



The Role of Vessel Biofouling in the Translocation of Marine Pathogens: Management Considerations and Challenges

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Vessel biofouling is a major pathway for the introduction, establishment, and subsequent spread of marine non-indigenous macro-organisms. As a result, national and international regulations and guidelines have been implemented to manage the risks associated with this pathway, yet widespread enforcement and uptake are still in their infancy. By comparison, translocation of marine pathogens by vessel biofouling has received little attention despite a mounting body of evidence highlighting the potential importance of this pathway. Using molluscan pathogens as a model, this paper examines the potential for translocation of marine pathogens via the vessel biofouling pathway by reviewing: (1) examples where vessel biofouling is suspected to be the source pathway of non-indigenous pathogen introduction to new areas, and (2) the association between pathogens known to have detrimental effects on wild and farmed mollusk populations with species known to foul vessels and anthropogenic structures. The available evidence indicates that vessel biofouling is a viable and important pathway for translocating marine pathogens, presenting a risk to marine values (i.e., environmental, economic, social, and cultural). While preventive measures to minimize the translocation of macro-organisms are the most efficient way to minimize the likelihood of associated pathogen translocation, the application of reactive management measures to biofouled vessels, including post-filtration treatment, requires further and explicit consideration.

Keywords: vessel biofouling, pathogens, mollusks, in-water cleaning, marine biosecurity

INTRODUCTION

The International Maritime Organization (IMO) defines biofouling as the growth and accumulation of organisms on immersed ship surfaces or structures (International Maritime Organization [IMO], 2011). Typically, any substrate placed in natural waters is quickly colonized by micro-organisms (creating a biofilm, also known as the slime layer) that is followed by a succession

of diverse sessile or sedentary micro- and macro-organisms (Flemming, 2002; Aldred and Clare, 2008; Amara et al., 2018). Vessel biofouling is acknowledged as a major, and perhaps the most important, pathway for the introduction, establishment, and subsequent spread of marine non-indigenous macro-organisms (Drake and Lodge, 2007; Hewitt and Campbell, 2010; Bell et al., 2011; Ruiz et al., 2015; Bailey et al., 2020). Similar to the concerns over the transport of human pathogens in ships' ballast water (McCarthy and Khambaty, 1994; Ruiz et al., 2000a; Cohen et al., 2012), the potential for vessel biofouling to act as a vector for non-indigenous pathogens has been highlighted for some time (e.g., Howard, 1994). Pathogens have been found in biofilms growing on vessel surfaces (e.g., Drake et al., 2007; Shikuma and Hadfield, 2010), and mature organisms within vessel biofouling assemblages are more likely to harbor pathogens than their younger or larval planktonic stages associated with ballast water (Hine, 1995). For the purpose of this document, the term pathogen is used to include viruses, bacteria, protists, and fungi that cause disease in other organisms; the term vessel is used to include every description of ship, boat, or other craft used in water navigation, i.e., both recreational and commercial vessels are included.

The role of anthropogenic vectors in global scale biotic exchange is largely based on patterns and processes linked to macro-organism translocations and biogeography (Ruiz et al., 2000b; Pagenkopp-Lohan et al., 2020), thus the magnitude and impacts of marine micro-organism translocations are likely "vastly underestimated" (Pagenkopp-Lohan et al., 2020). Practical difficulties in micro-organism identification and detection, and a lack of baseline knowledge about native organism geographical ranges, however, have hampered research efforts (Davidson et al., 2013; Pagenkopp-Lohan et al., 2020).

In the risk analysis to support implementation of New Zealand's biofouling regulations (Ministry for Primary Industries New Zealand [MPI], 2018), Bell et al. (2011) identified recent changes to shipping patterns including increased shipping volumes, expansion of trade routes, and increased vessel speeds were providing a greater likelihood of translocation of marine non-indigenous macro-organisms. The delivery rate of micro-organisms associated with vessel biofouling may be similarly increasing over time (Pagenkopp-Lohan et al., 2020). The role of shipping, particularly vessel biofouling, in pathogen translocations may, therefore, undermine regulations and improved management practices aimed at addressing the major anthropogenic vectors for historical pathogen translocations, such as aquaculture and fisheries stocking (Williams et al., 2013; Georgiades et al., 2016; Pagenkopp-Lohan et al., 2020). While vessel biofouling has also been subject to increased scrutiny and management in some jurisdictions (Georgiades et al., 2020; Scianni et al., in press), it remains a largely unregulated broad-scale vector of organisms both domestically and internationally.

The threats posed by non-indigenous pathogens are similar to marine non-indigenous macro-organisms: once established they are difficult to control, and eradication is often infeasible or unsuccessful (Centre for Environment, Fisheries and Aquaculture Science [CEFAS], 2009; Georgiades et al., 2020).

Prevention is therefore the only effective measure (Centre for Environment, Fisheries and Aquaculture Science [CEFAS], 2009; Georgiades et al., 2016). This is particularly the case for shellfish aquaculture and fisheries, where the introduction and establishment of novel pathogens can have devastating effects. For example, *Bonamia ostreae* and *Marteilia refringens* drastically reduced European production of cultured flat oysters (*Ostrea edulis*) from 29,595 t in 1961 to 5,921 t in 2000 (Culloty and Mulcahy, 2007). Between 1980 and 1983 alone, estimated losses in France included a 20% reduction of employment within the industry, US\$ 240 million turn-over, and US\$ 200 million of added value [Meuriot and Grizel (1984) in Arzul et al., 2006]. European flat oyster production has stabilized but at low levels (< 3,000 t; Gouletquer, 2004), using modified husbandry techniques with lower employment than the industry had historically provided (Arzul et al., 2006; Culloty and Mulcahy, 2007). Based on these impacts, introduction of *B. ostreae* to New Zealand in 2015 led to the pre-emptive depopulation of all farmed flat oyster (*O. chilensis*) stock to protect uninfected areas, particularly the Bluff wild oyster fishery at the southern tip of the South Island (Farnsworth et al., 2020).

The ostreid herpes virus microvariant 1 (OsHV-1) has caused mass mortalities in spat and juvenile Pacific oysters (*Crassostrea gigas*) in France (Ségarra et al., 2010), Australia (Paul-Pont et al., 2014), and New Zealand (Bingham et al., 2013). During initial outbreaks, stock losses of up to 100% were recorded (Keeling et al., 2014; European Food Safety Authority [EFSA], 2015), and the disease halved New Zealand's Pacific oyster production (Johnston, 2014). The immediate impacts to industry and biosecurity response costs for OsHV-1 and *B. ostreae* in New Zealand have far outweighed those related to marine non-indigenous macro-organisms (Georgiades et al., 2020).

The introduction of *Haplosporidium nelsoni* to the mid-Atlantic coast of the United States in the 1950s also had extensive impacts on *Crassostrea virginica* populations, with mortality exceeding 90% in Delaware and Chesapeake Bays (Haskin and Ford, 1982; Haskin and Andrews, 1988). Between 1958 and 1983, it was estimated that *H. nelsoni* had reduced oyster landings in Delaware Bay by two-thirds (Haskin and Ford, 1982). The introduction of *H. nelsoni*, combined with pollution and oyster overharvesting, drove large-scale ecological impacts on Chesapeake Bay (Kemp et al., 2005), and extensive management and restoration efforts have achieved relatively modest success (National Research Council, 2004). The fishery in Chesapeake Bay declined to 2% of its historical catch in 30 years, and introduction and culture of non-native oyster species (including *C. gigas* and *C. ariakensis*) was seriously considered (Mann et al., 1991; Calvo et al., 1999; Tamburri et al., 2008).

