Microorganisms in ballast water: Disinfection, community dynamics, and implications for management

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HIGHLIGHTS

• Special focus has been placed on bacteria and consequences for ecosystem processes.
• Disinfection involves an increase in biological availability of organic matter.
• In most cases, after a successful disinfection, subsequent recolonization takes place.
• Recolonization after disinfection induces a change of bacterial communities.
• Invasive potential might be affected by natural and anthropogenic changes in recipient waters.

GRAPHICAL ABSTRACT

Abstract

Increasing concerns have accelerated the development of international regulations and methods for ballast water management to limit the introduction of non-indigenous species. The transport of microorganisms with ballast water has received scientific attention in recent years. However, few studies have focused on the importance of organisms smaller than 10 μm in diameter. In this work, we review the effects of ballast water transport, disinfection, and the release of microorganisms on ecosystem processes with a special focus on heterotrophic bacteria. It is important to evaluate both direct and indirect effects of ballast water treatment systems, such as the generation of easily degradable substrates and the subsequent regrowth of heterotrophic microorganisms in ballast tanks. Disinfection of water can alter the composition of bacterial communities through selective recolonization in the ballast water or the recipient water, and thereby affects bacterial driven functions that are important for the marine food web. Dissolved organic matter quality and quantity and the ecosystem status of the treated water can also be affected by the disinfection method used. These side effects of disinfection should be further investigated in a broader context and in different scales (laboratory studies, large-scale facilities, and on the ships).

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1. Introduction

Despite the developments in the transport industry, seaborne trade is currently the main way of freight. More than 80% of the international trade is transported by the world’s oceans (UNCTAD, 2017). Since 1970, marine traffic has increased at an average rate of 2.1% per year, surpassing 10 billion tons of cargo in 2015 (UNCTAD, 2017).

Maritime traffic represents a series of environmental challenges with a range of consequences for marine life. Currently, uncontrolled discharge of ballast water is an increasing problem on a global scale with severe ecological, economic, and health related consequences through the unintentional transfer of aquatic species (Bax et al., 2003; Kim et al., 2016; Ruiz et al., 2000; Wan et al., 2016). There is also considerable transport in lakes and rivers with documented impacts related to ballast water in the United States and Canada (Bailey, 2015).

Plants, animals, and microorganisms enter the ballast tanks of ships when the surrounding water is pumped on board to maintain stability (Carlton, 1985; Davidson et al., 2017; Ruiz et al., 2000). The majority of organisms in the water are not considered harmful to the environment, but some may be pathogenic to humans and wildlife (by enabling rapid propagation of epidemics or epizootics) and others may severely impact ecosystem functioning and -services in the recipient water (Drillet, 2016; Wallentinus and Nyberg, 2007). Many species will not survive during uptake, transport, and discharge of ballast water due to altered physical and chemical conditions. However, many will survive, and non-indigenous species (NIS) have the potential to become invasive (Carlton, 1985; Carlton and Geller, 1993; Seebens et al., 2013). The invasiveness potential is influenced by a wide range of environmental factors, both natural and anthropogenic.

At a global scale, shipping is estimated to transfer three to five billion tonnes of ballast water annually (David and Gollasch, 2015; Endresen et al., 2004; Lloyd’s Register Maritime, 2017; Tsolaki and Diamadopoulos, 2010). As a consequence, thousands of species are transported by vessels each day and unwanted introduction of approximately 450 marine and estuarine NIS has been documented in North America (Bailey, 2015; Carlton et al., 1993; Carlton and Geller, 1993; Ruiz et al., 2015; Tsolaki and Diamadopoulos, 2010). Thus, aquatic invasive species, mostly from ballast water, are among the four largest global threats to the oceans (GEF-UNDP-IMO, 2017; Nunes et al., 2014; Seebens et al., 2013; Werschkun et al., 2012). Most introductions have likely been facilitated by the small size of the organisms (larval stage) or through vectors in the ballast water, as documented for cholera outbreaks (McCarthy and Khambaty, 1994). To date, the attention of ballast water researchers has been biased towards larger invertebrates, due to detection problems and lack of information on the inactivation of microorganisms (Carney et al., 2013; Rey et al., 2016; Ruiz et al., 2000).

The International Maritime Organization (IMO) has adopted an International Convention for the Control and Management of Ships’ Ballast Water and Sediments (the Ballast Water Management Convention or BWMC) (IMO, 2004). It was ratified by >75% of the global fleet by tonnage by September 2017 (IMO, 2018). With the treaty in force, all vessels must carry a ballast water management plan, a ballast water record book, and an international ballast water management certificate. Additionally, all vessels must either perform an oceanic ballast water exchange (D-1 Standard) or ballast water treatment that complies with a set of parameters according to Rule D-2 (Ballast Water Performance Standard). Moreover, ballast water treatment systems (BWTSs) should be developed and approved according to the IMO’s Guideline 8. In cases where active substances are used for treatment, additional procedures (IMO Guideline 9) must be established to control the possible negative effects of these substances, either on the vessel or in the receiving environment. The United States (US), through the US Coast Guard’s (USCG) Ballast Water Management Act of 2005, define the same limits for discharge as IMO, but with a more restrictive protocol for the approval of BWTSs (USEPA, 2010). Some states have even stricter standards, such as California (Falkner et al., 2006), New York and Minnesota (Albert et al., 2013).

