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University of Kalyani, India
Okko Outinen,
Finnish Environment Institute (SYKE), Finland

*CORRESPONDENCE
Stephen Loiacono
sloiacono@csum.edu

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Technical considerations for sampling ballast water to determine compliance with discharge performance standards

Stephen Loiacono[1]*, Lina Ceballos-Osuna[2], Chris Scianni[2] and Christopher W. Brown[1]

[1]Golden Bear Research Center, California State University, Maritime Academy, Vallejo, CA, United States, [2]Marine Invasive Species Program, California State Lands Commission, Sacramento, CA, United States

In order to discharge ballast in waters of the USA and Member States of the IMO, a vessel must comply with ballast water discharge standards (BWDS). In most cases, this involves use of a Type-Approved ballast water management system (BWMS). Although rigorous efficacy testing is required to gain Type-Approval status, there are currently no requirements for regular compliance checks after a BWMS has been commissioned. Routine compliance checks, to enumerate organisms in treated discharges, are currently the only way to know if a system is meeting a BWDS. This policy brief has two objectives: 1) Highlight the importance of routine collection and analysis of treated ballast water discharges, and 2) Present technical considerations to perform compliance assessments, highlighting good practices for sample collection and analysis and advising of potential obstacles. These assessments are necessary to inhibit the spread of non-indigenous species.

KEYWORDS

ballast water management systems, compliance, sample collection, sample analysis, commissioning test, discharge performance standards

Introduction

Ballast water discharges are a well-known vector for nonindigenous species (NIS) in aquatic environments (Carlton, 1985; Ruiz et al., 1997; Bailey, 2015). Many jurisdictions have adopted ballast water discharge standards (BWDS) to drastically reduce the concentration of living organisms in ballast water discharges and effectively lower the risk of NIS transfer (IMO, 2004; USCG, 2012; USEPA, 2013; California Code of Regulations, 2017).

The International Maritime Organization (IMO) adopted the International Convention for the Control and Management of Ships' Ballast Water and Sediments (BWM Convention; IMO, 2004) that requires vessels in international traffic to meet the IMO D-2 discharge standard. While vessels can use different strategies to meet the IMO D-2 BWDS, most vessels attempt to do so by installing an onboard IMO type-approved ballast water management system (BWMS). Beginning in June 2022, the IMO required all BWMSs installed on new build and existing vessels to undergo a commissioning test to ensure the BWMS is safely installed and operational (IMO, 2020). Once this commissioning test is complete, however, the IMO requires no additional tests to monitor and validate the continued efficacy of the BWMS during normal operations (Table 1 describes the different types of BWMS tests). However, the 81st meeting of the IMO Marine Environmental Protection Committee included discussions focused on working towards regular mandatory biological compliance testing during a vessel's intermediate and renewal surveys, but the details have not yet been finalized (MEPC, 2024).

The United States of America (USA, not a signatory to the IMO BWM Convention) has developed its own set of BWDS (USCG, 2012). The USA requires all vessels discharging ballast water in USA waters to use a U.S. Coast Guard (USCG) Type-Approved BWMS to meet USA federal BWDS (Code of Federal Regulations [CFR], 2017). However, the USCG does not require commissioning testing to evaluate the efficacy of a BWMS after installation and does not sample discharges to assess compliance with the USA federal BWDS (Table 1). Similarly, California adopted regulations that require vessels to comply with the California BWDS (identical to USA federal BWDS) for all discharges occurring in California waters (California Code of Regulations, 2017). California does not require a Type-Approved BWMS to meet the California BWDS; the regulations are based on meeting the BWDS, independent of the strategy used. For this reason, compliance assessment must be conducted with direct measurements of living organisms collected from the discharged water. California started developing and trialing compliance assessment protocols for this purpose in 2022. Aside from recent compliance sampling efforts in California, we are not aware of any jurisdictions assessing compliance against all organisms categories included in any of the BWDS using direct measurements of living organisms in discharged ballast water (Table 1). Without periodic regular compliance checks to assess the number of living organisms in a discharge, it is impossible to know if a BWMS continues to perform as intended during normal operations and if it is compliant with BWDS.