While vessel biofouling regulations have been enacted by some jurisdictions (California Code of Regulations, 2017; Ministry for Primary Industries New Zealand [MPI], 2018; Georgiades et al., 2020), these are focused largely on preventing the translocation of marine non-indigenous macro-organisms (e.g., Bell et al., 2011). New information on translocation of pathogens associated with biofouling is emerging, however (e.g., Lane et al., 2018; Itoh et al., 2019; Costello et al., 2020; Lane and Jones, 2020; Pagenkopp-Lohan et al., 2020). Biofouling management measures routinely

applied to vessels, such as in-water cleaning of macrofouling [i.e., reactive in-water cleaning (RIC)], are also cause for concern as they may increase the likelihood of pathogen release and establishment into new areas (Scianni and Georgiades, 2019).

In light of the major negative consequences novel pathogen introductions have thus far caused, understanding the risk of pathogen translocation by vessel biofouling is critical to inform biosecurity guidelines, regulations, and management approaches to protect marine values such as biodiversity, customary and recreational practices, fisheries, and aquaculture. This analysis reviews the literature to investigate the likelihood of this translocation pathway, including the ramifications for vessel maintenance, and discusses potential risk management options, as appropriate.

ANALYSIS

Analysis Scope

This analysis primarily focuses on the pathogens of mollusks as a model. There are numerous World Organization for Animal Health (OIE) listed or emerging molluscan pathogens that are of major concern for jurisdictions worldwide (Bower, 2017; OIE, 2020). The implications, conclusions, and recommendations drawn from the molluscan model may have broad application to other marine and human pathogens.

Pathogen Translocation Associated With Vessel Biofouling

Howard (1994) noted that the domestic transfer of *B. ostreae* from Cornwall to Plymouth (England) likely occurred with biofouling, which included live mollusks, on concrete barges. This conclusion was based on Plymouth having no history of the disease nor any reason to receive live oyster transfers for aquaculture purposes (Howard, 1994). The spread of *B. ostreae* has also been linked to vessel biofouling in the Netherlands (van Banning, 1991) and Ireland (Culloty and Mulcahy, 2007).

Bonamia ostreae was detected in the Southern Hemisphere (New Zealand) in 2015 (Georgiades, 2015; Lane et al., 2016) and was most likely introduced by vessel biofouling (Lane et al., 2020). The spread of *B. exitiosa*, from southern New Zealand to the Northern Hemisphere (Bishop et al., 2006; Abollo et al., 2008; Longshaw et al., 2013) and Argentina (Kroeck and Montes, 2005), has similarly been associated with shipping (Hill-Spanik et al., 2015; Lane et al., 2018). *B. ostreae* and other molluscan pathogens are associated with non-indigenous ascidians (Costello et al., 2020), highlighting the potential role of non-molluscan biofouling species as vectors of this pathogen.

Vessel biofouling is suggested as responsible for the introduction of OsHV-1 into New Zealand (Lane et al., 2020) and Australia (Fisheries Research and Development Corporation, 2011; Whittington et al., 2018), and its subsequent Australian spread to Tasmania and South Australia (Deveney et al., 2017). Fuhrmann and Hick (2020) found that laboratory transmission of OsHV-1 between donor and naive Pacific oysters via a simulated biofouling scenario was plausible but complex. While transmission from other biofouling species was not

observed by Fuhrmann and Hick (2020), the association of OsHV-1 with some biofouling organisms (i.e., bryozoan species) suggested that they may protect the virus from degradation (Martenot et al., 2015; Hick et al., 2016). OsHV-1 transmission to naive Pacific oysters has also been shown following cohabitation with exposed wild crabs (*Carcinus maenas*; Bookelaar et al., 2018) and mussels (*Mytilus* spp.; O'Reilly et al., 2018). These taxa have previously been identified within vessel biofouling assemblages (Visscher, 1928; Apte et al., 2000; Moshchenko and Zvyagintsev, 2001; Coutts et al., 2003). The mechanisms by which marine non-indigenous species can affect pathogen-host interactions are complex (Goedknecht et al., 2016), which has possible implications for their association and transport by vessels.

Vessel movements have also been linked to the introduction of *H. nelsoni* to the United States and Canada either through biofouling or release of *H. nelsoni* spores by ballast water discharges (National Research Council, 2004; Stephenson and Petrie, 2005). Hine (1996) highlighted the biofouling pathway as a risk for spreading *Perkinsus marinus*, and Pagenkopp-Lohan et al. (2018) and Itoh et al. (2019) further demonstrated the potential for shipping to contribute to the long-range dispersal of *Perkinsus* species.

In addition to pathogens, it is also noteworthy that parasitic invertebrates may be translocated by vessel biofouling (Davidson et al., 2013). Parasitic invertebrates were found to infect 7.8% of mussels sampled from 23 vessels operating on the U.S. West Coast, including the parasitic copepods *Pseudomyicola spinosus* and *Modiolicola gracilis*. Transport of infected mussels by international shipping has also been implicated in the intercontinental spread of a molluscan transmissible neoplasia (Yonemitsu et al., 2019).

In assessing pathogen risks associated with translocation of mollusk shells for reef restoration, Diggles (2020) noted the potential of molluscan pathogens, including iridoviruses, OsHV-1, and *Bonamia* species, to be introduced to Australia by vessel biofouling specifically and others, including *H. nelsoni* and *Perkinsus* species, by shipping more generally. The Australian Government Field Identification Guide for Aquatic Animal Diseases (Department of Agriculture, Water and the Environment, 2020) also recognizes vessel biofouling as a potential pathway for translocating various *Bonamia* species and OsHV-1. These examples show that the role of shipping, and more specifically vessel biofouling, in pathogen translocation is increasingly recognized as a serious risk factor for increasing the incidence of infection and disease outbreaks at various scales (within regions and over long-distance oceanic scales).

Associations of OIE-Listed and Other Important Pathogens With Known Biofouling Species

The OIE is an intergovernmental organization established to promote global animal health (OIE, 2019). To facilitate health certification and reduce risk in the trade of aquatic animals and their products, the OIE Aquatic Animal Health Standards Commission compiles the *Manual of Diagnostic Tests for Aquatic Animals* (the Aquatic Manual), records species known to be

susceptible to listed pathogens, and provides standardized, validated approaches for diagnosis.

The chapters of the OIE Aquatic Manual (OIE, 2019) specific to molluscan pathogens show that many known susceptible species are associated with biofouling of vessels, anthropogenic structures within harbors, hard substrates, or offshore installations (Table 1). Further, many of these pathogens either have poorly understood life cycles (e.g., *B. exitiosa*) or have been shown to survive outside the host for periods of weeks to months (e.g., *B. ostreae*, *P. marinus*, and *P. olseni*; OIE, 2019). There are also many molluscan pathogens that, although not listed by the OIE, cause substantial impacts and are associated with biofouling species (Table 2).

Pathogen translocation via the biofouling pathway adds further layers of complexity to factors that influence pathogen transmission and disease dynamics (Fuhrmann and Hick, 2020; Lane et al., 2020). These parameters include pathogen life cycles, host specificity and susceptibility, infective dose, survival outside host, and susceptible host life stages (OIE, 2019; Pagenkopp-Lohan et al., 2020). To understand the translocation dynamics of these pathogens, presence of host or carrier species on vessel submerged surfaces, exposure of those species to pathogens prior to transit, prevalence and intensity of infection, and time of year (i.e., both seasonal dynamics and environmental conditions encountered) need to be considered.

Introduction of pathogens to new environments is influenced by vessel itinerary (i.e., places visited and duration of stay) and the type and duration of exposure of hosts in recipient environments. For example, exposure may occur as a result of pathogen shedding from organisms on a vessel, release of macro-organisms from the vessel surface, or an uncontained release of biofouling and pathogens following in-water cleaning. Characteristics of the recipient environment also need to be considered, including temperature, salinity, pollution, and—importantly—the presence, proximity, and density of susceptible host or carrier species (OIE, 2019; Lane et al., 2020; Pagenkopp-Lohan et al., 2020).

