
Panasia Co.	Land-Based	-	-	-	-	-	-	-
	Shipboard	-	-	-	-	-	-	-
	Laboratory	-	-	-	-	-	-	-
Resource Ballast Tech	Land-Based	-	-	-	-	-	-	-
	Shipboard	-	-	-	-	-	-	-
	Laboratory	-	-	-	-	-	-	-
RWO Marine Water Tech	Land-Based	-	-	-	-	-	-	-
	Shipboard	-	-	-	-	-	-	-
	Laboratory	-	-	-	-	-	-	-
SeaKleen (Hyde)	Land-Based	-	-	-	-	-	-	-
	Shipboard	-	-	-	-	-	-	-
	Laboratory	-	-	-	-	-	-	-
Severn Trent	Land-Based	-	-	-	-	-	-	-
	Shipboard	-	-	-	-	-	-	-
	Laboratory	-	-	-	-	-	-	-
Techcross Inc.	Land-Based	-	-	-	-	-	-	-
	Shipboard	-	-	-	-	-	-	-
	Laboratory	-	-	-	-	-	-	-
Toagosei Group	Land-Based	-	-	-	-	-	-	-
	Shipboard	-	-	-	-	-	-	-

Unk = Unknown

APPENDIX C

California State Lands Commission
Advisory Panel Members

Ryan Albert	U.S. Environmental Protection Agency
Marian Ashe (2007 only)	California Department of Fish and Game
John Berge	Pacific Merchant Shipping Association
Dave Bolland	Association of California Water Agencies
Brad Chapman	Chevron Shipping Company
Andrew Cohen	San Francisco Estuary Institute
Tim Eichenberg (2007 only)	The Ocean Conservancy
Richard Everett	United States Coast Guard
Naomi Feger	San Francisco Bay Regional Water Quality Control Board
Andrea Fox	California Farm Bureau Federation
Marc Holmes	The Bay Institute
Dominic Gregorio	State Water Resources Control Board
Bill Jennings	The DeltaKeeper
Edward Lemieux	Naval Research Laboratory
Karen McDowell	San Francisco Estuary Project
Steve Morin	Chevron Shipping Company LLC
Allen Pleus	Washington Department of Fish & Wildlife
Kevin Reynolds	The Glosten Associates
Greg Ruiz	Smithsonian Environmental Research Center
Spencer Schilling	Herbert Engineering Corp.
Jon Stewart	International Maritime Technology Consultants Inc.
Lisa Swanson	Matson Navigation
Mark Sytsma	Portland State University
Drew Talley	San Francisco Bay National Estuarine Research Reserve
Kim Ward	State Water Resources Control Board
Nick Welschmeyer	Moss Landing Marine Laboratory

California State Lands Commission
2009 Treatment Technology Assessment Report
Technical Advisory Panel
October 1, 2008
Meeting Notes

Participants:

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* = Participated by phone

Meeting Notes:
Nicole welcomed everyone to the meeting and participants introduced themselves. Nicole mentioned that legislation passed [SB 1781] to extend the initial performance standards implementation date for newly built vessels with a ballast water capacity of less than 5000 MT from January 1, 2009 to 2010. The purpose of the meeting was to go over any major concerns with the report, not to wordsmith the document. She needed any written comments and feedback by Friday October 3rd. Marc asked when the public comment period takes place on this report. Maurya replied that the document will be available on the Commission website for public comment 10 days prior to the Commission meeting to be held on December 3rd.

Nicole went through the report, and indicated where the report had gone through substantial changes. This included the legislative section and the treatment assessment sections. More system developers are performing land-based and ship-based testing to comply with IMO system approval that for the last report. While more data has been submitted to MISP staff, it is still difficult to obtain all the necessary reports etc...

Nicole pointed out the new information in Table VI.1 regarding system efficacy. In previous TAG meetings, it became clear that the bacterial standard was an issue because of the lack of methods to both quantify numbers and assess viability. Nicole recommended that the standard should be changed from “bacteria per ml” to “culturable heterotrophic bacteria (colony forming units).” Viability needs to be determined using culturing techniques, and thus the change in standard would reflect that fact. The proposed bacteria standard would be preventative since there are estimated to be 10^3 bacteria/ml in the natural environment, and the performance standard is 10^3/100ml.

