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Title: 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.

Keywords:

ballast water management convention; non-indigenous species; early detection; genetic methods; metabarcoding

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 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).

Table 1. Regulations and associated Guidelines of the BWM Convention; shaded grey indicates Guidelines where genetic tools could provide relevant biological data

Regulations (R)	**Guidelines (G)**
Section A: General provisions	
R. A1: Definitions	
R. A2: General applicability	
R. A3: Exceptions	
R. A4: Exemptions	G7: Guidelines for Ballast Water Risk Assessment
R. A5: Equivalent Compliance	G3: Guidelines for Ballast Water Management Equivalent Compliance
Section B: Ballast Water Management and control requirements for ships	
R. B1: Ballast Water Management Plan	G4: Guidelines for Ballast Water Management and the Development of Ballast Water Management Plans
R. B2: Ballast Water Record Book	
R. B3: Ballast Water Management for ships	G5: Guidelines for Ballast Water Reception Facilities
R. B4: Ballast Water Exchange	G14: Guidelines on Designation of Areas for Ballast Water Exchange
	G6: Guidelines for Ballast Water Exchange
R. B5: Sediments Management for Ships	G1: Guidelines for Sediment Reception Facilities
	G12: Guidelines on Design and Construction to Facilitate Sediment Control on Ships
R. B6: Duties of officers and crew	
Section C: Special requirements in certain areas	
R. C1: Additional measures R. C2: Warning concerning Ballast Water uptake in certain areas and related flag state measures R. C3: Communication of information	G13: Guidelines for Additional Measures regarding Ballast Water Management including Emergency Situations

Section D: Standard for Ballast Water Management	
R. D1: Ballast Water Exchange standard	G2: Guidelines on Ballast Water Sampling G6: Guidelines for Ballast Water Exchange G11: Guidelines for Ballast Water Exchange Design and Construction Standards
R. D2: Ballast Water performance standard	G2: Guidelines on Ballast Water Sampling
R. D3: Approval requirements for Ballast Water Management Systems	G8: Guidelines for Approval of Ballast Water Management Systems G9: Procedure for Approval of Ballast Water Management Systems that Make Use of Active Substance
R. D4: Prototype of Ballast Water Treatment Technologies	G10: Guidelines for Approval and Oversight of Prototype Ballast Water Treatment Technology Programs
R. D5: Review of standards by the organization	
Section E: Survey and certification requirements for Ballast Water Management	
R. E1: Surveys	
R. E2: Issuance or Endorsement of a certificate	
R. E3: Issuance or Endorsement of a certificate by another party	
R. E4: Form of the certificate	
R. E5: Duration and validity of the certificate	
Article	**Guidelines**
Article 9: Inspection of ships	G15: Guidelines for Port State Control under the BWM Convention

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Testing the compliance with the BWM Convention requirements

Regulations: B1-D1-D2; Guidelines: G2-G4-G15

Aim: *Conduct a port state control inspection to verify compliance*

Actions

Initial inspection: Check the presence of ballast water documentation, inspection of the equipment, the training of officer

- **More detailed inspection:** Check in detailed the ballast water management operations
  - **Sampling for indicative analysis:** Quick direct or indirect measurement of a representative sample of ballast water
    - **Sampling for detailed analysis:** Direct measurement to determine the viable organism concentration

Risk assessment for granting exemptions

Regulation: A-4; Guidelines: G7

Aim: *Scientifically robust risk assessment to assess the feasibility of granting exemptions*

Actions

Environmental matching risk assessment
Species biogeograhical risk assessment
Species specific risk assessment

Additional measures for warning concerns

Regulations: C1-C2-C3; Guidelines: G13

Aim: *Parties may introduce additional measures and alert regarding the ballast water uptake in non recommanded areas*

Actions

Assess the need for and the nature of the measures:
-Identify harmful aquatic organisms and pathogens in the area;
-Description of the cause of the identified concern;
-Identification of the potential aditional measures;
-Identification of the potential effects and consequences, beneficial and detrimental, resulting from the introductuction of the measure

Development of Ballast Water Management strategies:

- **Ballast Water Treatment Systems (BWTS)**

Regulations: D2-D3-D4; Guidelines: G3-G8-G9-G10

Aim: *Provide the necessary framework for approval of BWTS (with and without use of active substances) to meet standards of Regulation D-2*

Actions

-Evaluation of documentation for Pre-Test Evaluation of the BWTS
-Land-based tests: Determine the biological efficacy of the BWTS under defined challenge water conditions (different salinities range and associated dissolved and particulate content, specific organism concentration)
-Ship-board tests: Check the practicability and the biological efficacy by comparing water from treated and non treated ballast tanks
-For BWTS using active substances: Check all the active substances, characterize the associated risk for environmental and human safety

- **Ballast Water Exchange (BWE)**

Regulations: B4-B5-D1; Guidelines: G6-G11-G12-G14

Aim: *Develop the best design for ballast water tank to meet standards of Regulation D-1 and conduct BWE in required and specifc areas*

Actions

-Design and construction of a ship:
Maximizing the efficiency of BWE, shortening the time of BWE, increasing the range of sea conditions under which BWE may be conducted safely and minimizing the accumulation of sediments
-Conduct BWE must be reported in the ballast water record book
-Design of BWE area when the distance from the nearest land and depth required can not be met: Risk assessment of biological, oceanographic, physico-chemical, environmental, important resources, ballast water operations parameters

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Figure 1: Highlights of the main actions with associated Regulations and Guidelines proposed by the BWM Convention grouped in four categories to prevent Harmful Aquatic Organisms and Pathogens (HAOP) introduction. The actions requiring biological analyses are shown in red.

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 mains 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 meter 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 designate 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 commercials 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 et al., 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 Brown et al., 2016), filtered water (Zaiko et al., 2015b) or sediment (Briski et al., 2011). From filtered water or sediment, it is possible to extract environmental DNA (eDNA) which refers to the genetic material released in the surrounding environment in form of feces, saliva, tissue, or simply free DNA (Lodge et al., 2012). Analyzed eDNA isolated from several liters of water seems very promising as it allows early identification of taxa present in low abundances such as recently introduced NIS, which are not detectable by traditional means (Lodge et al., 2012; Valentini et al., 2015).

(2) Genetic tools target mostly DNA as a molecule for species identification because it is stable, long-lived and can be easily preserved (Pereira et al., 2008). Yet, DNA is limited when detecting living organisms is required, so RNA is usually targeted instead (Cenciarini-Borde et al., 2009; Doblin et al., 2007).

(3) Most genetic tools described in Table 2 are based on the Polymerase Chain Reaction (PCR). This technique utilizes primers (short DNA fragments that bind to a specific region of the genome) to amplify a region of interest. Primers can be species-specific (Patil et al., 2005), target a given taxonomic group (Stehouwer et al., 2013a) or even the whole diversity (Zaiko et al., 2015b). In the conventional PCR, the amplified product is monitored at the end of the reaction, and can be either analyzed by electrophoresis to check presence/absence or to differentiate among closely related species (*e.g.* DGGE; see Darling and Blum, 2007), or sequenced. In the Quantitative PCR (qPCR), the amplified product is measured in real time during the PCR allowing to quantify the copy number of the targeted gene (Nathan et al., 2014; Wood et al., 2013). Amplicons from single species are generally obtained using non-specific primers and directly sequenced by Sanger in a process named barcoding (Hebert et al., 2003a). Mix of species can be amplified using species-specific primers to look for a given species and sequenced by Sanger or amplified using more generic primers to amplify several species at the same time and sequenced on High-Throughput Sequencing (HTS) platforms

(Shokralla et al., 2012) in a process called metabarcoding (Taberlet et al., 2012), which has been described as a powerful tool to detect invasive species (Comtet et al., 2015). At the end of the process, comparison of the sequences to a reference database such as BOLD (www.barcodinglife.org) to assign barcodes to species or taxonomic groups is required. The choice of the DNA barcode depends on the research question (*e.g.* species detection level or broad biodiversity assessment) and the taxonomic group of interest. Traditionally, the mitochondrial cytochrome c oxidase subunit I gene (COI) is used for metazoans (Hebert et al., 2003b), the 18S RNA gene for protists (Forster et al., 2016) and the 16S RNA for bacteria (Muyzer et al., 1993). The metabarcoding approach has been widely used in ballast water biodiversity monitoring to screen zooplankton (Ghabooli et al., 2016) and phytoplankton (Steichen and Quigg, 2015). Also, interestingly, the development of HTS is opening opportunities to assess the biodiversity of usually under looked microorganisms such as bacteria, virus (Kim et al., 2015; Ma et al., 2009; Xu et al., 2011) and eukaryotic microorganisms also called protists (Pagenkopp Lohan et al., 2015; Pagenkopp Lohan et al. 2017; Steichen et al., 2014). Many protists are toxic and pathogen species and are considered to be very abundant in ballast water, which underlines the importance of detecting them with a passive surveillance genetic approach such as developed in Pagenkopp Lohan and collaborators (2015).