Receiving environments for vessels are typically ports and marinas which, being heavily modified, offer a variety of habitats for colonization by sessile and mobile taxa, including non-indigenous species (Ruiz et al., 1997; Johnston et al., 2017). These environments are often enclosed, leading to high particle retention (Gadd et al., 2011; Morrisey et al., 2013), thus, if released, pathogens may stay in contact with host species that are present for longer periods at potentially infective doses. The presence of artificial and modified habitats, combined with relatively high retention, enhances conditions for introduction, establishment, and spread of new non-indigenous macro-organisms (Floerl et al., 2009; Ruiz et al., 2009; Johnston et al., 2017) and associated pathogens. Following arrival and colonization, domestic vessels provide a vital link for spread from primary infected areas via movement of associated ballast water (Inglis et al., 2013) and biofouling, including fouled vessels that transit aquaculture zones (Sim-Smith et al., 2016).

Not every mollusk that is translocated with vessel fouling will cause a novel pathogen to establish with subsequent consequences (e.g., Gias and Johnston, 2010; Davidson et al.,

2013; Fuhrmann and Hick, 2020). Mounting evidence from laboratory and field observations as well as documented consequences, however, indicates that the risks associated with this pathway are non-negligible and that risk management measures may be justified. The evidence chain outlined here is consistent with the criteria applied to assess the risks associated with vessel biofouling for marine non-indigenous macro-organism translocations (Bell et al., 2011), which was a key step that led to biofouling regulations in New Zealand (Georgiades et al., 2020).

Mollusks are, importantly, not the only taxa associated with vessel biofouling that may translocate pathogens of concern. The invasive crabs *Eriocheir sinensis* and *C. maenas* are associated with fouled vessels (Peters and Panning, 1933 in Herborg et al., 2003; Coutts et al., 2003) and are susceptible hosts or carriers of several pathogens with well-documented consequences to marine values, including fisheries and aquaculture (Table 3). Parasitic invertebrates can also be translocated by barnacles, including the castrating isopod *Hemioniscus balani* (Davidson et al., 2013). Human pathogens, such as *Vibrio cholerae*, *Vibrio parahaemolyticus*, and *Escherichia coli*, have also been found in the surface biofilms of vessels (Shikuma and Hadfield, 2010; Revilla-Castellanos et al., 2015).

Potential Management Options

Pathogens can be released from vessel biofouling by being: (a) sloughed from the attached biofilm, (b) dispersed by proactive in-water cleaning (PIC), (c) shed from macrofouling that remains intact on the vessel, (d) shed with macrofouling released during normal vessel operations (i.e., drop-off of attached species or active escape of mobile species), or (e) dispersed with or without their hosts during application of RIC. We have identified a two-pronged approach to protect marine values from pathogen introductions associated with vessel biofouling by: (1) limiting the volume and frequency of pathogen translocations via ongoing vessel transportation (i.e., propagule pressure; Lockwood et al., 2005) and (2) avoiding pathogen releases by reactive management activities. The methods used to achieve these approaches all have associated advantages and disadvantages (Table 4).

The maritime antifouling industry is established to prevent and manage biofouling on vessels. The primary focus has been on surface paints or coatings on the immersed surfaces of ships to prevent macrofouling growth (using biocides, such as copper- and zinc-based compounds) and/or restrict macrofouling adhesion (non-biocidal or fouling-release, such as silicone-based coatings; Dafforn et al., 2011; Lewis, 2020). The use of biocidal coatings represents a trade-off between vessel fuel efficiency, reduced exhaust emissions, and reduced translocation of non-indigenous species, versus environmental impacts of the biocides (Dafforn et al., 2011; Scianni and Georgiades, 2019; Richir et al., 2021). Even though these coatings have evolved toward more sophisticated, cost effective, and environmentally acceptable products, antifoulants do not prevent biofilm formation (Dobretsov, 2010) or incidental macrofouling that establishes during vessel in-service periods (i.e., the time between vessel dry-docking; Georgiades and Kluz, 2017). There are areas of ships that cannot be painted

TABLE 1 | OIE-listed molluscan pathogens associated with known fouling species and their size (Bower, 2017; OIE, 2019).

Pathogen and OIE chapter	Susceptible species associated with fouling	Fouling type	Fouling reference	Particle size (μm)
<i>Bonamia exitiosa</i> OIE (2019) Chapter 2.4.2	<i>Ostrea chilensis</i>	Vessels	Smith et al. (2016)	2.4 \pm 0.5
	<i>O. angasi</i>	Harbors	Lewis (1982, 1986)	
	<i>O. edulis</i>	Vessels	Howard (1994)	
	<i>O. stentina</i>	Settlement plates	Hamaguchi et al. (2017)	
<i>Bonamia ostreae</i> OIE (2019) Chapter 2.4.3	<i>O. edulis</i>	Vessels	Howard (1994)	3 \pm 0.3
	<i>O. chilensis</i>	Vessels	Coutts and Dodgshun (2007)	
	<i>O. puelchana*</i>	Harbors	Schwindt et al. (2014)	
	<i>O. angasi*</i>	Harbors	Lewis (1982, 1986)	
<i>Marteilia refringens</i> OIE (2019) Chapter 2.4.4	<i>O. edulis</i>	Vessels	Howard (1994)	4–40
	<i>Mytilus edulis</i>	Vessels	Visscher (1928)	
	<i>M. galloprovincialis</i>	Vessels	Apte et al. (2000)	
	<i>O. stentina</i>	Settlement plates	Hamaguchi et al. (2017)	
	<i>Xenostrobus securis</i>	Vessels	Barbieri et al. (2011)	
	<i>O. chilensis*</i>	Vessels	Coutts and Dodgshun (2007)	
	<i>O. puelchana*</i>	Harbors	Schwindt et al. (2014)	
	<i>O. angasi*</i>	Harbors	Lewis (1982, 1986)	
<i>O. denselamellosa*</i>	Offshore structures	Tao and Wenxia (2002)		
Ostreid herpesvirus 1 μ var OIE (2019) Chapter 2.4.5	<i>Crassostrea gigas</i>	Vessels	Brock et al. (1999)	0.07–0.35
	<i>C. angulata</i>	Vessels	Ojaveer et al. (2018)	
<i>Perkinsus marinus</i> OIE (2019) Chapter 2.4.6	<i>C. virginica</i>	Vessels	Woods Hole Oceanographic Institution (WHOI) (1952)	2–15
	<i>C. gigas</i>	Vessels	Brock et al. (1999)	
	<i>C. ariakensis</i>	Vessels	National Research Council. (2004)	
	<i>C. rhizophorae</i>	Vessels	Farrapeira et al. (2010)	
	<i>C. corteziensis</i>	Hard substrate	Angell (1986)	
	<i>Mya arenaria***</i>	Vessels	Carlton (1999)	
<i>Perkinsus olseni**</i> OIE (2019) Chapter 2.4.7	<i>C. ariakensis</i>	Vessels	National Research Council. (2004)	5–15
	<i>C. sikamea</i>	Hard substrate	Hamaguchi et al. (2013)	
	<i>Pinctada margaritifera</i>	Offshore structures	Yan et al. (2006)	
	<i>P. martensii</i>	Offshore structures	Yan et al. (2006)	
	<i>P. fucata</i>	Hard substrate	Alagarswami (1977)	
<i>Mikrocytos mackini</i> OIE (2019) Chapter 2.4.9	<i>C. gigas</i>	Vessels	Brock et al. (1999)	2–3
	<i>C. virginica</i>	Vessels	Woods Hole Oceanographic Institution (WHOI) (1952)	
	<i>O. edulis</i>	Vessels	Howard (1994)	
	<i>O. lurida</i>	Vessels	Brock et al. (1999)	

*Infected when deployed in a known infected area although pathogen identification not completed to molecular level.

***Perkinsus olseni* has an extremely wide host range. Members of the families Arcidae, Malleidae, Isognomonidae, Chamidae, and Veneridae are particularly susceptible.