Implementation of the IMO and US regulations will require efficient treatment systems, which is a major task for the industry and a challenge for researchers (David and Gollasch, 2015, 2008; Davidson et al., 2017; Gollasch et al., 2007; Lehtiniemi et al., 2015). All ships must eventually comply with the established discharge limits, D-2 standard (Table 1), which is based on the abundance of different size classes (>10 μm to ≥50 μm) of viable organisms and specified indicator microbes that are harmful to human health. However, the focus on human pathogens neglects the large number of organisms smaller than 10 μm, that may have a significant impact on the receiving environment (van der Star et al., 2011). There is an increasing scientific interest in microorganisms in ballast water and introduction of ‘invisible invaders’ (Drillet, 2016; Endresen et al., 2004; Litchman, 2010; Lymeropolou and Dobbs, 2017), and whether heterotrophic bacteria should be incorporated into...
the standards (Cohen et al., 2017; Cohen and Dobbs, 2015; Gjaveer et al., 2014).

Currently, only California has adopted general limits for the density of bacteria and viruses into their standard: zero detectable living organisms for all organism size classes, including virus-like particles (VLPs), starting in 2030 (California Aquatic Invasive Species Management Plan 2008; amended by Assembly Bill No. 1312; Chapter 644) (Balaji et al., 2014; Falkner et al., 2006; Kim et al., 2015). The Great Lakes states in North America also plan to implement the ultimate goal of zero discharge of viable organisms (Albert et al., 2013; Great Lakes Commission, 2017).

This review is based on international regulations and scientific literature (Fig. 1), and focuses on the effects of ballast water transport, disinfection, and release of microorganisms < 10 μm, with a special focus on bacteria and the consequences for ecosystem processes. We also discuss treatment and management strategies relevant for treatment of ballast water in the future.

2. The importance of microorganisms in ballast water

Oceanic and coastal waters contain a wide range of microorganisms which have an important functional role at the base of the food web in all aquatic ecosystems on earth (Azam, 1998). Ballast water holds a wide range of microorganisms with a large phylogenetic diversity. Easily recognized forms of microorganisms such as algae and protists are less abundant than prokaryotes (Archaea and Bacteria) and viruses, which may exceed the other taxonomic groups in number by several orders of magnitude (Carlton, 1985; Gasol and Kirchman, 2008). For instance, planktonic algae typically make up 0.1–10% of the microbial density in natural waters. Many bacteria can tolerate a broader range of environmental conditions than other organisms and may exhibit properties such as dormant resting stages, high reproduction capacity, diverse substrate utilization, and remineralisation functions. Whether bacteria more likely establish a population than eukaryotic microorganisms have not been detected before in ballast water. Previous studies suggest that it is necessary to discuss the detection problems and methodological aspects in quantifying the composition of bacteria, and whether the indicator bacteria implemented in the regulations are appropriate (Cohen and Dobbs, 2015; Lymperopoulou and Dobbs, 2017).

Viruses are key components in marine ecosystems (Lara et al., 2017; Suttle, 2005), but are little studied as potential invaders. They are extremely abundant, diverse, and ubiquitously distributed in aquatic ecosystems and infect all known forms of life. As with bacteria, most viruses are innocuous to humans. They mainly infect bacteria, phytoplankton, and other microbial hosts, and may cause significant mortality in their host populations (Suttle, 2007). Most viruses are very host specific (Fuhrman and Suttle, 1993). Viruses have the potential to alter ecosystem functionality by regulating the population density, community succession, primary production, and ultimately nutrient cycling in the oceans (Suttle, 2005). Some studies have confirmed the introduction of viruses through ballast water (Drake et al., 2007; Kim et al., 2015; Lovell and Drake, 2009).

Organisms, which are below 10 μm in size, have attracted more attention in ballast water management in recent years. Abundance and diversity make them an important fraction to monitor, as mentioned in several studies (Gollasch et al., 2012; Lundgreen et al., 2018; van der Star et al., 2011). Moreover, most bacteria and other microorganisms <10 μm in size with high abundance in ballast waters, are unregulated. These smaller organisms occasionally show higher resistance than microbial standards to different treatment methods (Liu et al., 2016; Romero-Martínez et al., 2016). This makes it difficult to assess the performance of different BWTSs. Some reports, such as Gollasch et al. (2012), expressed this concern and stated that the best solution would be to validate the treatments in bench-scale tests that consider the organisms with sizes 2–10 μm. Such tests should be performed with local assemblages, because their response to different inactivation techniques may differ (Gollasch et al., 2012; Moreno-Andrés et al., 2018a; Petersen et al., 2019). Also previous studies suggest that the D-2 standard should be amended to include limits for densities of organisms with sizes < 10 μm, including heterotrophic bacteria who are <1–2 μm.

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**Fig. 1.** Number of papers registered in Scopus (abstract and citation database of peer-reviewed scientific literature) for topic keywords “ballast water” and period 1990–2018 (Accessed: 11.10.2018). A. Publications. B. Document type. Total documents: 3182. Others include: Notes, Short surveys, Business article and Letters.
3. Technologies for disinfection of ballast water

In this section, we discuss some important variables that can influence different processes for ballast water disinfection (Table 2). We also summarize published data for both commercial (small- and large-scale tests and shipboard trials) and ongoing research on ballast water treatment methods (Tables 3 and 4).

3.1. Physicochemical variables influencing ballast water management

Different bodies of seawater vary with respect to environmental variables, e.g., salinity, particle content, temperature, and pH. This variability should be considered in the development of ballast water management systems (Nosrati-Ghods et al., 2017). Some of these variables are presented in Regulation D-3 (Approval requirements for ballast water management systems) and have to be adjusted according to G8 guidelines (IMO, 2004) (Table 2).