Table 2: Examples of studies applying genetic tools to ballast water management

Molecule	Techniques	Target gene(s)	Target organisms	Objectives	References
DNA RNA	qPCR NASBA	groEL and tcpA	*Vibrio cholerae*	To develop molecular methods for rapid monitoring of *V. cholerae* in ballast water according to the detection levels set by the IMO (1CFU/100 ml)	Fykse et al., 2012
DNA	qPCR	18S and ctxA	*Vibrio cholerae* and *Potamocorbula amurensis*	To test the efficiency of qPCR for detecting *V. cholerae* and *P.amurensis* in sediments, biofilm, benthic assemblage and seawater	Mountfort et al., 2012
DNA	qPCR Metabarcoding (Cloning)	18S, 16S, LSU, 23S, myc, hly, himA, lpaH, hsp, ctxA, vvhA	Harmful microalgae, cyanobacteria, Bacteria	To determine the tank variability resulting from ballast age, season, region, vessel characteristics, and exchange status	Burkholder et al., 2007
DNA RNA	qPCR Species-specific sequencing	16S and mcyE	Toxic cyanobacteria: *Microcystis sp* and *Anabaena sp*	To investigate harmful microalgae dynamics (viability and toxin production) in ballast water and to provide data for port to-port risk assessment	Doblin et al., 2007
DNA	Species-specific sequencing	SSU and LSU rDNA	Harmful algae: *Gymnodinium catenatum*	To specifically detect *G. catenatum* in cultures, in heterogeneous ballast water and environmental samples	Patil et al., 2005
DNA	Species-specific sequencing Metabarcoding (Cloning)	18S,ITS,5.8S, 28S	Nonnative bivalves, crustaceans and algae	To assess the presence of targeted species in ballast water and ocean environmental samples	Harvey et al., 2009
DNA	Metabarcoding (Cloning)	16S	Cultivable bacteria	To explore for the presence of novel micro-organisms species in ballast water	Xu et al., 2011
DNA	RFLP	COI	*Carcinus. maenas* and *C. aestuarii*	To detect *C. maenas* and *C. aestuarii* larvae in environmental samples	Darling et al., 2008

DNA	RFLP Metabarcoding (Cloning)	16S	Planktonic bacterial community	To compare bacterial community structure of ballast water to the local seawater; and to use this study as background information to risk assessment	Ma et al., 2009
DNA	DGGE Species-specific sequencing	COI and 18S	Asterias larvae	To specifically detect *A. amurensis* larvae in ballast water samples	Deagle et al., 2003
DNA	DGGE Barcoding	16S	Heterotrophic bacterial community	To study survival and succession of heterotrophic bacteria after disinfection by ultraviolet irradiation or ozonation of seawater	Hess-Erga et al., 2010
DNA	DGGE Barcoding	16S	Phytoplankton	To compare different analytical techniques for phytoplankton counting and identification	Stehouwer et al., 2013a
DNA	DGGE Barcoding	16S	Microbial community	To study the environmental impact of acetate compounds of Peracleans@Ocean Ballast Water Treatment System on microbial dynamic	Stehouwer et al., 2013b
DNA	DGGE Barcoding	18S	Eukaryotic microorganisms and dinoflagellates and diatoms	To examine the eukaryotic microorganism diversity being discharged into a port	Steichen et al., 2014
DNA	DGGE Barcoding	18S	Dinoflagellates and diatoms	To test the viability of phytoplankton transiting via ballast water	Steichen and Quigg, 2015
DNA	Barcoding	COI and 16S	Invertebrate dormant stages	To assess the effect of the saltwater flushing regulations on the density and diversity of invertebrate dormant stages in ballast sediment	Briski et al., 2010
DNA	Barcoding	COI and 16S	Diapausing eggs	To test the accuracy of DNA barcoding as a tool for species-level identification of diapausing eggs of invertebrates found in ballast sediment	Briski et al., 2011
DNA	Barcoding	COI and 16S	Adult macroinvertebrates	To explore the presence and species diversity of adult macroinvertebrates transported by transoceanic and coastal vessels	Briski et al., 2012
DNA	Barcoding	16S	*Pseudomonas sp* and *Vibrio sp*	To evaluate a rapid and cost-effective method for monitoring bacteria in ballast water	Emami et al., 2012
DNA	Barcoding	COI and 16S	Zooplankton	To compare the efficiency of ballast water exchange plus ballast water treatment versus ballast water treatment alone	Briski et al., 2015
DNA	Barcoding	COI and 16S	Mollusks	To investigate exotic mollusks in 3 ports of different intensities of maritime traffic in the Cantabrian Sea	Pejovic et al., 2015
DNA	Barcoding	COI	Zooplankton	To examine the genetic information of the known NIS *Acartia tonsa* in estuaries including the commercial port of Bilbao and to discover any novel invasive species	Albaina et al., 2016
DNA	Barcoding	18 and COI	Invertebrates	To inventory the fouling invertebrate communities from 8 ports in Northern Spain	Miralles et al., 2016
DNA	Barcoding	COI, 12S and 16S	Zooplankton	To identify coastal NIS present in the non-exchanged ballast water: assessment of the effectiveness of ballast water exchange	Ware et al., 2016
DNA	Metabarcoding	16S	Microbial community	To determine the effectiveness of NaOH treatment to reduce microbial community structure in ballast water	Fujimoto et al., 2014
DNA	Metabarcoding	COI	Mollusk *Peringia ulvae*	To detect mollusk species that could have survived in ballast water	Ardura et al., 2015a
DNA	Metabarcoding	16S	Viral community	To investigate the composition and taxonomic diversity of viruses in ballast water	Kim et al., 2015
DNA	Metabarcoding	V4 and V9 SSU	Protists	To identify microbial eukaryotes present in ballast water	Pagenkopp Lohan et al., 2015
DNA	Metabarcoding	COI	All organisms	To assess the performance of metabarcoding for species detection in ballast water and assess consistency of	Zaiko et al., 2015a

				different sequencing platforms; and possible biases due to DNA decay and/or degradation in ballast water	
DNA	Metabarcoding	RuBisCo and COI	All organisms	To assess the applicability of metabarcoding for detection of organisms in ballast waters during en-route survey	Zaiko et al., 2015b
DNA	Metabarcoding	18S	Zooplankton	To detect NIS with the application of metabarcoding in 16 Canadian ports	Brown et al., 2016; Chain et al., 2016
DNA	Metabarcoding	18S	Zooplankton	To assess community changes in zooplankton transiting in ballast water	Ghabooli et al., 2016
DNA	Metabarcoding	18S	Eukaryotic from early biofouling communities	To assess the potential of metabarcoding to detect the community structure of biofouling eukaryotic assemblages and the patterns of initial succession in ports	Zaiko et al., 2016
DNA	Metabarcoding	V4 SSU	Protists	To compare diversity and community composition of protists in ballast water among and between ports	Pagenkopp Lohan et al., 2017
DNA	Metabarcoding	16S	Bacteria	To analyze diversity and community composition of bacterial assemblages in ballast water of commercial ships following voyages in the North Atlantic Ocean.	Lymperopoulou and Dobbs, 2017
DNA	Metabarcoding	16S	Bacteria	To characterize changes in bacterial assemblages of ballast water and assess the effects of ballast water exchange, duration time of the voyage as well as season sampling	Johansson et al., 2017

4 Genetic data for helping the implementation of the BWM Convention

The potential of the most promising genetic tools for performing biological analyses required by the Guidelines of the Convention are exposed in Table 3, and are further described in sections 4.1 to 4.2. This analysis comes from crossing the biological analyses required for each Guidelines and the review of the studies presented in Table 2, where a genetic approach has been used to supply biological data relevant for BWM Convention.

Table 3: Main genetic techniques with their related characteristics for ballast and port water monitoring and BWM Convention's Guidelines where they could be integrated (see also Table 1).

	Monitoring application (and associated guidelines)			
	For ports and specific areas		**For ballast water discharges or in tank**	
	Biological baseline survey (G7 and G14)	**Target species identification (G7, G13 and G14)**	**Assessing viable community (G2, G8, G10 and G15)**	**Target species identification (G2, G8, G10 and G15)**
Genetic method proposed	**Metabarcoding**	**qPCR**	**Metabarcoding**	**qPCR**
Key requirements of the BWM Convention:				
Estimation of viability	Not required		RNA or DNA coupled with propidium monoazide: need experimental studies (*e.g.* mock communities)	
Estimation of abundance	Low: relative abundance, still limited	Yes	Low: relative abundance, still limited	Yes

Species level detection	Moderate to high	High	Moderate to high	High
Low abundance detection	Moderate to high	High	Moderate to high	High
Portability	Not required	Not required	Portable machines: PCR, sequencer	Portable qPCR: need further development
Time-Consumption per analysis	Weeks with traditional sequencing platform but could be reduced to hours with portable devices (*e.g.* MinION)	Hours	Weeks with traditional sequencing platform but could be reduced to hours with portable devices (*e.g.* MinION)	Hours
Cost: operating costs, consumable, personnel	Low for high number of samples processed	Low	Low for high number of samples processed	Low
Standardization	High potential: need developed protocol	High: need developed species specific primers for all targeted species	High potential: need developed protocol	High: need developed species specific primers for all targeted species

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206 ### 4.1 Compliance inspection: Guidelines G2, G8, G10 and G15

207 Testing the compliance with the discharge standards of Regulation D-2 does not require species
208 identification (except for indicator microbes) and only relies on the number of viable organisms.
209 Nonetheless, the Article 6 of the BWM Convention asks to each Party to promote relevant information
210 on the effectiveness of ballast water management obtained from any monitoring program (IMO,
211 2004). In this context, identification of organisms discharged is beneficial to assess the efficiency of
212 the ballast water management measures and thus reduce the events of introduction of Harmful Aquatic
213 Organisms and Pathogens, at the same time, early detect introduction of a potential NIS.

