BALLAST WATER TREATMENT TECHNOLOGY TESTING GUIDELINES

PREPARED BY THE CALIFORNIA STATE LANDS COMMISSION, MARINE INVASIVE SPECIES PROGRAM

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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.

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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: Bacteria Viruses	 CFU/100 ml Viruses/100 ml	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: • 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: 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.

CHAPTER 7. CONTACT INFORMATION

For more information or to submit documents for review and comment, please contact:

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APPENDIX A. ADVISORY PANEL MEMBERS AND MEETING NOTES

Advisory Panel

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John Berge Pacific Merchant Shipping Association	Dave Lawrence University of Washington
Andrea Copping Pacific Northwest National Laboratory	Henry Lee U.S. EPA
Annie Cox University of Rhode Island	Edward Lemieux Naval Research Laboratory
Fred Dobbs Old Dominion University	Lucie Maranda University of Rhode Island
Nicole Dobroski California State Lands Commission	Allen Pleus Washington Department of Fish and Wildlife
Richard Everett U.S. Coast Guard	Kevin Reynolds The Glosten Associates
Maurya Falkner California State Lands Commission	Andrew Rogerson Fresno State University
Ray Frederick ETV Program	Chris Scianni California State Lands Commission
Steve Foss CDFG/OSPR	Andrea Solow, Woods Hole Oceanographic Institution
Daphne Gehringer California State Lands Commission	Tom Stevens NSF International
Dominic Gregorio State Water Resources Control Board	Mario Tamburri University of Maryland
Russ Herwig University of Washington	Nick Welschmeyer Moss Landing Marine Laboratories

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

Participants

John Berge Pacific Merchant Shipping Association	Henry Lee** U.S. EPA
Andrea Copping** Pacific Northwest Laboratories	Lucie Maranda** University of Rhode Island
Fred Dobbs** Old Dominion University	Allen Pleus** WA Department of Fish and Wildlife
Nicole Dobroski CSLC	Kevin Reynolds** The Glosten Associates
Maurya Falkner CSLC	Chris Scianni CSLC
Steve Foss, CA Dept. Fish and Game, Office of Spill Prevention and Response	Tom Stevens** NSF International
Dominic Gregorio State Water Resources Control Board	Mario Tamburri** University of Maryland
Rian Hooff** Oregon Department of Environmental Quality	Nick Welschmeyer Moss Landing Marine Lab

** Indicates participation by phone

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, 3^{rd} 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 1st 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 B. 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 C. 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)

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

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.