CALENDAR ITEM
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A. Newsom
C. Scianni
D. Hermanson

CONSIDER AMENDMENTS TO BALLAST WATER PERFORMANCE STANDARDS REGULATIONS TO ESTABLISH PROTOCOLS FOR THE EVALUATION OF COMPLIANCE WITH CALIFORNIA BALLAST WATER DISCHARGE STANDARDS

PROPOSAL:

Pursuant to Public Resources Code (PRC) Section 71205.3, Commission staff is proposing to amend the California Code of Regulations (CCR), Title 2, Division 3, Chapter 1, Article 4.7, titled "Performance Standards and Assessment Protocols for the Discharge of Ballast Water for Vessels Operating in California Waters." The proposed amendments would amend CCR Sections 2291, 2292, and 2293, and amend, renumber and adopt Section 2297(a-g). These amendments would make some substantive and sufficiently related changes to existing regulations, amend the requirements for the installation of ballast water sampling ports, establish protocols to take ballast water samples and assess vessel compliance with California's performance standards for the discharge of ballast water. If adopted, the proposed changes would go into effect immediately, as vessels are already in operation that are subject to California's existing statutory performance standards for discharge of ballast water.

BACKGROUND:

The Ballast Water Management for Control of Nonindigenous Species Act (Chapter 849, Statutes of 1999) established a statewide multi-agency program to prevent and control aquatic nonindigenous species introductions from commercial vessels, and appointed the Commission with the responsibility to administer the program. In 2003, the Marine Invasive Species Act (Chapter 491, Statutes of 2003) expanded and renamed the program as the Marine Invasive Species Program. The Coastal Ecosystems Protection Act (Chapter 292, Statutes of 2006) further expanded the responsibilities of the Commission and added Section 71205.3 to the PRC, which required the Commission to adopt regulations implementing the interim and final performance standards for the discharge of ballast water, as identified in statute. CCR Title 2, Division 3, Chapter 1, Article 4.7 was approved by the Commission in June 2007 (Agenda Item C 56) and implements California's performance standards for the discharge of ballast water.

Public Resources Code Section 71206 requires the Commission to inspect at least 25% of arriving voyages for compliance with the Marine Invasive Species Act (PRC Section 71200 *et seq*.), which includes the performance standards adopted by the California Legislature.

The proposed changes to Article 4.7 in CCR Sections 2291 and 2292 are substantive and sufficiently related amendments to enhance the continuity and clarity of Article 4.7, and amendments that must be made to ensure that the regulation in CCR Section 2293 mirrors the requirements set forth in statute (PRC Section 71205.3).

More than 80% of vessel arrivals to California do not discharge ballast and would therefore be in compliance with the performance standards through this management practice. Commission staff recognizes that for many vessels, compliance with California standards will require the installation of a ballast water treatment system (BWTS), although such installation is not expressly required by California regulations. As BWTS installation represents a significant investment on the part of owners and operators, proposed amendments to Section 2297(b) establish a grandfathering provision. Under this provision, if a vessel installs a BWTS as part of a ballast water management plan, the protocols in place for evaluation of compliance with California's performance standards will be used to evaluate that vessels' compliance for up to 10 years following BWTS installation. For vessels that install a BWTS, the proposed regulations are essential to provide transparency to the regulated community. Vessel owners, operators, agents and treatment technology vendors can understand exactly how vessels would be inspected for compliance with California's performance standards. This transparency is necessary to help provide industry with the tools to assess BWTS performance and determine what option to choose, among those available systems, for their vessels to meet California's performance standards, as determined by the protocols being set forth in the proposed amendments.

California's existing statutory performance standards are discharge standards. Assessment of vessel compliance must be undertaken using ballast water samples from the discharge line (pipe). Vessels are not traditionally constructed with ports that allow ballast water to be sampled from the discharge line. The proposed amendments to Section 2297(c) modify the existing requirements for the installation of sampling ports. The regulated community informed staff that existing language in this section is complicated and confusing. The proposed changes streamline the requirements and provide clarity as to the types of sampling ports that will be allowed and where they may be located. The changes to Section 2297(c)(1-3) create prescriptive requirements for sample port installation as is necessary to ensure the precise fitting of vessel pipes and equipment with Commission sampling equipment that will be brought on board should a vessel operator choose not to retain all ballast on board or not to conduct other appropriate management practices such as discharging to a shoreside or barge-based treatment facility. Section 2297(c)(5) is not prescriptive and proposes general protocols for ballast water sample disposal, as there is no need for specific pipes and fittings to be

used for the disposal of samples. Vessels must simply provide Commission staff with access to a disposal mechanism.