214 #### 4.1.1 Determine viability of organisms

215 Viability is the key criteria to assess if a vessel will comply with Regulation D-2 and the universal
216 method to detect only viable organisms is yet to be developed. Nonetheless, genetic tools hold great
217 potential if their targets are linked with viability such as using RNA or if the technique used prevent
218 the amplification of DNA from dead cells such as with the application of propidium monoazide
219 (PMA) (Fujimoto et al., 2014). Doblin et al (2007) shows the potential of RNA analyses to reveal that
220 harmful algae species could synthesis active toxin after 11 days in a ballast water tank. To assess the
221 living community discharged with ballast water targeting RNA instead of DNA for metabarcoding
222 has been proposed as an appealing solution (Zaiko et al., 2015a, 2015b) since studies already
223 successfully developed protocols based on RNA metabarcoding to screen living protists (Pawlowski
224 et al., 2014; Visco et al., 2015). Yet, RNA is very difficult to preserve, and thus working with RNA
225 is more challenging when compared to DNA. Nonetheless, several studies have proposed optimized
226 protocols to extract high quality RNA (Asai et al., 2015; Zhang et al., 2013). Concerning the use of

propidium monoazide, it seems well defined for microbes as Fujimito et al (2014) used it to show alkali ballast water treatment efficacy to reduce alive microbial diversity. Nonetheless, its potential remains unclear for multicellular organisms such as for zooplankton tested in compliance (Lance and Carr, 2012; Zetsche and Meysman, 2012) and further research is needed.

4.1.2 Test the efficacy of ballast water management

Genetic methods have been successfully used to provide biological data on the effects of ballast water management by identifying the community found in the tanks or at the discharge line after the Ballast Water Exchange (Briski et al., 2015, 2010; Ware et al., 2016), by assessing the efficacy of Ballast Water Treatment Systems to reduce bacteria and phytoplankton diversity (Fujimoto et al., 2014; Stehouwer et al., 2013b, 2013b) and by identifying the composition of "recolonizer" organisms after the application of a treatment (Hess-Erga et al., 2010). A future interesting direction will be to develop RNA metabarcoding (see 4.1.1) to provide a better depiction of "the living organisms" after the application of the Ballast Water Treatment System.

4.1.3 Assess the representativeness of sampling to inspect compliance

Sampling for compliance testing requires that samples must be representative of the whole ballast water discharged (IMO, 2008). The large volumes of water, tank characteristics (shape, size and number) and heterogeneous distribution along with low density of organisms within tanks make very challenging to develop the best representative approach for compliance sampling and research is still going on (Basurko and Mesbahi, 2011; Carney et al., 2013; Hernandez et al., 2017). To our knowledge, genetic tools have never been applied to such use yet. Metabarcoding could be very useful to assess if there is a significant difference in the communities inferred using alternative sampling approaches. Indeed, the volume, number and frequency of samples, and the availability to sample from the discharged lines or directly from the tank are factors that need to be tested and optimized to get an accurate representation of the discharged community.

4.2 Target species detection and biological monitoring: Guidelines G7, G13 and G14

4.2.1 Risk assessment for granting exemptions

HELCOM/OSPAR Guidelines have been developed in line with the Guidelines G7 to provide advice on risk assessment procedures for granting exemptions from the port survey sampling to the decision support and administrative decisions (HELCOM/OSPAR, 2013). The biological methods proposed are based on considerable sampling effort of all types of organisms to detect Non-Indigenous Species (NIS) and native harmful species and on checking the presence of potential pathogens. The analysis of NIS is done with a visual taxonomic identification approach. The port of Rotterdam, for example,

has conducted such survey and 32 NIS were detected over 225 species identified to the species level recorded in more than 250 samples taken from 118 different locations (Gittenberger et al., 2014). The species accumulation curves indicated that more sampling would have resulted in scoring more species for several habitats of the port. So, despite such important sampling effort, the entire diversity of the habitats was not recovered. Genetic methods, by enhancing detection sensitivity, increasing specificity of target identification and reducing monitoring time and costs, hold great promise over traditional methods (Darling and Mahon, 2011) and has been proposed as a cost-effective method in this context (Comtet et al., 2015; Lehtiniemi et al., 2015).. Genetic-based background information to assess the risk associated with Harmful Aquatic Organisms and Pathogens in ports are numerous. Examples are Ma et al., 2009 for pathogens, Miralles et al., 2016; Pejovic et al., 2015 for invasive mollusks, Doblin et al., 2007 for harmful algae species, Brown et al., 2016 for zooplankton, Hirst and Bott, 2016 for fouling organisms and zooplankton, Pochon et al., 2015; Zaiko et al., 2016 for early fouling organisms. One very interesting study in Canadian ports shows that metabarcoding detected 24 NIS species of zooplankton of which 11 were firstly reported in this regions (Brown et al., 2016**)**. Also, the combination of sampling artificial settlement plates with DNA metabarcoding based species detection seems very promising to early detect potential invasive species in ports where species are at very low densities and morphologically undistinguishable early life stages occur (Pochon et al., 2015).

4.2.2 Specific ballast water uptake zone

As described in section 2. The preventing actions of the BWM Convention and their required biological analyses in Actions related to "Development of Ballast Water Management Strategies" and "Additional measures for warning concerns", biological monitoring is required when there is a need to design alternative ballast water exchange areas and when a Party shall warn about the uptake of ballast water in certain zones. In both cases, metabarcoding appear as sensitive methods to perform such biological monitoring as they allow to detect sewage contamination environment by identifying sewage-related bio-indicator microbial species (Tan et al., 2015). Also, qPCR has been demonstrated to play a major role in identifying harmful algae bloom species, understanding their ecology as well as facilitating the management of their outbreaks (Antonella and Luca, 2013).

5 Future investments for the use of genetic tools in ballast water management

5.1 Standardization and further development of genetic methods

Implementation of genetic methods for regular application in ballast water monitoring requires benchmarking against traditional methods and standardization of procedures (Aylagas et al., 2016;

Aylagas and Rodríguez-Ezpeleta, 2016). Each step, from sampling to taxonomic assignment, can be performed using alternative protocols and the choice of the most appropriate will depend on i) type of sample, *e.g.* water, sediment, fouling, ii) taxonomic group tackled, *e.g.* bacteria, protists, metazoa, iii) pursued aim, *e.g.* to obtain a comprehensive species inventory or look for a specific species, iv) and other specific conditions such as the need to detect only living organisms. It is thus crucial that reproducible protocols are defined, tested and broadly communicated so that results obtained can be comparable and usable for regular monitoring purposes (Cristescu, 2014). Also, to validate protocols, one key element is the use of artificial communities to i) develop rigorous laboratory workflows (Port et al., 2016) and standard bioinformatic pipelines (Brown et al., 2015; Flynn et al., 2015) and to ii) assess the sensitivity of genetic tools such as metabarcoding to detect invasive species in complex environmental samples (Pochon et al., 2013). Also, one important technical shortcoming of DNA metabarcoding is the PCR bias (amplification success is not equal for all taxa present in a sample) which can lead to false negatives and impede estimating taxa abundances based on sequences (Elbrecht and Leese, 2015). To overcome PCR bias, community genome sequencing (metagenomic) could be applied (Taberlet et al., 2012). Although this approach shows promising results regarding their use to species identification and estimate relative abundance (Gómez-Rodríguez et al., 2015; Srivathsan et al., 2015; Tang et al., 2015, 2014), it is much more expensive and requires more complicated data analyses that rely on the availability of large numbers of complete genomes in databases, which is currently only existing for bacteria**.**

5.2 Improvement of reference databases

Both DNA barcoding and metabarcoding rely on the availability of a well populated and curated reference database that associates DNA sequences with species names (*e.g.* BOLD www.barcodinglife.org, Silva www.arb-silva.de). Unfortunately, such databases are far from being complete (Aylagas et al., 2014; Comtet et al., 2015) and are usually biased towards certain organismal groups (Briski et al., 2016). Recently, significant effort has been devoted to increase reference databases, and the number of sequences, especially from eukaryotes, has notably increased during the past few years (Briski et al., 2016). Additionally, the AquaNIS database (www.corpi.ku.lt/databases/aquanis) which constitutes the reference information system on aquatic non-indigenous and cryptogenic species, is also gathering molecular information among all the information recorded for a species and has undertaken a promising step forward action to integrate molecular data into the management of NIS. Also, the metabarcoding research is moving forward to the use of a multiple marker approach (*e.g.* COI and 18S) instead of a single marker which help providing a better catalogue of biodiversity (Cowart et al., 2015; Marcelino and Verbruggen, 2016;

Zaiko et al., 2015b). This approach is thus, strongly encouraged for future studies assessing the ballast water discharged community.

