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Invasive Species and Aquaculture Pathogens in the Irish and Celtic Seas

Thesis presented by

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Declaration

This is to certify that the work I am submitting is my own and has not been submitted for another degree, either at University College Cork or elsewhere. All external references and sources are clearly acknowledged and identified within the contents. I have read and understood the regulations of University College Cork concerning plagiarism.

Signed:

Katie Ellen Costello

Bluefish Project

This research was funded by the Bluefish Project, part of the Ireland-Wales Programme 2014-2020 and designed to invest in communities across both countries in support of development and sustainability. The focus of Bluefish is the vulnerability of local aquaculture and fishery industries to predicted climate change, and involved cooperative work across six institutions; Aberystwyth, Bangor and Swansea Universities in Wales, and Bord Iascaigh Mhara, the Marine Institute and University College Cork in Ireland. The Project took the form of work packages, listed as follows: *i*) Ecosystem Understanding, *ii*) Ecosystem Resources, *iii*) Ecosystem Health, *iv*) Ecosystem Change and *v*) Communications. The research presented here is aligned with the Ecosystem Health work package, and provides an integrated study of the impacts of current and potential marine invasive species on cultured stock, primarily shellfish, in Irish and Celtic Sea coastal regions.

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Abstract

Invasive species represent a major threat to biodiversity and ecosystem functioning, however research into the interactions between invasive species and their parasites is lagging far behind research into general invasion biology. This thesis explores the relationship between invasive species, specifically those which impact the aquaculture sector through biofouling or predation on commercial species, and the parasites and pathogens with which they interact. Focus is paid to bivalve aquaculture, since species such as the Pacific cupped oyster *Crassostrea gigas* and the blue mussel *Mytilus edulis* are heavily cultured within the study regions. The first research chapter takes the form of a review which synthesises invasive host-parasite interactions using marine bivalves as a model group. The global aquaculture industry is discussed in detail, as often it is this industry that facilitates the spread of both invasive species and disease, but it is also this industry that is adversely impacted by subsequent disease outbreaks. The chapter then provides recommendations to enhance our understanding of marine diseases, and also addresses how climate change might influence invasive host-parasite complexes. The second data chapter investigates the impact of one particular group of invasive species - tunicates that can have a significant impact on aquaculture. The study looks at the impact of these tunicates on the maintenance of select pathogens that affect commercial bivalves, including the ostreid herpesvirus OsHV-1 µVar, the bacterium *Vibrio aestuarianus* and the haplosporidia *Bonamia ostreae* and *Minchinia* spp. PCR, Sanger sequencing and histology confirmed the presence of *B. ostreae* and *Minchinia mercenariae*-like in the leathery/club tunicate *Styela clava*, and *V. aestuarianus* was confirmed by qPCR in the orange sheath tunicate *Botrylloides violaceus* and the carpet sea squirt *Didemnum vexillum.* Furthermore, histology confirmed *M. mercenariae*-like sporonts in *S. clava* suggesting that the tunicate can facilitate replication of this species. The results indicate that tunicates can act as reservoirs of infection in areas where disease occurs and potentially transport diseases to uninfected sites. Microbial diversity in nearshore bivalve culture environments is well-documented, but less is known about offshore environments. The third data chapter of this thesis examined 65 plankton samples collected from the Irish and Celtic Seas in May 2018 to investigate zooplankton-associated microbial communities. Bacteriome sequencing was used to characterise the bacterial community structure and identify any potential pathogens, and PCR was also used to further screen for

haplosporidian and viral pathogens. The bacterium *Vibrio splendidus* was detected, as was a haplosporidian species (18_Haplo_BMVA_WEY) first detected off Weymouth, England between 2011-2012. The results also revealed distinct bacterial profiles arising from the Irish Sea, Celtic Front, Eastern Celtic and Southern Celtic Sea areas, suggesting that oceanic currents and fronts may act as barriers or facilitators to microbial dispersal thereby providing pathways for pathogens. The final empirical chapter of this thesis takes the form of a horizon scanning exercise utilising cargo shipping records from 2018-2019 (n = 9,291). The chapter focuses on the connectivity between four major ports in Ireland (Dublin, Cork, Rosslare and New Ross) and global shipping ports. Ballast water and hull fouling are vectors for invasive species movements, and regional management may be strengthened by identifying shipping networks as this will allow for targeted inspections. A targeted horizon scanning exercise for invasive species likely to arrive in Ireland was included, and seventeen incoming ports were highlighted as having high connectivity to Ireland. Furthermore, the focal invasive species are present in these ports, suggesting there is strong potential for invasion. Shipping routes within Ireland also demonstrate high connectivity, meaning the potential for secondary spread is strong. The thesis concludes with a discussion highlighting the main findings and emphasising the importance of integrating the fields of parasitology and invasion ecology to enhance our understanding of pathogen dispersal and transmission.

Chapter 1: General Introduction

Overview

Invasive species signify a major threat to ecosystem stability and are one of the primary drivers of biodiversity loss. In marine systems the ecological impacts of invasive species are well documented, as they may impede the sustainability and growth of the aquaculture and fishery sectors in a number of ways, for example: displacing native species, restructuring food webs, modifying habitat and acting as fouling organisms. However, research into the interactions between invasive species and their associated parasites is less developed than research into general invasion biology. Furthermore, parasites can have free-living life stages, indeed viral and bacterial pathogens may persist in the water column, thus maintaining disease reservoirs. The lack of empirical data relating to invasive species, parasites and pathogens is concerning, as biological invasions may be linked to both emerging and existing infectious diseases, thereby perpetuating disease-cycling within the marine environment.

The Invasion Pathway

To successfully complete the invasive process a potentially invasive species must move through a number of biotic and abiotic filters (Hellmann *et al*., 2008) known as the invasion pathway. In the marine environment, this movement may be facilitated by hull fouling, ballast water, rafting on anthropogenic debris, translocation of aquaculture gear and stock and recreational shipping (Hulme, 2015; Rech *et al*., 2018). The first stage of the invasion pathway is the crossing of a geographic barrier as the species moves to a new range, and this introduction to the extended range is heavily influenced by the propagule pressure. Propagule pressure is a composite measure of two factors (Figure 1): propagule size which is the number of individuals within the propagule and propagule number, the amount of propagule introductions within a given unit of time (Simberloff, 2009). High propagule pressure facilitated the invasion of the green porcelain crab *Petrolisthes armatus* in Eastern oyster *Crassostrea virginica* reefs around the southeast United States. The propagule pressure, facilitated by high larval recruitment, overcame biotic resistance from native mud crabs and resulted in *P. armatus* densities 10 – 37 times greater than ever recorded in its historic range (Hollebone & Hay, 2007).

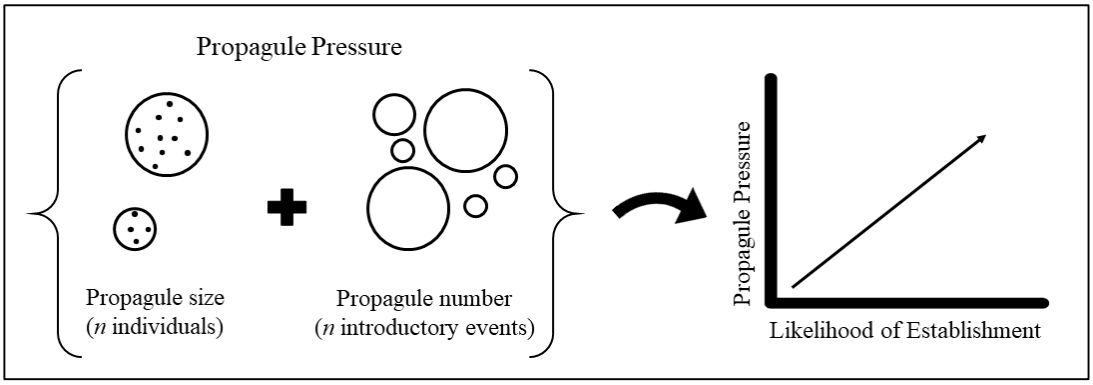

Figure 1: Propagule pressure – a composite factor combining the number of individuals with the number of introductory events. Higher propagule pressure leads to an increased likelihood of invasive species establishment.

The second stage of the invasion pathway occurs upon arrival, when the species must tolerate the biotic and abiotic conditions of the new range. Lenz *et al*. (2011) compared the response of native and non-native ascidians, bivalves and crustaceans to fluctuations in salinity, temperature and oxygen concentration and suggested that non-native species are more tolerant to environmental stress than native species. They found that non-native species demonstrated lower deviations from normal respiratory function and higher rates of survival under stress than native species, and Havel *et al*. (2015) also identified environmental tolerance as an important invasive characteristic.

The third stage of the invasion pathway is when the introduced species successfully reproduce to facilitate the expansion of the population. Whitney & Gabler (2008) noted a number of species traits that distinguish successful invasive species. These include consistent or uniparental reproduction without specialised requirements and a short generation time. An example is that of the European sea squirt *Ascidiella aspersa*, a species native to the northeast Atlantic but which demonstrates invasive tendencies in its introduced ranges of Australia, New Zealand, India and the northwest Atlantic. *A. aspersa* can alternate between a single sex and hermaphroditic state and has also been observed spawning year round in its native range (Lynch *et al*., 2016).

Once an invasive species has entered a local marine system it will likely persist in that area and many invasive species act as system engineers, modifying the environment and creating suitable conditions for further species to colonise and invade the locality. This synergistic relationship between invasive species is termed the ‘invasional meltdown’ (Bax *et al*., 2003; Havel *et al*., 2015; Hohenadler *et al*., 2018).

Invasive Host-Parasite Interactions

It is not only the invasive species that must survive the invasion pathway, but also the parasites to which they may play host (and parasites that are introduced during their free-living life stages are subject to the same biotic and abiotic filters). Accordingly, there is the potential for novel parasites to be co-introduced with invasive host species and these parasites may then spillover to native hosts. For spillover of invasive parasites to occur the parasite must encounter a suitable host among the native community to make a potential host switch (Goedknegt *et al*., 2016). An example of parasite spillover is the parasitic copepod *Mytilicola orientalis* moving from its principal host, the invasive Pacific cupped oyster *Crassostrea gigas*, to blue mussels *Mytilus edulis*, common cockles *Cerastoderma edule* and Baltic tellins *Macoma balthica* in northern Europe (Goedknegt *et al*., 2017). Both *M. orientalis* and *C. gigas* originate in Asia and this is also an example of how the invasive nature of an organism depends on its locality, as in some regions *C. gigas* are purposely introduced for aquaculture (Keightley *et al*., 2015).

Although a co-introduced parasite has a direct cost to the invader, it might also be of benefit to the invader in cases where the native competitors are susceptible to the parasite or more adversely affected (Dunn *et al*., 2012). Blackburn & Ewan (2017) suggested a mechanism known as the 'novel weapon hypothesis'. This hypothesis postulates that if parasite spillover from invasive species to native hosts results in a decline in native populations that otherwise could have competed with the invasive species, then the invasive host may be more likely to establish.

Spillback is a different parasite-host interaction whereby an invasive species proves to be a suitable host for endemic parasites, thus augmenting the number of available hosts and allowing parasites to spill back to native hosts (Kelly *et al*., 2009). Conversely if the invasive species is not a suitable host they may dilute the effects of parasitism by acting as sinks for the parasites and reducing the threat to native hosts (Poulin *et al*., 2011).

On occasion invasive parasites may be transported to a new range, for example in ballast water, during free-living life-history stages. Parasites with low host-specificity and low habitat-specificity may be suited then to establish in the new range (Taraschewski, 2006). Parasites and pathogens can also expand their geographic range

due to the movement of shellfish consignments for aquaculture purposes, despite the fact that strict legislation is involved in shellfish movement worldwide. It can then be the case that it is the parasite rather than the host that becomes invasive, for example the nematode *Anguillicola crassus* was transmitted successfully to the European eel *Anguilla anguilla* with consignments of Japanese eel *Anguilla japonica*, its native host. While the Japanese eel did not become invasive, the parasite spread through Europe, and impacted native hosts by causing structural changes to the swim bladder and increasing susceptibility to environmental stress (Barry *et al*., 2017).

Invasive species can alter ecosystem functioning or habitat structure and, in modifying these environmental stressors, indirectly affect the hosts' susceptibility or disease resistance (Poulin *et al*., 2011). Accordingly, invasive species may be neither host nor parasite but impact native parasite-host interactions by interfering with transmission, for example if invasive species out-compete native hosts this may reduce transmission of endemic parasites as hosts are less available. Conversely the Australian tubeworm *Ficopomatus enigmatus* is a reef-forming serpulid that aggregates in large densities and can foul pipes and artificial structures. This invasive species interacts with native trophic levels in coastal lagoons in Argentina, facilitating parasite transmission of larval digenean trematodes in molluscan hosts (Etchegoin *et al*., 2012).

One further hypothesis that is widely utilised to explain the success of an invasion is termed the 'enemy release hypothesis' (ERH). The ERH (also known as the 'escape-from-enemy hypothesis' and the 'enemy escape hypothesis') proposes that upon entering a new range an alien species will proliferate because parasites did not survive the invasion pathway, and invasive behaviour is facilitated by release from the predators, parasites and pathogens the species may be susceptible to in its native range (Roy *et al*., 2011; Dunn & Hatcher, 2015; Young *et al*., 2017). The European green/shore crab (*Carcinus maenas*) is a prolific invader globally (excluding the poles) and heavily predates commercially exploited soft shell clams and young oysters, with part of its success attributed to release from natural enemies, specifically the rhizocephalan barnacle *Sacculina carcini,* a parasitic castrator (Torchin *et al*., 2001).

Although the ERH is widely cited, empirical evidence testing the soundness of the hypothesis is limited (Roy *et al*., 2011; Heger & Jeschke, 2014). Given the complexity of interactive processes that underpin invasions, for example intrinsic species'

attributes and the composition and niche availability of the invaded community, the theorised relationship between enemy release and invasion success may be over-simplified (Colautti *et al*., 2004). Kellet's whelk *Kelletia kelletii* is a gastropod that has expanded its northward range along the United States west coast. Although this species is the subject of a commercial fishery and not considered invasive, it demonstrates the caveats associated with the enemy release hypothesis. This is because whelks from the expanded range were only 20% as likely to be infected by parasites as whelks in the historical native range. Furthermore, expanded-range whelks had on average 14% the number of parasite species than native-range whelks, but this release from parasitism has not resulted in increased fitness as the expanded-range whelk population still displays poor demographic performance, potentially due to predation by the sea otter *Enhydra lutris* (Hopper *et al*., 2014), and because recruitment for the northward expanded populations is still reliant on larvae from southern California (Hubbard, 2008).