The proposed regulatory changes to Section 2297(d-e) are necessary because Commission inspectors need to have protocols that will be used to collect ballast water samples and assess vessel compliance with California's performance standards as part of the vessel inspection process. Furthermore, it is necessary for proposed changes to Section 2297(d-e) to be largely prescriptive. These sections establish precise methods that will be used by Commission staff to collect and analyze ballast water samples to assess compliance with California's performance standards. These exact methods have been vetted by U.S. and international scientists as being based on the best available science and utilizing the best available technology to conduct this type of analysis.

The proposed amendments are based on the requirements of PRC Section 71205.3 and were developed in consultation with a Technical Advisory Group (TAG) consisting of scientists, marine engineers, and representatives from the shipping industry, environmental organizations, and state and federal government agencies. The TAG met four times between June and November 2011. During these meetings, the TAG discussed the best available scientific methods to collect and analyze ballast water samples for compliance with California's performance standards. During the last meeting in November 2011, the TAG discussed the contents of the draft regulations and made comments and suggestions. Written records of these meetings will be included as part of the final rulemaking package and are considered not only a source of technical information that support the development of the regulations, but also the main avenue by which alternatives to the regulation were discussed.

The proposed amendments will improve the clarity of Article 4.7 and will help fulfill the Commission's statutory mandate to sample ballast water and sediments from at least 25% of vessel arrivals. Establishing clear, detailed protocols for the collection, analysis and handling of ballast water samples is essential to fulfillment of PRC Section 71205.3. Vessels that are complying with the performance standards will significantly reduce the numbers of harmful nonindigenous species (e.g. harmful algae blooms, toxic dinoflagellates and disease causing bacteria such as *Vibrio cholerae*) being discharged into California waters, and therefore human health and welfare will benefit significantly by enforcement of this important law. The proposed amendments meet the purpose of the Marine Invasive Species Act (Public Resources Code Section 71201(d)) to "move the state expeditiously toward elimination of the discharge of nonindigenous species into the waters of the state…"

ISSUES OR CONCERNS:

Commission staff held one public hearing on April 17, 2012 at the Port of Long Beach in Long Beach, California. Two people presented oral statements at this hearing relevant to the proposed regulatory action. In addition, Commission staff received eight comment letters relevant to the proposed regulatory action. Comments were either

accepted for updated informational purposes without changing the regulatory text, or were rejected, as they were not sufficiently related to the regulatory text. Many comments were related to California's statutory performance standards under PRC Section 71205.3, rather than the proposed amendments to regulations. All comments and responses will be summarized in the Final Statement of Reasons per the Administrative Procedures Act.

STATUTORY AND OTHER REGULATIONS:

A. Public Resources Code Section 71200 through 71271

PERMIT STREAMLINING ACT DEADLINE:

N/A

OTHER PERTINENT INFORMATION:

1. The staff recommends that the Commission find that the subject amendments to regulations do not have a potential for resulting in either a direct or a reasonably foreseeable indirect physical change in the environment, and are, therefore, not a project in accordance with the California Environmental Quality Act (CEQA).

   Authority: Public Resources Code Section 21065 and California Code of Regulations, Title 14, sections 15060, subdivision(c)(3) and 15378.

2. The proposed regulatory amendments do not affect small businesses as defined in Government Code Section 11342, subsection (h), because all affected businesses are transportation and warehousing businesses having annual gross receipts of more than $1,500,000, as specified under Government Code Section 11342, subsection (h)(2)(I)(vii).

   Authority: Public Resources Code Section 21065 and California Code of Regulations, Title 14, sections 15060(c)(3) and 15378.

EXHIBIT:

A. TEXT OF PROPOSED MODIFIED REGULATIONS

IT IS RECOMMENDED THAT THE COMMISSION:

1. Find that the subject amendments to regulations are not subject to the requirements of CEQA pursuant to California Code of Regulations, Title 14, Section 15060, subdivision (c)(3), because the subject activities are not a project as defined by the Public Resources Code Section 21065 and California Code of Regulations, Title 14, Section 15378.

2. Find that the amendment will not affect small businesses as defined in Government Code Section 11342, subdivision (h), because all affected businesses are transportation and warehousing businesses having annual gross receipts of more than $1,500,000, as specified under Government Code Section 11342, subdivision (h)(2)(I)(VII).

3. Find that the amendment will not have a significant impact on the creation or elimination of jobs or new or existing businesses within California, nor will they have an adverse economic impact on business, including the ability of California businesses to compete with businesses in other states.

4. Find that no alternatives would be more effective in carrying out the purposes for which the amendments are proposed or would be as effective as and less burdensome to affected private persons than the proposed regulations.

5. Adopt the proposed amendments to Article 4.7, Sections 2291, 2292, 2293 and 2297 in California Code of Regulations, Title 2, substantially in the form of that set forth in Exhibit "A" to become effective January 1, 2013.

6. Authorize the Commission staff to make modifications to the proposed amendments in response to recommendations by the Office of Administrative Law.