In response to these global to local requirements, vessels are now using BWMS that have been type-approved either by USCG or through IMO processes to manage ballast water discharges to reduce the spread of NIS. However, BWMS performance information, specifically their biological efficacy to meet BWDS, during vessels' normal operations is very limited.

Despite differences between the implementation of international, federal, and state BWDS, the overarching goal of reducing the introduction of ballast-mediated NIS relies on the effectiveness of the ballast water management strategies used by vessels. As the field of compliance testing BWMS emerges, there is a

TABLE 1 Description of the different types of tests used to assess Ballast Water Management Systems' performance and the specific circumstances for which these tests are applied.

Type of testing	Main purpose	Current jurisdictions with regulations/requirements and vessel population applied to
Type-approval (Prior to installation)	To test if the BWMS can meet the BWDS, perform as it is specified by the manufacturer, and identify specific system design limitations. Tests designed and performed under controlled conditions.	International: All vessels discharging ballast waters in countries signatory to the IMO Convention, and vessels flagged in those countries, are required to have an IMO type-approved BWMS. US: All vessels discharging ballast water in U.S. are required to have a USCG type-approved BWMS California: Type-approved BWMS are not required
Commissioning testing (After installation)	To ensure proper installation and functionality of the BWMS on board the vessel. Biological efficacy is one component of commissioning tests. Test is performed under controlled conditions, not reflective of normal vessel operations. Indicative tools are often used instead of detailed analysis	International: IMO requires commissioning testing for all vessels flagged in countries signatory of the convention or that will discharge in those countries. The test does not require detailed analysis of all size categories (the use of indicative tools is accepted). US (including California): Does not require commissioning testing
Regular testing against BWDS to monitor performance	To assess the continuing functionality of the system, and its ability to satisfy BWDS, after installation. Test performed by an independent party as part of regular monitoring protocols.	International: Not required **US**: EPA requires an annual biological testing, but only for indicator microbes. California: Not required because California does not require the use of specific technologies to meet BWDS
Compliance testing to assess compliance with BWDS (during normal operations)	To assess if a vessel is compliant with the BWDS during discharge. Test performed by port control entities during regular inspections to assess compliance.	International: Not performed **US**: Not performed at federal level (to the best of our knowledge California: Opportunistic assessment based on resources availability.

new focus on the high rate of noncompliant discharges (Outinen et al., 2024). Without continuous monitoring and testing against BWDS during normal vessel operations (i.e., not under controlled conditions), it is impossible to know if the BWMS are performing as they are intended and protecting the environment against NIS introductions.

Preliminary BWDS assessment data obtained from detailed biological testing in California (unpublished data) and elsewhere (Outinen et al., 2024) during vessels' normal operations have shown that even vessels using a properly functioning BWMS can fail to meet BWDS for numerous reasons. Likely causes for these failures may include, but are not limited to:

- incorrect BWMS installation.
- untrained crew operating the BWMS.
- biofilms on ultraviolet light bulbs.
- lack of understanding of the parameters in which the system should be operated (system design limitations), including ballast water holding times post treatment.
- unreliable technical support.

Even if a BWMS is installed correctly and performing properly during commissioning, the potential for introductions of NIS remains due to human error and technical issues unknown to vessel crew. If these problems are not recognized, NIS can be introduced during ballast water discharge operations over the life of the vessel. Routine monitoring, collection, and analysis of treated water from BWMS is the primary way to ensure NIS introductions are severely reduced through this vector. This policy brief describes the technical considerations necessary for a surveyor to conduct a successful compliance check, highlighting the increasing awareness of potential obstacles that might be encountered, and good practices to employ by providing details on logistics, collection, and analysis of the samples (Table 2).