***Primarily an infaunal clam. Associated (nestled) in a biofouling community but not sessile or attached in this sense.

(e.g., anodes), are difficult to paint (e.g., dry-dock support strips), or experience sub-optimal coating performance because of surface or hydrodynamic issues (e.g., sea chests, gratings, rudders, and projections). These “niche areas” are hotspots of biofouling accumulation (Coutts and Taylor, 2004; Davidson et al., 2009, 2016) that require ongoing vigilance and maintenance (California Code of Regulations, 2017; Georgiades et al., 2018; Ministry for Primary Industries New Zealand [MPI], 2018). While biocide release rates from coatings can be estimated and environmental concentrations predicted (e.g., Morrissey et al., 2013), no quantitative assessments or estimates have been made of the release rates or quantities of live micro- or macrofouling organisms into coastal ecosystems as a result of normal vessel operations.

In-water cleaning has emerged as the principal approach to address limitations in coating performance and operational

impacts of biofouling that accumulate while in-service. In-water cleaning typically involves use of diver or remotely operated cleaning or cart systems that remove biofouling from hull surfaces (McClay et al., 2015; Morrissey and Woods, 2015; Tamburri et al., 2020). While in-water cleaning is often performed in response to fundamental operational factors, such as increasing fuel consumption (to reset hull surfaces to a more hydrodynamic state), it can have unintended consequences including: (a) increased release of antifouling biocides to ambient waters; (b) active liberation of live biofouling organisms or their propagules into local habitats, with increased risk of non-indigenous species introduction; and (c) diminished coating condition that reduces antifouling performance and longevity (Scianni and Georgiades, 2019; Tamburri et al., 2020). There is growing consensus internationally that steps should be taken to minimize these environmental impacts,

TABLE 2 | Examples of non-OIE listed molluscan pathogens that have caused substantial impacts and are associated with known fouling species (per Bower, 2017).

Pathogen and reference	Susceptible species associated with fouling	Fouling type	Fouling reference	Particle size (μm)
Oyster velar virus Bower (2001d)	<i>Crassostrea gigas</i>	Vessels	Brock et al. (1999)	0.228 + 0.007
Gill necrosis virus Bower (2001a)	<i>C. angulata</i> <i>C. gigas</i>	Vessels	Ojaveer et al. (2018) Brock et al. (1999)	0.350–0.380
Haemocytic infection virus Bower et al. (1994)	<i>C. angulata</i> <i>C. gigas</i>	Vessels Vessels	Ojaveer et al. (2018) Brock et al. (1999)	0.380
<i>Roseovarius crassostreae</i> Bower (2010)	<i>C. virginica</i>	Vessels	Woods Hole Oceanographic Institution (WHOI) (1952)	4.8 × 1.2
<i>Marteilioides chungmuensis</i> Itoh et al. (2004)	<i>C. gigas</i> <i>C. nippona</i>	Vessels Hard substrate	Brock et al. (1999) Wang and Li (2020)	6.3 × 4 Initial sporulation stage
Virus-like particles Bower (2001e)	<i>Perna canaliculus</i> <i>Mytilus galloprovincialis</i>	Vessels Vessels	Smith et al. (2016) Apte et al. (2000)	0.025–0.047
<i>Vibrio</i> spp. Bower (2009) Lopez-Joven et al. (2018)	<i>C. gigas</i> <i>C. virginica</i> <i>C. sikamea</i> <i>O. edulis</i> <i>O. conchaphila</i>	Vessels Vessels Hard substrate Vessels Hard substrate	Brock et al. (1999) Woods Hole Oceanographic Institution (WHOI) (1952) Hamaguchi et al. (2013) Howard (1994) Groth and Rumrill (2009)	<5
<i>Cytophaga</i> spp. Bower (2001b) Dungan et al. (1989)	<i>C. gigas</i> <i>C. virginica</i> <i>Ostrea edulis</i>	Vessels Vessels Vessels	Brock et al. (1999) Woods Hole Oceanographic Institution (WHOI) (1952) Howard (1994)	>5
<i>Nocardia crassostreae</i> Bower (2006)	<i>C. gigas</i> <i>O. edulis</i>	Vessels Vessels	Brock et al. (1999) Howard (1994)	<10
<i>Plectonema terebrans</i> <i>Hyella caespitose</i> <i>Mastigocoleus testarum</i> <i>Mastigocoleus</i> sp. (Nostochopsidaceae) <i>Pleurocapsa</i> sp.) Bower et al. (2002)	<i>M. galloprovincialis</i> <i>Choromytilus meridionalis</i> <i>Aulacomya ater</i>	Vessels Hard substrate Hard substrate	Apte et al. (2000) Barkai and Branch (1989) Barkai and Branch (1989)	<8
Kidney coccidia <i>Pseudoklossia semilunar</i> Bower (2001c)	<i>M. edulis/galloprovincialis/trossulus</i> species complex	Vessels	Visscher (1928) Apte et al. (2000) Moshchenko and Zvyagintsev (2001)	6 × 3 Sporocysts
<i>Haplosporidium costale</i> Bower (2014a)	<i>C. virginica</i>	Vessels	Woods Hole Oceanographic Institution (WHOI) (1952)	3–4 2.6 × 3.1
<i>Haplosporidium nelsoni</i> Bower (2014b)	<i>C. virginica</i>	Vessels	Woods Hole Oceanographic Institution (WHOI) (1952)	7.5 × 5.4 Spores 4–100 Multi-nucleate plasmodia
<i>Minchinia occulta</i> Bower (2014c)	<i>Saccostrea cucullata</i>	Vessels	Yan and Huang (1993)	4.5–6.7 × 3.3–4.1 Spores (micro) 4.5–5.0 × 3.5–4.1 Spores (EM)
<i>Marteilia sydneyi</i> Bower and Kleeman (2011) Green and Barnes (2010)	<i>S. glomerata</i>	Vessels	Ulman et al. (2017)	<10 diameter Mature sporonts
<i>Bonamia roughleyi</i> Bower (2015)	<i>S. glomerata</i>	Vessels	Ulman et al. (2017)	1–2
<i>Marteilia maurini</i> Bower (2019)	<i>M. galloprovincialis</i>	Vessels	Apte et al. (2000)	9.9 8.4
<i>Marteilia pararefringens</i> Bower (2019)	<i>M. edulis</i>	Vessels	Visscher (1928)	

by moving away from in-water cleaning of macrofouling that does not attempt to capture debris (i.e., RIC), and toward RIC that capture, contain, and treat debris (RICC), or PIC to prevent macrofouling establishment and growth. For all

in-water cleaning systems, there are two main processes that can release biological material including pathogens: (a) application of the cleaning unit to the vessel surface (either through incomplete or ineffective capture of debris by the cleaning head)

TABLE 3 | Notable pathogens and parasitic invertebrates associated with *Eriocheir sinensis* and *Carcinus maenas*.

Species	Pathogen	Particle size (μm)	References
<i>Eriocheir sinensis</i>	<i>Hepatospora eriocheir</i>	1.8 × 0.9	Stentiford et al. (2011)
	<i>Spiroplasma eriocheir</i>	0.1–0.35 diameter 0.1–0.2 diameter 3–12 length	Wang et al. (2004a,b) Wang et al. (2011)
	<i>E. sinensis</i> ronivirus	0.060– 0.110 × 0.024– 0.042	Zhang and Bonami (2007)
	White spot syndrome virus	0.080–0.150 diameter 0.250–0.380 length	Ding et al. (2015) OIE (2019) (Chapter 2.2.8)
	<i>Aphanomyces astasci</i> (freshwater)	Hyphae 7–9 width Secondary zoospores 8 × 12	OIE (2019) Chapter 2.2.2
<i>Carcinus maenas</i>	<i>Paragonimus westermani</i> (secondary host)	> 100 Metacercariae diameter	Peters and Panning (1933) Habe et al. (1993)
	<i>Hematodinium perezii</i>	15–100 length multinucleate plasmodium	Bower (2013a,b) Davies et al. (2019)
	<i>Hematodinium</i> sp.	6–22 diameter Single cell trophont	
	White spot syndrome virus	0.080–0.150 diameter 0.250–0.380 length	Bateman et al. (2012)
	Ostreid herpesvirus 1 μvar	0.07–0.35	Bookelaar et al. (2018) OIE (2019)

and (b) release of untreated or incompletely treated effluent (Scianni and Georgiades, 2019).