High salinity and low levels of organic matter, typical for oceanic waters, are reflected in ballast water management (BWM) regulations because they can affect the behaviour of microorganisms and the efficiency of BWTSs. High salinity can affect the survival of indicator bacteria established in BWMC because they are usually not adapted to a life in natural seawater (Belkin and Colwell, 2005; Byappanahalli et al., 2012). Faecal enterococci can survive longer in seawater than faecal coliforms and their survival is negatively correlated with salinity (Belkin and Colwell, 2005; Giannakis et al., 2014; Oguma et al., 2013). Therefore, this makes them not the best indicator organisms to evaluate disinfection efficiency (Aguilar et al., 2018; Romero-Martínez et al., 2014; Moreno-Andrés et al., 2018a). When ultraviolet (UV) treatment is applied to saltwater, inactivation rates may decrease because of the scattering effect or light absorption by inorganic compounds (Chen et al., 2016). However, some studies report an increase in the inactivation rates of indicator microorganisms attributed to the osmotic stress (Moreno-Andrés et al., 2017; Oguma et al., 2013). Accordingly, it is not clear whether high salinity has an effect on bacterial sensitivity or on treatment effectiveness (Chen et al., 2016; Liu et al., 2016; Moreno-Andrés et al., 2017; Rubio et al., 2013).

Ballast water can contain different types and concentrations of particles that may interfere with the disinfection process. Both biotic and abiotic particles are reported to reduce disinfection efficiency (Hess-Erga et al., 2008; Tang et al., 2011). Mechanisms reducing disinfection efficiency act differently depending on the disinfection method used. This is further discussed in Section 5.

Temperature is an important variable in the ballast water context for two reasons. First, temperature influences the distribution of organisms along the latitudes. Thus, large temperature differences between the uptake and the discharge areas might affect survival during transport and discharge. Second, inactivation efficiency increases with temperature in several methods, such as UV or deoxygenation (Chen et al., 2016; de Lafontaine and Despatie, 2014; Drillet et al., 2013; Jung et al., 2017). Temperature also affects the formation of by-products from chemical disinfection treatments (Drillet et al., 2013; Zhang et al., 2013). Increased formation of harmful by-products can result in an added chemical risk at discharge. Temperature is included as a factor in the verification of BWTSs in the US Coast Guard (USCG) regulations, but not in IMO G8-guidelines.

3.2. Commercially available treatment methods

Most systems for ballast water treatment use mechanical filtration or separation and a subsequent physical or chemical secondary treatment, or a combination of both (Balaji et al., 2014; Davidson et al., 2017; Gegg et al., 2009; Satir, 2014; Tsolek and Diamadopoulos, 2010). Currently, approximately 57 BWTSs are approved and/or commercially available (Lloyd’s Register, 2017; Mouawad Consulting, 2018). Most systems use UV (~48%) and electrochemical (~28%) treatment. The rest uses processes such as ozonation, ultrasound, the addition of biocides, or deoxygenation (~24%) (Fig. 2).

Ultrasound (Holm et al., 2008), deoxygenation (de Lafontaine and Despatie, 2014; First et al., 2015), or heat (Quilez-Badia et al., 2008) treatment methods have been previously evaluated as potential

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**Table 2**

Physical-chemical requirements for type approval testing according to IMO and USCG guidelines.

<table>
<thead>
<tr>
<th>Water quality parameters</th>
<th>IMO</th>
<th>USCG</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salinity (PSU)</td>
<td>28–36</td>
<td>10–20</td>
</tr>
<tr>
<td>Organic matter (mg·L⁻¹)</td>
<td>&gt;1</td>
<td>&gt;5</td>
</tr>
<tr>
<td>Suspended solid material (mg·L⁻¹)</td>
<td>&gt;1</td>
<td>&gt;5</td>
</tr>
<tr>
<td>Temperature</td>
<td>–</td>
<td>4–35 °C</td>
</tr>
</tbody>
</table>

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**Fig. 2.** Pie chart with different types of commercially available Ballast Water Treatment Systems (BWTSs) based on Chemical treatment or Physical treatment. The majority of BWTSs consist of a mechanical filtration or separation (represented by “Physical separation”) and a subsequent secondary treatment (represented as “Disinfection phase”). Disinfection phase is represented in the chart.

Source: Lloyd’s Register Maritime, 2017; Mouawad Consulting, 2018 (CUBE Tool. https://www.bwm.no/ (accessed 02.01.2018)).
BWTSSs. Interferences by environmental variables (mainly temperature) and low efficiency in bacterial inactivation, limit the use of these treatments as stand-alone methods to inactivate bacteria from the ballast water.

Some authors detected different levels of resistance to UV radiation depending on the evaluated organisms (First et al., 2015; Romero-Martínez et al., 2016). They concluded that bacteria are more sensitive to UV radiation than phytoplankton and zooplankton. When mechanical separation and UV are combined, the inactivation success increases significantly; this is also valid in large-scale (Sutherland et al., 2001; Waite et al., 2003) and shipboard trials (Wright et al., 2007). Previous works that applied UV radiation for ballast water treatment suggest that more studies should consider post-treatment recovery and the regrowth of the organisms (First et al., 2015; Hess-Erga et al., 2010; Stehouwer et al., 2015).

Chemical treatment methods, such as electro-chlorination and ozonation, involve the use of active substances to achieve a bactericidal effect in the water matrix. Ozonation, as a stand-alone method for ballast water treatment, has been studied in mesocosms (Perrins et al., 2006) and shipboard testing (Herwig et al., 2006; Wright et al., 2010). The inactivation of heterotrophic bacteria was effective (≥99.9%) at doses of 1.6 mg·L⁻¹. In shipboard testing, the highest reductions observed were ∼99% for cultivable bacteria at high doses (1.6 mg·L⁻¹). Although effective disinfection of plankton assemblages was achieved in these studies, regrowth was detected for few bacterial groups (Maranda et al., 2013).