Pre-sampling considerations

Vessel selection

Vessel selection for ballast water sampling depends on several factors relevant to each jurisdiction and enforcement entity. Ideally, vessels should be selected based on their risk of introducing invasive species during ballasting operations. Multiple factors contribute to the likelihood of successful species introductions (Lockwood et al., 2005; Leung et al., 2004; Drake and Jerde, 2009; Keller et al., 2011; Bailey, 2015), including the source and volume of the ballast water to be discharged, the environmental matching with the recipient water (Santagata et al., 2008), and the history of the BWMS functionality and malfunctions (either due to human error, operating conditions, or the type of system). All these factors should allow the enforcing entity to identify red flags and select the highest risk vessels for inspection and sampling for compliance with the BWDS. Each enforcement entity should have protocols in place to identify and select vessels for compliance assessment based on their resources, interests, and capabilities (Bradie and Bailey, 2020; Ceballos-Osuna et al., 2021).

Unfortunately, selecting vessels based on risk rating alone may not be the most logistically viable approach due to the variability of vessel schedules, the short timeframe required to analyze the samples, and the availability of trained personnel to perform sampling and analysis. Therefore, selecting vessels to sample for

TABLE 2 Summary of actionable recommendations to sample ballast water for compliance assessment.

Prior to sampling event	Vessel selection	Based on risk: BWMS malfunctions, BWMS alarms, failure to provide maintenance records.
		Opportunistic: based on logistics (vessel's schedule, discharging operations, qualified personnel availability, resources).
	Vessel details, communications, and logistics	Contact the vessel as early as possible to discuss sampling details • Ballast water discharging operations schedule. • Sampling point specifications. • Access and layout of the sampling location • Volume requirements and sampled water disposal options.
During sampling collection	Pre-established protocols	Use a sample collection device that allows for collecting a representative sample in a short period of time without compromising the organisms collected.
		Apply scientifically defendable protocols.
		Ensure proper sample storage and transportation conditions.
		Follow chain of custody protocols
Sample analysis	Pre-established protocols	Apply scientifically defendable protocols.
		Trained personnel and proper laboratory practices
		Follow chain of custody protocols
Defendable results and recordkeeping	Non-compliant vessels	• Prepare legal documentation in case of enforcement action. • Visual evidence (videos and photos) • Inform the vessel and other interested parties (e.g., owner, agent)

compliance may need to be done opportunistically. By adding randomness to the assessed vessel population, jurisdictions may uncover compliance and efficacy patterns that may otherwise be ignored because of perceived low risk.

Communication and information gathering

For effective assessments of BWMS, surveyors must gather as much pertinent information from selected vessels as early as possible, ideally prior to boarding. Often vessels of the same type (e.g., bulk vessels) can have major differences in their build, including the piping or arrangement of equipment and machinery, that can affect sample collection (Figure 1). We recommend surveyors communicate with vessel crews, using a standardized form, before the sampling event to explain the process and obtain the following information:

- Discharging operations schedule.
- Sample port specifications (e.g., sampling pipe diameter).
- Source and management of ballast water to be discharged and sampled.
- Sample collection protocols including volume requirements, water disposal, and sample port accessibility.

Sample port

Treated ballast water samples are collected from discharge sample ports. Although there are existing international requirements for the design and fitting arrangements (International Organization for Standardization, 2019, 2022) they are not standardized across vessels. These sample ports have fittings of various dimensions that need to be compatible with the surveyor's sampling device. If the connection between the sample port valve and the sampling device is incompatible, the sample collection will be delayed or canceled until modifications are made. To avoid disruptions, the sample collection device should have the adaptability necessary to fit most sample ports.

FIGURE 1
Examples of different vessels' sample port configurations that may affect sample collection.

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Access and layout

The equipment setup to collect the samples needs to be adjacent to the discharge sample port, which is typically located in the vessel's pump/engine room. The accessibility of the sample port can vary, and, in some cases, the space may be too confined for the sample collection setup. If so, the vessel's crew and surveyor will need to improvise the placement and orientation of the sample collection device to connect the discharge sample port to the sample collection device. Coordination with the vessel's crew prior to a sampling event is needed to understand the space layout and accessibility of the port to resolve potential issues related to accessibility.