Nicole clarified that in the Table VI.1, the <10 micron size category with cells indicating “unknown” was described as such because the bacteria were tested for a particular system but was done so using methods that didn’t allow for both counts and bacterial viability. In the viral category, Nicole discussed that it is difficult to test for viral viability/infectivity. CSLC staff suggested a change of wording to “concentrations of such additional indicator microbes and viruses as may be specified in regulations.” This change would provide CSLC staff with room to specify organisms of concern in the future. Thus there are two recommended changes for the performance standards - bacteria units of measurement and replacement of virus standard with clause about indicator microbes.

CSLC staff believes there will be technology available for the 2010 deadline since only a few vessels will need to comply (should be less than 50 total vessels arriving to CA with ballast water capacity <5000 MT). There could be less than 50 vessels needing treatment systems because not all of those vessels are necessarily discharging in CA waters. Maurya pointed out that a lot of these vessels are likely passenger vessels, since they carry small volumes of ballast and have a variety of options of managing their ballast.

Kevin pointed out that there will be some risk from bulk ships that are involved in the “tramp trade.” These vessels do not come to CA predictably, so it is unclear whether or not they will comply with CA standards. Maurya said that vessels such as these will be targeted for ballast water inspections. Brad thought that these tramp trade vessels might increase the number of estimated vessel arrivals to CA. However, he also thought that these operators may be more selective about what vessels they would send to CA. Realistically, operators would send their old vessels to CA until more systems were available for installation on vessels. Kevin thought that perhaps operators could actually benefit from having treatment systems on board because they could charge a significant fee for using vessels that will be compliant with CA standards.

Andy mentioned that onshore treatment would be a big solution for all of these discharge problems, especially in terms of the vessels involved in the tramp trade. Maurya agreed, but pointed out that the infrastructure does not yet exist for onshore treatment. John said that deballasting while underway is critical for vessels trying to get to ports such as Sacramento and Stockton, and that if onshore treatment was required for those vessels it would push the transport of those goods from ships to rail.

John then went on to discuss the problem resulting from the date change from 2009 to 2010. Since only the initial date of performance standards implementation was changed, the staggering of the implementation schedule is no longer in line with a 5 year dry dock cycle (i.e. by the Commission stating that no systems were available for 2009 that means that a vessel that goes into dry dock then won't have any systems installed and will thus be noncompliant with CA standards if that vessel doesn't go back into dry dock until after January 1, 2014). This will make it hard for some vessels to get dry dock times in order to install treatment systems before the 2014 date in the implementation schedule. John argued that even though the operators have known about the scheduled deadlines, they haven't had the legislation to enforce these dates until now. Maurya clarified that previous discussions did not involve changing the other implementation dates, and solely focused on the first deadline, with knowledge that additional decisions would need to be made later. The next technology report is due in 2010 and can discuss these matters. Also, not all vessels will have to go into dry dock to install the treatment systems.

Kevin wanted to clarify that the implementation schedule for IMO isn't as inline with CA as may seem. IMO has a clause that applies to existing ships that requires them to meet the performance standards at the point of the first vessel survey after the date of implementation of standards for that vessel size class. The table in the technology assessment report should clarify that the IMO and CA implementation dates are not identical.

Regarding the efficacy table, Andy wondered if a vessel had multiple treatment systems onboard, if CSLC would consider that as meeting the performance standard, for the purposes of this report. Maurya pointed out that it is not a CSLC mandate to assess how multiple systems would enable vessels to meet the performance standards. Kevin discussed the efficacy table as a function of treatment type, and wondered if he could assume that if one technology treatment (e.g. UV) met a standard that other manufacturers of that technology type would also meet that standard, even if they haven't sent in the data yet to MISP. Nicole agreed with the concept, but noted that operators need to make that type of decision and request the appropriate information. CSLC isn't going to indicate in the report that a technology meets the standard without data from that system available for review. Marc wondered if it would be helpful, nonetheless, to make some general statement in the report about the treatment types in terms of meeting performance standards. Nicole agreed to add a general statement about the possibility of a treatment type being effective, even if data had not been sent to MISP.