5.3 Increasing practicability of genetic tools in the field

The BWM Convention requires fast, portable and user-friendly sampling and analysis protocols which appears difficult to achieve with genetic tools because most require specialized equipment and training. Nonetheless, several alternatives have been designed and could be considered for detecting species in ballast water samples such as combining "minutes"-DNA/RNA extraction techniques with portable DNA/RNA amplification devices such as portable PCR or Loop-mediated isothermal amplification (LAMP) machine (Agrawal et al., 2007; Lee, 2017). LAMP is commonly used for food safety testing for pathogens and fungi as it has a very short reaction time, only requires a heating block to perform the amplification and holds high sensitivity for species detection (Niessen et al., 2013). Also, in the context of passive surveillance (*i.e.* screening the biodiversity) of ballast water, the development of the Oxford Nanopore Technologies® MinION device, a miniaturized and portable real-time high-throughput sequencer, shows great promises to reduce the time of samples processing and facilitate *in situ* monitoring for environmental research (Edwards et al., 2016; Mitsuhashi et al., 2017; Ramgren et al., 2015). Mitsuhashi et al (2017) shows the possibility of rapid sequencing and bacterial composition identification within 2 hours. Nonetheless, the in-field preparation of samples (homogenization, DNA/RNA extraction and library preparation) as well as the bioinformatic analyses still needs optimized protocols and further adjustments to be used routinely in ballast water monitoring.

5.4 Exploring alternative sampling strategies

eDNA appears very promising source from which to infer biodiversity that reduces sampling effort especially in a context of port baseline survey or targeted species identification where exhaustive sampling is usually required. The field of sampling eDNA for detecting invasive species is of growing interest as numerous studies show its great potential (Ardura et al., 2015b; Jerde et al., 2011; Simmons et al., 2015), and eDNA should be a focal point of research efforts to apply its use in ports monitoring in a near future. Yet, eDNA degradation appears to be influenced by environmental conditions and the approximate time range can go from less than one day to a couple of weeks (Barnes et al., 2014; Thomsen et al., 2012) challenging it use as a proxy for the living organisms present in an environment such as a ballast water tank. Future work is needed to disentangle the complex relationship between eDNA persistence in ballast tank and the actual presence of organisms, especially in a context of ballast water sampling for compliance. But, interestingly, monitoring eDNA in ballast water can detect species that survive the harsh conditions of a ballast water tank by showing an increase in

proportion of DNA signal during the journey (Ardura et al., 2015a; Zaiko et al., 2015b). These studies show the potential of eDNA sample to monitor ballast water biodiversity to potentially detect trend in species that could need a special attention to be monitored in case of discharge of ballast water into a port.

5.5 Efforts to integrate of genetic methods into monitoring programs

Despite the potential of genetic tools for ballast water monitoring and management, the application of these tools by stakeholders remains still experimental, as it has associated challenges (Bucklin et al., 2016; Darling, 2015; Darling and Mahon, 2011; Kelly et al., 2014; Wood et al., 2013). Only very few countries such as New Zealand or Australia started to integrate genetic tools to early detect invasive species and harmful algal blooms species (Hirst and Bott, 2016; Wood et al., 2013). One major issue is the difficult communication between researchers and stakeholders, which makes the former unaware of the real problems and the latter unknowledgeable of the potential of genetic tools (Darling, 2015). To provide an effective integration of genetic methods into monitoring programs, geneticists, environmental monitoring agencies and ballast water policy stakeholders (*e.g.* Port State Control) should together define standardized processes so that genetic methods can provide reliable data for decision making (*e.g*. interdisciplinary theoretical and hands-on workshops are encouraged).

6 Conclusions and future directions

The analyze of the research studies using genetic tools for ballast water monitoring exposed the great potentials of such tools to be applied from the risk assessment to the early detection and monitoring of Non-Indigenous Species and so, provide valuable information at all stages of the invasion process (Fig. 2).

Given their potential in terms of accuracy and cost-effectiveness along with their fast-growing evolution, genetic tools, with an emphasize on metabarcoding, should definitely continue to be developed for ballast water monitoring. DNA metabarcoding and qPCR standardized protocols need to be created for performing respectively, port baseline surveys and target species detection required in the BWM Convention in Guidelines G7, G13 and G14. Nonetheless, genetic tools are facing technical challenges associated to the techniques itself (*e.g.* lack of completed reference database, standardized protocols) or specific to ballast water monitoring. Indeed today, genetic tools do not fulfill all the key criteria of the BWM Convention such as portability and rapidity of results or viability assessment. Yet, extensive experimental research is needed to assess the potential of RNA to detect the viable community discharged via ballast water (*e.g.* use of artificial communities), as well as testing the feasibility for on-board genetic analyses. Also, the evaluation of the number and

minimum dimension of organisms discharged via ballast water remains very limited with genetic tools alone and needs to be combined with other appropriate tools. But we are confident that the worldwide burning development of DNA/RNA-based environmental monitoring is leading to better refinement of these tools for qualitative assessment of biodiversity discharged via ballast water.

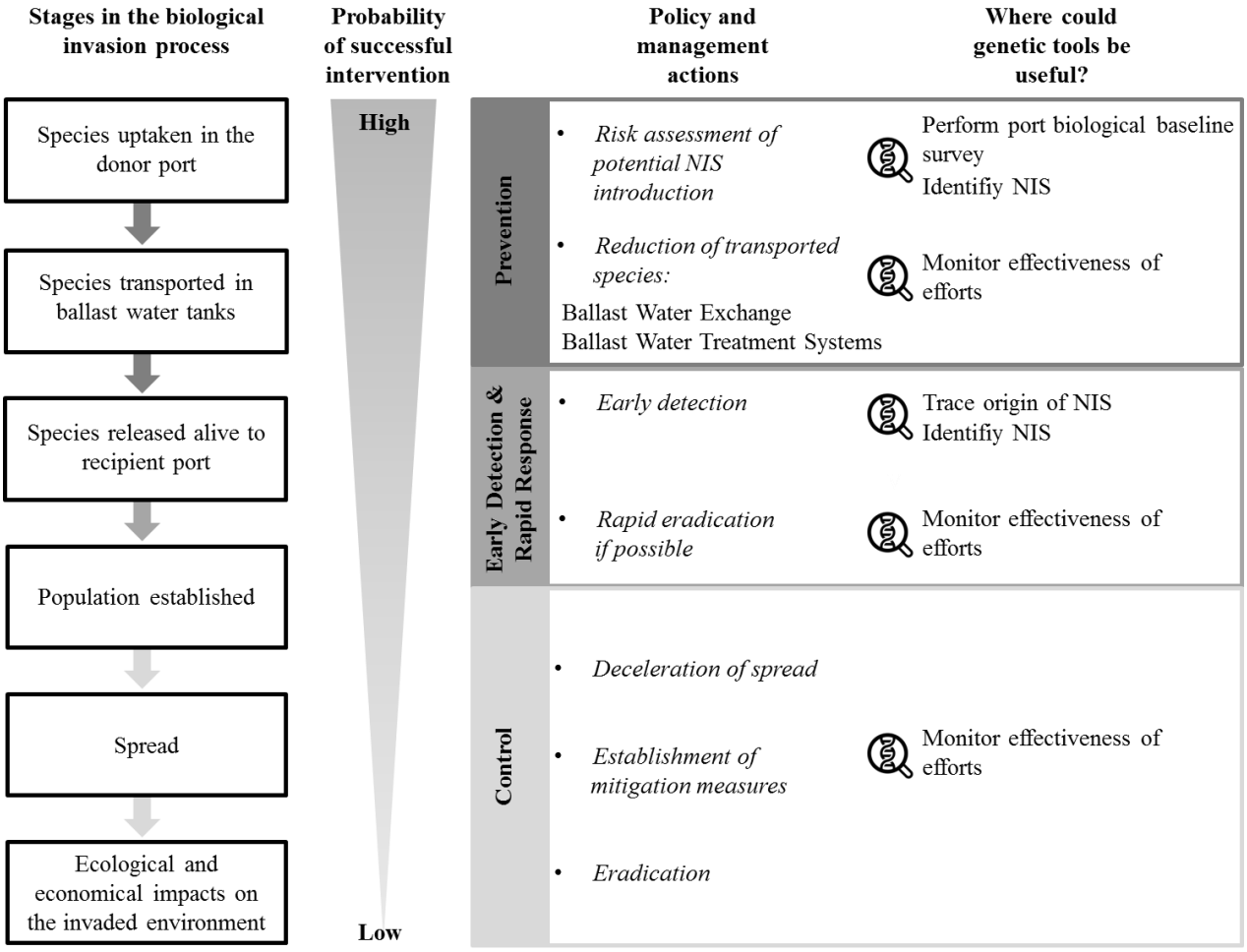

Figure 2: Stages of the ballast water induced invasion process with the corresponding management actions (adapted from Lodge et al., 2006) and usefulness of potential genetic tools

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8 References

Agnarsson, I., Kuntner, M., 2007. Taxonomy in a changing world: seeking solutions for a science in crisis. Syst. Biol. 56, 531–539. https://doi.org/10.1080/10635150701424546

Agrawal, N., Hassan, Y.A., Ugaz, V.M., 2007. A Pocket-Sized Convective PCR Thermocycler. Angew. Chem. Int. Ed. 46, 4316–4319. doi:10.1002/anie.200700306

Aguirre-Macedo, M.L., Vidal-Martinez, V.M., Herrera-Silveira, J.A., Valdés-Lozano, D.S., Herrera-Rodríguez, M., Olvera-Novoa, M.A., 2008. Ballast water as a vector of coral pathogens in the Gulf of Mexico: the case of the Cayo Arcas coral reef. Mar. Pollut. Bull. 56, 1570–1577. doi:10.1016/j.marpolbul.2008.05.022

Albaina, A., Uriarte, I., Aguirre, M., Abad, D., Iriarte, A., Villate, F., Estonba, A., 2016. Insights on the origin of invasive copepods colonizing Basque estuaries; a DNA barcoding approach. Mar. Biodivers. Rec. 9, 51. doi:10.1186/s41200-016-0045-2