Monitoring Invasive Species and Parasites

Traditionally the detection and subsequent monitoring of invasive species is reliant on classical survey work that includes visual taxonomic identification of samples (Borrell *et al*., 2017), and survey techniques worldwide can differ in their methods and intensity (Minchin, 2007). However, intensive surveys can prove costly and are only effective if surveyors have the appropriate levels of taxonomic expertise. Recording of new species can often reflect the distribution of scientists rather than the dispersal of the species itself, and it can be difficult to detect and identify larval stages (Zaiko *et al*., 2015), especially if molecular techniques are not used. Furthermore, visually identifying the invasive species does not necessarily result in identifying the parasites and pathogens to which it may play host.

To counteract the difficulties involved in the timely detection and mitigation of both invasive species and pathogens, research into molecular based detection methods is becoming more common, for example using eDNA to identify four species of invasive freshwater Mollusca (Clusa *et al*., 2017) or metabarcoding approaches to produce an inventory of fouling invertebrates in shipping ports (Borrell *et al*., 2017). Molecular and histological diagnostic techniques as per the World Organisation for Animal Health, known as the OIE due to its former designation as the Office International de Epizooties, may also be applied to enable the early detection of diseases, and also facilitate safe trade in sectors such as the aquaculture industry.

Cryptogenic Parasites

Given that there are already difficulties associated with the detection and monitoring of invasive species, complications also arise within studies of pathogen outbreaks which often go unnoticed unless they directly affect humans (Otterstatter & Thomson, 2008). Cryptogenic species, namely those whose biogeographic origin is uncertain, are not uncommon because human-mediated transport occurred long before the introduction of species monitoring programmes, and also because parasites may have ambiguous taxonomies (Lymbery *et al*., 2014). This can make it difficult to determine if a parasitic species is non-native or native to a region. Furthermore, lag times (the time between introduction and rapid population growth) for parasites are not well understood (Taraschewski, 2006). Accordingly, if historical data are insufficient or

inaccurate then identifying the pathways for potential introductions may help find the origin of cryptogenic species.

Climate Change, Invasive Species and Parasites

Currently many invasive or potentially invasive species and pathogens have range limits that are governed by thermal conditions (Hellmann *et al*., 2008) so spread is likely to relate to changes in temperature constraints (Walther *et al*., 2009), or altered hydrological conditions due to altered precipitation patterns. In the marine realm, the distribution and abundance of species could be impacted due to changes in survival, growth and fecundity (Perry *et al*., 2005). Furthermore, if climate change shifts native species out of the range to which they are endemic, then there could be less biotic resistance to invasive species colonisation, for example Gallagher *et al*. (2019) modelled the response of native (*Semibalanus balanoides*) and invasive (*Austrominius modestus*) barnacle species to climate change in the form of warmer water temperatures, and found that under conditions where *A. modestus* extends its reproductive interval so that it starts early and ends after the reproductive interval of *S. balanoides* it could render the native species extinct in certain geographic areas.

Sorte *et al*. (2013) noted that in aquatic ecosystems, environmental increases in CO_2 and temperature favoured non-native species over co-existing native organisms and Iacarella *et al*. (2015) suggested that the ecological impacts of invasive species are higher in habitats with temperatures matching their optimal thermal conditions. An interesting example is that of the landlocked sea lamprey *Petromyzon marinus* in the Laurentian Great Lakes, North America. This species, a parasitic vertebrate that feeds on native fish communities, has increased in body size corresponding with longer growing seasons of their preferred hosts due to warmer thermal conditions (Cline *et al*., 2014). It is important to note that a native species that has expanded its range is not considered invasive unless it has deleterious environmental or economic impacts. However, Hulme (2017) postulated that established non-native species that do not currently display invasive traits (known as ‘sleeper aliens’) may exhibit greater rates of establishment and invasion potential if climate change increases their competitive ability.

Given that climate change could influence the distribution and life-history of invasive hosts, it follows that the distribution of their associated parasites may also shift.

Elevated temperatures could also enable pathogenic organisms to rapidly complete their lifecycle and thus obtain greater population densities (Marcogliese, 2001). Furthermore, climate change may increase the virulence of non-native pathogens. An example of this is *Myxobolus cerebralis*, the causative agent of whirling disease in salmonids; this species, introduced from Europe to North America, increases in virulence with higher temperatures (Marcogliese, 2008), potentially resulting in more severe impacts on native salmonid populations (Rahel & Olden, 2008). Finally, higher temperatures may cause epizootic outbreaks in areas that had been disease-free, if pathogens from warmer climates colonise waters that were previously outside of their thermal limits (Rowley *et al*., 2014). Outbreaks with direct implications for human health are possible, for example Baker-Austin *et al*. (2012) documented that warming temperatures in the Baltic Sea area, Northern Europe, coincided with outbreaks of water-born *Vibrio* spp. diseases.

To predict the movement and impacts of invasive species and parasites in response to climate change, it is necessary to examine the crucial stages of the invasion pathway, as shifting conditions and climatic constraints may alter mechanisms of transport. It is also necessary to study factors such as localised or large-scale weather patterns, as extreme weather events or altered currents could transport larvae large distances and distribute propagules to places that they could not have previously reached. Mechanical, biological and chemical management techniques may need to change (Hellmann *et al*., 2008) and analyses of previous invasions and the characteristics that separate successful invaders from those that fail will be important in informing management practices (Zenni & Nuñez, 2013).

A useful management tool is to consult existing risk analyses that can determine whether eradication efforts should be undertaken. Of particular interest are species that have known invasiveness and recognised impacts elsewhere, and as such horizon scanning exercises that identify future threats are important. Analysis of the pathways and vectors that may facilitate the spread and dispersal of species is also necessary, for example classification and quantification of shipping routes and densities (David & Loveday, 2018). The development of synchronised approaches that compile accurate empirical evidence will then enable policy makers to enact measures that protect from potentially invasive species and the diseases they may transmit.

The research presented here highlights the mechanisms by which invasive host/parasite complexes interact and disperse, and the challenges to sustainable growth within the blue economy, primarily the bivalve aquaculture sector, caused by the dual threat of invasive species and parasites. In addition to this introduction (Chapter One), the thesis consists of four individual chapters (Chapters Two-Five) and a final discussion with closing remarks (Chapter Six). The second chapter takes the form of a review that synthesises the interactions between invasive species and parasites, and their intrinsic links to the bivalve aquaculture sector. The third chapter uses a series of field samples and laboratory cohabitation trials to investigate how species belonging to a model taxon, the Subphylum Tunicata, can maintain parasites known to impact the aquaculture sector. Chapter Four uses empirical data to investigate the offshore microbial community in marine plankton from the Irish and Celtic seas, identifying distinct communities and also the pathways of connectivity. The fifth chapter is a horizon scanning exercise that analyses potential vectors for invasive species that may impact bivalve aquaculture, specifically shipping routes and densities, in an effort to provide clarity on the pathways of introduction that may then be used to inform management policies. The final chapter takes the form of a discussion to summarise and contextualise the findings of the thesis.

With regards to terminology, the term ‘invasive species’ is consistently used throughout. When this term is used it is in line with the definition put forward by the European Commission *Regulation (EU) 1143/2014 on invasive alien species* (Regulation (EU) No 1143/2014 of the European Parliament and of the Council of 22 October 2014 on the prevention and management of the introduction and spread of invasive alien species). This regulation defines ‘invasive alien species’ (IAS) as those which through human-mediated transport, either deliberate or accidental, have been introduced to a geographic area outside of their natural range. IAS are recognised as having negative effects on their new environment by adversely affecting biodiversity and related ecosystem services. On occasions where an organism is discussed that has extended its native range but does not display invasive tendencies it is specified that it is non-native, not invasive. The need for defined terminology is particularly acute in invasion biology and has been highlighted as confusing in previous research, as the many available definitions can confuse both debate and management efforts (Colautti & MacIsaac, 2004).

Oceanography of Study Regions

This study focused specifically on the Irish and Celtic Sea regions which are dynamic areas with distinct oceanography. This oceanography is of relevance to the movement of invasive species and pathogens, both offshore whereby species may expand their range from continental Europe, and inshore whereby they may disperse around the coast.

There are three primary features of the water around Ireland that contribute to its oceanography. Firstly, the temperate climate means that waters thermally stratify in the summer (while becoming well-mixed in winter) and secondly the continental shelf is tidally energetic, meaning that conditions can range from fully mixed to thermally stratified. Lastly the waters are shallow (<200m in depth) which means that the general thermohaline circulation may be influenced by weather, specifically wind speed and direction. Tidal fronts are found at the boundaries between tidally mixed and thermally stratified waters (typically between May-September in the stratified season) and these fronts are often associated with elevated chlorophyll levels (Raine, 2014). One distinct front that is of relevance is that which builds up between the stratified waters of the Celtic Sea and the mixed waters of the Southern Irish Sea and Bristol Channel, known as the Celtic Sea Front (CSF).

Figure 2: Continental shelf edge surrounding the island of Ireland with prevailing currents (red arrows).

A major feature along the northwest European continental shelf is that of organised, thermohaline circulation consisting of narrow, fast-flowing jets. These jets are near-surface and located above large horizontal gradients in either bottom temperatures and/or salinity. These gradients form from May-October, when the European continental shelf seas stratify as the surface waters are warmed. A seasonal pycnocline is maintained by winds and tide, as dense offshore pools of cold, saline (35.3) oceanic water carry over from winter and become trapped underneath this pycnocline. The bottom density gradients drive the near-surface geostrophic jets with a cyclonic flow. The density-driven currents are connected on a shelf-wide scale and provide a

continuous route of transport from the French coast to the Celtic Sea and along the west coast of Ireland to the Scottish shelf. This pathway is a potential conveyor belt for plankton, contaminants and harmful algal blooms (Hill *et al*., 2008).

The Celtic Sea acts as a transition area between the Atlantic waters at the edge of the northwest European continental shelf to the coastal waters of the Bristol Channel and the Irish Sea. Analysis of the flow within the region reveals a seasonal baroclinic circulation that draws saline Atlantic water up to St. George's Channel. Westward flow across St. George's Channel is then directed south into the Celtic Sea and then around the southwest tip of Ireland (Brown *et al*., 2003).

Along the southwest of Ireland, the start of the continental slope lies within 55 km of the coast, and north of this slope lies the shelf region where the boundary between the Celtic Sea and the Irish Shelf Sea occurs (Figure 2). A major feature of this shelf region is a haline front that separates oceanic and coastal water, known as the Irish Shelf Front. The surface of the front is variable, lying between 20-35 km from the coast, however its proximity to land is important in determining southwest coastal currents. Inshore the flows are northwestwards in a clockwise direction around the coast and offshore of the front the current is to the south. When the front lies close to the shore current is constricted, but as the front moves away a stronger coastal flow develops. In between these two extremes a 'mid-water jet' of northern Celtic Sea water (NCSW) lies at a 50 m depth over eastern North Atlantic Water and this NCSW is that which is advected around the southwest coast of Ireland to continue along the west coast (Raine & McMahon, 1998).

There is a strong northwards flow along the west coast of Ireland. In combination with features from the Celtic Sea, specifically the cyclonic anti-clockwise flow from the Celtic Sea and St. George's Channel that extends around the southwest tip of Ireland, there is evidence for a continuous pathway from the English Cornish coast, along the west coast of Ireland and then north to Malin Head and beyond (Fernand *et al*., 2006). Indeed, a drogued Argos buoy demonstrated that water from the Celtic Shelf region near the Goban Spur could reach western Norway over a timeframe of eight months, covering a distance of approximately 1,600 kilometres (Pingree *et al*., 1999).

Bivalve Aquaculture in the Irish and Celtic Seas

The Irish seafood sector has an estimated GDP of €1.25 billion as of 2018 and provides employment for over 14,000 people, many of whom work in rural coastal areas (BIM, 2018). The industry is of significant importance to regional economies, but shellfish culture in particular plays a role in the introduction and spread of invasive species. The two primary shellfish species within this sector are the Pacific cupped oyster *Crassostrea gigas* and the blue mussel *Mytilus edulis* (the Mediterranean mussel *M. galloprovincialis* is also present, as are hybrids of both parent species). Indeed *C. gigas* has seen a ten-year (2009-2018) production increase, and in 2018 exceeded production of 10,000 tonnes for the first time (BIM, 2018). The success of *C. gigas* is due in part to the fact that the market has switched to this species from the native European flat oyster *Ostrea edulis* due to a lack of *O. edulis* supply. From a production perspective, 51% of farmed oyster output is from the Irish and Celtic Sea regions, and the oysters are primarily grown on trestles, however floating, shelved and suspended baskets are increasingly used. Small seed (6-8 mm) is mainly imported from French and UK hatcheries, and larger seed procured from Irish hatcheries. The market is primarily EU, and to a lesser extent Canada, the United Arab Emirates and Southeast Asia, although exports to China (including Hong Kong) are increasing (BIM, 2019).

Eighty percent of *M. edulis* production is in the form of rope mussel cultivation and production varies between 8,500-10,000 tonnes (BIM, 2019). The rope industry is concentrated in the southwest of Ireland, in Cork and Kerry. Seabed-cultured mussel production is still in operation with a total output of 4,697 tonnes (2018); however, production has declined due to several years of poor seed settlement. Ninety percent of seabed-culture occurs in the northeast and southeast. While *C. gigas* and *M. edulis* are the dominant cultured species, there is a small industry for cultured scallop, urchins (echinoids) and abalone (univalves), however the combined production for these latter species measured just 60 tonnes in 2018 (BIM, 2019). There are also local shellfisheries for common cockles *Cerastoderma edule*, for example 44.5 km^2 of fished cockle beds in Dundalk Bay, Louth (Mahony *et al*., 2020), razor clams *Ensis* spp. (particularly fished in the Irish Sea region) and surf clams *Spisula solida*.

Aquaculture licencing in Ireland is ministered through the Aquaculture and Foreshore Management Division of the Department of Agriculture, Food and the Marine. The

Sea Fisheries Protection Authority (SFPA) is then the authority responsible for regulation of the seafood sector, and also oversees compliance with food safety legislation. The SFPA works closely with other bodies including Inland Fisheries Ireland (IFI), the Marine Institute (MI) and Bord Iascaigh Mhara (BIM). While BIM focuses mostly on the technical, financial and sustainable aspects of the seafood sector, the MI holds the Fish Health Unit, which is the national reference laboratory for molluscs, crustaceans and finfish diseases in Ireland. The laboratory is responsible for the development and management of methodologies used to test for fish and shellfish diseases. The MI also works with the SFPA to conduct frequent monitoring of phytoplankton to detect any species that may cause shellfish toxicity.

The primary legislation governing animal diseases in Ireland is the EU Animal Health Regulation 2016/429, which has applied since April 21st 2021. This Regulation legislates for the prevention and control of animal diseases which are transmissible to animals or humans, and is informed by the OIE (Office International des Epizooties) World Organisation for Animal Health. OIE-listed diseases are those of international concern, for which Ireland is obliged to submit information such as the presence, absence or number of cases in the country to the OIE annually.