The studies reviewed in this section are not exhaustive and not necessarily comparable because treatment efficacies were evaluated differently. Most disinfection methods demonstrate problems with the inactivation efficiency at high particle concentrations. This problem can be mitigated with increased doses or by the inclusion of a pretreatment method that involves particle removal. Improved detection methods and stricter or altered treatment standards may enhance the efficiency of some of these methods in the future (Batista et al., 2017).

### 3.3. Disinfection methods under development

New technologies and disinfection methods (Table 4) focus on a combination of treatment methods with different properties (Wang et al., 2018). Some studies investigated the feasibility of new technologies to disinfect a wide range of microorganisms (Bai et al., 2016, 2012; Dang et al., 2016; Feng et al., 2015a, 2015b; van Slooten et al., 2015a). UV-based systems have been developed to promote the production of radical species with high oxidation rates and thus higher cell damage. Such methods have been tested for photochemical and photocatalytic treatments against standard indicator organisms and phytoplankton species (Moreno-Andrés et al., 2016; Penru et al., 2012; Romero-Martínez et al., 2014, 2018; Rubio et al., 2013; Wu et al., 2011a, 2011b; Yang et al., 2014; Zhang et al., 2014). Improvements of the EC processes with different anode materials can favour the generation of reactive oxygen species with higher inactivation rates (Lacasa et al., 2013; Moreno-Andrés et al., 2018; Petrucci et al., 2013). The combination of EC treatments with reagents such as CO₂ or parallel generation of hydroxyl radicals resulted in improved inactivation rates for all plankton fractions (Cha et al., 2015; Zhang et al., 2012). New chemical processes, such as the use of persulfates, which agree with the chemistry of typical ballast waters, have been recently tested and can be a promising alternative for the disinfection of ballast water (Ahn et al., 2013; Moreno-Andrés et al., 2019).

All the studies cited in this section have been performed only in the laboratory scale. Large-scale studies are required to evaluate their applicability for BWM. The complexity of ballast water treatment suggests that different methods should be thoroughly tested under realistic conditions, specifically by assessing the efficacy on marine bacteria and viruses naturally present in oceanic waters. Problems such as cost, environmental acceptability, and economic costs of installation and operation must also be evaluated (Ren, 2018; Satir, 2014).

### Table 3: Evaluation of ballast water treatment technologies, small-scale/laboratory (S), large-scale (L) and shipboard (V). The questions are answered by yes (Y), no (N) or not answered (−) and graded by slightly less (–). The evaluation is based on statements and conclusions from the literature.

<table>
<thead>
<tr>
<th>Methods</th>
<th>Scale</th>
<th>Influenced by particles</th>
<th>Successful inactivation</th>
<th>Residual toxicity</th>
<th>Corrosion</th>
<th>Expensive</th>
<th>Safe</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Physical</td>
<td>V</td>
<td>Y</td>
<td>Y– Y– Y–</td>
<td>N– N– N– Y–</td>
<td>–</td>
<td>–</td>
<td>N– N–</td>
<td>(Wright et al., 2007)</td>
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<tr>
<td>Ultrasound</td>
<td>S</td>
<td>–</td>
<td>Y</td>
<td>N– N– –</td>
<td>–</td>
<td>–</td>
<td>N– N–</td>
<td>(Holm et al., 2008)</td>
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<tr>
<td>Cyclone/Filter + UV</td>
<td>L</td>
<td>N</td>
<td>Y</td>
<td>Y– Y– Y–</td>
<td>–</td>
<td>–</td>
<td>Y– N–</td>
<td>(Waite et al., 2003)</td>
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<tr>
<td>Cyclone + UV</td>
<td>L</td>
<td>Y–</td>
<td>Y</td>
<td>Y– Y– Y–</td>
<td>–</td>
<td>–</td>
<td>Y– N–</td>
<td>(Sutherland et al., 2001)</td>
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<tr>
<td>Filter + UV</td>
<td>V</td>
<td>Y</td>
<td>Y</td>
<td>Y– Y– Y–</td>
<td>–</td>
<td>–</td>
<td>Y– N–</td>
<td>(Wright et al., 2007)</td>
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<tr>
<td>Electro-chlorination</td>
<td>S</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>–</td>
<td>N– N– Y</td>
<td>Y– Y–</td>
<td>(Maranda et al., 2013)</td>
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<tr>
<td>Ozone</td>
<td>V</td>
<td>Y</td>
<td>Y– Y–</td>
<td>Y</td>
<td>–</td>
<td>N– N– Y</td>
<td>Y– Y–</td>
<td>(First et al., 2015)</td>
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<tr>
<td></td>
<td>L</td>
<td>Y</td>
<td>Y– Y–</td>
<td>Y</td>
<td>–</td>
<td>N– N– Y</td>
<td>Y– Y–</td>
<td>(Tsolaki et al., 2010)</td>
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<td></td>
<td>V</td>
<td>Y</td>
<td>Y– Y–</td>
<td>Y</td>
<td>–</td>
<td>N– N– Y</td>
<td>Y– Y–</td>
<td>(Herwig et al., 2006; Wright et al., 2010)</td>
</tr>
</tbody>
</table>

Methods: Y, yes; N, no; –, not answered; V, vessel; L, laboratory scale.

Influence of methods on particles: Y, yes; N, no; –, not answered.

Successful inactivation: Y, yes; N, no; –, not answered; –, not applicable.

Residual toxicity: Y, yes; N, no; –, not applicable.

Corrosion: Y, yes; N, no; –, not applicable.

Expensive: Y, yes; N, no; –, not applicable.