Pathway Management Approach

Few jurisdictions have enacted biofouling regulations to limit the translocation of marine non-indigenous macro-organisms (Georgiades et al., 2020; Scianni et al., in press). New Zealand's Craft Risk Management Standard for Biofouling on Vessels Arriving to New Zealand (CRMS-BIOFOUL) defines "clean hull" thresholds which are governed by the vessel's itinerary (Ministry for Primary Industries New Zealand [MPI], 2018). These thresholds, while acknowledging issues of feasibility and practicality, were designed to limit macro-organism species richness and density, constraining reproduction, and limiting establishment (Georgiades and Kluza, 2017). The holistic "level of fouling" approach applied by New Zealand manages risk but avoids difficulties, costs, and time associated with taxonomic identifications (Bell et al., 2011). This approach also protects against species not yet known to be invasive and does not require formation and ongoing maintenance of lists of "risk species" or the ongoing cost of surveillance and reporting against such lists.

The likelihood of pathogen translocation from vessels is most efficiently reduced by limiting the amount of macrofouling, which includes susceptible hosts and carrier species, on incoming international or inter-regional vessels. Similar to predicting invasive marine macro-organisms (Bell et al., 2011),

identifying future high-risk marine pathogens is difficult; further, the relative dearth of information in this area (Lane et al., 2020; Pagenkopp-Lohan et al., 2020) and the complexities related to pathogen introduction and establishment (section "Associations of OIE-Listed and Other Important Pathogens With Known Biofouling Species") create challenges and uncertainty in determining risk. A pathway management approach to holistically manage pathogen translocations associated with vessel biofouling is therefore likely to be more effective than a pathogen-specific approach.

In-water cleaning plays an important role in managing risks associated with the vessel biofouling pathway (Georgiades et al., 2018; Scianni et al., in press). There are, however, multiple approaches to consider in advancing a "cleaning strategy" to explicitly minimize the transfer of pathogens and micro-organisms. The following sections build on existing tools while identifying challenges, knowledge gaps, and possible solutions.

Proactive In-Water Cleaning (PIC) to Support Ongoing Vessel Maintenance

Proactive in-water cleaning (PIC) is an emerging approach used to prevent biofilm formation, to remove it from the hull (including microscopic life stages of macrofouling organisms), and ultimately to prevent or reduce the establishment and growth of macrofouling (Scianni and Georgiades, 2019). Because of its application early in the biofouling process, less abrasive techniques that are more consistent with the recommendations of antifouling system manufacturers may be used. While a substantial amount of microscopic material is released into the marine environment, PIC is viewed as a relatively low-risk activity because it minimizes the translocation of macrofouling species (Department of Agriculture [DOA] et al., 2015) and therefore minimizes potential translocation of pathogens replicating in macrofouling organisms (Hine, 1995; Lallias et al., 2008; Fuhrmann and Hick, 2020).

Harmful microalgae (including diatoms and dinoflagellates) and pathogens *can* occur in the biofilm of vessels (Drake et al., 2005, 2007; Molino and Wetherbee, 2008; Shikuma and Hadfield, 2010; Revilla-Castellanos et al., 2015). Biofilm formation and its subsequent sloughing from submerged vessel areas during normal operations (including interactions with tugs, bunkering barges, pilot boats, fenders, lines, etc.), however, cannot be prevented (Dobretsov, 2010; Morrissey et al., 2013) without near continual maintenance (Tribou and Swain, 2017; Amara et al., 2018). It may be conceivable to treat liberated biofilm associated micro-organisms during PIC [e.g., exposing the cleaning unit effluent to ultraviolet (UV) radiation], however, the role of the biofilm in pathogen and non-indigenous species translocations requires clarification, thus decisions about the utility and efficacy of PIC require consideration of this uncertainty.

The "Clean Before You Leave" Approach

To further limit the potential for pathogen translocation, adopting the reactive in-water cleaning (RIC) approach of "clean before you leave" could be especially useful, considering that the geographic origin of accumulated fouling dictates the

TABLE 4 | High-level approach for management of pathogen translocation via vessel biofouling.

Approach	Advantages	Disadvantages	Level of maturity/ availability	Key references
Pathway management	Protects against known and unknown risks Consistent with international fuel efficiency and emissions reduction policies Practicality/feasibility and low cost of implementation Not reliant on species lists and ongoing international surveillance and reporting activities	Some areas on vessels are difficult to access and maintain	High	Bell et al. (2011) Georgiades et al. (2018) Georgiades et al. (2020)
Proactive cleaning	Prevents or reduces establishment of macrofouling Consistent with some antifouling system (AFS) manufacturer's recommendations More convenient and cost effective than fouling penalties and dry-docking Lower environmental risk both chemically and biologically (macrofouling) than reactive cleaning	Can release substantial amounts of biological material (pathogens and other microbes) of unknown risk into the environment Some areas on vessels are difficult to access and maintain Upfront costs for predicted future benefits Some potential for increased release of biocides and macrofouling into the environment	Low to medium* *Depending on the need for recapture	Schultz et al. (2011) Inglis et al. (2012) Morrisey et al. (2013) Tribou and Swain (2017) Hunsucker et al. (2019) Scianni and Georgiades (2019)
Reactive cleaning				
<i>Clean before you leave</i>	Little or no biological risk depending on vessel history/itinerary Potentially more convenient and cost effective than dry-docking Generally applies to a wide range of vessel types (includes most recreational boats) and a subset of other vessels that are high-risk (work barges/platforms, idle/lay-ups, etc.)	Potential release of biocides into the environment Some areas on vessels are difficult to access and maintain Removal of hard fouling associated with AFS damage Does not apply to a majority of commercial vessels	High	Inglis et al. (2012) Department of Agriculture [DOA] et al. (2015) Scianni and Georgiades (2019)
<i>Reactive in-water cleaning with capture</i>	Potentially more convenient and cost effective than dry-docking Costs only incurred if/when macrofouling removal is clearly beneficial	Some potential release of macrofouling and pathogens into the environment Some potential release of biocides into the environment Some areas on vessels are difficult to access and maintain Removal of hard fouling associated with AFS damage	Low	Schultz et al. (2011) Inglis et al. (2012) Morrisey et al. (2013) Morrisey and Woods (2015) Scianni and Georgiades (2019) Tamburri et al. (2020)

biosecurity risk (Department of Agriculture [DOA] et al., 2015). This approach recognizes that the most effective focal point for management is prevention, and thus looks to manage the risk of pathogen translocation at the source to limit spread and downstream impacts (Ricciardi et al., 2020). The applicability of this practice depends on the vessel's prior itinerary, pathogen status of the recipient area and areas visited earlier, and proximity to high-value areas. Management of environmental

contamination by antifouling biocides is a key consideration if such an approach is to be used (Scianni and Georgiades, 2019).

Reactive In-Water Cleaning With Capture (RICC)

Reactive in-water cleaning of vessel biofouling includes methods to capture, contain, and treat associated organisms [i.e., RICC, also referred to as in-water cleaning with capture (IWCC)]. The efficacy of RICC systems is uncertain in many cases and

may vary by organism size and local conditions (Davidson et al., 2008; Scianni and Georgiades, 2019; Tamburri et al., 2020). To date, cleaning units are designed primarily to capture macrofouling organisms, with some attention to treatment of chemical effluent (Scianni and Georgiades, 2019). Vessel surveys to assess if RICC of macro-organisms is required are limited to detection and identification of macrofouling, while pathogens that may be associated with biofouling organisms have seldom been considered (Georgiades et al., 2020).