7. Direct the Commission staff to take whatever action is necessary and appropriate to comply with provisions of the Government Code regarding adoption of regulations and amendments and to ensure that the regulation becomes effective.

8. Direct Commission staff to take whatever action is necessary and appropriate to implement the amendments to the regulation at such time as they become effective.

EXHIBIT A

STATE LANDS COMMISSION

TEXT OF MODIFIED REGULATIONS

The Commission has illustrated changes to the original text noticed to the public in the following manner: proposed language is underlined; deletions from the original text are shown in strikeout using a "-".

Title 2, Division 3, Chapter 1, Article 4.7 Performance Standards and Assessment Protocols for the Discharge of Ballast Water for Vessels Operating in California Waters

Section 2291. Purpose~~,~~ and Applicability.~~, and Date of Implementation.~~

(a) The purpose of the regulations in Title 2, Division 3, Chapter 1, Article 4.7 of the California Code of Regulations is to move the state expeditiously toward elimination of the discharge of nonindigenous species into the waters of the state or into waters that may impact the waters of the state, based on the best available technology economically achievable.

(b) The provisions of Article 4.7 apply to all vessels that discharge ballast water in California waters except those that are exempt under Section 71202, Public Resources Code.

Authority Cited: Sections 71201.7~~, 71202~~ and 71205.3, Public Resources Code.

Reference: Sections 71201, 71201.7, 71202 and 71205.3, Public Resources Code.

Section 2292. Definitions

Unless the context otherwise requires, the following definitions shall govern the construction of this Article:

(a) "Ballast Water Capacity" means the total volumetric capacity of all ~~any~~ tanks, spaces, or compartments on a vessel used for carrying, loading or discharging ballast water, including any multi-use tank, space or compartment designed to allow carriage of ballast water.

(b) "Ballast Water Sample" means a unit of ballast water that may be collected and assessed for compliance ~~verification~~ purposes.

(c) “Ballast Water Treatment System” means a prefabricated, commercial-ready, treatment system designed to remove, kill or inactivate (prior to discharge) organisms in ballast water.

(d) ~~(c)~~ “Board” means the State Water Resources Control Board

(e) ~~(d)~~ “Colony Forming Unit” is a visible bacteria colony and is ~~means~~ a measure of viable bacterial cells ~~numbers~~.

(f) ~~(e)~~ “Commission” means the California State Lands Commission.

(g) ~~(f)~~ “Constructed” means a stage of vessel construction where:
(1) the keel is laid; or
(2) construction identifiable with a specific vessel begins; or
(3) assembly of the vessel has commenced comprising at least 50 tons ~~nes~~ or 1 percent of the estimated mass of all structural material, whichever is less; or
(4) the vessel undergoes a major conversion.

~~(g) “Isokinetic Sampling Facility” means a sampling apparatus in which the velocity (or speed) of the sample stream does not change from the pipe being sampled to the sample pipe itself.~~

~~(h) “Isokinetic Diameter” assumes a circular main flow pipe and circular sampling pipe of which the diameter is designed to maintain the fluid velocity from the main flow to the sample flow.~~

(h) ~~(i)~~ “Major Conversion” means a conversion of a vessel;
(1) ~~which~~ that changes its ballast water carrying capacity by 15 percent or greater; or
(2) ~~which~~ that changes the vessel type; or
(3) ~~which~~ that, in the opinion of the Commission, is projected to prolong its life by ten years or more; or
(4) ~~which~~ that results in modifications to its ballast water system other than component replacement-in-kind. Conversion of a vessel to meet the provisions of this Article shall not be deemed to constitute a major conversion for the purposes of this Section.

(i) ~~(j)~~ “Photomicrograph” means a photograph taken through a microscope.

(j) “Sampling Port” means the piping connection that provides sampling access into the ballast water main pipe.

~~(j) “Sampling Facilities” means the equipment installed to take the ballast water sample.~~

~~(k) "Sampling Point" means that place in the ballast water piping where the sample is taken.~~

(k) "Sedgewick Rafter Counting Chamber" means a type of microscope slide designed specifically for the quantitative enumeration of particles in a precise volume of fluid.

(l) "Vessel" means a vessel of 300 gross registered tons or more.

Authority Cited: Sections 71201.7 and 71205.3, Public Resources Code.

Reference: Sections 71200, 71201.7, ~~and~~ 71205.3, and 71207, Public Resources Code.

Section 2293. Interim Performance Standards for Ballast Water Discharges.