Safe: Y, yes; N, no; –, not applicable.
4. Methodological challenges

Examination of microorganisms in their natural environment is challenging due to their small size and heterogeneity. Currently there is no consensus protocol for ballast water sampling (Carney et al., 2013; Gollasch and David, 2017). The distinction between live and dead bacteria also involves large methodological uncertainties because of the variable physiology of the cells and the non-culturable nature of most cells. Numbers, biomass, and metabolic activity are the variables of interest, but methodological problems may force us to measure less relevant variables as proxies. This presents a critical challenge in ballast water treatment (Cullen and MacIntyre, 2016; Lundgreen et al., 2018; Romero-Martínez et al., 2016), because international ballast water discharge standards are based either on viable organisms (IMO, 2004) or live organisms (USCG, 2012).

Several scientists have proposed the establishment of consensus protocols for enumerating viable organisms to better evaluate disinfection efficiency and allow comparisons between different experiments. Phenotypic identification and confirmation of viability is possible for some of the larger microorganisms, but for the majority of organisms, this requires a combination of different methods. Previous research has mainly focused on the fraction of organisms in the 10–50 μm size range and have tested different methodologies such as adenosine triphosphate (ATP) measurements (van Slooten et al., 2015b; Wright et al., 2015), fluorescence methods (Drake et al., 2014; Gollasch et al., 2015), flow cytometry (Olsen et al., 2016; Romero-Martínez et al., 2017) and ‘Most Probable Number’ (Cullen, 2018; Cullen and MacIntyre, 2016). Others compared different techniques because the use of a combination of both direct and indirect techniques can be more reliable (Brade et al., 2016; Casas-Monroy et al., 2016). Enumeration of viable bacteria is probably the most challenging task and it is the least studied method.

Most marine bacteria resist cultivation (one certain indicator of viability) on synthetic media (Joint et al., 2010), whereas most molecular methods detect DNA from both viable and dead cells (Darling and Frederick, 2016). Therefore, a combination of culture dependent and culture independent techniques could be needed. Recent advances in instrumentation have improved precision and resolution and reduced analysis time (David and Gollasch, 2015; Outinen and Lehtiniemi, 2017). The live/dead assessment of individual cells frequently exploits fluorescent dye exclusion or metabolic activity as the criteria for viability. The exclusion of some fluorescent dyes (e.g., propidium iodide) indicates membrane integrity and viability. Metabolic activity by viable cells may also be indicated by the uptake and intracellular modification of dyes (e.g., 5-cyano-2,3-ditolyl tetrazolium chloride, CTC). The use of such physiological probes is not straightforward as indicated by the contradictory results in the literature (Gasol and Del Giorgio, 2000) and only estimate viability at the community level. Live cells with damaged membranes or a low metabolic activity are frequently interpreted as dead, but they have the potential to resume growth. This illustrates the necessity of using several independent techniques to evaluate the disinfection efficiency. The staining based techniques can be automated by flow cytometry.

The analysis of nucleic acids can resolve phylogenetic affiliation and is highly sensitive, rapid, and specific (Darling and Frederick, 2016). Deoxyribonucleic acid (DNA) of the dead cells may persist for a long time and give false positives, whereas ribonucleic acid (RNA) persists for a shorter period and has the potential to assess viability. Although RNA analysis is not widely used owing to some disadvantages such as the complexity of the RNA decay or the low metabolic activity, it is an effective approach to link the community structure with viability. At present, the extraction and analysis of environmental DNA encoding ribosomal RNA is the most widespread molecular method to study microbial populations despite the risk of detection of the DNA of the dead cells. There exist a few techniques to reduce the influence of DNA from the dead cells, such as the binding of ethidium monooxaide (EMA, membrane impermeant dye) to the DNA, which inhibits polymerase chain reaction (PCR) amplification (Rudi et al., 2005).

DNA marker genes (mainly ribosomal genes) or their RNA amplified by PCR or RT-PCR, respectively, were mainly assessed using fingerprinting techniques such as the denaturing gradient gel electrophoresis (DGGE) and terminal restriction fragment length polymorphism (T-RFLP) and they reflect the composition of the predominant PCR-targeted members (Darling and Blum, 2007; Larsen et al., 2001; Marzorati et al., 2008). In recent years, fingerprinting methods have been replaced by high-throughput sequencing of PCR amplicons. These methods allow the high-resolution (detection limit ~0.001%) identification of microbes present at the genus level and sometimes at the species level (Amato, 2016; Lee et al., 2012; Vestrum et al., 2018). High-throughput sequencing can also be used to sequence the metagenome (the sum of all genes in a community) (Venter et al., 2004). This makes it possible to look for genes or gene families relevant

<table>
<thead>
<tr>
<th>Methods</th>
<th>Scale</th>
<th>Influenced by particles</th>
<th>Successful inactivation of Zoopl Phytoph Bact</th>
<th>Residual toxicity</th>
<th>Corrosion</th>
<th>Expensive</th>
<th>Safe</th>
<th>Reference</th>
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<tbody>
<tr>
<td>UV-based</td>
<td>UV/H2O2</td>
<td>S</td>
<td>Y</td>
<td>–</td>
<td>–</td>
<td>Y</td>
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<td></td>
<td>UV/ TiO2</td>
<td>L</td>
<td>Y</td>
<td>Y</td>
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<td>N</td>
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<td>UV/O3</td>
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<td>Y</td>
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<td></td>
<td>UV + Heat + US</td>
<td>S</td>
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a BDD: Boron Doped Diamond.
b DDAC: Didecyldimethylammonium chloride.
c IPL: Intense Pulse Light.
to the evaluation of ballast water treatment (e.g., genes for the production of toxic substances or relevant for pathogenesis). High-throughput methods have revolutionized the amount of information gained and the speed of processing of environmental samples. Also microarray technology has been applied to microbial communities and is a potential solution for monitoring invasive species (Darling and Blum, 2007).