Sample volume

The recommended minimum volume to assess compliance with the BWDS of ≥50 µm size category organisms is ≥1 m^3 (IMO, 2020). To sample this volume, the vessel will need to have enough ballast water to last the duration of the sample collection, typically 20-60 minutes, depending on flow rates. We advise surveyors to communicate this need with vessel crew in advance to make sure the vessel intends to discharge enough volume to meet the minimum requirements.

Considerations for collecting samples

Surveyor

The vessel's crew must not collect samples independently and provide them to the surveyor or laboratory. Samples must be collected by a surveyor or other independent sample collector to maintain an appropriate chain of custody.

Sampling device

Sampling devices are still in an early phase of development, each with their advantages and disadvantages. Sampling devices can be separated into two categories: closed and open.

Closed sampling devices create a loop where treated water is taken from the discharge valve, sent through a housed plankton net, and returned into the discharge pipe (downstream of the sample) before going overboard, resulting in no need to manage disposal of the processed water on board the vessel. The vessel's crew may find this design desirable because they do not need to assist with the disposal of the ≥1 m^3 of sampled ballast water, which requires either dumping it into the bilge or using a pump to send the water into a holding tank. Closed sampling devices typically have a smaller plankton net to fit inside the housing. This reduction causes a considerable decrease in the surface area of the net that can increase the chance of clogging and potentially cause physical stress to any living organisms inside. Another limitation of closed systems is that, in some cases, the discharge pipe must be depressurized and emptied of water before the device can be connected to the sampling port or flange. This can pose an operational issue for many vessels, as some may not be able to accommodate a change in ballasting operations while loading and unloading cargo. In addition, closed devices require a more complicated design to create the loop and return the water to the discharge port, making them more expensive and less adaptable to various sample port sizes.

Open sampling devices do not return the sample water back into the discharge pipe. The sample hose connects to the discharge valve, which leads to a flow meter, before flowing into a plankton net suspended in a clean container which acts as a "soft landing" for any remaining live organisms. Prior coordination with the vessel's crew is crucial, as the water that is being filtered through the plankton net will have to be redirected to the bilge or into a nearby holding tank. The plankton nets used with open sampling devices tend to be much larger than those in closed sampling devices, typically with a mouth diameter of 30-50 cm. Plankton nets used with open sampling devices are used to create a more benign environment due to their increase in surface area, which decreases crowding of organisms in the cod end that collects and concentrates the sample at the end of the plankton net. The hoses, flow meters, and plankton nets used in open sampling devices are typically light-weight and easy to transport, making it the preferred sampling design if only one surveyor is available for sample collection. Open sampling devices also typically cost less than closed sampling devices, are easier to construct, and adaptable to different sample port fittings and vessel designs.

Regardless of the type of sample collection device used, the device should have a side stream or drip hose (before the plankton net) to allow the collection of an unconcentrated sample for the enumeration of the ≥10 and <50 µm size category organisms.

Prior to collecting a ballast water discharge sample, we recommend that the surveyor run ballast water through the sampling device prior to sending it through the plankton net. Often, there is a buildup of rust and fine particulates in the ballast water pipes that can block and/or damage sampling equipment, as well as contaminate the entire sample with debris which can clog plankton nets and obstruct enumerations using microscopy. As little as 1 L of particulate laden water can cause significant difficulty for sample processing/analysis if it flows into the plankton net and sampling container.

Disposal of sampled water

Collecting and disposing the recommended volume of treated ballast water can pose an issue for vessels. Some vessels can easily accept 1 m^3 or more into their bilgewater system, while others cannot. In latter cases, the vessel's crew may need to set up a hose and pump system to dispose of the excess sampled water and direct it into a nearby holding tank, delaying the sampling process. How the processed water is to be handled must be understood by both the surveyor and the vessel crew before the sampling event begins.