Subject to the Implementation Schedule in Section 2294, before discharging ballast water in waters subject to the jurisdiction of California, the master, owner, operator, or person in charge of a vessel to which this section applies shall conduct ballast water treatment so that ballast water discharged will contain:

(a) No detectable living organisms that are greater than 50 micrometers in minimum dimension;

(b) ~~Less than~~ 0.01 or fewer living organisms per milliliter that are equal to or less than 50 micrometers in minimum dimension and equal to or greater ~~more~~ than 10 micrometers in minimum dimension;

(c) For living organisms that are less than 10 micrometers in minimum dimension:
  (1) ~~less than~~ 1,000 or fewer bacteria per 100 milliliters;
  (2) ~~less than~~ 10,000 or fewer viruses per 100 milliliters;
  (3) concentrations of microbes that are equal to or less than:
    (A) 126 colony forming units per 100 milliliters of *Escherichia coli*;
    (B) 33 colony forming units per 100 milliliters of Intestinal enterococci; and
    (C) 1 colony forming unit per 100 milliliters or 1 colony forming unit per gram of wet weight of zoological samples of Toxicogenic *Vibrio cholerae* (serotypes O1 and O139)

Authority Cited: Sections 71201.7 and 71205.3, Public Resources Code.

Reference: Sections 71201.7 and 71205.3, Public Resources Code.

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Section 2297. Protocols for the Assessment of Vessel Compliance with the Ballast Water Performance Standards

(a) Designation of Compliance Protocol Version

(1) The protocols set forth in Section 2297 shall be hereafter referred to as "Vessel Compliance Protocol Version 2012."

(2) As part of any and all future amendments to Section 2297, the protocol version shall be renamed according to the year of revision (e.g. protocols revised in the year 2040 would be renamed "Vessel Compliance Protocol Version 2040").

(b) Compliance Period Provision (Grandfathering)

(1) Vessels subject to Article 4.7 shall be inspected by Commission Staff for compliance with the provisions set forth in Article 4.7 using the version of the Vessel Compliance Protocol established by Section 2297 in effect at the time of the inspection.

(2) Notwithstanding the provisions of sub.(b)(1), and applicable only to vessel's that use ballast water treatment systems to comply with Article 4.7, once Commission Staff using the Vessel Compliance Protocol applicable at the time of an inspection, determines, for the first time after commissioning of the vessel's ballast water treatment system, that a vessel is compliant with Article 4.7, then that same Vessel Compliance Protocol shall be used in determining compliance for that vessel until ten years following the date that the ballast water treatment system was first commissioned on the vessel or until the ballast water treatment system is substantially modified or replaced, whichever is earlier.

(c) Sampling Port for the Collection and Disposal of Ballast Water Samples.

Subject to the implementation schedule in Section 2294 and taking into account the following considerations, ballast water cannot be discharged into waters of the state from a vessel to which this section applies unless that vessel has installed ~~shall install~~ sampling port(s) ~~facilities~~ to enable collection and disposal of ballast water samples in order to assess compliance with Section 2293.

1) The sample port(s) provided by the vessel shall consist of a piping connection into the ballast main, a shut-off valve, and a flange. All other sampling and test equipment is to be provided by the Commission staff performing the sampling and testing.

2) ~~a)~~ Technical specifications for design of sampling ports ~~in-line sampling facilities~~:

A) ~~1)~~ ~~The sampling facility shall not damage and/or induce substantial incidental mortality to organisms to be collected in ballast water.~~ The sampling port shall provide clear access into the ballast water main pipeline.

B) ~~2)~~ ~~The isokinetic sample port diameter shall be determined according to the equation:~~

$$D_{iso} = D_m \sqrt{\frac{Q_{iso}}{Q_m}}$$

~~where D_{iso} and D_m are the diameters of the sample port opening and the main flow in the discharge line, respectively; and Q_{iso} and Q_m represent the respective volumetric flow rates through the two pipes.~~

~~Sample port size shall be based on the combination of maximum sample flow rate and minimum ballast flow rate that yields the largest isokinetic diameter.~~ All sample port components shall be at least four inches (100 mm) in nominal dimension and comply with the materials schedule of the existing ballast water system.

C) ~~3)~~ ~~The opening of the sampling pipe shall be chamfered to provide a smooth and gradual transition between the inside and outside pipe diameters.~~ The sample port shall consist of the following components (see Figure 1 – Sampling port components and dimensions):