In a discussion about environmental assessment and toxicity testing, Dominic pointed out that the different measurements for chlorine residues (Total Residual Chlorine (TRC) vs. Total Residual Oxidant) are pretty comparable, and suggested not getting caught up in the type of measurement, but just to indicate what measurement was used with an asterisk. John pointed out that for the TRC compliance for OptiMarin should be changed from "yes" to "NA."

Nicole wrapped up introductory discussion of the report by commenting that AB 169 was vetoed, so the report will be changed accordingly to add the recommendation that the Commission requires the authority to revise the reporting requirements to include information about treatment system usage. Also, the testing guidelines will be given to technology developers for testing their systems for possible compliance with CA standards in next couple of weeks and any relevant info from the completion of this document will be added to the report.

The conversation moved to discuss larger issues that meeting participants had with the report. Participants first listed their main concerns with the report and then discussion later retuned to each item of concern:

- Andy stated that changing the bacteria terminology weakens the standard because with the change in units comes with a change in the number of bacteria that can be screened for. Therefore, if the units are to change then the number of bacteria used in the count (colony forming units) should be changed appropriately. Also, he pointed out that adding the indicator microbes wording does not substitute for the existing viral standards.

- Marc's concern was in terms of the organization and tone of the report. He would like for an executive summary to be included to make the objectives of the report more clear. He also felt that there was a disconnect between the efficacy section and the conclusions because the report states on page 34 that there is not enough information to make a conclusions, but then the conclusion states that several systems will be available and ready to go. The connection between these two points can be addressed in an executive summary. Also, the conclusions that were addressed are buried.

- Brad agreed about the continuity and, in reading the report, thought that CSLC was going to extend the first deadline back another year, until he got to the very end of the report. Brad was also concerned that if the changes to the bacteria and viral standards get rejected, then how would the conclusions of the report change?

- Dominic had technical comments on the toxicity table and a general comment about a statement on page 15 about the State Water Board's authority.

- John was curious if there was any reference to actual physical parameters when discussing system performance and availability (e.g. what type of water flow rates can these systems handle while meeting the standards). Nicole mentioned that these items were not explicitly discussed in the report, but that developers are well aware of necessary flow rates.

- Kevin made a point about the tone of the report in terms of the availability of these treatment systems. He felt like treatment development looks promising and on track, but the systems are not commercially available. Kevin said he would send specific comments via email.

- Allen, Karen, and Lisa had no comments.

BREAK

After the break, Nicole started a discussion about the concerns regarding changing the units to bacteria in the performance standards. Andy stated that changing the units for the bacteria substantially weakens the regulation because approximately 1% (or so, although no specific numbers were available) of bacteria are culturable, so CSLC would need to change the number of bacteria in the performance standard from 1000 to 10 to make the two regulations comparable. Also, the reasoning for changing the units in the regulation is not clear. The report states that there are methods to quantify bacteria, but they are not necessarily appropriate. Dominic agreed with Andy's point about changing the concentration of bacteria. The concentration in the law might be more stringent than the existing assessment methodology, but that's not unusual. Chlorine measurements have a similar limitation. CSLC should keep the strict standard, but use assessment methods to the best of technology's limitations. Dominic suggested that if the bacteria units are going to change to colony forming units (CFU), then the units should be changed to CFU or MPN because then it would allow for the use of the IDEXX system, which is the most effective method available. Nicole stated that the number that they chose was based on what Fred Dobbs and Russ Herwig suggested was found in a natural environment.

Also, John was concerned about how to comply with CA standards, and if there is a dispute, will the enforcement be brought into administrative court? Maurya expressed concern for that as well, and wants to push these treatment systems forward. Kevin was concerned that if the methods of measuring organisms advance beyond today's technology, and these systems (that should pass the regulations) end up failing, is CSLC going to have strong enforcement against those systems, requiring those systems to be removed? Or is there an element of good faith for the systems that CSLC is reviewing, and industry won't be penalized for systems that fail under future enforcement programs? Without system approval, and with uncertain but progressive sampling techniques, there isn't much security that the vessels will pass inspections in the future. Maurya replied that CSLC would have to go back to the legislature since we don't have the authority to address that. Marc reiterated a comment by Dominic that these standards are intended to be about reducing an effect in nature, rather that how to quantify the organisms. Changing the standards opens a can of legislative worms.