Removal of macrofouling by RICC is likely to kill the hosts and, where appropriate treatment of waste is not applied, release pathogens if present. Infected mollusks that are dead or moribund are known pathogen sources, for example: OsHV-1 (see Sauvage et al., 2009), *P. olsenii* (see Raynard et al., 2007), and *P. marinus* (see Bobo et al., 1997). The reactive removal of macrofouling can release infective material, potentially in large amounts, into receiving environments with high particle retention. A single oyster can contain 4.4×10^8 *B. ostreae* parasites (Lallias et al., 2008), and Arzul et al. (2009) observed 58% survival of *B. ostreae* parasites after 7 days exposure to seawater at 15°C. OsHV-1 has been observed at 10^7 DNA copies per mg of clinically affected Pacific oyster tissue (Pepin et al., 2008). Hick et al. (2016) found that OsHV-1 remained infectious in seawater for 2 days at 20°C and in non-viable oyster tissues (wet or dry) for at least 7 days at 20°C. Vigneron et al. (2004) detected OsHV-1 DNA released into seawater from macerated larvae for 22 days at 4°C and 12 days at 20°C, although it was not determined if this DNA was viable. *P. olsenii* loads in infected host tissues can exceed 2×10^6 parasites per gram (Choi and Park, 2010), and *P. olsenii* can survive outside a host for at least several months (Casas et al., 2002). All life stages of *P. olsenii* are considered infective (Villalba et al., 2004). While elevated concentrations of antifouling biocides in the treatment stream (Tamburri et al., 2020) may render some pathogens non-viable (Dupont et al., 2011), these can also impact the health of organisms in the receiving environment (Dafforn et al., 2011; Amara et al., 2018), and potentially increase their susceptibility to disease (Moreau et al., 2015).

Direct releases of biofouling from the cleaning head can be assessed by analyzing the total suspended solids (TSS) in water sampled from the surrounding environment during equipment operation (Alliance for Coastal Technologies Maritime Environmental Resource Center [ACT/MERC], 2019; Tamburri et al., 2020). Inclusion of this analysis is a useful addition to the technical advice released by MPI on testing in-water cleaning and treatment systems for external hull and niche areas (Morrisey et al., 2015) and internal seawater systems (Growcott et al., 2019). Similar to direct video observations of the cleaning unit during operations, TSS may serve as a proxy for propagule release into the marine environment.

Recommendations for biosecure effluent treatment standards for surface-based waste processing systems associated with RICC are typically based on physical separation (typically settling tanks followed by filtration) to remove live organisms and propagules associated with macro-organisms (Scianni and Georgiades, 2019). Targeted particle size thresholds range between 2 µm (Morrisey et al., 2013), 5 µm (California Water Boards,

2013), 10 µm (Lewis, 2020), 12.5 µm (Morrisey et al., 2015; Growcott et al., 2019), 50 µm (Department of Agriculture [DOA] et al., 2015), and 60 µm (Morrisey et al., 2013). Assessments of filtration technologies associated with ballast water management systems (BWMS) that use similar designs and functions as those incorporated in RICC systems have shown that they are far from 100% effective at removing live organisms above the stated target particle size, or even the specific physical mesh or sieve size employed (e.g., Gregg et al., 2009; Briski et al., 2014; Cangelosi et al., 2014). Although filtration and settlement can be effective in removing the proportion of pathogens contained within infected material, physical separation of particles, even down to 2–5 µm, is unlikely to completely remove many known pathogens, including smaller protists, bacteria, and viruses (Tables 1–3), or dissolved biocides (Terraphase Engineering Inc, 2012; Tamburri et al., 2020), from the effluent. Additional effluent treatment options (i.e., a disinfection step) should therefore be considered where the prevention of pathogen translocation associated with vessel biofouling is a concern.

Additional effluent treatment options for reactive in-water cleaning

For high risk international vessels or vessels from regions with different biosecurity conditions, additional measures to the physical separation of captured debris alone include, *but are not limited to*, treatment using biocides, UV radiation, or heat to render propagules non-viable (Table 5), or direct disposal of the liquid effluent into municipal sewerage where permitted (Morrisey and Woods, 2015). Pre-filtration to reduce the particulate and organic material present is still required to improve the treatment efficacy of UV, ozone, or oxidants (Chahal et al., 2016; Hess-Erga et al., 2019). Depending on the treatment purpose, pre-filtration recommendations vary between 7 µm (Fraser et al., 2006), 20 µm (Sassi et al., 2005), and 50 µm (Department of Agriculture, Fisheries and Forestry [DAFF], 2008). High flow velocities, however, such as those associated with fouling removal by RICC systems, may decrease filtration efficacy by reducing the contact time between pathogens and particles, and increasing hydraulic shear which can disrupt pathogen-to-particle binding (Chahal et al., 2016). While this may lead to increase in the exposure to subsequent treatments resulting improved efficacy, it also results increased load on this stage of the treatment process.

The concept of appropriate effluent treatment from RICC systems is challenging because:

- a) The macrofouling species and pathogens present on submerged surfaces of any given vessel will be largely unknown, therefore any treatments employed should have a wide range of efficacy across a number of pathogen types (e.g., viruses, bacteria, and protists).
- b) The efficacy of some treatments (e.g., biocides and UV) is dictated by the amount of organic and particulate matter present and the size of some pathogens (i.e., viruses) is far smaller than any filter size that can be practically achieved. The main purpose of physical separation will, therefore, be to reduce the amount of particulate and

TABLE 5 | Recommended dose/fluence for efficacy of common water treatment agents.

Disinfecting agent	Application	Recommended dose/fluence	References
Chlorine	Drinking water	1 mg/L, 30 min	Henze et al. (2008)
	Effluent (aquaculture)	2 mg/L, 5 min	Meyers (2010)
	Effluent (Processing facilities)	5 mg/L, 30 min An initial concentration of 1,000 mg/L of sodium hypochlorite is sufficient	Fraser et al. (2006)
	Wastewater (aquaculture) (Decontamination)	30 mg/L, 24 h (maintain residual 5 mg/L)	Department of Agriculture, Fisheries and Forestry [DAFF] (2008)
	Wastewater	20–40 mg/L	Henze et al. (2008)
	Wastewater (Cryptosporidium)	20–40 mg/L, 90 min	Henze et al. (2008)
	OSHV-1	50 mg/L, 15 min	Hick et al. (2016)
	<i>Marteilia sydneyi</i>	200 mg/L, 4 h	Wesche et al. (1999)
	<i>Perkinsus marinus</i>	300 mg/L, 30 min	OIE (2019) Chapter 2.4.6
	<i>P. olsenii</i>	6 mg/L, 30 min	OIE (2019) Chapter 2.4.7
<i>Bonamia exitiosa</i>	40 g/L, 10 min	Buss et al. (2020)	
Ultraviolet radiation	Effluent (aquaculture)	> 25 mJ/cm ²	Department of Agriculture, Fisheries and Forestry [DAFF] (2008)
	Effluent (aquaculture) (including Mxyosporideans)	> 35 mJ/cm ²	Department of Agriculture, Fisheries and Forestry [DAFF] (2008)
	OsHV-1	42 mJ/cm ²	Stavrakakis et al. (2017)
	<i>P. marinus</i>	> 28 mJ/cm ²	OIE (2019) Chapter 2.4.6
	<i>P. olsenii</i>	60 mJ/cm ²	OIE (2019) Chapter 2.4.7
	Influent (freshwater aquaculture) Infectious pancreatic necrosis virus (IPNV)	122 mJ/cm ²	Fraser et al. (2006)
	Effluent (freshwater aquaculture) (Nodavirus)	290 mJ/cm ²	Fraser et al. (2006)
	Effluent (processing facilities)	120 mJ/cm ²	Fraser et al. (2006)
	Influent/effluent (aquaculture) High flow/heavy particulates (99.9% reduction fish viruses)	175 mJ/cm ²	Meyers (2010)
	Ozone	Influent/effluent (aquaculture)	8 mg/L, 3 min (Corresponding to redox potential 600–750 mV)
Emergency disease events (Residual level)		0.5 mg/L, 10 min 1 mg/L, 1 min	Department of Agriculture, Fisheries and Forestry [DAFF] (2008)
OSHV-1		1 mg/L, 30 min	Stavrakakis et al. (2017)
Heat	Wastewater (aquaculture) (Decontamination)	60°C, 10 min 70°C, 6 min 75°C, 5 min 80°C, 4 min Most pathogens Enveloped viruses and some bacteria may be resistant	Department of Agriculture, Fisheries and Forestry [DAFF] (2008)
	Treatment of hard surfaces and equipment	Steam cleaning at 115–130°C for 5 min	Department of Agriculture, Fisheries and Forestry [DAFF] (2008)
	Treatment of gear and steam cleaning non-porous surfaces	70°C, 2 h (IPNV)	Fraser et al. (2006)
	<i>P. marinus</i>	50°C, 1 h (Filtered seawater) 60°C, 1 h (Tissues)	OIE (2019) Chapter 2.4.7