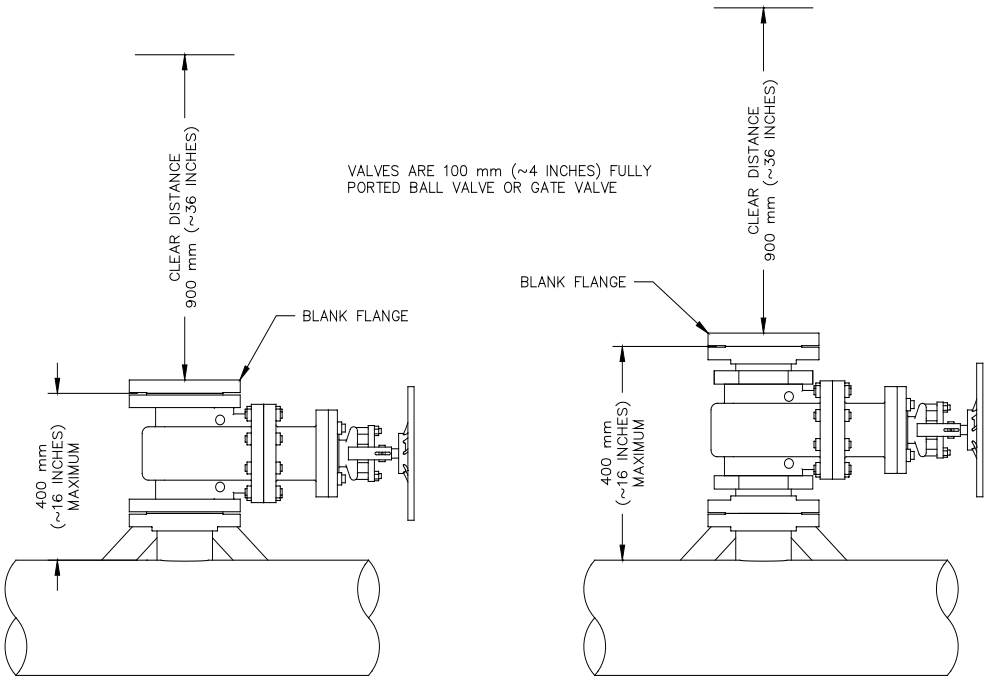
i. A branch connection, using a tee fitting, pipe boss, saddle, or other approved method shall be used to access the inside of the ballast water main pipeline. The inside of the access shall be unobstructed by the ballast main pipe, internal edges filed smooth, and de-burred.

ii. An isolation valve that meets marine vessel regulatory requirements as mandated by the relevant flag state and/or marine classification society shall be provided to allow the sample port to be closed off from the ballast main pipeline. The isolation valve shall be fully ported, such that a clear and straight opening equal to the pipe inside diameter is provided from the access flange into the ballast main. Suitable valves include fully ported ball valves and gate valves. Butterfly, globe, and similar valves that do not provide a clear and straight opening are not acceptable.

iii. An access flange shall be provided to allow connection of Commission equipment to the access port. The access flange shall be of standard design such as ANSI B16.5 or DIN equivalents. It is acceptable to utilize a flanged isolation valve as the access flange. The access flange must be paired with a blank flange, gasket, and bolting suitable to close the sample port. The access flange must be maintained such that Commission gaskets can fit tightly when sampling equipment is attached.

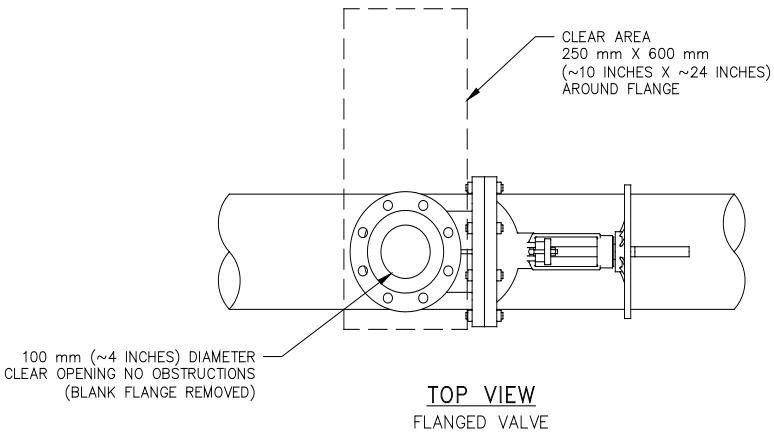
iv. The distance from the ballast main pipe inside diameter to the face of the access flange shall not exceed 400 millimeters (mm) (approximately 16 inches).

v. A minimum of 900 mm (approximately 36 inches) of clear operating area above the access shall be available for the attachment of Commission sampling equipment.



EXAMPLE A
FLANGED VALVE

EXAMPLE B
THREADED VALVE



TOP VIEW
FLANGED VALVE

Figure 1. Sampling port components and dimensions

D) 4) The length of the straight sample pipe facing into the flow can vary, but shall not be less than one diameter of the sampling pipe. The sampling port shall be oriented such that the opening is facing upstream and its lead length is parallel to the direction of flow and concentric to the discharge pipe, which may require sampling pipes to be "L" shaped with an upstream facing leg if installed along a straight section of discharge pipe.

E) 5) The design of the sample facility shall allow for the servicing and/or cleaning of the sampling facility without impacting the safety of the vessel. The sampling pipe should be retrievable either manually or mechanically, or it should be in a system which can be isolated.