Considerations for sample analysis

Sample analysis to detect organisms for all BWDS fall into two categories: indicative and detailed. Indicative analyses typically involve Compliance Monitoring Devices (CMD) that utilize various technologies (e.g., chlorophyll fluorescence, ATP) to estimate organism concentrations. Although portable and designed for ease of use, CMDs can generate false positives or negatives if they are not calibrated properly or if a sensor fails, and they do not provide a true count of living organisms within a sample. Due to these reasons, some CMDs are not recognized as valid testing devices by certain flag states. Although more complex and time consuming, detailed analysis provides direct measurements of organism concentrations. Detailed analyses involve highly trained technicians and sophisticated instruments (e.g., fluorescence microscope) that may not be feasible for onboard analysis.

Regardless of the type of sampling device used, its purpose is to produce the most unaltered sample of treated ballast water possible. Prompt sample analysis is invaluable, as time has a positive correlation with organism mortality (Riley et al., 2006) as conditions in the sample bottle deviate from the physical or chemical *in situ* conditions. As specified in the requirements for IMO/USCG BWMS Type-Approval testing, enumeration of both ≥10 µm to <50 µm and ≥50 µm size category organisms for compliance testing, must be conducted within six hours of collection (USEPA, 2010). An even shorter time between collection and enumeration is desirable, especially for the ≥50 µm size category because these organisms are concentrated in a smaller volume and will be more sensitive to the stress associated with increased holding times due to dissolved oxygen depletion from respiration. For detailed analyses, the samples should be analyzed by a qualified laboratory, ideally ISO 17025:2017 (International Organization for Standardization, 2017) certified or equivalent, that uses standardized protocols to complete the sample analysis in the shortest time possible.

Policy recommendations for proper recordkeeping and defendable results

Transparent and strict protocols must always be followed to support enforcement actions. The samples must be accompanied by a chain of custody form from the moment of collection to the end of the analysis. Each person handling the samples during collection, transport, and analysis must sign the form, noting the time, and any other relevant information.

Legally defensible scientific protocols must be followed at each step. For this, Standard Operating Procedures (SOP) must be established prior to the collection event. The SOP must include sampling collection protocols for all organism size categories, analytical procedures, equipment required, timeframe requirements, data collection forms, chain of custody form, personnel training expectations, and protocols for reporting results.

Evidence for enforcement cases must include, at a minimum, field collection data, chain of custody form, results report from a qualified laboratory, and ideally visuals if live organisms are observed (e.g., videos and photos).

Discussion

The proper collection, handling, and analysis of treated ballast water samples for biological enumeration is a significant undertaking by a surveyor. Collecting a sample of treated ballast water to assess a BWMS for compliance with BWDS first requires thoughtful communication between the vessel crew and surveyor. We recommend surveyors send a standardized form to vessel crew to collect information on relevant ballasting operations, vessel layout, and engineering specifications. Next, surveyors must be trained in appropriate and vetted sample collection protocols to ensure samples are uncontaminated and handled in a manner that reduces sampling-induced mortality to any possible living organisms. Last, sample analysis for ≥10 µm and <50 µm and ≥50 µm size category organisms should be conducted by an authorized lab or analyst with ISO 17025:2017 accreditation, or equivalent, so results are accurate, tracible, and defendable.

Currently, commissioning testing is only required by IMO's D-2 Commissioning Testing requirements (IMO, 2020). Although these guidelines can be used to assess if a system is safely installed and operational, it does not necessarily demonstrate a BWMS's ability to satisfy BWDS because commissioning tests are not representative of the various conditions that a vessel will face during typical ballasting operations. From our experience, vessels often prepare for IMO commissioning tests by cleaning their ballast tanks and targeting source water from oligotrophic bodies of water (e.g., open ocean), due to low organism concentrations (Gregg et al., 2005). If source water has a concentration below the discharge standard, a faulty system will still pass even if it is not working properly. Once the commissioning test has been passed, the BWMS can then operate, possibly ineffectively, for the life of the vessel, undetected without further testing. This represents a serious management gap, given the high rate of noncompliant discharges described by Outinen et al. (2024).