The OIE list is reviewed on a regular basis and the molluscan diseases are follows: abalone herpesvirus, the protistans *Bonamia exitiosa*, *Bonamia ostreae*, *Marteilia refringens*, *Perkinsus marinus*, *Perkinsus olseni* and the bacterium *Xenohaliotis californiensis.* Of these, only *B. ostreae* is considered established in Ireland (although *X. californiensis* and *B. exitiosa* have been recorded). The ostreid herpesvirus OsHV-1 µVar, considered an OIE emerging disease, is also present in Ireland and associated with *Crassostrea gigas* mortality events (OIE, 2021). Bacteria within the *Vibrio* genus, particularly *Vibrio aestuarianus* and *V. splendidus* but also *V. splendidus*-like and *V. gallicus*, have also been detected in *C. gigas* populations in Ireland and these too are associated with mortality events (Bookelaar, 2018). *V. tapetis*, causative agent of brown ring disease, has also been detected in Manila clams *Ruditapes philippinarum* from north-west coasts (Drummond *et al*., 2010). Lastly, the Phylum Haplosporidia contains a number of molluscan parasites, many of which are considered pathogens of concern for the aquaculture and shellfishery sectors worldwide (Arzul & Carnegie, 2015). The previously mentioned *Bonamia ostreae*, causative agent of bonamiosis in

Ostrea edulis, is one such haplosporidian, but other species present in Ireland include *Minchinia tapetis* and *Minchinia mercenariae*-like, *Haplosporidium nelsoni* and *Haplosporidian* sp., likely H. *armoricanum* (Lynch *et al*., 2013; Lynch *et al*., 2019).

Surveillance is considered the key element of disease control policy as early detection of transmissible diseases, coupled with efficient notification, will facilitate timely prevention and control. In Ireland, all movements of aquaculture animals for on-growing must be notified to the MI at least three days in advance for movements within Ireland, and five days in advance of the planned movement for imports/exports. The movement orders include information on the species, categories (e.g. age/size range) and quantities (in numbers, volume or weight). Cultured stock may only be imported from regions that are not subject to disease restrictions, and health certificates are required to facilitate trade. Ireland is also a member of ICES and is therefore encouraged to follow ICES Code of Practice for aquaculture, re-stocking and enhancement purposes, which requires risk assessments and documented periodic inspections (ICES, 2005). A useful resource is the OIE aquatic animal health code which provides standards and sanitary measures for aquatic animal health worldwide and is also used to inform the ICES Code of Practice (OIE, 2019). In addition to health certificates and consistent disease monitoring, this health code recommends disinfection of aquaculture establishments and equipment, use of appropriate containers to ensure there is no water leakage and appropriate treatment of water to ensure no pathogens are transferred.

Research Objectives

When considering the dispersal and impact of invasive species in the marine environment there are three major vectors to consider, namely aquaculture, current-mediated dispersal and oceanic shipping. This research focuses on aquaculture at both global and local scales, in the form of a review and empirical chapter respectively. The focus is then broadened to look at what potential pathogens are present in the waters surrounding Ireland. Finally, the focus shifts to international routes of connectivity which may facilitate invasive species movement.

Chapter two synthesises available research on marine invasive bivalves and their associated parasites and presents an overview of current research into the diseases associated with invasive species. The piece also considers how the interactions between non-native and/or invasive bivalves and parasites are intrinsically linked to aquaculture, as the movement of stock consignments often provides the vector for host and/or parasite, and aquaculture is one of the sectors most vulnerable to disease. Finally, the review discusses how climate change may impact the distribution of invasive species and the potential outcomes for parasite transmission and disease outbreaks.

Chapter three examines a model invasive taxon, the Subphylum Tunicata, focusing on species that are invasive in coastal regions and determining their potential to act as carriers or reservoirs of pathogens that are known to affect commercial oyster culture, thereby impacting the blue economy. This subphylum was selected as an appropriate model group due to the invasive tendencies displayed by many tunicate species, namely a wide tolerance to fluctuations in temperature, *r*-selected traits and competition with other species for resources. The research experimentally examines the ways in which invasive tunicates maintain pathogens when cohabiting with diseased oysters under controlled laboratory conditions.

Chapter four examines samples gathered from a research cruise on the RV *Celtic Voyager* (Marine Institute) conducted in May 2018 to ascertain the community composition of marine plankton within two offshore study regions, the Irish and Celtic Seas, thus ascertaining how oceanic fronts and currents act as barriers and facilitators to larval dispersal and consequently the dispersal of the associated microbial community. The study uses amplicon based metagenomic sequencing to determine the

bacteriome of both holo- and meroplankton across the two study regions, and polymerase chain reaction to screen for diseases associated with aquaculture, in particular bivalve culture, that may be cycling offshore. This analysis provides novel insight into the microbial community diversity associated with plankton from the aforementioned regions, and additionally the potential role of plankton as carriers or vectors of problematic microbes.

Chapter five utilises and analyses cargo shipping data from four major ports in Ireland spanning 2018-2019. The study is a horizon scanning exercise that explores the connectivity between Ireland and other international ports, with a focus on connections to ports in regions with confirmation of the presence of selected invasive species. The piece also analyses journey times and residence times in port, as well as consistencies in pathways at varying temporal scales to assess the pathways of highest concern.

Chapter six, the final section of this thesis, discusses the research as a whole, noting the key findings and highlighting the potential mechanisms to control the spread of invasive species or mitigate their impacts when already established, with recommendations for the direction of future research.

References

Arzul, I. & Carnegie, R. (2015). New perspective on the haplosporidian parasites of molluscs. *Journal of Invertebrate Pathology*, 131, 32-42.

Baker-Austin, C., Trinanes, J.A., Taylor, N.G.H., Hartnell, R., Siitonen, A. & Martinez-Urtaza, J. (2013). Emerging *Vibrio* risk at high latitudes in response to ocean warming. *Nature Climate Change*, 3(1), 73–77.

Barry, J., Newton, M., Dodd, J.A., Evans, D., Newton, J. & Adams, C.E. (2017). The effect of foraging and ontogeny on the prevalence and intensity of the invasive parasite *Anguillicola crassus* in the European eel *Anguilla anguilla*. *Journal of Fish Diseases*, 40, 1213-1222.

Bax, N., Williamson, A., Aguero, M., Gonzalez, E. & Geeves, W. (2003). Marine invasive alien species: a threat to global biodiversity. *Marine Policy*, 27, 313-323.

Blackburn, T.M & Ewan, J.G. (2017). Parasites as Drivers and Passengers of Human-Mediated Biological Invasions. *EcoHealth*, 14, 61-73.

Bookelaar, B. (2018). Understanding and minimizing the impacts of host-pathogen-environment interactions in the Pacific oyster *Crassostrea gigas*. PhD Thesis, University College Cork

Borrell, Y.J., Miralles, L., Mártinez-Marqués, A., Semeraro, A., Arias, A., Carleos, C.E. & García-Vazquez. (2017). Metabarcoding and post-sampling strategies to discover non-indigenous species: A case study in the estuaries of the central south Bay of Biscay. *Journal for Nature Conservation*, 42, 67-74.

Brown, J., Carrillo, L., Fernand, L., Horsburgh, K.J., Hill, A.E., Young, E.F. & Medler, K.J. (2003). Observations of the physical structure and seasonal jet-like circulation of the Celtic Sea and St. George's Channel of the Irish Sea. *Continental Shelf Research*, 23, 533-561.

BIM. (2018). The Business of Seafood 2018; A Snapshot of Ireland's Seafood Sector. *Bord Iascaigh Mhara*, 1-48.

BIM. (2019) National Seafood Survey: Aquaculture Report 2019. *Bord Iascaigh Mhara*, 1-48.

Cline, T.J., Kitchell, J.F., Bennington, V., McKinley, G.A., Moody, E.K. & Weidel, B.C. (2014). Climate impacts on landlocked sea lamprey: Implications for host-parasite interactions and invasive species management. *Ecosphere*, 5(6):68.

Clusa, L., Miralles, L., Basanta, A., Escot, C. & García-Vázquez, E. (2017). eDNA for detection of five highly invasive molluscs. A case study in urban rivers from the Iberian Peninsula. *PLoS ONE*, 12(11), e0188126, 1-14.

Colautti, R.I. & MacIsaac, H.J. (2004). A neutral terminology to define 'invasive' Species. *Diversity and Distributions*, 10, 135-141.

Colautti, R.I., Ricciardi, A., Grigorovich, I.A. & MacIsaac, H.J. (2004). Is invasion success explained by the enemy release hypothesis? *Ecology Letters*, 7, 721-733.

David, A.A. & Loveday, B.R. (2018). The role of cryptic dispersal in shaping connectivity patterns of marine populations in a changing world. *Journal of the Marine Biological Association of the United Kingdom*, 98(4), 647-655.

Drummond, L., Mulcahy, M.F. & Culloty, S. (2010). A survey of the health status of the Manila clam *Ruditapes philippinarum* in Ireland with specific reference to brown ring disease. *Aquaculture International*, 18, 787-800.

Dunn, A.M., Torchin, M.E., Hatcher, M.J., Kotanen, P.M., Blumenthal, D.M., Byers, J.E., Coon, C.A.C., Frankel, V.M., Holt, R.D., Hufbauer, R.A., Kanarek, A.R., Schierenbeck, K.A., Wolfe, L.M. & Perkins, S.E. (2012). Indirect effects of parasites in invasions. *Functional Ecology*, 26, 1262-1274.

Dunn, A.M. & Hatcher, M.J. (2015). Parasites and biological invasions: parallels, interactions, and control. *Trends in Parasitology*, 31(5), 189-199.

Etchegoin, J.A., Merlo, M.J. & Parietti, M. (2012). The role of the invasive polychaete *Ficopomatus enigmaticus* (Fauvel, 1923) (Serpulidae) as facilitator of parasite transmission in Mar Chiquita coastal lagoon (Buenos Aires, Argentina). *Parasitology*, 139, 1506-1512.

Fernand, L., Nolan, G.D., Raine, R., Chambers, C.E., Dye, S.R., White, M. & Brown, J. (2006). The Irish coastal current: A seasonal jet-like circulation. *Continental Shelf Research*, 26, 1775-1793.

Gallagher, M.C., Arnold, M., Kadaub, E., Culloty, S., O'Riordan, R.M., McAllen, R. & Rachinskii, D. (2019). Competing barnacle species with a time dependent reproduction rate. *Theoretical Population Biology*, https://doi.org/10.1016/j.tpb.2019.11.001.

Goedknegt, M.A., Feis, M.E., Wegner, K.M., Luttikhuizen, P.C., Buschbaum, C., Camphuysen, K.C.J., van der Meer, J. & Thieltges, D.W. (2016). Parasites and marine invasions: Ecological and evolutionary perspectives. *Journal of Sea Research*, 113, 11-27.

Goedknegt, M.A., Schuster, A.K., Buschbaum, C., Gergs, R., Jung, A.S., Luttikhuizen, P.C., van der Meer, J., Troost, K., Wegner, K.M., Thieltges, D.W. (2017). Spillover but no spillback of two invasive parasitic copepods from invasive Pacific oysters (*Crassostrea gigas*) to native bivalve hosts. *Biological Invasions*, 19, 365-379.

Havel, J.E., Kovalenko, K.E., Thomaz, S.M., Amalfitano, S. & Kats, L.B. (2015). Aquatic invasive species: challenges for the future. *Hydrobiologia*, 750, 147-170.

Heger, T. & Jeschke, J.M. (2014). The enemy release hypothesis as a hierarchy of hypotheses. *Oikos*, 123, 741-750.

Hellmann, J.J., Byers, J.E., Bierwagen, B.G. & Dukes, J.S. (2008). Five Potential Consequences of Climate Change for Invasive Species. *Conservation Biology*, 22(3), 534-543.

Hill, A.E., Brown, J., Fernand, L., Holt, J., Horsburgh, K.J., Proctor, R., Raine, R. & Turrell, W.R. (2008). *Geophysical Research Letters*, 35(L11605) 1-5.

Hohenadler, M.A.A., Honka, K.I., Emde, S., Klimpel, S. & Sures, B. (2018). First evidence for a possible invasional meltdown among invasive fish parasites. *Scientific Reports*, 8(1), 1–5.

Hollebone, A.L. & Hay, M.E. (2007). Propagule pressure of an invasive crab overwhelms native biotic resistance. *Marine Ecology Progress Series*, 342, 191-196.

Hopper, J.V., Kuris, A.K., Lorda, J., Simmonds, S.E., White, C. & Hechinger, R.F. (2014). Reduced parasite diversity and abundance in a marine whelk in its expanded geographical range. *Journal of Biogeography*, 41, 1674-1684.

Hubbard, K. (2008.) Kellet's whelk, *Kelletia kelletii*. Status of the Fisheries Report 2008. California Department of Fish and Wildlife. https://nrm.dfg.ca.gov/FileHandler.ashx?DocumentID=34437&inline=true. Accessed 20/07/2021.

Hulme, P.E. (2015). Invasion pathways at a crossroad: policy and research challenges for managing alien species introductions. *Journal of Applied Ecology*, 52, 1418-1424.

Hulme, P.E. (2017). Climate change and biological invasions: evidence, expectations, and response options. *Biological Reviews*, 92, 1297-1313.

Iacarella, J.C., Dick, J.T.A., Alexander, M.E. & Ricciardi, A. (2015). Ecological impacts of invasive alien species along temperature gradients: testing the role of environmental matching. *Ecological Applications*, 25(3), 706-716.

ICES (2005). ICES Code of Practice on the Introductions and Transfers of Marine Organisms 2005. 1-30.

Keightley, J., von der Heyden, S. & Jackson, S. (2015). Introduced Pacific oysters *Crassostrea gigas* in South Africa: demographic change, genetic diversity and body condition. *African Journal of Marine Science*, 37(1), 89-98.

Kelly, D.W., Paterson, R.A., Townsend, C.R., Poulin, R. & Tompkins, D.M. (2009). Parasite Spillback: A Neglected Concept in Invasion Ecology? *Ecology*, 90(8), 2047-2056.

Lenz, M., da Gama, B.A.P., Gerner, N.V., Gobin, J., Gröner, F., Harry, A., Jenkins, S.R., Kraufvelin, P., Mummelthei, C., Sareyka, J., Xavier, E.A. & Wahl, M. (2011). Non-native marine invertebrates are more tolerant towards environmental stress than taxonomically related native species: Results from a globally replicated study. *Environmental Research*, 111, 943-952.