F) 6) The sample facility and all associated parts of the sampler that come into contact or near proximity with the ballast piping shall be constructed of galvanically compatible materials and generally corrosion resistant.

G) 7) When control of the sample flow rate is required, appropriate valves shall be used that do not result in organism mortality due to sharp velocity transitions. Ball, gate or butterfly valves shall not be used.

H) 8) If a pump must be used to sample the discharge side of a tank, an appropriate sampling pump shall be used to minimize organism mortality.

I) 9) The Master of the vessel must maintain positive control (e.g. tamper evident lock-out seals) over the ballast water sampling facility when compliance verification or scientific sampling is not being conducted.

3) b) Technical specifications for installation location of a sample port point in the ballast water discharge line:

A) 1) The sample port shall be accessible from a working area of about 2 square meters (m^2) (approximately 21.53 square feet) and with clear head room of about 2 m (approximately 6.56 feet). The working area shall be on a flat, platform or deck, have suitable lighting, and allow for safe access and conditions for two persons and their sampling/test equipment.

B) 2) The sampling port point shall be installed in a straight part of the discharge line, downstream of the last treatment process, as near to the ballast water overboard discharge as practicable.

3) The sample shall be removed from the main pipeline at a location where the flowing stream at the sample point is representative of the contents of the stream. The sample facility should be placed at a point where the flow in the main pipe is

~~fully mixed and fully developed.~~

C) ~~4)~~ As many sample ports ~~points~~ shall be provided as necessary to draw a ballast water sample during ~~typical~~ deballasting of any ballast water tank on the vessel.

D) ~~5)~~ In cases where the ballast system design does not enable sampling from the discharge pipeline, other arrangements for a sampling port ~~point~~ may be made on a vessel-specific basis with prior approval of Commission staff.

4) ~~c)~~ Existing sampling ports ~~facilities~~

Vessels may use existing sampling ports ~~facilities~~, installed prior to ~~January 1, 2010~~ July 1, 2012, to fulfill the requirements of this Section with prior approval of Commission staff.

5) Disposal of ballast water samples

The sample port may serve the dual function of obtaining and returning (disposing of) the ballast water sample from the ballast water main. Alternative means of disposal of the ballast water sample (not to exceed 5 m^3 for each sampling event) is at the vessel's option. It may be collected in the bilge, returned to the ballast system, or pumped overboard as long as compliance with all vessel discharge requirements is maintained.

Authority Cited: Sections 71201.7, 71205.3 and 71206, Public Resources Code.

Reference: Sections 71201.7, 71205.3 and 71206, Public Resources Code.

(d) Sample Collection

1) Samples shall be collected by Commission staff or designated representatives as part of the process to assess vessel compliance with the ballast water discharge performance standards set forth in 2CCR Section 2293.

2) All samples shall be collected from the sampling port, as required by 2CCR Section 2297(c).

3) Samples shall be collected continuously so that they are representative of the discharge at the time of collection.

4) Organisms greater than 50 micrometers (µm) in minimum dimension

A) Sample volume – A total of 3 cubic meters (m^3) of ballast water to be discharged shall be collected.

B) Concentration – Samples shall be concentrated at the time of collection using a 35 µm (no greater than 50 µm in diagonal dimension) plankton net. Samples may be further concentrated using a 35 µm (no greater than 50 µm in diagonal dimension) mesh sieve in order to reach a final sample volume of between 1 – 4 liters (L), depending on the concentration of the sample. Artificial seawater, filtered seawater, or freshwater, as appropriate, free of any organisms greater than 50 micrometers (µm) in minimum dimension shall be added to maintain oxygen levels for the living organisms to be counted.

C) Storage – Samples shall be stored in a flask or carboy with a capacity of up to 4 L. Storage containers shall be cleaned and rinsed with deionized water prior to use. The temperature of the sample shall be maintained as close as possible, plus or minus 5 degrees Celsius, to the original ambient temperature of the discharged ballast water.

5) Organisms equal to or less than 50 µm in minimum dimension and equal to or greater than 10 µm in minimum dimension

A) Sample volume – A total of 3 L of ballast water to be discharged shall be collected.

B) Concentration - Samples shall not be concentrated at the time of collection. Whole water samples shall be collected. Any concentration shall take place during sample analysis.

C) Storage - Samples shall be stored in an acid-washed, opaque 3 L HDPE bottle during transport from ship to the site of analysis. The temperature of the sample shall be maintained as close as possible, plus or minus 5 degrees Celsius, to the original ambient temperature of the discharged ballast water.

6) Bacteria (culturable, aerobic, heterotrophic bacteria)

A) Sample volume – 1000 mL of ballast water to be discharged shall be collected.

B) Concentration - No concentration is necessary. Use whole water samples.

C) Storage – Sample shall be collected and stored in sterile glass or HDPE plastic bottles at least 1000 mL in volume. Samples shall be stored on ice during transit to the laboratory and until analysis begins.