Albert, R.J., Lishman, J.M., Saxena, J.R., 2013. Ballast water regulations and the move toward concentration-based numeric discharge limits. Ecol. Appl. 23, 289–300. doi:10.1890/12-0669.1

Antonella, P., Luca, G., 2013. The quantitative real-time PCR applications in the monitoring of marine harmful algal bloom (HAB) species. Environ. Sci. Pollut. Res. Int. 20, 6851–6862. doi:10.1007/s11356-012-1377-z

Ardura, A., Zaiko, A., Martinez, J.L., Samuiloviene, A., Borrell, Y., Garcia-Vazquez, E., 2015a. Environmental DNA evidence of transfer of North Sea molluscs across tropical waters through ballast water. J. Molluscan Stud. eyv022. doi: https://doi.org/10.1093/mollus/eyv022

Ardura, A., Zaiko, A., Martinez, J.L., Samulioviene, A., Semenova, A., Garcia-Vazquez, E., 2015b. eDNA and specific primers for early detection of invasive species–A case study on the bivalve Rangia cuneata, currently spreading in Europe. Mar. Environ. Res. 112, 48–55. doi: https://doi.org/10.1016/j.marenvres.2015.09.013

Asai, S., Ianora, A., Lauritano, C., Lindeque, P.K., Carotenuto, Y., 2015. High-quality RNA extraction from copepods for Next Generation Sequencing: A comparative study. Mar. Genomics, SI: Structure Regulation Evolution 24, Part 1, 115–118. doi:10.1016/j.margen.2014.12.004

Aylagas, E., Borja, Á., Irigoien, X., Rodríguez-Ezpeleta, N., 2016. Benchmarking DNA Metabarcoding for Biodiversity-Based Monitoring and Assessment. Front. Mar. Sci. 3, 96. doi:10.3389/fmars.2016.00096

Aylagas, E., Borja, Á., Rodríguez-Ezpeleta, N., 2014. Environmental status assessment using DNA metabarcoding: towards a genetics based marine biotic index (gAMBI). PloS One 9, e90529. doi:https://doi.org/10.1371/journal.pone.0090529

Aylagas, E., Rodríguez-Ezpeleta, N., 2016. Analysis of Illumina MiSeq Metabarcoding Data: Application to Benthic Indices for Environmental Monitoring. Methods Mol. Biol. Clifton NJ 1452, 237–249. doi:10.1007/978-1-4939-3774-5_16

Barnes, M.A., Turner, C.R., Jerde, C.L., Renshaw, M.A., Chadderton, W.L., Lodge, D.M., 2014. Environmental conditions influence eDNA persistence in aquatic systems. Environ. Sci. Technol. 48, 1819–1827. doi: 10.1021/es404734p

Basurko, O.C., Mesbahi, E., 2011. Statistical representativeness of ballast water sampling. Proc. Inst. Mech. Eng. Part M J. Eng. Marit. Environ. 225, 183–190. doi:10.1177/1475090211412682

449 Bott, N.J., 2015. Detection of Invasive Species, in: Kim S-K. (Ed.), Springer Handbook of Marine
450 Biotechnology. Springer Berlin Heidelberg, pp. 441–453. doi:10.1007/978-3-642-53971-
451 8_16
452 Briski, E., Bailey, S.A., Cristescu, M.E., MacIsaac, H.J., 2010. Efficacy of "saltwater flushing" in
453 protecting the Great Lakes from biological invasions by invertebrate eggs in ships' ballast
454 sediment. Freshw. Biol. 55, 2414–2424. doi:10.1111/j.1365-2427.2010.02449.x
455 Briski, E., Cristescu, M.E., Bailey, S.A., MacIsaac, H.J., 2011. Use of DNA barcoding to detect
456 invertebrate invasive species from diapausing eggs. Biol. Invasions 13, 1325–1340.
457 doi:10.1007/s10530-010-9892-7
458 Briski, E., Ghabooli, S., Bailey, S.A., MacIsaac, H.J., 2016. Are genetic databases sufficiently
459 populated to detect non-indigenous species? Biol. Invasions 18, 1911–1922.
460 doi:10.1007/s10530-016-1134-1
461 Briski, E., Ghabooli, S., Bailey, S.A., MacIsaac, H.J., 2012. Invasion risk posed by
462 macroinvertebrates transported in ships' ballast tanks. Biol. Invasions 14, 1843–1850.
463 doi:10.1007/s10530-012-0194-0
464 Briski, E., Gollasch, S., David, M., Linley, R.D., Casas-Monroy, O., Rajakaruna, H., Bailey, S.A.,
465 2015. Combining Ballast Water Exchange and Treatment To Maximize Prevention of
466 Species Introductions to Freshwater Ecosystems. Environ. Sci. Technol. 49, 9566–9573.
467 doi:10.1021/acs.est.5b01795
468 Brown, E.A., Chain, F.J., Crease, T.J., MacIsaac, H.J., Cristescu, M.E., 2015. Divergence thresholds
469 and divergent biodiversity estimates: can metabarcoding reliably describe zooplankton
470 communities? Ecol. Evol. 5(11), 2234–2251. doi:10.1002/ece3.1485
471 Brown, E.A., Chain, F.J.J., Zhan, A., MacIsaac, H.J., Cristescu, M.E., 2016. Early detection of aquatic
472 invaders using metabarcoding reveals a high number of non-indigenous species in Canadian
473 ports. Divers. Distrib. 22(10), 1045–1059 doi:10.1111/ddi.12465
474 Bucklin, A., Lindeque, P.K., Rodriguez-Ezpeleta, N., Albaina, A., Lehtiniemi, M., 2016.
475 Metabarcoding of marine zooplankton: prospects, progress and pitfalls. J. Plankton Res.
476 fbw023. doi:10.1093/plankt/fbw023
477 Burkholder, J.M., Hallegraeff, G.M., Melia, G., Cohen, A., Bowers, H.A., Oldach, D.W., Parrow,
478 M.W., Sullivan, M.J., Zimba, P.V., Allen, E.H., Kinder, C.A., Mallin, M.A., 2007.
479 Phytoplankton and bacterial assemblages in ballast water of US military ships as a function
480 of port of origin, voyage time, and ocean exchange practices. Harmful Algae 6, 486–518.
481 doi:10.1016/j.hal.2006.11.006
482 Carney, K.J., Basurko, O.C., Pazouki, K., Marsham, S., Delany, J.E., Desai, D.V., Anil, A.C.,
483 Mesbahi, E., 2013. Difficulties in obtaining representative samples for compliance with the
484 Ballast Water Management Convention. Mar. Pollut. Bull. 68, 99–105.doi:
485 10.1016/j.marpolbul.2012.12.016
486 Cenciarini-Borde, C., Courtois, S., La Scola, B., 2009. Nucleic acids as viability markers for bacteria
487 detection using molecular tools. Future Microbiol. 4, 45–64. doi:10.2217/17460913.4.1.45
488 Chain, F.J.J., Brown, E.A., MacIsaac, H.J., Cristescu, M.E., 2016. Metabarcoding reveals strong
489 spatial structure and temporal turnover of zooplankton communities among marine and
490 freshwater ports. Divers. Distrib. 22, 493–504. doi:10.1111/ddi.12427
491 Comtet, T., Sandionigi, A., Viard, F., Casiraghi, M., 2015. DNA (meta) barcoding of biological
492 invasions: a powerful tool to elucidate invasion processes and help managing aliens. Biol.
493 Invasions 17, 905–922. doi: 10.1007/s10530-015-0854-y
494 Cowart, D.A., Pinheiro, M., Mouchel, O., Maguer, M., Grall, J., Miné, J., Arnaud-Haond, S., 2015.
495 Metabarcoding is powerful yet still blind: a comparative analysis of morphological and
496 molecular surveys of seagrass communities. PloS One 10, e0117562.
497 doi:10.1371/journal.pone.0117562

Cristescu, M.E., 2014. From barcoding single individuals to metabarcoding biological communities: towards an integrative approach to the study of global biodiversity. Trends Ecol. Evol. 29, 566–571. doi: 10.1016/j.tree.2014.08.001

Darling, J.A., 2015. Genetic studies of aquatic biological invasions: closing the gap between research and management. Biol. Invasions 17, 951–971. doi:10.1007/s10530-014-0726-x

Darling, J.A., Blum, M.J., 2007. DNA-based methods for monitoring invasive species: a review and prospectus. Biol. Invasions 9, 751–765. doi:10.1007/s10530-006-9079-4

Darling, J.A., Frederick, R.M., 2017. Nucleic acids-based tools for ballast water surveillance, monitoring, and research. J. Sea Res. doi:10.1016/j.seares.2017.02.005

Darling, J.A., Mahon, A.R., 2011. From molecules to management: adopting DNA-based methods for monitoring biological invasions in aquatic environments. Environ. Res. 111, 978–988. doi:https://doi.org/10.1016/j.envres.2011.02.001

Darling, J.A., Tepolt, C.K., 2008. Highly sensitive detection of invasive shore crab (Carcinus maenas and Carcinus aestuarii) larvae in mixed plankton samples using polymerase chain reaction and restriction fragment length polymorphisms (PCR-RFLP). Aquat Invasions 3, 141–152. doi:10.3391/ai.2008.3.2.4

David, M., Gollasch, S. (Eds.), 2015. Global Maritime Transport and Ballast Water Management. Springer Netherlands, Dordrecht.