Lymbery, A.J., Morine, M., Kanani, H.G., Beatty, S.J. & Morgan, D.L. (2014). Co-invaders: The effects of alien parasites on native hosts. *International Journal for Parasitology: Parasites and Wildlife*, 3, 171-177.

Lynch, S.A., Villalba, A., Abollo, E., Engelsma, M., Stokes, N.A. & Culloty, S.C. (2013a). The occurrence of haplosporidian parasites, *Haplosporidium nelsoni* and

Haplosporidium sp., in oysters in Ireland. *Journal of Invertebrate Pathology*, 112:208-212.

Lynch, S.A., Darmody, G., O'Dwyer, K., Gallagher, M.C., Nolan, S., McAllen, R. & Culloty, S.C. (2016). Biology of the invasive ascidian *Ascidiella aspersa* in its native habitat: Reproductive patterns and parasite load. *Estuarine, Coastal and Shelf Science*, 181, 249-255.

Lynch, S.C., Lepée-Rivero, S., Kelly, R, Quinn, E., Coghlan, A., Bookelaar, B., Morgan, E., Finarelli, J.A., Carlsson, J. & Culloty, S.C. (2020). Detection of haplosporidian protistan parasites supports an increase to their known diversity, geographic range and bivalve host specificity. *Parasitology*, 147, 584-592.

Mahony, K.E., Lynch, S.A., Egerton, S., Cabral, S., de Montaudouin, X., Fitch, A., Magalhães, L., Rocroy, M. & Culloty, S.C. (2020). Mobilisation of data to stakeholder communities. Bridging the research-practice gap using a commercial shellfish species model. *PLoS ONE*, 15(9), e0238446.

Marcogliese, D.J. (2001). Implications of climate change for parasitism of animals in the aquatic environment. *Canadian Journal of Zoology*, 79(8), 1331-1352.

Marcogliese, D.J. (2008). The impact of climate change on the parasites and infectious diseases of aquatic animals. *Revue Scientifique et Technique (International Office of Epizootics)*, 27(2), 467–484.

Minchin, D. (2007). Rapid coastal survey for targeted alien species associated with floating pontoons in Ireland. *Aquatic Invasions*, 2(1), 63-70.

OIE (2019). https://www.oie.int/en/what-we-do/standards/codes-and-manuals/aquatic-code-online-access/. Accessed 12/07/2021.

OIE (2021) https://www.oie.int/en/what-we-do/animal-health-and-welfare/animal-diseases/?_tax_animal=aquatics%2Cmolluscs. Accessed 12/07/2021.

Otterstatter, M.C. & Thomson, J.D. (2008). Does Pathogen Spillover from Commercially Reared Bumble Bees Threaten Wild Pollinators? *PLoS ONE*, 3(7), e2771, 1-9.

Perry, A.L., Low, P.J., Ellis, J.R. & Reynolds, J.D. (2005). Climate Change and Distribution Shifts in Marine Fishes. *Science*, 308(5730), 1912-1915.

Pingree, R.D., Sinha, B. & Griffiths, C.R. (1999). Seasonality of the European slope current (Goban Spur) and ocean margin exchange. *Continental Shelf Research*, 19, 929-975.

Poulin, R., Paterson, R.A., Townsend, C.R. & Tompkins, D.M. (2011). Biological invasions and the dynamics of endemic diseases in freshwater ecosystems. *Freshwater Biology*, 56, 676-688.

Rahel, F.J. & Olden, J.D. (2008). Assessing the Effects of Climate Change on Aquatic Invasive Species. *Conservation Biology*, 22(3), 521-533.

Raine, R. & McMahon, T. (1998). Physical dynamics on the continental shelf off southwestern Ireland and their influence on coastal phytoplankton blooms. *Continental Shelf Research*, 18, 883-914.

Raine, R. (2014). A review of the biophysical interactions relevant to the promotion of HABs in stratified systems: The case study of Ireland. *Deep-Sea Research II*, 101, 21-31.

Rech, S., Salmina, S., Borrell Pichs, Y.J. & García-Vazquez, E**.** (2018). Dispersal of alien invasive species on anthropogenic litter from European mariculture areas. *Marine Pollution Bulletin*, 131, 10-16.

Rowley, A.F., Cross, M.E., Culloty, S.C., Lynch, S.A., Mackenzie, C. L., Morgan, E., O'Riordan, R.M., Robins, P.E., Smith, A.L., Thrupp, T.J., Vogan, C.L., Wootton, E.C. & Malham, S.K. (2014). The potential impact of climate change on the infectious diseases of commercially important shellfish populations in the Irish Sea - A review. *ICES Journal of Marine Science*, 71(4), 741–759.

Roy, H.E., Lawson Handley, L.-J., Schönrogge, K., Poland, R.L. & Purse, B.V. (2011). Can the enemy release hypothesis explain the success of invasive alien predators and parasitoids? *BioControl*, 56, 451-468.

Simberloff, D. (2009). The Role of Propagule Pressure in Biological Invasions. *Annual Review of Ecology, Evolution, and Systematics*, 40, 81-102.

Sorte, C.J.B., Ibáñez, I., Blumenthal, D.M., Molinari, N.A., Miller, L.P., Grosholz, E.D., Diez, J.M., D'Antonio, C.M., Olden, J.D., Jones, S.J. & Dukes, J.S. (2013).

Poised to prosper? A cross-system comparison of climate change effects on native and non-native species performance. *Ecology Letters*, 16, 261-270.

Taraschewski, H. (2006). Hosts and parasites as aliens. *Journal of Helminthology*, 80, 99-128.

Torchin, M.E., Lafferty, K.D. & Kuris, A.M. (2001). Release from parasites as natural enemies: increased performance of a globally introduced marine crab. *Biological Invasions*, 3, 333-345.

Walther, G. R., Roques, A., Hulme, P. E., Sykes, M. T., Pyšek, P., Kühn, I., Zobel, M., Bacher, S., Botta-Dukát, Z., Dauber, J., Hickler, T., Jarošík, V., Kenis, M., Klotz, S., Minchin, D., Moora, M., Nentwig, W., Ott, J., Panov, V.E., Reineking, B., Robinet, C., Semenchenko, V., Solarz, W., Thuiller, W., Vilà, M., Vohland, K. & Settele, J. (2009). Alien species in a warmer world: risks and opportunities. *Trends in Ecology and Evolution*, 24(12), 686–693.

Whitney, K.D. & Gabler, C.A. (2008). Rapid evolution in introduced species, 'invasive traits' and recipient communities: challenges for predicting invasive potential. *Diversity and Distributions*, 14, 569-580.

Young, H.S., Parker, I.M., Gilbert, G.S., Guerra, A.S. & Nunn, C.L. (2017). Introduced Species, Disease Ecology, and Biodiversity-Disease Relationships. *Trends in Ecology & Evolution*, 32(1), 41-54.

Zaiko, A., Samuiloviene, A., Ardura, A. & García-Vázquez, E. (2015). Metabarcoding approach for nonindigenous species surveillance in marine coastal waters. *Marine Pollution Bulletin*, 100, 53-59.

Zenni, R.D. & Nuñez M.A. (2013). The elephant in the room: the role of failed invasions in understanding invasion biology. *Oikos*, 122, 801-815.

Chapter 2: The Importance of Marine Bivalves in Invasive Host-Parasite Introductions

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Abstract

Although research into the ecology and impacts of invasive species is prevalent, there are knowledge gaps relating to the role of invasive species in parasite transmission. This work synthesises invasive host-parasite interactions and impacts, using marine bivalves as a model group. We consider how the global movement of shellfish consignments for aquaculture purposes facilitates the unintentional transfer of invasives. We then discuss how invasive species can act as both hosts or parasitic organisms themselves, and introductions may lead to diseases within the bivalve aquaculture sector. This review highlights the importance of interdisciplinary research, with particular regard to the fields of parasitology and invasion ecology. We suggest that further integrating these fields will enhance critical knowledge of marine diseases, parasite-invasive-bivalve interplay dynamics, and potential mitigation strategies, including temperature-based disease surveillance models. We also address how climate change might impact invasive species, again with a focus on marine bivalves, and the potential outcomes for parasite transmission, including changes in host/parasite distribution, life-history and virulence. We acknowledge the importance of horizon scanning for future invasive host-parasite introductions and note that increased screening of invasive species, both in their native and invaded ranges, will provide clarity on invasion dynamics and potential impacts.

Keywords: Invasion Ecology, Parasites, Marine Bivalves, Management, Transmission Trials, Climate Change

Introduction

The purpose of this review is to highlight the intrinsic links between marine bivalves and invasive host-parasite interactions, and the subsequent impacts on the aquaculture sector. There is a particular focus on the cyclical nature of these links, as commercial bivalve culture often provides a pathway for invasive host-parasite complexes and yet these same bivalves are then impacted by disease outbreaks caused by the parasites. Throughout the existing literature a number of synonyms exist for species that have extended their geographic distribution outside of their native range, primarily 'alien', 'exotic', 'non-indigenous' and 'non-native'. However, these terms must be carefully distinguished from 'invasive species', defined here as having spread widely beyond the point of initial establishment (Lodge *et al*., 2006) and likely to have deleterious effects on biodiversity and associated ecosystem services in their new range (EU regulation 1146, 2014). Here, we distinguish between non-native species, which although introduced do not necessarily have negative impacts, and invasive species (as defined above) and use these terms and definitions throughout.

The movement of invasive species in the marine environment has been exacerbated in recent decades due to increased globalisation resulting from trade and travel (Hulme, 2009; Katsanevakis *et al*., 2016). Introductions can be mediated by deliberate human intervention but vectors of introduction are primarily accidental, with transport of the invasive species secondary to primary purposes such as trade and tourism (Lodge *et al*., 2006; Hulme, 2015). The relative importance of vectors for accidental introductions differs both spatially and temporally. However, aquaculture, ballast water and hull fouling are consistently cited as prominent vectors (Minchin *et al*., 2009; Williams *et al*., 2013, Hulme, 2015; Laeseke *et al*., 2020). Anthropogenic debris is another vector, and may be influenced by aquaculture activities due to the contribution of aquaculture gear to what is termed 'abandoned, lost or otherwise discarded fishing gear' (ALDFG) (Rech *et al*., 2018). Accidental introductions may also arise through escapes from captivity (Hulme, 2009), from recreational water use (Hulme, 2015) and from static marine structures (Laeseke *et al*., 2020).

Global production of marine bivalves, particularly oysters, mussels, clams and scallops, measures over 15 million tonnes per year, with 89% of this production coming from aquaculture rather than wild fisheries (Wijsman *et al*., 2019). Indeed,

culture of shelled molluscs accounts for 58.8% of the combined production of marine and coastal aquaculture (FAO, 2018). Over 85% of bivalve production originates in Asia, particularly China (Wijsman *et al*., 2019), and the sector is projected to increase globally to provide food security as a source of affordable protein (Steeves *et al*., 2018). It is therefore important to fully elucidate the potential risks and challenges to growth in an effort to develop sustainable practices. Intentional translocation of bivalves for aquaculture is common practice, for example Atlantic European countries regularly engage in local, national and regional transfer throughout the European economic zone, along with occasional international transfers (Muehlbauer *et al*., 2014). These transfers can lead to one of the primary risks to sustainable growth; the movement of invasive species and their associated parasites.

Aquaculture is one of the primary anthropogenic activities that results in the unintentional movement of invasive species, as they may be inadvertently shipped with consignments of cultured species (Naylor *et al*., 2001). Potentially invasive organisms can be transported in a number of ways, for example as epifauna on the shells, internally within the shell cavity, in water, sediment or equipment, or in association with other fouling epifauna. The three major groups that may be introduced are phytoplankton, macrofauna and macroalgae (McKindsey *et al*., 2007). It follows therefore that parasitic introductions will increase, as parasites travel with invasive hosts, or are unintentionally introduced with purposefully translocated cultured hosts (Peeler *et al*., 2011). The term parasite is often used in its broadest sense to include macroparasites, defined as those in which direct multiplication in the definitive host is low or entirely absent, and microparasites, specifically protozoa, bacteria and viruses that display high rates of direct reproduction within the host (Anderson & May, 1979). Both macro- and microparasites affect naturally occurring aquatic species and also the commercial bivalve species utilised both in cultured systems and in local, often traditional, shellfisheries.

Climate change will influence the geographic range, abundance and impacts of invasive species (Walther *et al*., 2009; Beaury *et al*., 2020). Warming temperatures will likely allow invasive species to expand into regions where previously they could not survive and reproduce. Altered climatic factors might also facilitate extended spawning times, increases in reproductive output and growth rates and altered dispersal due to modified hydrodynamic conditions (Mellin *et al*., 2016). However,

the impact of climate change on invasive-parasite interactions, and the subsequent potential for disease transmission in the marine environment is still a developing field of research. Climate change may enhance the virulence of introduced pathogens, or cause disease outbreaks in new ranges if pathogens are introduced (Conn, 2014; Rowley *et al*., 2014). Furthermore, susceptible hosts may already be under increased thermal or osmotic stress (Harvell *et al*., 2002; Burge *et al*., 2016a), rendering them vulnerable to infection and potential mortality events, which can in turn negatively impact the aquaculture sector.

Awareness of the links between parasitology and invasion ecology is still not fully reflected in research output. Indeed Poulin (2017) noted circa 4000 studies per year on invasive species, but circa 50 per year only on parasitism in the context of biological invasions. However, the potential role of parasites in marine invasions is increasingly recognised as an important factor in invasion biology (Lagrue, 2017). The importance of marine bivalves in invasive parasite/host interactions and their influence on native community dynamics are synthesised in the following sections.

Aquaculture and Parasite Introductions: Impacts on Commercial Bivalves

Bivalves are an interesting model group when considering invasion ecology as they can enter new regions via accidental introductions or as a result of deliberate translocations for aquaculture (McKindsey *et al*., 2007) where they can be stocked at high densities (Wijsman *et al*., 2019), spread quickly from initial introduction points, are susceptible to co-infections (Zannella *et al*., 2017) and can transfer parasites to native bivalves. They may also introduce other cohabiting and potentially invasive species (McKindsey *et al*., 2007). Ruesink *et al*. (2005) noted, in a study of nine global regions, that the culturing of non-native oysters introduced 78 species of invasive marine algae, invertebrates and protozoa. The role of bivalve culture in species introductions holds true across other studies, as an estimated 40% of non-native species in the North Sea arrived via oyster culture (Gallardi, 2014). Furthermore, an analysis of current and historical introductions of both marine and estuarine species in California found that 126 introductions arose from aquaculture practices, and of these 106 species became established. A number of the species that established are also bivalve predators, specifically the Atlantic oyster drill *Urosalpinx cinerea* and

Japanese oyster drill *Ocenebra inornata*, and are now considered severe oyster pests (Grosholz *et al*., 2015).