In summary, none of the methods discussed in this section, fully satisfy all the key criteria for BWM in terms of accuracy, feasibility, and reliability. The cost, analysis time, and availability of easy-to-operate equipment are important for practical on-board applications. The new nucleic acid based methods have the potential to identify a larger fraction of the microbial community than the traditional methods, and are promising tools for monitoring ballast water (Darling and Frederick, 2016; Rey et al., 2016). Increased knowledge of the effects of disinfection on the microbial community structure and functionality is crucial for the development of improved ballast water management. The unpredictable presence of harmful bacteria, detection difficulties, and potential effects on ecosystem functioning suggest further research on bacteria in ballast water management and on methodological challenges.

5. Modifying factors and side effects

Most studies on disinfection methods focus on disinfection and inactivation efficacy. However, disinfection of ballast water may induce modifying factors and side effects. Bacterial regrowth and recolonization always take place after disinfection (Grob and Pollet, 2016). However, the time needed to reach substantial population densities depend on the size of the seed population. A few studies examining heterotrophic bacteria and indicated regrowth within hours to days after a successful disinfection treatment (Hess-Erga et al., 2010; Moreno-Andrés et al., 2018b; Petersen et al., 2019). These recolonizing bacteria may originate from surviving bacteria or from a point downstream of the disinfection system. It is important to distinguish between damage repair and regrowth.

Repair of moderate damage after UV-based disinfection is common for many bacteria and involves both dark and photo-repair mechanisms (Nebot et al., 2007). It usually happens within 24 h and is mainly evaluated for faecal microbiological indicators (mostly _E. coli_). Published studies indicate that percent repair does not exceed 4% (Moreno-Andrés et al., 2016; Nebot et al., 2007; Rubio et al., 2013; Vélez-Colmenares et al., 2012). Regrowth, however, mostly depends on the environmental conditions and vary for different bacteria. Only a few studies have focused on the inactivation and post-irradiation regrowth of bacteria in natural communities (Hess-Erga et al., 2010; Moreno-Andrés et al., 2018a; Wennberg et al., 2013; Williams et al., 2007). All these studies detected high recolonization rates and suggest that marine bacteria have a great capacity for growth when discharged to natural bodies of water (Hess-Erga et al., 2010; Williams et al., 2007). This is because they are returned to their natural environment with favourable conditions for growth, in contrast to faecal bacteria (Giannakis et al., 2014; Moreno-Andrés et al., 2018a). The significance of recovery by both repair and regrowth is not known for bacteria in ballast water and should be further studied (Grob and Pollet, 2016; Liltved and Landfald, 1996).

The disinfection of water and the subsequent recolonization can alter the composition of the bacterial community through selective inactivation and selective recolonization of the ballast water or the recipient water. This may affect the bacterial driven functions important for the marine food web. Recent studies have demonstrated quantitative and qualitative changes in the bacterial community after treatment with UV irradiation, ozonation, and electro-oxidation of seawater (Hess-Erga et al., 2010; Moreno-Andrés et al., 2018b; Petersen et al., 2019); e.g., members of _Gamma-proteobacteria_ dominate after recolonization (Hess-Erga et al., 2010; Petersen et al., 2019; Vadstein et al., 2018). _Gamma-proteobacteria_ include many genera with species that are pathogenic to many animals, including humans. Several species within this group have high maximum growth rates (Kirchman, 2016) and thus they are r-strategists. Opportunistic r-strategic bacteria become dominant during the first period after disinfection and create a low-diversity community (Vadstein et al., 2018). It has also been documented experimentally that r-selection after disinfection may result in increased mortality of the larval stages of lobster (Vadstein et al., 2018). These findings should be verified under full-scale conditions to confirm their relevance for the treatment of ballast water.

Protection of particle-associated and free-living bacteria in the shadow of other particles is rarely discussed in published studies, although it has significant impacts on both the disinfection efficiency and regrowth (Hess-Erga et al., 2008; Mamane and Linden, 2006; Tang et al., 2011; Wu et al., 2005). The latter is due to the higher seed density for regrowth. Many bacteria are particle-associated and this could be a strategy to survive under unfavourable conditions. Mamane and Linden (2006) estimated that 30–50% of the spores from _Bacillus subtilis_ located in aggregates were protected from UV irradiation. Hess-Erga et al. (2008) showed that a six-fold increase in the UV dose was required to obtain 99.9% inactivation of particle-associated bacteria compared to free-living bacteria, but particles provided less protection during disinfection by ozone. These results indicate the different protections kinetics and mechanisms for different disinfection methods, and illustrate the need for monitoring the properties and concentrations of particles as a basis for setting a sufficient disinfection dose.

A third factor, which is rarely mentioned in connection with the ballast water treatment, is that many disinfection methods have the potential to generate easily degradable substrates and nutrients. These can be organic matter (e.g., organic acids, aldehydes and DNA) and inorganic nutrients serving as important sources of carbon, nitrogen, and phosphorus for the microorganisms (Ibañez de Aldecoa et al., 2017; Keil and Kirchman, 1993; Świerlik et al., 2009). These substrates increase the magnitude of regrowth. The products formed depend on the disinfection method and the constituents in the ballast water. UV irradiation and ozonation of seawater result in increased availability of labile substrates for the survival of heterotrophic bacteria and they can induce increased growth and succession in the bacterial community (Eiler et al., 2007; Hess-Erga et al., 2010; Sulzberger and Durisch-Kaiser, 2009).

