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# The challenges and promises of genetic approaches for ballast water management

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## ABSTRACT

Ballast water is a main vector of introduction of Harmful Aquatic Organisms and Pathogens, which includes Non-Indigenous Species. Numerous and diversified organisms are transferred daily from a donor to a recipient port. Developed to prevent these introduction events, the International Convention for the Control and Management of Ships' Ballast Water and Sediments will enter into force in 2017. This international convention is asking for the monitoring of Harmful Aquatic Organisms and Pathogens. In this review, we highlight the urgent need to develop cost-effective methods to: (1) perform the biological analyses required by the convention; and (2) assess the effectiveness of two main ballast water management strategies, *i.e.* the ballast water exchange and the use of ballast water treatment systems. We have compiled the biological analyses required by the convention, and performed a comprehensive evaluation of the potential and challenges of the use of genetic tools in this context. Following an overview of the studies applying genetic tools to ballast water related research, we present metabarcoding as a relevant approach for early detection of Harmful Aquatic Organisms and Pathogens in general and for ballast water monitoring and port risk assessment in particular. Nonetheless, before implementation of genetic tools in the context of the ballast water management convention, benchmarked tests against traditional methods should be performed, and standard, reproducible and easy to apply protocols should be developed.

## 1. Introduction

Ballast water discharges are recognized as critical sources of pathogens, harmful algae blooms and Non-Indigenous Species (NIS) introduction (Aguirre-Macedo et al., 2008; Drake and Lodge, 2004; Hallegraeff, 2007; Molnar et al., 2008). To prevent potential environmental, human health and socioeconomic impacts of these introductions, the International Convention for the Control and Management of Ships' Ballast Water and Sediments (referred after as “BWM Convention”) was adopted in February 2004 by the International Maritime Organization (IMO, 2004). The BWM Convention will enter into force in September 2017 as the required ratification by at least 30 States representing 35% of world merchant shipping tonnage has finally been reached (IMO, 2016). One of the many challenges of the BWM Convention is ballast water monitoring in commercial ports, *i.e.* screening the whole biodiversity discharged from ballast water to guarantee the prevention and control of Harmful Aquatic Organisms and Pathogens (HAOP) which includes NIS, in recipient ports, which are also monitored for presence of HAOP. Traditionally, ballast water biological inventories, which should preferably be done to the lowest taxonomic level, have relied upon morphological identification, which is costly,

time-consuming (Ji et al., 2013) and requires a high level of taxonomy expertise, skill that is becoming rare (Agnarsson and Kuntner, 2007). Besides, one of the most crucial issues associated with this traditional approach is the difficulty to identify early developmental stages (*e.g.* larvae and eggs), broken organisms or morphologically indistinguishable species; all are common in ballast water (Gollasch et al., 2002). Thus, alternative fast, cost-effective, accurate and broadly applicable methods need to be developed in order to improve ballast water monitoring (Lehtiniemi et al., 2015). Genetic methods overcome some of the main limitations of morphological identification and have been described as a challenging revolution in the assessment and management of species diversity (Fonseca et al., 2010; Wood et al., 2013). Specially, numerous reviews highlight the promises of these tools for studying marine biological invasions processes (Rius et al., 2015; Viard et al., 2016), including early detection (Bott, 2015; Comtet et al., 2015) and provide recommendations regarding the associated technical challenges and possible solutions of using High-Throughput Sequencing technologies in such context (Xiong et al., 2016). As a result, a great number of ballast water related studies already use the advantages of genetic tools to describe the in-tank biodiversity of ballast water (*e.g.* eukaryotes, Zaiko et al., 2015b and viruses, Kim et al., 2015) and

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sediments (e.g. diapausing eggs of invertebrates, [Briski et al., 2011](#)) as well as to provide helpful biological data for assessing the efficiency of ballast water managements ([Briski et al., 2015](#); [Hess-Erga et al., 2010](#)). The rapid development of genetic tools applied to ballast water management have prompted the need for detailed assessments of their relevance and synthetic views of the available tools, as emphasized by [Darling and Frederick \(2017\)](#). While their work provides a comprehensive analysis of the genetic tools for ballast water monitoring in a general context, here, we give a thorough assessment and analyze the suitability of genetic tools to provide data for the BWM Convention and their feasibility in real conditions. For that aim, we meticulously studied the BWM Convention to detect opportunities where genetic tools could provide biological data while evaluating and discussing their great promise as valuable alternatives or additions by early detecting Harmful Aquatic Organisms and Pathogens introduced *via* ballast water.

## 2. The preventing actions of the BWM Convention and their required biological analyses

The BWM Convention is based on regulations defining the specific legal preventive actions to manage the introduction of Harmful Aquatic Organisms and Pathogens (HAOP), and on Guidelines providing technical guidance for all stakeholders to help the implementation of these regulations ([Table 1](#)).

To understand the common requirements for ballast water monitoring and facilitate the detection of opportunities where genetic tools could provide relevant biological data for the BWM Convention, all preventive actions are grouped in four main categories ([Fig. 1](#)) and further described below. To prevent new introduction of HAOP, biological analyses (in red in [Fig. 1](#)) are required and the nature of the analyses and method used will be different depending on the targeted action.