(Continued)

TABLE 5 | Continued

Disinfecting agent	Application	Recommended dose/fluence	References
	Iridoviruses of mollusks (VL) OsHV-1 Malacoherpesviruses Oyster edema disease <i>Haplosporidium nelsoni</i> (VL?) <i>Marteilia</i> spp. (?) <i>M. sydneyi</i> (VL?) <i>Marteiloides</i> spp. (?) <i>M. chungmuensis</i> (VL?) <i>Perkinsus</i> spp. (VL) <i>P. olseni</i> (VL) <i>P. chesapeakei</i> (VL)	55°C, > 10 min VL = very low risk (= appropriate level of protection) ? = uncertainty due to lack of information	Diggles (2020)
	OsHV-1 Malacoherpesviruses Oyster edema disease <i>Bonamia</i> spp. (VL?) <i>B. exitiosa</i> (VL?) <i>B. ostreae</i> (VL?) <i>Haplosporidium nelsoni</i> (?) <i>Marteilia</i> spp. (?) <i>M. refringens</i> (VL?) <i>M. sydneyi</i> (?) <i>M. roughleyi</i> (VL?) <i>Marteiloides</i> spp. (?) <i>M. chungmuensis</i> (?) <i>Mikrocytos</i> spp. (VL?) <i>M. mackini</i> (VL?) <i>Minchinia</i> spp. (VL?) <i>M. occulta</i> (VL?) <i>Perkinsus</i> spp. (VL?) <i>P. olseni</i> (VL?) <i>P. chesapeakei</i> (VL?) <i>P. marinus</i> (VL?) Akoya oyster disease (VL?)	80°C, > 5 min VL = very low risk (= appropriate level of protection) ? = uncertainty due to lack of information	Diggles (2020)

organic matter present to assist the efficacy of any subsequent treatment(s).

- c) The efficacy of some subsequent treatments (e.g., biocides and UV) will be difficult to ascertain without some form of indicator. That is, approaches for disinfecting effluent prior to discharge should demonstrate their efficacy in removing viral, bacterial, and protistan pathogens (or surrogates for pathogens).
- d) Biocidal treatments may be subject to local water quality regulations and may require neutralization prior to effluent discharge.

Effluent treatment for the in-water removal of high-risk biofouling can be guided by lessons learned from municipal sewage treatment (Henze et al., 2008; Chahal et al., 2016), the influent and effluent treatment for land-based aquaculture (Fraser et al., 2006; Department of Agriculture, Fisheries and Forestry [DAFF], 2008; Meyers, 2010; Baulch et al., 2013; Whittington et al., 2020), and the development of BWMS (Balaji et al., 2014; First and Drake, 2014; Batista et al., 2017; Hess-Erga et al., 2019).

While the fundamental goal for management of both the biofouling and ballast water pathways is to minimize the

risk of non-indigenous species introductions from vessels, discharge thresholds and management approaches for ballast water regulations (e.g., International Maritime Organization [IMO], 2004) are not directly applicable to RICC of vessel biofouling because:

- a) Ballast water brought onboard ships has fewer suspended solids and smaller particle sizes than wastewater produced and treated by RICC.
- b) The planktonic organisms treated by BWMS are dissimilar in many ways to micro- and macro-organisms observed within biofouling assemblages.
- c) The IMO ballast water regulations do not consider organisms less than 10 µm in size, apart from a few target taxa (*E. coli*, Enterococci, and toxigenic *V. cholerae*).
- d) RICC systems use large volumes of ambient water in the cleaning process, and RICC effluent therefore requires treatment of biofouling organisms removed from ship surfaces and substantial biomass of local planktonic organisms.
- e) Ballast water effluent treatment methods are unlikely to address the environmental risk of releasing antifouling biocides associated with vessel coatings.

In-water cleaning systems do not have the same physical space and power limitations as shipboard BWMS, which could facilitate broader treatment options, such as those used in land-based water treatment, including sewage treatment facilities and aquaculture establishments. While all RICC systems incorporate some form(s) of physical separation (e.g., settling tank, filter, hydrocyclone, and flocculation), decisions about the incorporation of subsequent treatment to either remove dissolved antifouling biocides (e.g., selective metal-binding or adsorption media) or kill pathogens should carefully consider a number of factors including, but not limited to: the marine values requiring protection, risk reduction outcomes, treatment feasibility and practicality, and cost (see Inglis et al., 2012). The following are examples of possible subsequent treatment and disinfection approaches, but not an exhaustive list.

Chlorine. A common disinfectant for wastewater, municipal water, and ballast water (see Ghernaout, 2017), “chlorine”—used here to represent the suite of reactive halogens derived from chlorine—may be suited to disinfect effluent from in-water cleaning operations (Table 5). Chlorine can be injected from an external source, or it may be produced on-site, through electrolytic chlorine generation. Regardless of the chlorine compound, and irrespective of whether it is introduced as a solid, liquid, or a gas, a series of cascading reactions occur based upon the water characteristics following the injection of the compound into the effluent. For example, an electrolytic chlorine generator introduces chlorine as a gas, which upon dissolution in water, reacts to form hypochlorous acid (HOCl). In seawater, HOCl is unstable and quickly dissociates to form hypochlorite (OCl^-) and then hypobromous acid, which is also an effective germicide and more stable than HOCl at the pH of seawater (Wong and Davidson, 1977; Abarnou and Miossec, 1992). When ammonia is present in seawater, the oxidants produced through electrolysis form mono-, di-, or tri-chloramines. While these are not considered the primary biocidal agent, some organisms (including invertebrates such as copepods and crab larvae) are sensitive to chloramines (Capuzzo, 1979).

Some BWMS use chlorine to treat large volumes of natural water (Joint Group of Experts on the Scientific Aspects of Marine Environmental Protection [GESAMP], 2019). Generally, ballast water is treated upon uptake, i.e., entry into the ship prior to transit. Treated water is typically held in ballast tanks for at least 1 day, and residual oxidants are neutralized prior to discharge into the environment. In-water cleaning operations require rapid treatment of large volumes, but—relative to ships’ ballasting operations—reservoir volumes are small and hold times are minimal. Adapting a BWMS-like approach for in-water cleaning operations would require large tanks and other infrastructure, which may not be practicable. For active substances, treatment would have to occur following the final filtration, as to minimize organic particulates that consume oxidants. In an in-water cleaning operation, treated water would almost immediately be neutralized thus limiting system efficacy, as short treatment times limit the dispersion of chlorine and the reactivity of the primary oxidizing species. Biocidal concentrations required for high flow, small reservoir systems will exceed those for treating ballast water,

and the effective doses would vary based upon a variety of factors including temperature, pH, light, salinity, presence of organic matter (Chahal et al., 2016; Batista et al., 2017; Hess-Erga et al., 2019), and contact time. These factors emphasize the importance of measuring the residual dose of chlorine in the system and assessing efficacy using validated methods.