Deagle, B.E., Bax, N., Hewitt, C.L., Patil, J.G., 2003. Development and evaluation of a PCR-based test for detection of Asterias (Echinodermata: Asteroidea) larvae in Australian plankton samples from ballast water. Mar. Freshw. Res. 54, 709–719.doi:10.1071/MF03031:

Doblin, M.A., Coyne, K.J., Rinta-Kanto, J.M., Wilhelm, S.W., Dobbs, F.C., 2007. Dynamics and short-term survival of toxic cyanobacteria species in ballast water from NOBOB vessels transiting the Great Lakes—implications for HAB invasions. Harmful Algae 6, 519–530. doi: 10.1016/j.hal.2006.05.007

Drake, J.M., Lodge, D.M., 2004. Global hot spots of biological invasions: Evaluating options for ballast–water management. Proc. R. Soc. Lond. B Biol. Sci. 271, 575–580.doi: 10.1098/rspb.2003.2629

Edwards, A., Debbonaire, A.R., Sattler, B., Mur, L.A., Hodson, A.J., 2016. Extreme metagenomics using nanopore DNA sequencing: a field report from Svalbard, 78 N. bioRxiv 73965. doi:10.1101/073965

Elbrecht, V., Leese, F., 2015. Can DNA-Based Ecosystem Assessments Quantify Species Abundance? Testing Primer Bias and Biomass—Sequence Relationships with an Innovative Metabarcoding Protocol. PLoS ONE 10, e0130324. doi:10.1371/journal.pone.0130324

Emami, K., Askari, V., Ullrich, M., Mohinudeen, K., Anil, A.C., Khandeparker, L., Burgess, J.G., Mesbahi, E., 2012. Characterization of bacteria in ballast water using MALDI-TOF mass spectrometry. PloS One 7, e38515. doi:10.1371/journal.pone.0038515

Flynn, J.M., Brown, E.A., Chain, F.J., MacIsaac, H.J., Cristescu, M.E., 2015. Toward accurate molecular identification of species in complex environmental samples: testing the performance of sequence filtering and clustering methods. Ecol. Evol. 5(11), 2252–2266. doi: 10.1002/ece3.1497

Fonseca, V.G., Carvalho, G.R., Sung, W., Johnson, H.F., Power, D.M., Neill, S.P., Packer, M., Blaxter, M.L., Lambshead, P.J.D., Thomas, W.K., Creer, S., 2010. Second-generation environmental sequencing unmasks marine metazoan biodiversity. Nat. Commun. 1, 98. doi:10.1038/ncomms1095

Forster, D., Dunthorn, M., Mahé, F., Dolan, J.R., Audic, S., Bass, D., Bittner, L., Boutte, C., Christen, R., Claverie, J.-M., Decelle, J., Edvardsen, B., Egge, E., Eikrem, W., Gobet, A., Kooistra, W.H.C.F., Logares, R., Massana, R., Montresor, M., Not, F., Ogata, H., Pawlowski, J., Pernice, M.C., Romac, S., Shalchian-Tabrizi, K., Simon, N., Richards, T.A., Santini, S., Sarno, D., Siano, R., Vaulot, D., Wincker, P., Zingone, A., de Vargas, C., Stoeck, T., 2016.

548 Benthic protists: the under-charted majority. FEMS Microbiol. Ecol. 92, fiw120.
549 doi:10.1093/femsec/fiw120
550 Fujimoto, M., Moyerbrailean, G.A., Noman, S., Gizicki, J.P., Ram, M.L., Green, P.A., Ram, J.L.,
551 2014. Application of Ion Torrent Sequencing to the Assessment of the Effect of Alkali Ballast
552 Water Treatment on Microbial Community Diversity. PLoS ONE 9(9): e107534.
553 doi:10.1371/journal.pone.0107534
554 Fykse, E.M., Nilsen, T., Nielsen, A.D., Tryland, I., Delacroix, S., Blatny, J.M., 2012. Real-time PCR
555 and NASBA for rapid and sensitive detection of Vibrio cholerae in ballast water. Mar. Pollut.
556 Bull. 64, 200–206. doi: 10.1016/j.marpolbul.2011.12.007
557 Ghabooli, S., Zhan, A., Paolucci, E., Hernandez, M.R., Briski, E., Cristescu, M.E., MacIsaac, H.J.,
558 2016. Population attenuation in zooplankton communities during transoceanic transfer in
559 ballast water. Ecol. Evol. 6(17), 6170–6177. doi:10.1002/ece3.2349
560 Gittenberger, A., Rensing, M., Niemantsverdriet, P., Schrieken, N., Stegenga, H., 2014. Port of
561 Rotterdam survey and monitoring non-native species conform HELCOM/OSPAR protocol.
562 Gollasch, S., MacDonald, E., Belson, S., Botnen, H., Christensen, J.T., Hamer, J.P., Houvenaghel,
563 G., Jelmert, A., Lucas, I.A.N., Masson, D., McCollin, T., Olenin, S., Persson, A.,
564 Wallentinus, I., Wetsteyn, L.P.M.J., Wittling, T., 2002. Life in ballast tanks, in: Invasive
565 Aquatic Species of Europe. Distribution, Impacts and Management. Springer, pp. 217–231.
566 doi:10.1007/978-94-015-9956-6_23
567 Gómez-Rodríguez, C., Crampton-Platt, A., Timmermans, M.J., Baselga, A., Vogler, A.P., 2015.
568 Validating the power of mitochondrial metagenomics for community ecology and
569 phylogenetics of complex assemblages. Methods Ecol. Evol. 6, 883–894. doi:10.1111/2041-
570 210X.12376
571 Hallegraeff, G.M., 2007. Foreword. Harmful Algae 6, 461–464. doi:10.1016/j.hal.2007.02.001
572 Harvey, J.B.J., Hoy, M.S., Rodriguez, R.J., 2009. Molecular detection of native and invasive marine
573 invertebrate larvae present in ballast and open water environmental samples collected in
574 Puget Sound. J. Exp. Mar. Biol. Ecol. 369, 93–99. doi:10.1016/j.jembe.2008.10.030
575 Hebert, P.D., Cywinska, A., Ball, S.L., deWaard, J.R., 2003a. Biological identifications through DNA
576 barcodes. Proc. R. Soc. Lond. B Biol. Sci. 270, 313–321. doi: 10.1098/rspb.2002.2218
577 Hebert, P.D., Ratnasingham, S., de Waard, J.R., 2003b. Barcoding animal life: cytochrome c oxidase
578 subunit 1 divergences among closely related species. Proc. R. Soc. Lond. B Biol. Sci. 270,
579 96–99.doi:10.1098/rsbl.2003.0025
580 HELCOM/OSPAR, 2013. Joint HELCOM/OSPAR Guidelines for the Contracting Parties of OSPAR
581 and HELCOM on the granting of exemptions under International Convention for the Control
582 and Management of Ships' Ballast Water and Sediments, Regulation A-4 (OSPAR
583 Agreement 2013-09), 45 pp.
584 Hernandez, M.R., Johansson, M.L., Xiao, Y., Lewis, M.A., MacIsaac, H.J., 2017. Modeling sampling
585 strategies for determination of zooplankton abundance in ballast water. Mar. Pollut. Bull.
586 115, 80–85. doi:10.1016/j.marpolbul.2016.11.050
587 Hess-Erga, O.-K., Blomvågnes-Bakke, B., Vadstein, O., 2010. Recolonization by heterotrophic
588 bacteria after UV irradiation or ozonation of seawater; a simulation of ballast water treatment.
589 Water Res. 44, 5439–5449. doi:10.1016/j.watres.2010.06.059
590 Hirst, A., Bott, N.J., 2016. Marine pests in the Gippsland Lakes: Existing threats and future
591 monitoring. doi: 10.13140/RG.2.2.28197.70886
592 IMO, 2016. Global treaty to halt invasive aquatic species to enter into force in 2017.
593 http://www.imo.org/en/MediaCentre/PressBriefings/Pages/22-BWM-.aspx (Accessed:
594 8/9/2016).
595 IMO, 2015. Guidance on ballast water sampling and analysis for trial use in accordance with the
596 BWM Convention and Guidelines (G2), BWM.2/Circ.42.Rev.1 International Maritime
597 Organization, 13 Feb 2004, London.