Neither IMO nor the USCG currently have regulations in place that requires subsequent performance tests to assess if the BMWS effectively satisfies the BWDS, leaving a gap in the collective effort to protect the environment from NIS introductions. Discussion during the 81st meeting of IMO Marine Environmental Protection Committee initiated a process toward regular compliance testing, but the plan is not yet formalized and the proposed schedule, at 2.5 and 5-year surveys (MEPC, 2024) may not be frequent enough to effectively reduce noncompliant discharges. These issues illustrate the need for routine compliance checks at an appropriate frequency and to move away from the assumption that a Type-Approved system with a successful commissioning test means a properly functioning system under normal vessel operations for the life of the BWMS.

Recommendations

The protection of our aquatic ecosystems from ballast-mediated NIS introductions has two parts: the installation of Type-Approved treatment technology and the routine assessment of that technology throughout its working life to ensure it performs as intended. We recommend that compliance assessments become a regular part of regulatory programs (Table 2). This can be accomplished by one or both of the following approaches:

- Implement random or targeted sampling of discharging vessels for compliance with BWDS, similar to the state of California.
- Require mandatory testing and reporting on a regular basis (e.g., every 12 or 18 months), similar to testing required by the United States Environmental Protection Agency's Vessel General Permit (USEPA, 2013), but including all organism categories.

Whether a vessel's discharge is determined to be compliant or not, the results of compliance assessments should be used to incentivize future compliance. Vessels found to be noncompliant with BWDS must be made aware of the noncompliance and the owner or operator should investigate the reason for noncompliance (e.g., equipment failure, challenging water, operator error). At a minimum, a violation notice from the relevant jurisdiction will offer an appropriate notification, but penalties may also be warranted in some situations. Regardless of the level of response, vessels found to be noncompliant should be prioritized for future inspections because of their compliance history.

Vessels found to be compliant with BWDS should likewise be incentivized to continue that compliance into the future. This positive reinforcement can be in the form of less frequent inspections or longer periods of being a reduced priority for inspection.

Regardless of how jurisdictions decide to assess compliance with BWDS and the performance of BWMS to achieve these standards, it is clear that these biological assessments are necessary to ensure that these management strategies continue to protect aquatic environments from NIS introductions.

Author contributions

SL: Conceptualization, Methodology, Project administration, Supervision, Writing – original draft, Writing – review & editing. LC-O: Visualization, Writing – original draft, Writing – review & editing. CS: Writing – review & editing. CB: Conceptualization, Funding acquisition, Project administration, Supervision, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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References

Bailey, S. (2015). An overview of thirty years of research on ballast water as a vector for aquatic invasive species to freshwater and marine environments. *Aquat. Ecosyst. Health Manage.* 18, 261–268. doi: 10.1080/14634988.2015.1027129

Bradie, J. N., and Bailey, S. A. (2020). A decision support tool to prioritize ballast water compliance monitoring by ranking risk of non-indigenous species establishment. *J. Appl. Ecol.* 58, 587–595. doi: 10.1111/1365-2664.13822

California Code of Regulations. (2017). *Biofouling Management Biofouling Management to Minimize the Transfer of Nonindigenous Species from Vessels Arriving at California Port, sections 2291-2297*. Sacramento, CA: California Office of Administrative Law

Carlton, J. T. (1985). Transoceanic and interoceanic dispersal of coastal marine organisms: the biology of ballast water. *Oceanogr. Mar. Biol. Ann. Rev.* 23, 313–371.

Ceballos-Osuna, L., Scianni, C., Falkner, M., Nedelcheva, R., and Miller, W. (2021). Proxy-based model to assess the relative contribution of ballast water and biofouling's potential propagule pressure and prioritize vessel inspections. *PloS One* 16, e0247538. doi: 10.1371/journal.pone.0247538

Code of Federal Regulations [CFR] (2017). *Ballast Water Management Requirements. Title 33, Part 151.1510* (Washington, D.C: National Archives).

Drake, J., and Jerde, C. (2009). "Stochastic models of propagule pressure and establishment," in *Bioecon Inv Spec*. Eds. R. Keller, D. Lodge, M. A. Lewis and J. F. Shogren (Oxford University Press, New York), 83–102.