Labile substrates can be generated by two different mechanisms: 1) Rupturing or killing the cells with a concurrent release of cellular matter and further degradation by released enzymes into dissolved organic matter (DOM). 2) Increased bioavailability of existing DOM due to chemical modification. The two mechanisms are connected because enzymes released by killed organisms also degrade existing DOM. Both mechanisms may increase the availability of labile DOM. Some disinfectants (e.g., residues of peracetic acid and acetic acid) also result in an increase in labile DOM (Rojas-Tirado et al., submitted). The relative importance of these two mechanisms for the production of labile DOM was addressed by Hess-Erga (2010), who demonstrated the direct physical or chemical action of UV irradiation with modification of both natural DOM and a combination of particulate and dissolved organic matter (POM-DOM). POM and DOM were also modified, indicating the cutting of polymers and production of simpler compounds (labile DOM). A consequence of ballast water disinfection may thus be increased availability of labile substrates and increased bacterial production. Elevated substrate concentrations and little competition due to low cell densities result in the rapid growth of relatively few opportunistic species (r-strategists) and thus low diversity.

Fourthly, many BWTSs can result in the formation of disinfection by-products (DBPs) (Shah et al., 2015; Delacroix et al., 2013; Werschkun et al., 2012). For example, systems that involve chlorination can generate substantial amounts of trihalomethanes (THMs), halogenated acetic acids (HAAs), and ozonation can result in the production of bromate (Shah et al., 2015). Formation of these DBPs are affected by the type/dose of the oxidant and salinity, concentration, and the type of dissolved organic matter, i.e., the initial properties of the ballast water (Shah et al.,
DBPs can have acute and chronic toxic effects on different organisms (e.g., algae) (Delacroix et al., 2013; Ziegler et al., 2018) and some DBPs may pose a risk for the local aquatic environment at concentrations detected in the discharged ballast water (David et al., 2018; Gregg et al., 2009).

The four groups of modifying factors and side effects of disinfection should be further investigated in a controlled environment and in large-scale facilities or onboard. These factors and side effects indicate the problems with the ballast water treatment, which are discussed to a limited extent in the scientific literature and are not considered in the design of ballast water treatment systems. Increased focus on the bacterial disinfection processes as a whole (inactivation + regrowth) is needed, especially in light of the announced stricter treatment standards (Albert et al., 2013; Balaji et al., 2014).

6. Ecosystem consequences

Ballast water literature has mostly focused on the properties of single species and less on ecosystem effects of NIS. Biological invasion in marine environments involves the introduction of NIS that become non-proportionally abundant in their new location and pose severe threats to the structure, functioning, and services of the ecosystem (Carlton, 1985; Carlton and Geller, 1993; Lovell and Drake, 2009; Ruiz et al., 2000; Wallentinus and Nyberg, 2007). Marine ecosystems are more open than terrestrial and limnetic ones, with the potential for long-distance dispersal of organisms. This might enable rapid propagation of epidemics (humans) or epizootics (animals).

If free-living microorganisms are distributed globally, they have no biogeography and cannot be invasive. However, numerous reports suggest that ballast water transport is the main mechanism of the introduction of NIS. This indicates at least some degree of biogeography and that environmental heterogeneity and biogeography are essential to understand microbial invasion ecology (Gollasch, 2006; Kim et al., 2016; Litchman, 2010; Martiny et al., 2006; Seebens et al., 2013). Invasive microorganisms can directly or indirectly influence the ecosystem when they disturb the local patterns of decomposition, symbiosis, predation, and pathogenicity (van der Putten et al., 2007; Wallentinus and Nyberg, 2007). Some of these processes may be enhanced by the introduction of novel properties through gene transfer (Lv et al., 2018). Such invasions are not always visible in the short term, not easy to forecast, or not well understood (Ojaveer et al., 2014).

When organisms are discharged into a new location, their invasion success is a function of their inoculation density (propagule pressure) and ability to survive and reproduce (ecological fitness). Specialized microorganisms probably have difficulties in establishing and spreading, unless the environmental conditions are similar to their native habitat. To establish in the new recipient ecosystem, such microorganisms must compete efficiently, avoid strong predation/parasitism, or exploit empty niches. On the contrary, opportunistic microorganisms might have problems in settling in stable and crowded environments unless the ecosystem status (uncrowded, limited competition, resource-rich) favours such generalists. Microbial communities degrade DOM that come from their native habitats more efficiently than DOM from other sources (Young et al., 2005). This suggest that specialization takes place locally; i.e., the establishment of NIS is more likely in habitats similar to their original habitat (Occipinti-Ambrogi and Savini, 2003; Drake et al., 2007). However, data on microbial invasion patterns are sparse despite the widespread introduction of NIS.

Major natural and human-induced perturbations induce instability in the ecosystem and may thus alter the invasion potential significantly (Nogales et al., 2011; Occipinti-Ambrogi and Savini, 2003). Overexploitation of many coastal areas has led to increased water runoff with a large content of particulate and dissolved matter (Nogales et al., 2011). This may affect the biological production in the local ecosystems and facilitate the establishment of NIS introduced by ballast water (Occipinti-Ambrogi and Savini, 2003). Contrary to robust and native communities with high resistance and resilience, a sudden and dramatic perturbation may lead to diversity loss and result in fragile communities with low resilience and increased vulnerability to invasions by NIS (Litchman, 2010; Occipinti-Ambrogi and Savini, 2003). For example, anthropogenic eutrophication may induce phytoplankton or cyanobacterial blooms, reducing diversity. Consequently, the ecosystem status of the water in the ballasting and the discharge area may either enhance or reduce the invasiveness potential of ballast organisms. Communities with high resistance and resilience might represent a natural impediment to invasion by NIS compared to communities exposed to an anthropogenic disturbance with the loss of ecosystem functions.