598 IMO, 2008. Guidelines for ballast water sampling (G2), IMO Resolution MEPC.173(58).
599 International Maritime Organization, 13 Feb 2004, London.
600 IMO, 2007. Guidelines for risk assessment under regulation A-4 of the BWM Convention (G7).
601 Marine Environment Protection Committee, Resolution MEPC.162(56), 13 July 2007.
602 International Maritime Organization, 13 Feb 2004, London.
603 IMO, 2004. International Convention for the Control and Management of Ships' Ballast Water and
604 Sediments, 2004. International Maritime Organization, 13 Feb 2004, London.
605 Jerde, C.L., Mahon, A.R., Chadderton, W.L., Lodge, D.M., 2011. "Sight-unseen" detection of rare
606 aquatic species using environmental DNA. Conserv. Lett. 4, 150–157.doi:10.1111/j.1755-
607 263X.2010.00158.x
608 Ji, Y., Ashton, L., Pedley, S.M., Edwards, D.P., Tang, Y., Nakamura, A., Kitching, R., Dolman, P.M.,
609 Woodcock, P., Edwards, F.A., Trond H. Larsen, W. Hsu, W.W., Benedick, S., Hamer, K.C.,
610 Wilcove, D.S., Bruce, C., Wang, X., Levi, T., Lott, M., Emerson, B.C., Yu. D.W., 2013.
611 Reliable, verifiable and efficient monitoring of biodiversity via metabarcoding. Ecol. Lett.
612 16, 1245–1257. doi: 10.1111/ele.12162
613 Johansson, M.L., Chaganti, S.R., Simard, N., Howland, K., Winkler, G., Rochon, A., Laget, F.,
614 Tremblay, P., Heath, D.D., MacIsaac, H.J., 2017. Attenuation and modification of the ballast
615 water microbial community during voyages into the Canadian Arctic. Divers. Distrib. 23,
616 567–576. doi:10.1111/ddi.12552
617 Kelly, R.P., Port, J.A., Yamahara, K.M., Martone, R.G., Lowell, N., Thomsen, P.F., Mach, M.E.,
618 Bennett, M., Prahler, E., Caldwell, M.R., Crowder L.B., 2014. Harnessing DNA to improve
619 environmental management. Science 344, 1455–1456. doi: 10.1126/science.1251156
620 Kim, Y., Aw, T.G., Teal, T.K., Rose, J.B., 2015. Metagenomic investigation of viral communities in
621 ballast water. Environ. Sci. Technol. 49, 8396–8407.doi:10.1021/acs.est.5b01633
622 King, D.M., Tamburri, M.N., 2010. Verifying Compliance with Ballast Water Discharge Regulations.
623 Ocean Dev. Int. Law 41, 152–165. doi:10.1080/00908321003733139
624 Lance, R. F., Carr, M. R., 2012. Detecting eDNA of Invasive Dreissenid Mussels, Report on Capital
625 Investment Project. ANSRP Technical Notes Collection. ERDC/TN ANSRP-12-2.
626 Vicksburg, MS: US Army Engineer Research and Development Center. Available at:
627 http://el. erdc. usace. army. mil/publications. cfm.
628 Lee, P.L.M., 2017. DNA amplification in the field: move over PCR, here comes LAMP. Mol. Ecol.
629 Resour. 17, 138–141. doi:10.1111/1755-0998.12548
630 Lehtiniemi, M., Ojaveer, H., David, M., Galil, B., Gollasch, S., McKenzie, C., Minchin, D.,
631 Occhipinti-Ambrogi, A., Olenin, S., Pederson, J., 2015. Dose of truth—Monitoring marine
632 non-indigenous species to serve legislative requirements. Mar. Policy 54, 26–35.
633 doi:10.1016/j.marpol.2014.12.015
634 Lodge, D.M., Turner, C.R., Jerde, C.L., Barnes, M.A., Chadderton, L., Egan, S.P., Feder, J.L.,
635 Mahon, A.R., Pfrender, M.E., 2012. Conservation in a cup of water: estimating biodiversity
636 and population abundance from environmental DNA. Mol. Ecol. 21, 2555–2558.
637 doi:10.1111/j.1365-294X.2012.05600.x
638 Lodge, D.M., Williams, S., MacIsaac, H.J., Hayes, K.R., Leung, B., Reichard, S., Mack, R.N., Moyle,
639 P.B., Smith, M., Andow, D.A., James T. Carlton, J.T., McMichael, A., 2006. Biological
640 invasions: recommendations for US policy and management. Ecol. Appl. 16, 2035–2054.
641 doi:10.1890/1051-0761(2006)016[2035:BIRFUP]2.0.CO;2
642 Lymperopoulou, D.S., Dobbs, F.C., 2017. Bacterial Diversity in Ships' Ballast Water, Ballast-Water
643 Exchange, and Implications for Ship-Mediated Dispersal of Microorganisms. Environ. Sci.
644 Technol. 51, 1962–1972. doi:10.1021/acs.est.6b03108
645 Ma, Y., Xiong, H., Tang, S., Yang, Q., Li, M., 2009. Comparison of the community structure of
646 planktonic bacteria in ballast water from entry ships and local sea water in Xiamen Port. Prog.
647 Nat. Sci. 19, 947–953.https://doi.org/10.1016/j.pnsc.2008.09.007

Marcelino, V.R., Verbruggen, H., 2016. Multi-marker metabarcoding of coral skeletons reveals a rich microbiome and diverse evolutionary origins of endolithic algae. Sci. Rep. 6. doi:10.1038/srep31508

Miralles, L., Ardura, A., Arias, A., Borrell, Y.J., Clusa, L., Dopico, E., de Rojas, A.H., Lopez, B., Muñoz-Colmenero, M., Roca, A., Valiente, A.G., Zaiko, A., Garcia-Vazquez, E., 2016. Barcodes of marine invertebrates from north Iberian ports: Native diversity and resistance to biological invasions. Mar. Pollut. Bull. 112, 183–188. doi:10.1016/j.marpolbul.2016.08.022

Mitsuhashi, S., Kryukov, K., Nakagawa, S., Takeuchi, J.S., Shiraishi, Y., Asano, K., Imanishi, T., 2017. A portable system for metagenomic analyses using nanopore-based sequencer and laptop computers can realize rapid on-site determination of bacterial compositions. bioRxiv 101865.doi: https://doi.org/10.1101/101865

Molnar, J.L., Gamboa, R.L., Revenga, C., Spalding, M.D., 2008. Assessing the global threat of invasive species to marine biodiversity. Front. Ecol. Environ. 6, 485–492. doi:10.1890/070064

Mountfort, D., Smith, K.F., Kirs, M., Kuhajek, J., Adamson, J.E., Wood, S.A., 2012. Development of single and multispecies detection methods for the surveillance and monitoring of marine pests in New Zealand. Aquat. Invasions 7, 125–128.doi:10.3391/ai.2012.7.1.013

Muyzer, G., De Waal, E.C., Uitterlinden, A.G., 1993. Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S rRNA. Appl. Environ. Microbiol. 59, 695–700.

Nathan, L.M., Simmons, M., Wegleitner, B.J., Jerde, C.L., Mahon, A.R., 2014. Quantifying environmental DNA signals for aquatic invasive species across multiple detection platforms. Environ. Sci. Technol. 48, 12800–12806.doi: 10.1021/es5034052

Niessen, L., Luo, J., Denschlag, C., Vogel, R.F., 2013. The application of loop-mediated isothermal amplification (LAMP) in food testing for bacterial pathogens and fungal contaminants. Food Microbiol. 36, 191–206. doi:10.1016/j.fm.2013.04.017

Pagenkopp Lohan, K.M., Fleischer, R.C., Carney, K.J., Holzer, K.K., Ruiz, G.M., 2017. Molecular characterisation of protistan species and communities in ships' ballast water across three U.S. coasts. Divers. Distrib. n/a-n/a. doi:10.1111/ddi.12550

Pagenkopp Lohan, K.M., Fleischer, R.C., Carney, K.J., Holzer, K.K., Ruiz, G.M., 2015. Amplicon-Based Pyrosequencing Reveals High Diversity of Protistan Parasites in Ships' Ballast Water: Implications for Biogeography and Infectious Diseases. Microb. Ecol. 1–13. doi: 10.1007/s00248-015-0684-6.

Patil, J.G., Gunasekera, R.M., Deagle, B.E., Bax, N.J., Blackburn, S.I., 2005. Development and evaluation of a PCR based assay for detection of the toxic dinoflagellate, Gymnodinium catenatum (Graham) in ballast water and environmental samples. Biol. Invasions 7, 983–994. doi:10.1007/s10530-004-3119-8

Pawlowski, J., Esling, P., Lejzerowicz, F., Cedhagen, T., Wilding, T.A., 2014. Environmental monitoring through protist next-generation sequencing metabarcoding: assessing the impact of fish farming on benthic foraminifera communities. Mol. Ecol. Resour. 14, 1129–1140. doi:10.1111/1755-0998.12261

Pejovic, I., Ardura, A., Miralles, L., Arias, A., Borrell, Y.J., Garcia-Vazquez, E., 2015. DNA barcoding for assessment of exotic molluscs associated with maritime ports in northern Iberia. 12, 168–176. doi:10.1080/17451000.2015.1112016

Pereira, F., Carneiro, J., Amorim, A., 2008. Identification of species with DNA-based technology: current progress and challenges. Recent Pat. DNA Gene Seq. 2, 187–199. doi: 10.2174/187221508786241738

Pochon, X., Bott, N.J., Smith, K.F., Wood, S.A., 2013. Evaluating detection limits of next-generation sequencing for the surveillance and monitoring of international marine pests. PLoS One 8, e73935. doi:https://doi.org/10.1371/journal.pone.0073935

Pochon, X., Zaiko, A., Hopkins, G.A., Banks, J.C., Wood, S.A., 2015. Early detection of eukaryotic communities from marine biofilm using high-throughput sequencing: an assessment of different sampling devices. Biofouling 31, 241–251. doi:10.1080/08927014.2015.1028923

Port, J.A., O'Donnell, J.L., Romero-Maraccini, O.C., Leary, P.R., Litvin, S.Y., Nickols, K.J., Yamahara, K.M., Kelly, R.P., 2016. Assessing vertebrate biodiversity in a kelp forest ecosystem using environmental DNA. Mol. Ecol. 25, 527–541. doi:10.1111/mec.13481

Ramgren, A.C., Newhall, H.S., James, K.E., 2015. DNA barcoding and metabarcoding with the Oxford Nanopore MinION, in: GENOME. Canadian Science Publishing, NRC research press, pp. 268–268.