- Actions related to “Testing the compliance with the BWM Convention requirements”: Ships will be first inspected for compliance with the BWM Convention by the port state control through an administrative control of the documentations required on board (*i.e.* ballast water record book) ([King and Tamburri, 2010](#); [Wright and Welschmeyer, 2015](#)); then, if evidences of non-appropriate management are found, ballast water may be sampled for a first and quick indicative biological analysis, and for a more detailed one if additional support for non-compliance is found. The biological methods to assess the compliance must meet certain criteria to be applicable ([David and Gollasch, 2015](#); [IMO, 2015](#)); for example, they shall be designed to take into account organismal minimum size, abundance and viability but also to be fast, applicable onboard, and usable by a non-specialist ([David and Gollasch, 2015](#); [Wright and Welschmeyer, 2015](#)). Regarding the type of analysis and organisms, the method can be qualitative, semi-quantitative, and quantitative. For now, the recommended methods for detailed analysis are visual counting including mobility test for zooplankton, counting chamber with epifluorescence microscopy as well as machine counts coupled with viability stains for phytoplankton, and grow of bacterial colonies for indicator microbes ([David and Gollasch, 2015](#)).
- Actions related to “Development of Ballast Water Management strategies”: A major concern of the BWM Convention is to test and approve the efficacy of the two main ballast water management actions, which are the interim Ballast Water Exchange (BWE) in open sea, at least from 200 nautical miles from the nearest land and at 200 m depth, and the installation of on-board Ballast Water Treatment Systems (BWTS) such as filtration combined with chemical (e.g. chlorination, use of biocides) or physical treatments (e.g. UV radiation, deoxygenation) ([David and Gollasch, 2015](#); [Stehouwer et al., 2015](#); [Tsolaki and Diamadopoulos, 2010](#)). When

the Ballast Water Exchange is not possible because the distance and depth requirements cannot be met, alternative area to do the exchange needs to be designated and biological assessment of the area should be performed to check the presence of Harmful Aquatic Organisms and Pathogens (HAOP). To test the performance of these measures, Regulations D-1 and D-2 standards ([IMO, 2004](#)) were set; the former by exchanging ballast water in a rate enough that guarantees “almost” clean waters, and the later by limiting the amount of viable individuals (zooplankton and phytoplankton) and concentration of indicator microbes that can be discharged ([Albert et al., 2013](#)). To assess the compliance with Regulation D-2, Ballast Water Treatment Systems must be rigorously tested before approval with similar biological methods as the one used “in real” for testing the compliance of a vessel. During the land-based and ship-board tests for approval, biological methods are used to detect, enumerate, identify viable organisms, and these methods need to take into account the organisms' rarity in treated water.

- Actions related to “Risk assessment for granting exemptions”: Shipping companies will likely seek exemptions from applying the BWM Convention to avoid the extra time and investment required for BWE and BWTS. Regulation A-4 states that granting exemptions for 5 years is possible if the process follows Guidelines G7, which provides advice regarding scientifically robust risk assessments ([IMO, 2007](#)). Three risk assessment approaches have been outlined based on comparison of environmental factors such as temperature and salinity or distribution of HAOP, to assess the likelihood of survival of a transferred species between recipient and donor regions: the environmental matching, the species biogeographical, and the species-specific approaches ([IMO, 2007](#)). Biological methods are required for doing such risk assessments and are based on comprehensive port baseline surveys and identification of target species ([HELCOM/OSPAR, 2013](#)).
- Actions related to “Additional measures for warning concerns”: The BWM Convention encourages monitoring ballast water uptake zones, especially in areas known to contain populations of HAOP such as harmful algae bloom species near sewage outfalls ([IMO, 2004](#)). Also, parties may develop a higher level of protection against species introduction if they prove the nature of their concern (*i.e.* the potential consequences of the introduction of harmful organisms in the concerned area) and detail the additional measures required. If identification of species is needed, it must be done following a scientific risk assessment at least to the same level of rigor as in Guidelines G7.

## 3. Overview of the genetic tools and their application in ballast water management

Genetic tools have been increasingly developed to screen ballast water biodiversity and, tightly related, also for monitoring commercial ports ([Table 2](#)). In general, genetic tools provide more accurate and cost-effective taxonomic identification compared to a visual taxonomy approach at all life stages such as eggs, spores, larvae, resting stages, juveniles and broken/incomplete adults; this is particularly relevant for Non-Indigenous Species (NIS) planktonic life-history stages ([Darling and Tepolt, 2008](#); [Harvey et al., 2009](#); [Mountfort et al., 2012](#)), benthic invertebrates and resting stages from ballast tanks sediments ([Briski et al., 2011, 2010](#)). Besides, genetic tools proved to be highly sensitive to early detect organisms in very low abundance ([Pochon et al., 2013](#)). The workflow of the genetic-based studies includes (1) sampling for target species, (2) extraction of the molecule of interest (DNA or RNA), (3) amplification of a particular region of the genome or transcriptome and analysis of the amplified product(s).

- (1) Genetic analyses have been performed on all types of samples retrieved from ballast tanks or ports such as sorted individual specimens ([Miralles et al., 2016](#)), mixed specimens (e.g. zooplankton

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