Gregg, W. W., Casey, N. W., and McClain, C. R. (2005). Recent trends in global ocean chlorophyll. *Geophysical Res. Lett.* 32, L03606. doi: 10.1029/2004GL021808

IMO. (2004). *International Maritime Organization. International Convention for the Control and Management of Ships' Ballast Water and Sediments; adopted 16 February 2004, BWM/CONF/36* (London, UK: International Maritime Organization).

IMO. (2020). *International Maritime Organization. 2020 Guidance for the commissioning testing of ballast water management systems. Approved November 2020, BWM.2/Circ.70/Rev.1* (London, UK: International Maritime Organization).

International Organization for Standardization. (2017). *General requirements for the competence of testing and calibration laboratories. Edition 3, 2017.* Geneva, Switzerland: International Organization for Standardization.

International Organization for Standardization. (2019). *Ships and marine technology — Aquatic nuisance species. Part 1: Ballast water discharge sample port. Edition 2, 2019.* Geneva, Switzerland: International Organization for Standardization.

International Organization for Standardization. (2022). *Ships and marine technology — Aquatic nuisance species. Part 2: Ballast water sample collection and handling. Edition 1, 2022.* Geneva, Switzerland: International Organization for Standardization.

Keller, R. P., Drake, J. M., Drew, M. B., and Lodge, D. M. (2011). Linking environmental conditions and ship movements to estimate invasive species transport across the global shipping network. *Divers. Distrib.* 17, 93–102. doi: 10.1111/ddi.2010.17.issue-1

Leung, B., Drake, J. M., and Lodge, D. M. (2004). Predicting invasions: Propagule pressure and the gravity of allee effects. *Ecol.* 85, 1651–1660. doi: 10.1890/02-0571

Lockwood, J. L., Cassey, P., and Blackburn, T. (2005). The role of propagule pressure in explaining species invasions. *Trends Ecol. Evol.* 20, 223–228. doi: 10.1016/j.tree.2005.02.004

MEPC. (2024). *Harmful aquatic organisms in ballast water: Report of the Ballast Water Review Group. MEPC 81/WP.9.* London, UK: International Maritime Organization.

Outinen, O., Bailey, S. A., Casas-Monroy, O., Delacroix, S., Gorgula, S., Griniene, E., et al. (2024). Biological testing of ships' ballast water indicates challenges for the implementation of the Ballast Water Management Convention. *Front. Mar. Sci.* 11, 1334286. doi: 10.3389/fmars.2024.1334286

Riley, S. C., Grant, J. F., Lemieux, E. J., Robbins, S. H., and Hyland, W. B. (2006). "Biological in-tank sampling and sample degradation for standardized ballast water treatment technology sampling," in *Abstract in the Proc 14th International Conference on Aquatic Invasive Species*, Key Biscayne, FL.

Ruiz, G. M., Carlton, J. T., Grosholz, E. D., and Hines, A. H. (1997). Global invasions of marine and estuarine habitats by non-indigenous species: Mechanisms, extent, and consequences. *Am. Zool.* 37, 621–632. doi: 10.1093/icb/37.6.621

Santagata, S., Gasiunaite, Z., Verling, E., Cordell, J., Eason, K., Cohen, J., et al. (2008). Effect of osmotic shock as a management strategy to reduce transfers of nonindigenous species among low salinity ports by ships. *Aquat. Invasions.* 3, 61–76. doi: 10.3391/ai

USCG. (2012). *United States Coast Guard. Standards for Living Organisms in Ships' Ballast Water Discharged in U.S. Waters. Code of Federal Regulations, 33 CFR Part 151 (2012).* Washington, D.C.: United States Coast Guard.

USEPA. (2010). *Generic protocol for the verification of ballast water treatment technology* - Version 5.1. EPA/600/R-10/146 September, 2010.

USEPA. (2013). Vessel general permit for discharges incidental to the normal operation of a vessel. Available online at: www3.epa.gov/npdes/pubs/vgp_permit2013.pdf (Accessed September 2021).