Selective inactivation and production of labile DOM by disinfection induce recolonization by opportunistic bacteria and strongly alter the community composition (Section 5). Such bacteria might have a different invasion potential from the untreated community. If ballast water is treated during discharge, labile DOM might induce a similar but a significantly weaker recolonization by the opportunists in the recipient ecosystem than in the ballast tank because of the higher population density and more competition. However, such recolonization has the potential to influence the community composition through bacterial driven processes. Therefore, aquatic habitats with low concentrations of labile DOM will likely be more affected by the release of labile DOM from the treated ballast water; i.e., increased substrate production might affect the survival and release of NIS from ballast tanks and potentially alter the bacterial driven processes in the recipient water (Fig. 3).

This section illustrates the possible microbial ecosystem consequences and highlights the insufficient number of studies on ecosystem effects of ballast water discharge. However, invasion biology is a rapidly developing field that includes theory and modelling (Fagan et al., 2002; van Kleunen et al., 2010; Courchamp et al., 2017; Young et al., 2017) and ballast water research may benefit from the rapid development of new knowledge within this field. The effects of treated and discharged ballast water on local ecosystems should be further investigated, especially substrate generation and bottom-up interactions affecting ecosystem functioning and the factors that facilitate the success of invasive species.

7. Management strategies

The mid-ocean ballast water exchange is the most widely used method in certain regions to avoid the spread of NIS with ballast water and comply with BWMC. Exchange between the coastal waters and open ocean water limits the establishment of organisms owing to the mismatch of selective factors in open-ocean and coastal habitats. However, rough weather and stability concerns can prevent the mid-ocean ballast water exchange; thus, this method may also prove insufficient for stopping the introduction of NIS (Molina and Drake, 2016; Pereira et al., 2014). IMO considers the ballast water exchange an interim measure, not a final solution. In 2024, installing BWTSs will become essential to meet the D-2 standard.

Fig. 3. The pelagic food web with the microbial loop as a major path for organic matter flux. Based on Azam, 1998 and Sutie, 2005.
All BWTSs are developed for onboard treatment; however, the convention mentions the possibility that ballast water can be treated in facilities located onshore or can be assisted from the land. This strategy has been rarely studied because it is difficult to implement. However, few studies suggest that under certain conditions, this strategy can be effective and applicable; it may reduce the number of BWTSs and utilize a more professional staff. Several studies carried out in specific ports, the Port of Milwaukee (Brown and Caldwell, 2007), the Port of Tubarao-Brasil (Pereira and Brinati, 2012), and the Port of Baltimore (King and Hagan, 2013), conclude that in certain types of ports, treatment on land may be the most viable option, economically and technically. The choice of the disinfection method(s) and timing can be crucial for treatment success, but the treatment strategy should be evaluated as well. If the ballast water is treated at uptake, there will be a recolonization of the surviving or already present bacteria in the ballast tanks (seed reservoir). This recolonization can be substantial due to high nutrient concentrations and low predation and competition for a long period in the ballast tanks. Depending on the disinfection method and the incubation time, this might increase the discharge of bacteria compared to the water not treated by disinfection. However, such a succession might be different if the ballast water is treated at discharge. Increased abundance of heterotrophic organisms and dilution may reduce selection for opportunists in the recipient ecosystem and the effects of substrate production. In contrast, substrate production and repair mechanisms may affect the survival of the treated bacteria in the recipient ecosystem. However, to the best of our knowledge, no small- or large-scale studies examining this strategy and possible negative effects have been performed to date.

Alternative treatment strategies such as disinfection during voyage should be compared with the regular treatment design (water treatment at uptake and/or discharge) and evaluated to determine the best treatment practice. Mid-ocean ballast water exchange in combination with disinfection may additionally reduce the environmental consequences (Paolucci et al., 2017). The possibility of generating potable water has also been studied for small vessels (Albert et al., 2017). Naval architects are trying to develop ballast-free ships, where a constant flow of local seawater runs through a network of large pipes from the bow to the stern below the waterline (Doblin et al., 2007; Drake et al., 2005). This might be an alternative to installing ballast water treatment systems on new vessels in the future for eliminating the ballast water problem.

8. Conclusions

The information we have compiled and analysed in this review demonstrates the need for further research on ballast water treatment. There is a growing interest in the critical role of microorganisms and potential ecological consequences of their release in connection with the ballast water discharge. The concerns originate from inadequate inactivation, recolonization with quantitative and qualitative changes of heterotrophic bacteria in ballast tanks, and the production of labile substrates, which increase the availability of organic matter for the recipient ecosystem. However, to the best of our knowledge, no small- or large-scale studies examining this strategy and possible negative effects have been performed to date.

An increasing number of available treatment systems comply with the ballast water standards. However, factors such as improved detection methods and the necessity to include organisms smaller than 10 μm in size in the treatment standards may necessitate certain improvements in the future. The unpredictable presence of harmful bacteria, side effects of disinfection, and potential negative effects on ecosystem functionality complicate the problem.

A major area for further research is to improve the understanding of the relative importance of microbial communities in facilitating the success of invasive species. One of the potential research questions is whether the interactions with higher trophic levels will be more important in stressed environments and environments with low diversity. Future research should address the problem of insufficient data, especially on microbial invasion patterns, despite the widespread introduction of NIS. Invasiveness has mainly been studied from the perspective of human pathogens or organisms causing economic losses, with less attention paid to the effects on the ecosystems. In addition, there are many unresolved important questions about the technological, biological, and economical aspects of different treatment methods, which should be addressed in future research efforts.

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References