Maurya and Nicole agreed that clarifying the regulations to include a statement about the best available measurement technique would provide vessel operators some certainty. Under best available techniques OptiMarin still meets the performance standards. Kevin suggested that having language about grandfathering these systems so that the vessels do not have to go through major retrofits would be helpful. Maurya will talk to Legal about this and see if it can be put into a rule. Marc suggested that if “effects in nature” is left in place, then CSLC wouldn’t need changes in statute. Also, there are provisions in current regulations to include what Kevin is concerned about, and statements can be made so that the treatment systems are “good” until a deadline (e.g. 2020, the life of the system). This would allow installations of these systems to be sped up since operators would have some security that the systems wouldn’t have be removed. Also, this would allow for the systems to be tested by techniques that were available when the system was installed. Maurya concluded that the idea of grandfathering treatment systems can be dealt with later, and isn’t as pressing as the microbial concerns.

Moving on to the concerns about the virus standards, Nicole pointed out that there are ways to count viruses, but part of the definition of viruses are whether or not they are viable and able to infect cells. She is not aware of methods that test this for viruses in general. It would not be feasible to use the terminology used for bacteria on viruses because the methods for bacteria are much more advanced that that of viruses. Karen suggested that if the standard isn’t practical because it cannot be measured, then it would be better to remove it and not hold back this process.

After some additional discussion, Maurya and Nicole agreed that the recommendations in the draft report to change the bacteria and virus standard would be dropped and instead information would be added to the looking forward section to clarify how Commission staff would use the best available techniques/methods in order to assess vessel compliance with the standards. The issue of possible grandfathering of vessels under an existing set of compliance verification methods would be brought up.

In terms of the tone of the report, Marc suggested that it would be helpful to characterize treatment system technology as emerging, rather than apologizing for not having all of the data. There are 30 systems, so there is a lot going on, and it shows that technology isn’t being developed from scratch. Brad thought that it would be important to discuss how out of sync the standards are between IMO, federal government, and California because if federal laws don’t change, vessels will have to exchange their ballast water and treat to meet the CA performance standards. Those big conflicts should be up front. There was discussion about the role of the EPA NPDES. Andy suggested that there could be alternatives added to the permit so that vessels wouldn’t have to meet both regulations. Dominic stated that those comments could be provided to the EPA.

John turned the discussion to the impact if a vessel doesn’t have a sample port? Are there alternative ways of sampling? Maurya and Nicole clarified that while there is some room for variability, sampling in the tank will not work for systems that treat ballast water downstream.

Regarding the toxicity table, Dominic pointed out that it should be clear in the report that the developers did not test for CA species. This is important because not all species respond the same to toxins and that there are strong regional components as well. Also, he suggested removing the N/A under toxicity testing for systems that use UV because exposing water to UV creates chemicals, such as H_2O_2, which is bad for the environment. Toxicity testing should be applicable for all systems. Kevin pointed out that there are guidelines for toxicity testing of substances in the G9 guidelines.

Dominic also suggested changing the question marks in the table (indicating total residual oxidants) to the appropriate yes or no, with an asterisk to show that technically the systems were tested for total residual oxidants. The difference between total residual chlorine and total residual oxidants is just semantics. Andy suggested that Dominic provide a reference to CSLC showing that these two methods are comparable. Dominic was also concerned about all of the other chemicals that he had discussed with Nicole, and suggested adding the Testing Guidelines document be added as an appendix to this report. Nicole stated that it could be added.

Overall, Andy and Marc said they were impressed with the report and the new information.

In conclusion Nicole said that she would move the recommendations to remove the bacterial and virus standards into the “looking forward” section and reword to discuss how Commission staff will make use of the best available methods to assess vessel compliance the standards. Nicole said she could send the new text out to the TAG. Andy wanted to see language regarding best available measurement techniques and also regarding the grandfathering of systems. John would like a legal opinion, and would like information on the scope of the vessels that this report would be impacting, as per Kevin’s comments. Marc stated that he’ll have to comment during the public hearing, and that the comments will not be too significant. Nicole requested any written comments by Friday, or Monday if need be. Because the report is available for public comment before the Commission meeting, the group decided that they did not need to immediately review the new text for the report.

Nicole thanked everyone for their comments, and the meeting was adjourned.