Chlorine may be effective for effluent treatment if on-site or nearby reservoirs are used to hold treated water, allowing time for the reaction and dissipation of chlorine prior to neutralization. Other strategies to improve the biocidal efficacy of chlorine will allow for shorter residence times in the treatment system. For example, combinations of chlorine and other reagents (e.g., CO_2) have shown improved efficacy (Growcott et al., 2017; Hess-Erga et al., 2019). Likewise, mixed-oxidant systems have demonstrated improved biocidal efficacy relative to chlorine alone (Venczel et al., 1997). Finally, an approach for the in-water treatment of hull surfaces has been envisioned: this suggestion would use chlorine produced directly on the hull surfaces by electrolytic chlorination as an antifouling treatment (Iliopoulos et al., 2014). For this approach, ships’ existing cathodic protection systems would be reengineered so that chlorine—naturally produced at the anodes—is dispersed to prevent fouling on the hull and other wetted surfaces. This approach would guard against both macrofouling organisms and their pathogens. However, “in-water chlorination” would potentially introduce high concentrations of chlorine (and disinfection byproducts) into natural water systems which would likely violate the local water quality standards of many jurisdictions.

While chlorination appears to be an obvious candidate for secondary treatment of in-water cleaning effluent, its efficacy remains unresolved (Cahill et al., 2019a), the effluent would need to be neutralized prior to discharge to satisfy local water quality standards, and it is not yet incorporated in any commercially available RICC system.

Ultraviolet (UV) radiation. Ultraviolet radiation is used to disinfect drinking, waste, and ballast water (see Chen et al., 2006). By contrast to chlorine, UV radiation is readily adaptable for an in-line system for effluent treatment: treatment occurs during the short period that suspended organism transits through a chamber. Reactive chemicals are not required, nor are neutralizing agents. Effluent water could be discharged immediately following treatment, given that the dose is sufficient. To achieve the prescribed ballast water discharge standards, Oemcke et al. (2004) and Kim et al. (2019) recommended doses of 60–70 mJ/cm^2 to treat most bacteria and viruses (Table 5). Oemcke et al. (2004) further recommended that a dose of 120 mJ/cm^2 would remove most micro-organisms except for resistant cysts and viruses. Much higher doses are required to treat the cysts of *Cryptosporidium* spp. (> 200 mJ/cm^2) and diatoms such as *Gymnodinium catenatum* (> 1,600 mJ/cm^2) (Gregg et al., 2009). For many micro-organisms, the effect of UV irradiation may not be immediate, raising the issue of organism viability (i.e., treatment efficacy). This issue has introduced a variety of complications when determining the efficacy of BWMS (First and Drake, 2014; Batista et al., 2017; Peperzak et al., 2020). For example, damage to organisms, in certain cases,

may be repairable (e.g., Zimmer and Slawson, 2002). Similar to chlorine, UV radiation efficacy varies based upon the water characteristics, particularly the concentration of particulate and dissolved material (primarily organic matter), which reduce the UV transmissivity (%UVT), but may also scavenge reactive oxidation species (Ou et al., 2011). At least one commercially available RICC system includes the option of secondary UV treatment of captured debris (Lewis, 2013).

Ozone. Ozone is an established water treatment (see Langlais et al., 1991) and is effective against a range of micro-organisms (Gregg et al., 2009; Chahal et al., 2016). Similar to chlorine, ozone is an oxidizing compound that may be generated on-site. For ballast water treatment > 5 mg/L, total residual oxidants for 10 h appear to be a broad-spectrum treatment for free-living bacteria, dinoflagellates, and diatoms; however, 8–14 mg/L for 24 h was required to effectively treat the spore-forming bacteria *Bacillus subtilis* (see Gregg et al., 2009; **Table 5**). Ozone—when used as a disinfectant for effluent from a land-based RICC treatment system—has some of the same limitations and caveats as chlorine. Treatment efficacy is a product of both dose concentration and exposure time, and for a flow-through system, exposure time will be limited. Likewise, residual oxidants will require neutralization. Ozone is not yet incorporated into RICC systems.

Heat. Some in-water cleaning technologies use heat as an approach for removing fouling, particularly the algal and biofilm fouling on easily accessible areas of ships' hulls (Morrissey and Woods, 2015). In this case, heat treatment would be effective against free-living pathogens. Heat may also be used in shore-side treatment systems to kill organisms remaining prior to discharge. For treatment of macrofouled vessel internal seawater systems, Cahill et al. (2019b) and Growcott et al. (2019) recommended exposure to 60°C for 60 min. Such a treatment would appear to be effective against all but the hardiest pathogens, e.g., birnaviruses such as infectious pancreatic necrosis virus (IPNV; Fraser et al., 2006). Aquatic birnaviruses have been found in oysters and mussels located near salmon farms (Mortensen, 1993; Rivas et al., 1993). IPNV has had substantial impacts on global salmonid aquaculture (Munro and Midtlyng, 2011) and has been experimentally transmitted from *Mytilus edulis* to *Salmo salar* by cohabitation (Molloy et al., 2013). When considering the range of pathogens of biosecurity relevance to Australia, Diggles (2020) concluded that heating (80°C in water, > 5 min) would meet the acceptable level of protection (i.e., an annual probability of establishment between 1 in 20 and 1 in 100 years) for all identified risk pathogens despite uncertainty from data deficiency (**Table 5**).

For BWMS, chlorine and UV radiation are common disinfectants, but heat is also used in some cases. For one particular BWMS, the heat source is primarily waste heat from the ship engines and cargo pumps. Treatment occurs in a section of heated pipe, designed so that flowing water meets minimal hold time and temperature limits for disinfection. Note that this system, while shown in Type Approval testing to meet limits on regulated groups of organisms (e.g., Coast Guard Maritime Commons, 2020), was not evaluated for its efficacy in treating pathogenic bacteria, protists < 10 μm, and viruses. While heat treatment is not sensitive to most water characteristics (such

as dissolved organic material, salinity, etc.), its efficiency does depend upon the temperature of the input water, i.e., more energy is required to meet the minimum effective temperature in cold and temperate waters relative to tropical waters. On ships, heat-based BWMS may be coupled with heat generating systems, but on a shore-side operation, it is likely that a dedicated, intentional heat source is necessary. The feasibility for using heat to treat the effluent from in-water cleaning operations, therefore, rests on the characteristics of a specific location, including water temperatures, access to fuel or energy for boilers, and opportunities for capturing waste heat. Heat is not yet incorporated into RICC systems.

CONCLUSION

The long history of devastating impacts to marine values caused by cross-boundary translocation of marine pathogens has resulted in improved practices from regulatory and non-regulatory controls that apply to established pathways, such as fisheries and aquaculture. Available evidence indicates that vessel biofouling is also a viable and important pathway for translocating marine pathogens which presents a risk to marine values. This largely unmanaged pathway, therefore, represents a considerable gap in the biosecurity measures of jurisdictions committed to the prevention and control of aquatic disease. Preventive measures, such as those used in New Zealand and Californian biofouling regulations, lower the likelihood of pathogen translocation by reducing diversity, population size, and total mass of susceptible hosts and carrier species on vessels. Reactive measures, such as in-water removal of macrofouling, may, however, exacerbate the problem and will likely need modification to manage the risk associated with pathogens. While lessons learned from ballast water management, sewage treatment, and aquaculture industries should be considered when developing cleaning and effluent treatment criteria for reactive approaches, preventing or greatly reducing the translocation of pathogens using proactive measures for vessel biofouling is likely to be more effective. Both proactive and reactive solutions, however, have their own unique challenges. The balance between marine protection and risk reduction versus treatment feasibility and cost requires careful consideration.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article. Further inquiries can be directed to the corresponding author/s.

AUTHOR CONTRIBUTIONS

EG and DK conceived the idea for the manuscript which was drafted by EG. DK, CS, ID, MT, MF, GR, KE, and MD revised the manuscript draft and contributed sections based on their areas of expertise. All authors contributed to manuscript revision and read and approved the submitted version.

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

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