7) *Escherichia coli*

A) Sample volume – 1000 mL of ballast water to be discharged shall be collected.

B) Concentration – No concentration is necessary. Use whole water samples.

C) Storage – Samples shall be collected and stored in sterile glass or HDPE plastic bottles at least 1000 mL in volume. Samples shall be stored on ice during transit to the laboratory and until analysis begins.

8) Intestinal enterococci

A) Sample volume - 1000 mL of ballast water to be discharged shall be collected.

B) Concentration – No concentration is necessary. Use whole water samples.

C) Storage – Samples shall be collected and stored in sterile glass or HDPE plastic bottles at least 1000 mL in volume. Samples shall be stored on ice during transit to the laboratory and until analysis begins.

9) *Vibrio cholerae* (serotypes O1 and O139)

A) Sample volume - 1000 mL of discharged ballast water shall be collected.

B) Concentration - No concentration is necessary. Use whole water samples.

C) Storage - Samples shall be collected in sterile glass or HDPE plastic bottles at least 1000 mL in volume. Samples shall be stored on ice during transit to the laboratory and until analysis begins.

(e) Sample Analysis

(1) Organisms greater than 50 micrometers (µm) in minimum dimension

A) Holding Time - Samples shall be processed as soon as possible to prevent organism die-off. Processing must be complete no more than six hours after collection.

B) Concentration – If the sample has a low concentration of zooplankton, the sample may need to be further concentrated before analysis. If necessary, the sample shall be gently concentrated using a 35 µm (no greater than 50 µm in diagonal dimension) mesh sieve.

C) Sample Analysis – The entire sample shall be counted. Samples shall be observed with a dissecting microscope at a magnification of 10x – 40x. Organisms shall be observed for movement. If the organism is not moving, it may be gently touched with a dissecting needle or probe to elicit movement. Organisms not moving shall be observed for a minimum of 10 seconds and a maximum of 20 seconds for any sign of movement or vital organ activity.

(2) Organisms equal to or less than 50 µm in minimum dimension and equal to or more than 10 µm in minimum dimension

A) Holding Time - Samples shall be processed as soon as possible to prevent organism die-off. Processing must be complete no more than six hours after collection.

B) Sample concentration – The 3L sample shall be concentrated down to 1 L. Concentrate samples very gently on a sieve with less than or equal to 10 micrometer (µm) mesh on the diagonal. Care shall be taken to gently sieve organisms to ensure that they are not killed or lost through the sieve in the concentration process.

C) Sample Analysis – Samples shall be gently homogenized to ensure organisms are well-mixed before taking each subsample. A 1 mL sub-sample shall be placed in a microfuge tube and stained with 5 microliters (µl) of 1 millimolar (mM) fluorescein diacetate (FDA) and 10 µl of 250 micromolar (µM) 5-chloromethylfluorescein diacetate (CMFDA) for a final concentration of 5 and 2.5 µM, respectively. Stained samples shall be incubated in the dark at room temperature for 10 minutes. The sample shall then be loaded into a Sedgewick Rafter Counting Chamber and examined using epifluorescence microscopy using a Fluorescein Isothiocyanate (FITC) narrow pass filter

cube. Samples shall be examined for a maximum of 20 minutes. A photomicrograph shall be taken to create a visual record of viable cells. A minimum of 4 subsamples shall be analyzed in this fashion.

(3) Bacteria (culturable, aerobic, heterotrophic bacteria)

A) Holding Time - Sample analysis shall begin as soon as possible but no later than six hours after sample collection.

B) Sample concentration - None necessary. Use whole water.

C) Sample Analysis – Samples shall be gently homogenized to ensure organisms are well-mixed before taking each subsample. Prepare a 10-fold dilution series using sterile Phosphate Buffered Saline or sterile ambient water such that concentrations of the original water are 10^{0}, 10^{-1}, and 10^{-2}. Spread 100 µL of sample onto each of two general-purpose media for culturable aerobic heterotrophic bacteria (media type is dependent on the salinity of the water at the time of collection: For marine water use 1 marine agar (2216 Marine Agar) and 1 nutrient agar (R2A agar modified with sodium chloride according to the salinity of the sample); For freshwater use Plate Count Agar and Nutrient broth (plus agar (15 g/L). Use at least 3 replicate plates (100 mm wide) for each media type and dilution. The total number of plates (3 dilutions x 2 agars x 3 replicates) should equal 18. Plates shall be incubated in the dark at 20 degrees Celsius and monitored to ensure overgrowth does not occur. Colonies shall be monitored and counted after 5 days (or after 3-5 days, if colony overgrowth appears imminent on all plates) and recorded as colony forming units (CFUs) per 100 mL of sample water.