In the marine environment the introduction of bivalves for aquaculture, similar in function to the expansion of invasive species to new ranges, may be considered a vector for unintentional parasite introduction and can be used as a proxy to investigate how the movement of invasive species may transport parasites. A number of these heavily cultured species, specifically the Pacific cupped oyster *Crassostrea gigas* (Goedknegt *et al*., 2017), the Mediterranean mussel *Mytilus galloprovincialis* (Lynch *et al*., 2020) and the Manila clam *Ruditapes philippinarum* (Cordero *et al*., 2017) are considered to display invasive tendencies despite also being commercially important species. The American razor clam *Ensis directus* (Gollasch *et al*., 2015) underwent accidental introductions to Europe and also displays invasive tendencies, despite being the subject of commercial fisheries (Table 1).

Parasites and diseases in the marine environment are currently a challenge to bivalve aquaculture practices (Solomieu *et al*., 2015). For example, microvariants of ostreid herpesvirus OsHV-1 threaten global production of *C. gigas* by inducing mass mortalities in early life-stages (Carnegie *et al*., 2016; Bookelaar *et al*., 2018). Macroparasites, often in the form of trematode species, can compromise the immunology and physiology of the hosts, thereby impacting the ecology of the species (Morley, 2010). Microparasites may include bacterial diseases, often belonging to the genus *Vibrio*, protistans and viruses. A number of protistan species, particularly within the genera *Bonamia*, *Haplosporidium*, *Marteilia* and *Perkinsus*, are recognised as threats to bivalve populations (Fernández-Robledo *et al*., 2014), as are viruses (Coen & Bishop, 2015) and bacterial species such as *Vibrio aestuarianus* and *V. splendidus* (Solomieu *et al*., 2015).

Table 1: A sample of introduced marine bivalves and/or parasites demonstrating the diversity of bivalve-parasite interactions and the impacts on commercial species, both wild and cultured.

Host Species	Parasite	Parasite Group	Impacts
Oysters			
Crassostrea ariakensis (Suminoe oyster)	*Bonamia* sp.	Protistan	The introduction of Asian *C. ariakensis* to mid-Atlantic United States was considered for aquaculture, but detection of *Bonamia* sp. in *C. ariakensis* (cultured in a hatchery and deployed in N. Carolina) cast doubt on the feasibility of culturing it in the US (Carnegie *et al*., 2008). Previous study suggested the *Bonamia* sp. had not originated from the hatchery but rather from the deployment site and, as native *C. virginica* were not infected, the movement of the introduced oysters revealed the previously unknown local parasite (Burreson *et al*., 2004). Molecular work suggested the *Bonamia* sp. was genetically similar to Australasian species and had been introduced locally via ballast water (Bishop *et al*., 2006).
Crassostrea virginica (Eastern oyster)	*Perkinsus marinus*	Protistan	*C. virginica* is native to the United States but the origin of *P. marinus* is unknown. The parasite was initially detected in the Gulf of Mexico in the 1950s before spreading up the east coast United States. *P. marinus* is the causative agent of 'Dermo' disease in *C. virginica* (Smolowitz, 2013).
Crassostrea gigas (Pacific cupped oyster)	*Haplosporidium nelsoni*	Protistan	The origins of *H. nelsoni* in USA are not conclusive, but it was likely introduced from Asia with introduced consignments of infected *C. gigas* spat. The parasite is the causative agent of MSX disease and mortality in native *C. virginica* (Burreson & Stokes, 2000).
Crassostrea gigas	OsHV-1 µVars	Virus	*C. gigas* was deliberately introduced to Europe in the 1960/70s and is now heavily cultured, but can also display invasive tendencies. Since 2008 massive mortalities originating in France have spread through Europe and are attributed to OsHv-1 µVar. Closely related microvariants have also been detected in *C. gigas* mortality events in Australia, New Zealand and Asia (Pernet *et al*., 2016).
Crassostrea gigas	*Ostracoblabe implexa*	Fungus	The shell structure of *C. gigas* introduced to the Wadden Sea in Europe is negatively affected by the widely distributed fungus (Thieltges *et al*., 2013). Oyster translocation spreads the fungus increasing the risk of *C. gigas* shell-disease (Blakeslee *et al*., 2013).

Crassostrea gigas	*Mytilicola orientalis*	Parasitic Copepod	Spillover of the introduced *M. orientalis* (Red worm, native to Japan) from introduced *C. gigas* to native *Mytilus edulis, Cerastoderma edule* and *Macoma balthica* (Goedknegt *et al*., 2017).
Clams			
Ensis directus (American razor clam)	*Renicola roscovita* & *Himasthla* spp.	Trematode	*E. directus* (native to the North American Atlantic coast) acquired infection with native trematodes when accidentally introduced to the Wadden Sea, dominated by *R. roscovita* but also included *Himasthla elongata*, *H. continua* and *H. interrupta* (Krakau *et al*., 2006).
Ruditapes philippinarum (Manila clam)	*Vibrio* sp.	Bacterium	*R. philippinarum* is endemic to the western Pacific and was accidentally introduced to North America in 1936, and intentionally introduced to Europe in the 1970s (Cordero *et al*., 2017). The clam is heavily cultured but can also display invasive tendencies. Brown ring disease (BRD), caused by *Vibrio tapetis* or *V. tapetis*-like strains, was first detected in 1987 in French clam beds before spreading along the European Atlantic coast and also appearing in South Korea. The disease is also known to occur in native *R. decussatus* (Paillard, 2004; Park *et al*., 2006).
Mussels			
Mytilus galloprovincialis	*Mytilicola intestinalis*	Copepod	*M. galloprovincialis* is native to southern Europe, and spreading northwards. However, it is also listed on the 'World's Worst 100 Invasive Species' (Lynch *et al*., 2020). *M. intestinalis* (mussel red worm) was first described in *M. galloprovincialis* in the Adriatic Sea (Elsner *et al*., 2011). The parasite was then detected in *M. edulis* in the North Sea in the 1930s. It has been suggested that the parasite spread with hull-fouling mussels from the Mediterranean, but ultimately the north-westwards spread is not fully understood (CABI datasheet 73758, 2020).
Mytilus galloprovincialis (Mediterranean mussel)	*Marteilia* spp.	Protistan	*M. refringens* causes marteiliosis in oysters, mussels and other bivalve species, and is responsible for mass mortalities of *O. edulis* in Europe. The full taxonomy and lifecycle has not been clearly evaluated to date, however it is included here as an important pathogen of commercial species. The parasite was originally considered two species, *M. refringens* and *M. maurini*, but synonymised in 2007 and written as *M. refringens* O-type and M-Type. However, recent work has proposed that there are in fact two distinct species, termed *M refringens* (formerly O-type and *M. pararefringens* (formerly M-type) (Carrasco *et al*., 2015; Kerr *et al*., 2018).

Interactions Between Parasites, Invasive Species and Native Communities

Parasites associated with freshwater biological invasions are well-documented. For example, upon introduction to the Great Lakes, the invasive zebra mussel *Dreissena polymorpha* introduced the parasitic platyhelminth *Bucephalus polymorphus* to cyprinid fish (Crowl *et al*., 2008). Furthermore, the zebra mussel has been the subject of numerous baseline parasitological studies early in its invasion, partly in an effort to detect viable biological controls, confirming the presence of helminths, protistans and bacteria (Toews *et al*., 1993). However, although the interactions between parasites and marine invasive species are increasingly recognised (Goedknegt *et al*., 2016), information relating to marine ecosystems is less available (Vignon & Sasal, 2010). Accordingly, questions about the co–introduction of parasites with invasive species, for example their origin or impacts, or the potential role of invasive species in the transmission of native parasites still arise (Lagrue, 2017; Poulin, 2017; Lucy *et al*., 2020). Please see Goedknegt *et al*. (2016) for an in-depth review of invasive species-parasite interactions across multiple taxa in the marine environment.

Marine diseases are a field important to both aquaculture research and management, particularly given the well-documented diversity of parasites and pathogens as seen in Table 1 (Lafferty & Hofmann, 2016). Aquaculture practices have long been associated with repeated introductions of infectious diseases (Lafferty & Kuris, 1999), particularly as high stocking densities under aquaculture conditions can encourage the spread of parasitic infections (Cheng & Combes (1990). An increased understanding of how invasive species also influence disease dynamics in bivalve aquaculture is necessary, as rapid detection of pathogens is important in preventing wide scale disease outbreaks (Telfer & Bowen, 2012). Interactions between parasites, invasive species and native communities are complex as invaders may benefit from parasite loss, transmit novel parasites to native hosts (spillover) or acquire new generalist parasites native to the invaded range, which may then spillback to native hosts (Figure 1) (Dunn *et al*., 2012).

Parasite spillover from an invasive species can occur when a parasite that co-invades with the invasive host infects a new susceptible native host species. For example, the parasitic copepod *Myicola ostreae* travelled from Asia (native to Korea and Japan) with *C. gigas*

consignments to France where it succeeded in becoming established and infecting the European flat oyster *Ostrea edulis*. The copepod can reduce the growth rate of the host, impacting the market quality and also potentially leading to mortality (CABI datasheet 110220, 2020). Mechanisms to address the questions relating to parasite spillover in the marine environment include screening invasive species in their native and invaded ranges to compare the parasitic burden in each locality, and then comparing the parasitic assemblages of invasive species in the invaded range with those of native species (Krakau *et al*., 2006).

Parasite spillback is when a parasite in a native host infects an invasive host, with the presence of an additional host increasing parasite abundance and/or dispersal thereby providing more opportunities to encounter and infect native species (Kelly *et al*., 2009; Chalkowski *et al*., 2018). Previous research conducted laboratory transmission trials between oysters and other invertebrate species to investigate whether they can act as carriers or vectors of parasites and confirmed that the native brittle star *Ophiothrix fragilis* was capable of transmitting *Bonamia ostreae* to naive *O. edulis* (Lynch *et al*., 2007). This principle could also be utilised to investigate parasite spillback, as transmission trials could be conducted between native and invasive species to determine if parasites from the native species can infect the invasive species and then in turn be transmitted back to native hosts.

Conceptually, parasite spillover and spillback influence community dynamics through the process of 'host-switching' and may be considered augmentative interactions. However, it is important to note that there is also the possibility for parasite dilution, when the addition of novel hosts decreases infection intensity in the native hosts (Blakeslee *et al*., 2020). Invasive hosts may reduce infection intensity in bivalves by acting as pathogen sinks, if hosts become infected but do not transmit the pathogen to other hosts (Burge *et al*., 2016a) or if they prey on free-living infectious stages (Thieltges *et al*., 2009). Alternatively, invasive hosts can be pathogen sources if they act as carriers or reservoirs of disease.

Non-bivalve taxa can serve as carriers or reservoirs of bivalve disease, maintaining bivalve pathogens even when bivalve hosts are absent. For example, Costello *et al*. (2020)

confirmed that invasive tunicates can harbour oyster pathogens, both protistan and bacterial. One such species (the club tunicate *Styela clava*) maintained the protistan *B. ostreae* with no oyster hosts present, indicating it can potentially act as a reservoir of the parasite. Additionally, protistan *M. mercenariae*-like sp. sporulation in *S. clava* suggested the tunicate can facilitate replication of this parasite. Non filter-feeders have also been found to transmit bivalve diseases; the European shore crab *Carcinus maenas* can effect transmission of ostreid herpesvirus-1 microvariant (OsHV-1 μVar) from wild-caught *C. maenas* (from oyster trestles where the presence of the virus has been confirmed) to uninfected *C. gigas*, demonstrating the role of the crab as a carrier and reservoir of the virus (Bookelaar *et al*., 2018). The presence of pathogens in these diverse taxa demonstrates the necessity of screening bivalve-associated organisms when investigating pathogen maintenance and transmission.

In addition to maintaining their own suite of parasites, bivalves may also serve as habitat for a multitude of fouling species that have their own associated parasites. Thus, bivalves can carry numerous species that may serve as intermediate or definitive hosts for parasites/pathogens. The relationship between oysters, sponges and parasites is note-worthy, as sponges have networks of canals permeating the interior and are home to a diverse array of invertebrates in relationships that vary from symbiosis to parasitism (Ďuriš *et al*., 2011). Invertebrates associated with sponges can number up to thousands of individuals and encompass amphipods, crustaceans, isopods, ophiuroids, ostracods and polychaetes. Even microbial communities within host sponges may differ from the habitat-specific bacteria present in the surrounding environment (Pierce & Ward, 2018). Essentially, the movement of bivalves also facilitates the movement of their associated fouling organisms and potentially the flora and fauna harboured within these fouling pests. Fouling organisms may then potentially interact with parasites resulting in further instances of spillover or spillback.

The strong association between fouling organisms and bivalves means that fouling organisms acting as pests or parasites may also influence the degree of parasitism within the bivalve host. For example, boring sponges in the genus *Cliona* are considered macroparasites that reduce oyster growth, condition and recruitment. The sponges

compromise the integrity of the oyster shells, thus decreasing fitness and potentially increasing susceptibility to predators and parasites (Hanley *et al*., 2019). Indeed, a study of two different sample sites on the east coast of the United States found that *Crassostrea virginica* fouled with these sponges were more likely to have a second pest, the pea crab *Zaops* (*Pinnotheres*) *ostreum*. Furthermore, when both sponge and pea crab were present their effects were additive, demonstrating that interactions between bivalve host and fouling pest/parasite can increase susceptibility to other parasites (Watts *et al*., 2018). While these species are native to the study region, interactions such as these may also be of relevance to invasive bivalve/parasite complexes, if associated fouling organisms influence the degree of parasitism.

With processes such as spillover, spillback and introductions of fouling organisms the invasive host directly interacts with the parasites or fouling species, with subsequent effects on native organisms. However, invasive bivalves can also indirectly affect native community host-parasite dynamics. For example, habitat modifications by the reef-building *C. gigas* influence parasitism in blue mussels *Mytilus edulis*, as mussels at the top of the reefs have a higher prevalence of parasitic copepods (*Mytilicola* spp.) compared to mussels seeking refuge at the bottom of the reef. Conversely the trematode *Renicola roscovita*, known to reduce blue mussel condition, displays higher prevalence in mussels at the bottom of the reef rather than mussels on the top. The study suggests indirect effects may be more common than currently understood and warrant further research (Goedknegt *et al*., 2020). There are also examples of the indirect effects of invasive parasites on native hosts, as in separate studies of *M. edulis*, individuals infected with the parasitic copepod *Mytilicola intestinalis* were more susceptible to secondary infections with a virulent bacterial *Vibrio orientalis*/*tubiashii* strain, thus highlighting the role of indirect effects arising from what was initially parasite spillover of copepod to mussel (Demann & Wegner, 2019).