Rius, M., Bourne, S., Hornsby, H.G., Chapman, M.A., 2015. Applications of next-generation sequencing to the study of biological invasions. Curr. Zool. 61, 488–504.

Shokralla, S., Spall, J.L., Gibson, J.F., Hajibabaei, M., 2012. Next-generation sequencing technologies for environmental DNA research. Mol. Ecol. 21, 1794–1805. doi: 10.1111/j.1365-294X.2012.05538.x

Simmons, M., Tucker, A., Chadderton, W.L., Jerde, C.L., Mahon, A.R., Taylor, E., 2015. Active and passive environmental DNA surveillance of aquatic invasive species. Can. J. Fish. Aquat. Sci. 73, 76–83. doi:10.1139/cjfas-2015-0262

Srivathsan, A., Sha, J.C.M., Vogler, A.P., Meier, R., 2015. Comparing the effectiveness of metagenomics and metabarcoding for diet analysis of a leaf-feeding monkey (Pygathrix nemaeus). Mol. Ecol. Resour. 15, 250–261. doi:10.1111/1755-0998.12302

Stehouwer, P.P., Buma, A., Peperzak, L., 2015. A comparison of six different ballast water treatment systems based on UV radiation, electrochlorination and chlorine dioxide. Environ. Technol. 36, 2094–2104. doi:10.1080/09593330.2015.1021858

Stehouwer, P.P., Liebich, V., Peperzak, L., 2013a. Flow cytometry, microscopy, and DNA analysis as complementary phytoplankton screening methods in ballast water treatment studies. J. Appl. Phycol. 25, 1047–1053. doi:10.1007/s10811-012-9944-8

Stehouwer, P.P., van Slooten, C., Peperzak, L., 2013b. Microbial dynamics in acetate-enriched ballast water at different temperatures. Ecotoxicol. Environ. Saf. 96, 93–98. doi: 10.1016/j.ecoenv.2013.06.027

Steichen, J.L., Quigg, A., 2015. Assessing the viability of microorganisms in the ballast water of vessels transiting the North Atlantic Ocean. Mar. Pollut. Bull. 101, 258–266. doi: 10.1016/j.marpolbul.2015.09.055

Steichen, J.L., Schulze, A., Brinkmeyer, R., Quigg, A., 2014. All aboard! A biological survey of ballast water onboard vessels spanning the North Atlantic Ocean. Mar. Pollut. Bull. 87, 201–210. doi: 10.1016/j.marpolbul.2014.07.058

Taberlet, P., Coissac, E., Pompanon, F., Brochmann, C., Willerslev, E., 2012. Towards next-generation biodiversity assessment using DNA metabarcoding. Mol. Ecol. 21, 2045–2050. doi: 10.1111/j.1365-294X.2012.05470.x

Tan, B., Ng, C., Nshimyimana, J.P., Loh, L.L., Gin, K.Y.-H., Thompson, J.R., 2015. Next-generation sequencing (NGS) for assessment of microbial water quality: current progress, challenges, and future opportunities. Front. Microbiol. 6, 1027. doi:10.3389/fmicb.2015.01027

Tang, M., Hardman, C.J., Ji, Y., Meng, G., Liu, S., Tan, M., Yang, S., Moss, E.D., Wang, J., Yang, C., Bruce, C., Nevard, T., Potts, S.G., Zhou, X., Yu, D.W., 2015. High-throughput monitoring of wild bee diversity and abundance via mitogenomics. Methods Ecol. Evol. 6, 1034–1043. doi:10.1111/2041-210X.12416

Tang, M., Tan, M., Meng, G., Yang, S., Su, X., Liu, S., Song, W., Li, Y., Wu, Q., Zhang, A., Zhou, X., 2014. Multiplex sequencing of pooled mitochondrial genomes–a crucial step toward biodiversity analysis using mito-metagenomics. Nucleic Acids Res. 42, 166–166. doi: 10.1093/nar/gku917

747 Thomsen, P., Kielgast, J.O.S., Iversen, L.L., Wiuf, C., Rasmussen, M., Gilbert, M.T.P., Orlando, L.,
748 Willerslev, E., 2012. Monitoring endangered freshwater biodiversity using environmental
749 DNA. Mol. Ecol. 21, 2565–2573. doi: 10.1111/j.1365-294X.2011.05418.x.
750 Tsolaki, E., Diamadopoulos, E., 2010. Technologies for ballast water treatment: a review. J. Chem.
751 Technol. Biotechnol. 85, 19–32. doi:10.1002/jctb.2276
752 Valentini, A., Taberlet, P., Miaud, C., Civade, R., Herder, J., Thomsen, P.F., Bellemain, E., Besnard,
753 A., Coissac, E., Boyer, F., Gaboriaud, C., Jean, P., Poulet, N., Roset, N., Copp, G.H., Geniez,
754 P.,Pont, D., Argillier, C., Baudoin, J-M., Peroux, T., Crivelli, A.J., Olivier, A., Acqueberge,
755 M., Le Brun, M., Møller, P.R., Willerslev, E., Dejean, T., 2015. Next-generation monitoring
756 of aquatic biodiversity using environmental DNA metabarcoding. Mol. Ecol. 25, 929–942.
757 doi: 10.1111/mec.13428.
758 Viard, F., David, P., Darling, J., 2016. Marine invasions enter the genomic era: three lessons from the
759 past, and the way forward. Curr. Zool. zow053. doi:10.1093/cz/zow053
760 Visco, J.A., Apothéloz-Perret-Gentil, L., Cordonier, A., Esling, P., Pillet, L., Pawlowski, J., 2015.
761 Environmental Monitoring: Inferring the Diatom Index from Next-Generation Sequencing
762 Data. Environ. Sci. Technol. 49, 7597–7605. doi:10.1021/es506158m
763 Ware, C., Berge, J., Jelmert, A., Olsen, S.M., Pellissier, L., Wisz, M., Kriticos, D., Semenov, G.,
764 Kwaśniewski, S., Alsos, I.G., 2016. Biological introduction risks from shipping in a warming
765 Arctic. J. Appl. Ecol. 53, 340–349. doi:10.1111/1365-2664.12566
766 Wood, S.A., Smith, K.F., Banks, J.C., Tremblay, L.A., Rhodes, L., Mountfort, D., Cary, S.C., Pochon,
767 X., 2013. Molecular genetic tools for environmental monitoring of New Zealand's aquatic
768 habitats, past, present and the future. N. Z. J. Mar. Freshw. Res. 47, 90–119. doi:
769 10.1080/00288330.2012.745885
770 Wright, D.A., Welschmeyer, N.A., 2015. Establishing benchmarks in compliance assessment for the
771 ballast water management convention by port state control. J. Mar. Eng. Technol. 14, 9–18.
772 doi: http://dx.doi.org/10.1080/20464177.2015.1022380
773 Xiong, W., Li, H., Zhan, A., 2016. Early detection of invasive species in marine ecosystems using
774 high-throughput sequencing: technical challenges and possible solutions. Mar. Biol. 163, 1–
775 12. doi:10.1007/s00227-016-2911-1
776 Xu, X.-W., Huo, Y.-Y., Bai, X.-D., Wang, C.-S., Oren, A., Li, S.-Y., Wu, M., 2011. Kordiimonas
777 lacus sp. nov., isolated from a ballast water tank, and emended description of the genus
778 Kordiimonas. Int. J. Syst. Evol. Microbiol. 61, 422–426. doi: 10.1099/ijs.0.018200-0.
779 Zaiko, A., Martinez, J.L., Ardura, A., Clusa, L., Borrell, Y.J., Samuiloviene, A., Roca, A., Garcia-
780 Vazquez, E., 2015a. Detecting nuisance species using NGST: methodology shortcomings and
781 possible application in ballast water monitoring. Mar. Environ. Res. 112, 64–72. doi:
782 10.1016/j.marenvres.2015.07.002
783 Zaiko, A., Martinez, J.L., Schmidt-Petersen, J., Ribicic, D., Samuiloviene, A., Garcia-Vazquez, E.,
784 2015b. Metabarcoding approach for the ballast water surveillance–An advantageous solution
785 or an awkward challenge? Mar. Pollut. Bull. 92, 25–34. doi:
786 10.1016/j.marpolbul.2015.01.008
787 Zaiko, A., Schimanski, K., Pochon, X., Hopkins, G.A., Goldstien, S., Floerl, O., Wood, S.A., 2016.
788 Metabarcoding improves detection of eukaryotes from early biofouling communities:
789 implications for pest monitoring and pathway management. Biofouling 32, 671–684.
790 doi:10.1080/08927014.2016.1186165
791 Zetsche, E.-M., Meysman, F.J.R., 2012. Dead or alive? Viability assessment of micro- and
792 mesoplankton. J. Plankton Res. 34, 493–509. doi:10.1093/plankt/fbs018
793 Zhang, H., Finiguerra, M., Dam, H.G., Huang, Y., Xu, D., Liu, G., Lin, S., 2013. An improved method
794 for achieving high-quality RNA for copepod transcriptomic studies. J. Exp. Mar. Biol. Ecol.
795 446, 57–66. doi:10.1016/j.jembe.2013.04.021
796