(4) *Escherichia coli*

A) Holding Time – Sample analysis shall begin no later than six hours after sample collection.

B) Sample concentration – None necessary. Use whole water.

C) Sample Analysis – Samples shall be analyzed using U.S. EPA Method 1603.

D) Alternatives - The defined enzyme substrate test, Standard Method 9223, or the IDEXX Colilert kit (Westbrook, ME; used according to the manufacturer's protocol) may be used in place of EPA Method 1603.

(5) Intestinal enterococci

A) Holding Time – Sample analysis shall begin no later than six hours after sample collection.

B) Sample concentration – None necessary. Use whole water.

C) Sample Analysis – Samples shall be analyzed using a modified version of US EPA method 1106.1. 10 mL and 100 mL water samples shall each be passed through 0.45 µm membrane filters, the filters transferred onto mEnterococcus agar (mEA) plates, and the plates incubated at 35 ± 2ºC for 24 hours. Membranes with light and dark red colonies shall be transferred to bile esculin agar (BEA) plates, which shall be incubated for 4 hours at 35 ± 2ºC. After incubation, colonies with black halos shall be scored and data reported as enterococci per 100 mL of sample water.

D) Alternatives – Standard Method 9230(D) or the IDEXX Enterolert kit (Westbrook, ME; used according to the manufacturer's protocol) may be used in place of EPA Method 1106.1.

(6) *Vibrio cholerae* (serotypes O1 and O139)

A) Holding Time – Sample analysis shall begin no later than six hours after sample collection.

B) Sample Concentration – None necessary. Use whole water.

C) Sample Analysis - 1 mL, 10 mL, and 100 mL water samples shall each be passed through 0.45 µm membrane filters, the filters transferred onto Thiosulfate Citrate Bile Salts Sucrose (TCBS) agar plates, and the plates incubated at 35 ± 2ºC for 24 hours. Sucrose-positive (yellow) colonies shall be purified, and inoculated with 2.5% yeast extract and nalidixic acid and fixed after incubation overnight. Viable *Vibrio cholerae* O1 and O139 cells are

detected using a direct-fluorescent antibody kit (New Horizons Diagnostics, Columbia, MD) for serogroups O1 and O139 using monoclonal antibodies tagged with fluorescein isothiocyanate (FITC) under an epifluorescence microscope. If cells are positive for serogroups O1 or O139, then a DNA colony blot hybridization method should be used to determine whether the corresponding isolates carry the *ctxA* gene. The following method is hereby incorporated by reference as appropriate for the detection and analysis of *Vibrio cholerae* samples: "Huq. et al. 2006. Detection, isolation, and identification of *Vibrio cholerae* from the environment."

(f) Chain of Custody

(1) Written records must accurately trace the custody of each sample through all phases of the sampling and analysis process.

(2) Sample seals must be used to protect the sample's integrity from the time of collection to the time of analysis. The seal shall indicate the collector's name, the date and time of sample collection, and the sample identification number.

A) The sample transport container shall be sealed or locked to readily detect any evidence of tampering.

B) Every sample shall be accompanied by a sample tag and a chain-of-custody record that has been completed, signed, and dated using indelible ink. The chain-of-custody record shall include the names of sample collectors, sample identification numbers, date and time of sample collection, location of sample collection, and names and signatures of all persons handling the sample in the field and in the laboratory.

C) When transferring possession of samples, the transferee must sign and record the date and time on the chain-of-custody record. Custody transfers shall be made for each sample. Each person who takes custody must fill in the appropriate section of the chain-of-custody record.

(g) Use of Laboratories

1) All bacteriological analyses shall be performed at a laboratory that will verify adherence to United States Environmental Protection Agency Quality Assurance/Quality Control (QA/QC) criteria. Bacterial analyses shall be

performed according to the most recent version of EPA Standard Methods or in accordance with an approved sampling and analysis plan.

2) Laboratories shall properly calibrate and maintain all monitoring instruments and equipment to ensure accuracy of measurements.

3) For each sample analysis, laboratories must document all laboratory results and procedures and shall provide a report to the Commission which includes:

   A) A laboratory statement of results of analyses;

   B) Descriptions of analytical methods used (note, if methods other than EPA approved methods or Standard Methods are used, the exact methodology shall be submitted for review and approval by the Commission prior to use); and

   C) Actual detection limits for each sample result (note, detection limits shall be appropriate for the expected concentrations).

4) The director of the laboratory shall supervise all analytical work in his/her laboratory and shall sign all reports of such work submitted to the Commission.

5) Laboratories must follow all chain-of-custody procedures as detailed in Section 2297(f), Chain of Custody.

Authority Cited: Sections 71201.7, 71205.3, ~~and~~ 71206, and 71207, Public Resources Code.

Reference: Sections 71201.7, 71205.3, ~~and~~ 71206, and 71207, Public Resources Code.

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