Invasive bivalves can also interact with other extrinsic factors to influence parasitism in native bivalve communities. For example, a study of invasive *C. gigas* investigated its role in determining parasitic infection levels in native blue mussel *M. edulis* in relation to other biotic factors such as salinity, tidal exposure and host densities (both oyster and

mussel) (Goedknegt *et al*., 2019). The results suggested that the presence of an invasive species could affect parasite interactions with native hosts in different ways. For example, when oyster density was high, the prevalence and abundance of the parasitic copepod *M. intestinalis* decreased in its native mussel host, while prevalence of the trematode *R. roscovita* increased. The study did note that interactions may be further mediated by environmental and biotic factors, as invasive species are not the sole drivers of infection levels (Goedknegt *et al*., 2019).

Parasites are particularly relevant to invasion ecology, as escape from parasites may facilitate host invasion via a mechanism known as the enemy release hypothesis (ERH) (Figure 1) (Torchin *et al*., 2001). Invasive species may lose their parasites as they colonise a new range, perhaps due to unsuitable environmental conditions, or the absence of a required secondary host. It may also be because the source population of invasive hosts has low parasite prevalence and only a subset of these hosts, some of which may be uninfected, extend their range, meaning that upon entry to the new range parasite abundance is low. Infected hosts may also be more likely to die during transfer due to the additional stress of parasitism. The hypothesis may be applied in both terrestrial and aquatic systems, for example the invasive fire ant *Solenopsis invicta* demonstrated a loss of microbial pathogens (microsporidia, bacteria and RNA viruses) in its invaded range compared to its native range (Yang *et al*., 2010).

Investigations of the ERH are broadly split into biogeographical studies, which examine native and introduced populations of a specific host, and community studies which compare parasitism between cohabiting native and introduced species (Colautti *et al*., 2004). One such study identified low levels of digenean trematode infection in introduced Manila clams *R. philippinarum* compared to native sympatric bivalves. However, digenean infection is also low in native clam populations, potentially due to characteristics intrinsic to the *Ruditapes* genus (specifically tough epithelial tissue that is difficult for cercariae to penetrate) rather than a classical enemy escape, further highlighting the complexities underpinning bivalve host-parasite interactions (Dang *et al*., 2009).

If invasive hosts do escape their parasites when colonising a new area, it is unlikely that they will remain free of parasites, especially if native generalist parasites are present. For

example, Miller *et al*. (2008) used laboratory choice-chambers to demonstrate that generalist native pea crabs, *Pinnotheres novaezelandiae*, could adopt the introduced Asian date mussel *Musculista senhousia* (now listed on the Global Invasive Species Database), although their preference was for the native New Zealand green lipped mussel *Perna canaliculus* and the little black mussel *Xenostrobus pulex*. The impact of native parasites on introduced hosts is host-parasite specific. For example, if the parasite has negative effects it could potentially act as a control and slow the expansion of the host. Furthermore, if the introduced species is not a competent host it may reduce the disease risk for native species by acting as a sink for parasites, but if it is a competent host this amplifies the potential for spillback (Poulin *et al*., 2011).

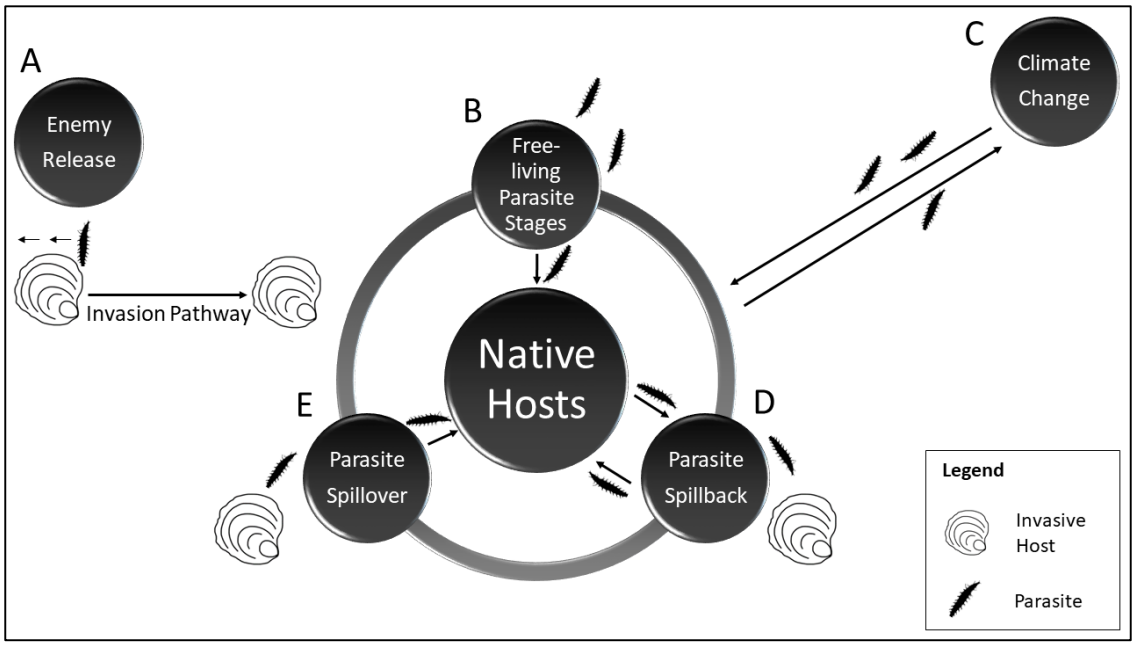

Figure 1: Major interactions between invasive hosts, parasites and native hosts featuring: (Clockwise) **A.** Enemy release hypothesis whereby an invasive host escapes some or all of its natural parasites as it moves through a series of biotic and abiotic filters (the invasion pathway) to a new range, **B.** The introduction of free-living parasite stages, **C.** Climate change and the potential for changes in disease prevalence/virulence, **D.** Spillback whereby an invasive species proves a suitable host for native parasites thus amplifying the load and **E.** Spillover whereby an invasive host spills its parasites to native hosts.

The previous examples have focused on invasive species as hosts, however it is important to note that invasive species can also act as bivalve parasites themselves rather than hosts. For example, invasive shell-boring polychaete worms are parasitic to commercial molluscs and impact host condition by damaging the protective shell and thus reducing the commercial viability (David *et al*., 2016). The spionid polychaete *Polydora hoplura* is one such species, an invasive pest that burrows into the shells of commercial bivalves and univalves including Cape rock oysters *Striostrea margaritacea* and South African abalone *Haliotis midae*, creating tubes and mud blisters, and reducing the growth rate of the mollusc (Coen & Bishop, 2015).

There is also the potential for parasites to be introduced to an area without a host (e.g. in free-living life stages) and these novel parasites could severely impact native hosts with whom they have no evolutionary history who therefore lack immune defenses (Poulin, 2017). Ostreid herpesvirus OsHV-1 (Evans *et al*., 2014) and the protistan *B. ostreae* (Arzul *et al*., 2009) are known to persist in the water column and, in the case of *B. ostreae*, within larvae brooded in the adult pallial cavity even though parents were negative for infection (Flannery *et al*., 2016). The fact that free living life stages of bacteria, viruses and protistans are potentially present in coastal waters highlights the intrinsic link between disease and filter-feeding bivalves, as consumption is a major pathway for parasite transmission in the bivalve hosts (Ben-Horin *et al*., 2015).

Introductions of free-living life-stages of parasites without an obvious associated host may give rise to cryptogenic invasions, meaning the origin and biogeographic status of the invasive species is unknown. For example, the cryptogenic protistan parasite *Haplosporidium pinnae* is the causative agent of mass-mortality events of the protected fan mussel *Pinna nobilis* in the western Mediterranean (Katsanevakis *et al*., 2019). These mortality events first occurred in 2016 and are continuing to spread, and the study noted that it is probable that this parasite is alien to the Mediterranean Sea. The fan mussel is Critically Endangered (IUCN Red List) due to historical exploitation and ongoing poaching, and the parasite poses a further threat to the conservation of the species (Katsanevakis *et al*., 2019). Of additional concern is the fact that *H. pinnae* is not the only parasite present in fan mussels undergoing mortality events. A gram-positive bacteria

belonging to the genus *Myobacterium* (considered invasive within the study) has also been detected, resulting in co-infections within fan mussels in Greek waters and causing high mortalities (Lattos *et al*., 2020).

Parasite-host interactions can be increasingly complex. For example, the digenean trematode *Parvatrema duboisi* uses the Manila clam *R. philippinarum* as an intermediate host (Yanagida *et al*., 2009). However, the trematode is in turn hyperparasitised by a protistan *Urosporidium* sp. and the *Urosporidium* burden on its trematode host causes mortality, thus decreasing the trematode load on the clam host (Cuong Le *et al*., 2015). While hyperparasitism in this instance is not causing deleterious effects to the clam itself, it can be the case that hyperparasitism can lead to decreased market value of commercial species (Cuong Le *et al*., 2015) and as such parasitological studies may be enhanced by elucidating all forms of parasitism within a host.

Disentangling host-parasite relationships may also lead to novel forms of monitoring and control, particularly when the functional role of bivalves as filter-feeders is taken into consideration. Ford *et al*. (2009) postulated that as bivalves are filter-feeders, both hosts and non-hosts take in parasites from the water column, but while susceptible hosts then become infectious, non-susceptible hosts discard the parasites in faeces. This principle was applied to investigate the environmental distribution of the protistan *Haplosporidium nelsoni* using faecal samples from both a non-host bivalve, the ribbed mussel *Guekensia demissa*, and a host with developed resistance, the Eastern oyster *C. virginica*. Data from faecal samples was then used in conjunction with tissue samples to describe pathogen distribution and temporal patterns that were not evident using traditional histology. Bivalves have also been posited as having the ability to remove or reduce pathogen concentrations. However, while filtration can remove pathogens from the water column thereby reducing transmission it can also have the converse effect of increasing disease risk if the filter-feeder acts as a reservoir. The outcome is contingent on factors such as the selectivity of the filter-feeder, mechanisms of pathogen transmission, susceptibility of that pathogen to degradation and the degree of infectivity (Burge *et al*., 2016a)

The control of parasitic diseases can benefit from knowledge of the entire lifecycle of the focal parasite. Blasco-Costa & Poulin (2017) used helminths as a model group to

demonstrate that many species are known only from their adult stage and neither juvenile stages or intermediate hosts are confirmed, and that this issue may be applied more broadly to other taxa. This is important for integrated ecology, as predicting and mitigating against invasive introductions and their effects on parasites requires knowledge of potential hosts, not only to confirm potential reservoirs but to know what life history stage to target if attempting eradication. Ultimately, understanding the mechanisms by which invasives transmit parasites and extending studies to different phyla, particularly those with links to aquaculture, may inform management strategies and minimise the risk of disease cycling.

Climate Change and Bivalve Diseases: Potential Outcomes for Parasite Transmission

The occurrence of disease requires synergy between three factors; the host, the pathogen and the environment. The interaction between these three factors is termed the epidemiological triangle (Zannella *et al*., 2017), and the impacts of climate change have the potential to radically change the characteristics of this triangle, particularly the environment (Figure 2). Environmental change increases physiological stress in bivalves in a number of ways, leaving them susceptible to pathogens. Warming water can enhance microbial growth, thereby increasing the content of organic matter in the water, with the subsequent microbial decomposition reducing dissolved oxygen levels (Soon & Zheng, 2019). Hypoxic conditions can impact bivalve immune systems and interact with diseases, for example rendering bivalve hosts less efficient at eliminating bacterial cells (Macey *et al*., 2008). Higher water temperatures have also led to concerns about the spread of Harmful Algal Blooms (HABs), for example the dinoflagellate *Prorocentrum minimum* has been implicated in mass mortalities of oysters, scallops and hard clams in North America. For oysters in particular, blooms of this dinoflagellate were found to alter immune system competence in juveniles, thereby comprising their disease resistance. In addition to low dissolved oxygen levels and HABs, toxins and pollutants have been shown to promote infection by the bacterium *Vibrio splendidus*, the pathogen responsible for juvenile summer mortality in *C. gigas* spat, also known as bacillary necrosis disease (Soon & Zheng, 2019).

The ecological traits that facilitate the movement of invasive species, for example plastic life-histories coupled with the tendency to occupy generalist and opportunistic niches, are disproportionately favoured under climate change (Mellin *et al*., 2016). As a result, non-native organisms in the aquatic environment may often be more resilient than native organisms when faced with environmental change, and appear to be at a performance advantage relative to co-occurring native species when subjected to warming or acidification (Sorte *et al*., 2013). Increased temperatures can cause persistent stress and mortality of native species, thus facilitating the establishment and spread of invasive species that are more tolerant to the higher temperatures or can occupy environmental niches previously held by native species (Diez *et al*., 2012). Furthermore, although reduction in body size has been suggested as a response to warming in aquatic systems, the inverse effect has been observed in bivalves introduced to the Mediterranean Sea, leading to their competitive dominance over native species. Not only are the introduced bivalves larger than cohabiting native species, there is evidence to suggest that some invasives, for example the mussel *Brachidontes pharaonis*, are larger in their invaded range than their native range (Nawrot *et al*., 2017).

Climatic vectors have implications for the movement of invasive species, with hurricanes and storm surges enhancing the dispersal of invasive propagules in the water column (Hellmann *et al*., 2008). An association between marine disease outbreaks and storm activity is also recognised (Burge *et al*., 2014). Climate change has increased extreme sea level events such as flooding and heat waves, and is predicted to increase the magnitude of storm surges in coastal areas (IPCC, 2019), where aquaculture occurs. Coastal marine environments are therefore extremely vulnerable to change (Holt *et al*., 2010) and an altered marine environment can in turn impact the distribution, life-history and physiological status of pathogens, hosts and vectors (Gallana *et al*., 2013). Furthermore, poleward advances of subtropical species into Europe, referred to as ‘African Creep’ are occurring in the eastern Atlantic (Canning-Clode & Carlton, 2017) and this phenomenon is mirrored by ‘Caribbean Creep’ in the western Atlantic, including the extension of species such as the invasive Asian green mussel *Perna viridis* (Canning-Clode *et al*., 2011).

The impacts of climate change on parasite transmission in the marine environment is in the early stages of our understanding (Burge *et al*., 2014). However, marine invertebrates demonstrate strong links between disease and climate (Marcogliese, 2008) and there is increasing evidence that climate change will affect the ecology of infectious diseases and the physiology of bivalve species and their resistance to infection (Soon & Zheng, 2019). Climate change could affect parasites by influencing the distribution and life-history of hosts (Callaway *et al*., 2012) and potentially increase host susceptibility to infection due to thermal stress (Harvell *et al*., 2002). Alterations in water temperature may also lead to osmotic stress, again rendering hosts more susceptible to disease (Burge *et al*., 2016a).

Warmer temperatures may allow disease-causing organisms to complete their lifecycle more rapidly, thus attaining higher population densities (Marcogliese, 2001; Lõhmus & Björklund, 2015; Schade *et al*., 2016). Furthermore, climate change may increase the virulence of pathogens, for example *Vibrio* spp. may demonstrate increased growth and an upregulation of virulence genes, or cause epizootic outbreaks in areas that were previously free from pathogens if disease-carrying organisms from warmer climates colonise waters at colder latitudes (Conn, 2014; Rowley *et al*., 2014). Historically pathogens have been known to maintain their pathogenicity when expanding. For example, the protistan oyster pathogen *Perkinsus marinus* first expanded northwards in the United States in the early 1990s and was well established in the new range by 1996-97, suggesting that range expansion had not limited its prevalence, infection capacity or proliferation (Ford & Smolowitz, 2001). Although rising temperatures are posited to be favourable to parasites, it is important to note that parasites with complex life-cycles may be adversely affected, as if secondary or definitive hosts breach their environmental thresholds for survival and undergo mortality events this will in turn negatively affect parasite abundance (Byers, 2020). Similarly, if there is a mismatch in range shifts between intermediate hosts and parasites, the parasites may not be able to complete their life-cycle.

In the marine environment, the synergistic effects of global warming and ocean acidification are forecast as two of the primary threats to bivalve health (Soon & Zheng, 2019). Mackenzie *et al*. (2014) investigated the effects of these coinciding stressors, acidification and warming, on the blue mussel *M. edulis* in a laboratory setting and

confirmed that exposure to these conditions led to altered host pathological conditions, in addition to changes in parasite diversity and prevalence. Species-specific environmental pH tolerances may alter previously stable parasite-host relationships, as either host or parasite may prove more susceptible to the stressors associated with acidification. For example, trematode cercarial longevity and metacercarial survival may be reduced in acidified water (MacLeod & Poulin, 2015). It is therefore important for future studies to measure the response of both host and parasite to acidification, particularly if the host is molluscan, as that acidification affects the ability of molluscs to lay down calcium carbonate, potentially imposing a further stress on the host (MacLeod & Poulin, 2015).

From an aquaculture perspective, warming temperatures may facilitate the spread of bacterial and viral diseases associated with bivalve mortality events. *Vibrio* species are ubiquitous in the aquatic environment and may become more prevalent in the context of a warming climate, as higher temperature enhances *Vibrio* growth. This may impact bivalve culture, as species including *V. aestuarianus* and *V. splendidus* are linked to mortalities of *C. gigas* in western Europe (Vezzulli *et al*., 2013). Mortality events have also been linked to outbreaks of ostreid herpesvirus OsHV-1, variants of which have spread through Australia, New Zealand and Asia, in addition to Europe (Pernet *et al*., 2016). Mortensen *et al*. (2016) recorded high and sudden mortalities of *C. gigas* associated with OsHV-1 μVar in both a hatchery in Sweden and wild populations in its expanded Scandinavian range. This study represented the first time this variant was detected in Scandinavia and demonstrates how range extension of bivalve species, whether deliberately for aquaculture purposes or via the establishment of wild populations, can facilitate the spread of disease. The ostreid herpesvirus OsHV-1 is lacking in host-specificity, meaning that it can also be found in scallops and clams including the invasive *R. philippinarum* (Ben-Horin *et al*., 2015), as is the microvariant which can be found in *Mytilus* spp. (O'Reilly *et al*., 2018) and *C. maenas* (Bookelaar *et al*., 2018), and this could further extend dispersal capability.

Temperature-based surveillance models have been used to model disease outbreaks and, given the sensitivity of many parasite-host systems to temperature change, may be useful for predicting the spread of bivalve diseases. Maynard *et al*. (2016) tested temperature

modelling as a disease surveillance technique by modelling the maximum monthly mean ocean floor temperature (using 12°C as the threshold under which outbreaks are minimised) and using it to predict epizootic shell disease (ESD) in American lobster *Homarus americanus* in southern New England. The results suggested outbreaks may continue for years especially in sheltered warm water bays. They also postulated that the model may be suitable to predict the spread of bivalves and pathogens, including the spread of *C. gigas*/*V. splendidus* in western Europe. Monitoring of bivalve aquaculture farms may also present an opportunity to create a network to map disease outbreaks, similar in function to the NOAA Mussel Watch Program, an ecosystem-based approach to monitoring that measures the concentration of chemical contaminants in sediment and bivalve tissues (NOAA, 2017). Screening for virulence genes, for example within *Vibrio* spp., may also enhance the understanding of pathogenesis and facilitate the development of new strategies to control diseases (Xu *et al*., 2018).

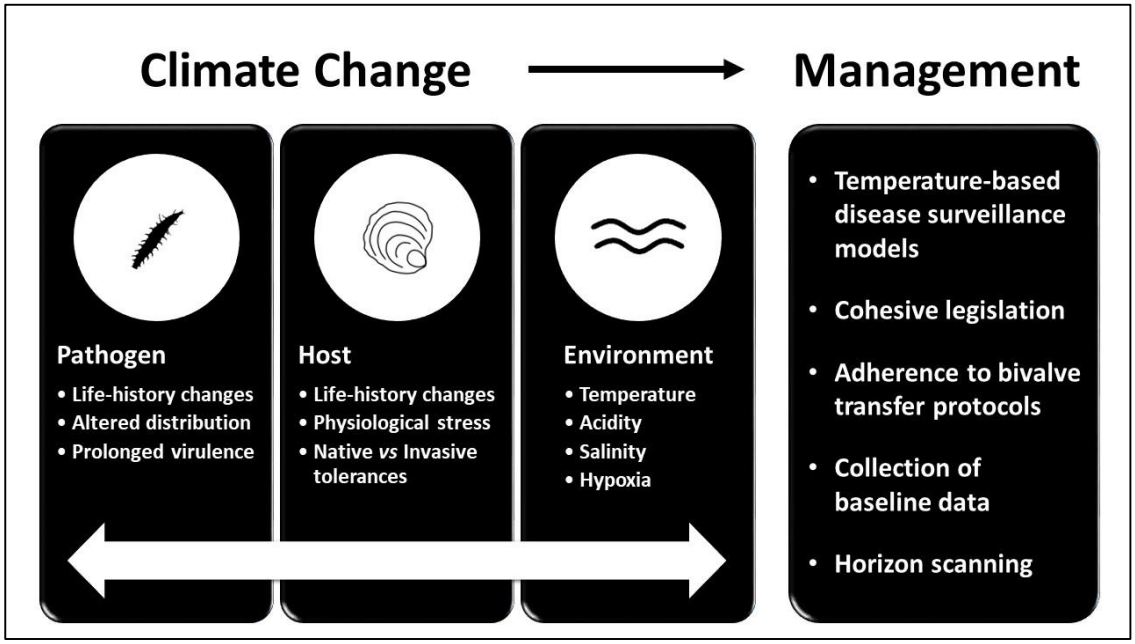

Figure 2: Potential outcomes of climate change and associated management strategies.

Discussion and Concluding Remarks

In the context of a changing environment, the impacts of invasive species and diseases may pose greater risks to bivalve aquaculture in the future. When diagnosing marine disease outbreaks, it is important to combine classic techniques such as histopathology with more modern techniques such as metagenomics (Burge *et al*., 2016b). However, it is equally important to trace the source and pathways of the diseases, thus creating a comprehensive understanding of parasite-host dynamics, particularly when facilitated by invasive species.

Population genetic analyses may be useful to elucidate the source and pathways of invasive parasites. For example, a recent study combined genetic sequencing of the two parasitic copepods, *Mytilicola intestinalis* and *Mytilicola orientalis*, with an in-depth literature review to identify native and invaded ranges. In the case of *M. orientalis* there was strong overall population differentiation between the native Japanese range and the invaded North American and European ranges, with the invasion history of this copepod species reflecting the movement of its principal host, *C. gigas* (Feis *et al*., 2019). However, although population sequencing proved effective for *M. orientalis*, the native range of *M. intestinalis* remained unclear due to a lack of population genetic structure, suggesting that for some species extrapolation of the invasion history using genetic analyses is not feasible.

Combining the fields of invasion ecology and parasitology will provide clarity on their impacts in the context of climate change. Rowley *et al*. (2014) noted that even with comprehensive models relating to climate change in marine systems, a lack of understanding as to how invertebrates transfer infectious diseases, for example uncertainties about the host range of pathogens (Carnegie *et al*., 2016), creates difficulties in understanding disease dynamics. These difficulties are exacerbated by the fact that parasite-host interactions are fluid, with a number of accepted hypotheses as to whether invasive hosts travel with parasites to a new range or whether the host escapes its native parasites. Pernet *et al*. (2016) highlighted the need for an integrated approach using ostreid herpesvirus OsHV-1 as a case study, and noted that a primary knowledge gap is the lack of information as to how other animals are involved in the transfer, as often host and

parasite are considered in a vacuum but there is a need to consider both sources of dilution and reservoirs when developing management strategies. The need for monitoring of both farmed and wild hosts has also been highlighted, in an effort to quantify the exchange between cultured stocks and the surrounding wildlife, as this may provide valuable information on the extent of disease transmission (Bouwmeester *et al*., 2020).

When coupled with the environmental stressors of warming and acidification, the impacts of invasive species and parasites may make bivalve species more susceptible to infection and disease. This has implications for the aquaculture sector and as such merits further discussion, particularly if commercial bivalves are purposely moved to new locations thus creating a vector for cohabiting invasive species to also move (McKindsey *et al*., 2007). Ensuring that relevant legislation is accessible and consistent is a vital management aspect when considering the impacts of invasive species (Shannon *et al*., 2020). Furthermore, if bivalve movements are increased to meet growing consumer demand, this necessitates augmented monitoring by management bodies to enhance biosecurity measures to ensure only the commercial target species is shipped and prevent the movement of associated invasive species. Strict implementation of the ICES Code of Practice on the Introduction and Transfers of Marine Organisms will minimise transfer of invasive species (Figure 2), and ICES member and non-member states are both encouraged to follow this Code. This protocol requires that before an introduction occurs the species undergo quarantine in the recipient region, and organisms that are to be released require documented examinations, including microscopic inspection, to confirm that there are no associated invasives (ICES, 2005). Additionally, David & Loveday (2018) highlighted global trade routes and the continuous movement of species as a factor in cryptic species dispersal and suggested that tracking the movement of shellfish consignments and investigating their associated cohabiting species is a feasible mechanism to monitor the movement of potentially invasive species.

One issue relating to invasive species and parasites in the marine environment is that their presence often goes undetected for long periods of time, and as such it is difficult to determine the mode and timing of arrival. This can in turn lead to uncertainty as to whether disease outbreaks arise from new introductions or just changes in the environmental

conditions (Pagenkopp Lohan *et al*., 2020). Epizootics of previously undocumented parasites should not instantly be considered exotic parasites, because it is possible they were present at a low prevalence but may be able to infect established invasive hosts and reach the required prevalence to be pathogenic (Torchin *et al*., 2002).

A useful mechanism to determine the current health status of populations is the collection of baseline data in potentially sensitive regions, for example potential aquaculture sites or coastal areas vulnerable to climate change. This will ensure managers and policy-makers fully understand the current breadth of host and parasite species and alleviate any future uncertainty about the timing of emerging diseases. One such baseline study, conducted in Patagonia, sampled both wild and cultured populations of the edible mussels *Mytilus platensis* and *Mytilus chilensis*. The study detected a number of parasites including prokaryote inclusions, protozoa and metazoa but concluded that none currently pose a problem to the industry. However, the benefits of conducting such a survey were also evident as there was a low incidence of the cancerous disease disseminated neoplasia present, and the authors stressed this required further monitoring (Vázquez *et al*., 2020)

Horizon scanning of future potential invasive species and pathogens, along with identification of new methods of detection, management and control acts as an early warning system, which can inform management strategies to prevent or mitigate future introductions (Dunn & Hatcher, 2015; Lucy *et al*., 2020). Targeted workshops to assess the status of emerging diseases, for example focusing on the ecology and pathogenesis of vibrios as conducted by le Roux *et al*. (2015) will also contribute to ocean health and food security. In the context of bivalve aquaculture, regional management could entail maintaining watch lists of species with known deleterious impacts, monitoring potential pathways of introduction and networks of connectivity, eliminating vectors of introduction for hitchhiking species, for example biological packaging material (Haska *et al*., 2012), and engaging in citizen science to augment observer numbers as early intervention is key to preventing species establishments. Documenting the environmental tolerance range of invasive species and their parasites may also provide clarity on which environments outside their native range are most at risk, i.e. are most suitable, with both abiotic and biotic drivers present to facilitate successful establishment. Furthermore, an

increased effort and focus on the screening of invasive species, both in their native and invaded range, will also provide clarity on co-invasions of hosts and their parasites. This may then be extended to investigate transmission between invasive and native hosts, and further describe aspects of disease-cycling in the marine environment.

References

Anderson, R.M. & May, R.M. (1979). Population biology of infectious diseases: Part I. *Nature*, 280, 361-367.

Arzul, I., Gagnaire, B., Bond, C., Chollet, B., Morga, B., Ferrand, S., Robert, M. & Renault, T. (2009). Effects of temperature and salinity on the survival of *Bonamia ostreae*, a parasite infecting flat oysters *Ostrea edulis*. *Diseases of Aquatic Organisms*, 85, 67-75.

Beaury, E.M., Fusco, E.J., Jackson, M.R., Laginhas, B.B., Morelli, T.L., Allen, J.M., Pasquarella, V.J. & Bradley, B.A. (2020). Incorporating climate change into invasive species management: insights from managers. *Biological Invasions*, 22, 233-252.

Ben-Horin, T., Bidegain, G., Huey, L., Narvaez, D.A. & Bushek, D. (2015). Parasite transmission through suspension feeding. *Journal of Invertebrate Pathology*, 131, 155-176.

Blakeslee, A.M.H., Fowler, A.E. & Keogh, C.L. (2013). Marine Invasions and Parasite Escape: Updates and New Perspectives. (Ed.) Michael Lesser, In: *Advances in Marine Biology*, 66, 87-169.

Blakeslee, A.M.H., Barnard, R.B., Matheson, K. & McKenzie, C.H. (2020). Host-switching among crabs: species introduction results in a new target host for native parasites. *Marine Ecology Progress Series*, 636, 91-106.

Blasco-Costa, I. & Poulin, R. (2017). Parasite life-cycle studies: a plea to resurrect an old parasitological tradition. *Journal of Helminthology*, 91, 647-656.

Bookelaar, B., O'Reilly, A.J., Lynch, S.A. & Culloty, S.C. (2018). Role of the intertidal predatory shore crab *Carcinus maenas* in transmission dynamics of ostreid herpesvirus-1 microvariant. *Diseases of Aquatic Organisms*, 130, 221-233.

Bouwmeester, M.M, Goedknegt, M.A., Poulin, R. & Thieltges, D. (2020). Collateral diseases: Aquaculture impacts on wildlife infections. *Journal of Applied Ecology*, 1-12.

Burge, C.A., Eakin, C.M., Friedman, C.S., Froelich, B., Hershberger, P.K., Hofmann, E.E., Petes, L.E., Prager, K.C., Weil, E., Willies, B.L., Ford, S.E. & Harvell, C.D. (2014). Climate Change Influences on Marine Infectious Diseases: Implications for Management and Society. *Annual Review of Marine Science*, 6, 249-277.

Burge, C.A., Closek, C.J., Friedman, C.S., Groner, M.L., Jenkins, C.M., Shore-Maggio, A. & Welsh, J.E. (2016a). The Use of Filter-feeders to Manage Disease in a Changing World. *Integrative and Comparative Biology*, 56, 537-587.

Burge, C.A., Friedman, C.S., Getchell, R., House, M., Lafferty, K.D., Mydlarz, L, D., Prager, K.C., Sutherland, K.P., Renault, T., Kiryu, I. & Vega-Thurber, R. (2016b). Complementary approaches to diagnosing marine diseases: a union of the modern and the classic. *Philosophical Transactions of the Royal Society B*, 371(1689), 20150207.

Burreson, E.M. & Stokes, N.A. (2000). Increased Virulence in an Introduced Pathogen: *Haplosporidium nelsoni* (MSX) in the Eastern Oyster *Crassostrea virginica*. *Journal of Aquatic Animal Health*, 12, 1-8.

Burreson, C.A., Stokes, N.A. & Carnegie, R. (2004). *Bonamia* sp. (Haplosporidia) Found in Nonnative Oysters *Crassostrea ariakensis* in Bogue Sound, North Carolina. *Journal of Aquatic Animal Health*, 16, 1-9.

Bishop, M.J., Carnegie, R.B., Stokes, N.A., Peterson, C.H. & Burreson, E.M. (2006). Complications of a non-native oyster introduction: facilitation of a local parasite. *Marine Ecology Progress Series*, 325, 145-152.

Byers, J.E. (2020). Marine Parasites and Disease in the Era of Global Climate Change. *Annual Review of Marine Science*, 13, 397-420.

CABI *Myicola ostreae* (2020). https://www.cabi.org/isc/datasheet/110220. Accessed 12/08/2020.

CABI *Mytilicola intestinalis* (2020). https://www.cabi.org/isc/datasheet/73758. Accessed 12/08/2020.

Callaway, R., Shinn, A.P., Grenfell, S.E., Bron, J.E., Burnell, G., Cook, E.L., Crumlish, M., Culloty, S., Davidson, K., Ellis, R.P., Flynn, K.J., Fox, C., Green, D.M., Hays, G.C.,

Hughes, A.D., Johnston, E., Lower, C.D., Lupatsch, I., Malham, S., Mendzil, A.F., Nickell, T., Pickerell, T., Rowley, A.R., Stanley, M.S., Tocher, D.R., Turnbull, J.F., Webb, G., Wootton, E. & Shields, R.J. (2012). Review of climate change impacts on marine aquaculture in the UK and Ireland. *Aquatic Conservation*, 22, 389-421.

Canning-Clode, J., Fowler, A.E., Byers, J.E., Carlton, J.T. & Ruiz, G.M. (2011). 'Caribbean Creep' Chills Out: Climate Change and Marine Invasive Species. *PLoS ONE*, 6(12), e29657, 1-5.

Canning-Clode, J. & Carlton, J.T. (2017). Refining and expanding global climate change scenarios in the sea: Poleward creep complexities, range termini, and setbacks and surges. *Diversity and Distributions*, 23, 463-473.

Carnegie, R.B., Stokes, N.A., Audemard, C., Bishop, M.J., Wilbur, A.E., Alphin, T.D., Posey, M.H., Peterson, C.H. & Burreson, E.M. (2008). Strong seasonality of *Bonamia* sp. infection and induced *Crassostrea ariakensis* mortality in Bogue and Masonboro Sounds, North Carolina, USA. *Journal of Invertebrate Pathology*, 98, 335-343.

Carnegie, R.B, Arzul, I. & Bushek, D. (2016). Managing marine mollusc diseases in the context of regional and international commerce: policy issues and emerging concerns. *Philosophical Transactions of the Royal Society B*, 371(1689), 20150215.

Carrasco, N., Green, T. & Itoh, N. (2015). *Marteilia* spp. parasites in bivalves: A revision of recent studies. *Journal of Invertebrate Pathology*, 131, 43-57.

Chalkowski, K., Lepczyk, C.A. & Zohdy, S. (2018). Parasite Ecology of Invasive Species: Conceptual Framework and New Hypotheses. *Trends in Parasitology*, 34, 655-663.

Cheng, T.C. & Combes, C. (1990). "Influence of environmental factors on the invasion of molluscs by parasites: with special reference to Europe" in Biological Invasions in Europe and the Mediterranean Basin, eds. f. di Castri, A.J. Hansen and M. Debussche (Kluwer Academic Publishers, Dordrecht), 307-332.

Coen, L.D. & Bishop, M. (2015). The ecology, evolution, impacts and management of host–parasite interactions of marine molluscs. *Journal of Invertebrate Pathology*, 131, 177-211.

Colautti, R.I., Ricciardi, A., Grigorovich, I.A. & MacIsaac, H.J. (2004). Is invasion success explained by the enemy release hypothesis? *Ecology Letters*, 7, 721-733.

Conn, D.B. (2014). Aquatic invasive species and emerging infectious disease threats: A One Health perspective. *Aquatic Invasions*. 9, 383-390.

Cordero, D., Delgado, M., Liu, B., Ruesink, J. & Saavedra, C. (2017). Population genetics of the Manila clam (*Ruditapes philippinarum*) introduced in North America and Europe. *Scientific Reports*, 7, 39745.

Costello, K.E., Lynch, S.A., McAllen, R., O'Riordan, R.M. & Culloty, S.C. (2020). The Role of Invasive Tunicates as Reservoirs of Molluscan Pathogens. *Biological Invasions*, 23, 641-655.

Crowl, T.A., Crist, T.O., Parmenter, R.R. Belovsky, G. & Lugo, A.E. (2008). The spread of invasive species and infectious disease as drivers of ecosystem change. *Frontiers in Ecology and the Environment*, 6, 238-246.

Cuong Le, T., Kang, H.-S., Hong, H.-K., Park, K.-J. & Choi, K.-S. (2015). First report of *Urosporidium* sp., a haplosporidian hyperparasite infecting digenean trematode *Parvatrema duboisi* in Manila clam, *Ruditapes philippinarum* on the west coast of Korea. *Journal of Invertebrate Pathology*, 130, 141-146.

Dang, C., de Montaudouin, X., Bald, J., Jude, F., Raymond, N., Lanceleur, L., Paul-Pont, I. & Caill-Milly, N. (2009). Testing the enemy release hypothesis: trematode parasites in the non-indigenous Manila clam *Ruditapes philippinarum*. *Hydrobiologia*, 630, 139-148.

David, A.A., Matthee, C.A., Loveday, B.R. & Simon, CA. (2016). Predicting the Dispersal Potential of an Invasive Polychaete Pest along a Complex Coastal Biome. *Integrative and Comparative Biology*, 56, 600-610.

David, A.A. & Loveday, B.R. (2018). The role of cryptic dispersal in shaping connectivity patterns of marine populations in a changing world. *Journal of the Marine Biological Association of the United Kingdom*, 98, 647-655.

Demann, F. & Wegner, K.M. (2019). Infection by invasive parasites increases susceptibility of native hosts to secondary infection via modulation of cellular immunity. *Journal of Animal Ecology*, 88, 427-438.

Diez, J.M., D'Antonio, C.M., Dukes, J.S., Grosholz, E.D., Olden, J.D., Sorte, C.J.B., Blumenthal, D.M., Bradley, B.A., Early, R., Ibáñez, I., Jones, S.J., Lawler, J.J. & Miller, L.P. (2012). Will extreme climatic events facilitate biological invasions*? Frontiers in Ecology and the Environment*, 10, 249-257.

Dunn, A.M., Torchin, M.E., Hatcher, M.J., Kotanen, P.M., Blumenthal, D.M., Byers, J.E., Coon, C.A.C., Frankel, V.M., Holt, R.D., Hufbauer, R.A., Kanarek, A.R., Schierenbeck, K.A., Wolfe, L.M. & Perkins, S.E. (2012). Indirect effects of parasites in invasions. *Functional Ecology*, 26, 1262-1274.

Dunn, A.M. & Hatcher, M.J. (2015). Parasites and biological invasions: parallels, interactions, and control. *Trends in Parasitology*, 31, 189-199.

Ďuriš, Z., Horká, I., Juračka, P.J., Petrusek, A. & Sanford, F. (2011). These Squatters Are Not Innocent: The Evidence of Parasitism in Sponge-Inhabiting Shrimps. *PLoS ONE*, 6(7), e21987, 1-10.

Elsner, N.O., Jacobsen, S., Thieltges, D.W. & Reise, K. (2011). Alien parasitic copepods in mussels and oysters of the Wadden Sea. *Helgoland Marine Research*, 65, 299-307.

European Commission Regulation (EU) 1143/2014 on invasive alien species. (2014) https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32014R1143&rid=5. Accessed 29/01/2021.

Evans, O., Paul-Pont, I., Hick, P. & Whittington, R.J. (2014). A simple centrifugation method for improving the detection of Ostreid herpesvirus-1 (OsHV-1) in natural seawater samples with an assessment of the potential for particulate attachment. *Journal of Virological Methods*, 210, 59-66.

FAO (2018). The State of World Fisheries and Aquaculture 2018 - Meeting the sustainable development goals. Rome, Licence: CC BY-NC-SA 3.0 IGO.

Feis, M.E., Goedknegt, M.A., Arzul, I., Chenuil, A., den Boon, O., Gottschalck, L., Kondo, Y., Ohtsuka, S., Shama, L.N.S., Thieltges, D.W., Wegner, K.M. & Luttikhuizen, P.C. (2019). Global invasion genetics of two parasitic copepods infecting marine bivalves. *Scientific Reports*, 9:12730.

Fernández-Robledo, J.A., Vasta, G.R. & Record, N.R. (2014). Protozoan Parasites of Bivalve Molluscs: Literature Follows Culture. *PLoS ONE*, 9(6), e100872, 1-9.

Flannery, G., Lynch, S.A. & Culloty, S.C. (2016). Investigating the significance of the role of *Ostrea edulis* larvae in the transmission and transfer of *Bonamia ostreae*. *Journal of Invertebrate Pathology*, 136, 7-9.

Ford, S.E. & Smolowitz, R. (2001). Infection dynamics of an oyster parasite in its newly expanded range. *Marine Biology*, 151, 119-133.

Ford, S.E., Allam, B. & Xu, Z. (2009). Using bivalves as particle collectors with PCR detection to investigate the environmental distribution of *Haplosporidium nelsoni*. *Diseases of Aquatic Organisms*, 83, 159-168.

Gallana, M., Ryser-Degiorgis, M.-P., Wahli, T. & Segner, H. (2013). Climate change and infectious diseases of wildlife: Altered interactions between pathogens, vectors and hosts. *Current Zoology*, 59, 427-437.

Gallardi, D. (2014). Effects of Bivalve Aquaculture on the Environment and Their Possible Mitigation: A Review. *Fisheries and Aquaculture Journal*. 5, 1-8.

Goedknegt, M.A., Feis, M.E., Wegner, K.M., Luttikhuizen, P.C., Buschbaum, C., Camphuysen, K. (CJ), van der Meer, J. & Thieltges, D.W. (2016). Parasites and marine invasions: Ecological and evolutionary perspectives. *Journal of Sea Research*, 113, 11-27.

Goedknegt, M.A., Schuster, A.K., Buschbaum, C., Gergs, R., Jung, A.S., Luttikhuizen, P.C., van der Meer, J., Troost, K., Wegner, K.M. & Thieltges, D.W. (2017). Spillover but no spillback of two invasive parasitic copepods from invasive Pacific oysters (*Crassostrea gigas*) to native bivalve hosts. *Biological Invasions*, 19, 365-379.

Goedknegt, M.A., Nauta, R., Markovic, M., Buschbaum, C., Folmer, E.O., Luttikhuizen, P.C., van der Meer, J., Waser, A.M, Wegner, K.M. & Thieltges, D.W. (2019). How invasive oysters can affect parasite infection patterns in native mussels on a large spatial scale. *Oecologia*, 190, 99-113.

Goedknegt, M.A., Buschbaum, C., van der Meer, J., Wegner, K.M & Thieltges, D.W. (2020). Introduced marine ecosystem engineer indirectly affects parasitism in native mussel hosts. *Biological Invasions*, 22, 3223-3237.

Gollasch, S., Kerckhof, F., Craeymeersch, J., Goulletquer, Jensen, K., Jelmert, A. & Minchin, D. (2015). Alien Species Alert: *Ensis directus*. Current status of invasions by the marine bivalve *Ensis directus*. *ICES Cooperative Research Report*. No. 323, 1-32.

Grosholz, E.D., Crafton, R.E., Fontana, R.E., Pasari, J.R., Williams, S.L. & Chabin, C.J. (2015). Aquaculture as a vector for marine invasions in California. *Biological Invasions*, 17, 1471-1484.

Hanley, T.C., White, J.W., Stallings, C.D. & Kimbro, D.L. (2019). Environmental gradients shape the combined effects of multiple parasites on oyster hosts in the northern Gulf of Mexico. *Marine Ecology Progress Series*, 612, 111-125.

Harvell, C.D., Mitchell, C.E., Ward, J.R., Altizer, S., Dobson, A.P., Ostfeld, R.S. & Samuel, M.D. (2002). Climate Warming and Disease Risks for Terrestrial and Marine Biota. *Science*, 296(5576), 2158-2162.

Haska, C.L., Yarish, C., Kraemer, G., Blaschik, N., Whitlatch, R., Zhang, H. & Lin, S. (2012). Bait worm packaging as a potential vector of invasive species. *Biological Invasions*, 14, 481-493.

Hellmann, J.J., Byers, J.E., Bierwagen, B.G. & Dukes, J.S. (2008). Five Potential Consequences of Climate Change for Invasive Species. *Conservation Biology*, 22, 534-543.

Holt, J., Wakelin, S., Lowe, J. & Tinker, J. (2010). The potential impacts of climate change on the hydrography of the northwest European continental shelf. *Progress in Oceanography*, 86, 361-379.

Hulme, P.E. (2009). Trade, Transport and Trouble: Managing Invasive Species Pathways in an Era of Globalization. *Journal of Applied Ecology*, 46, 10-18.

Hulme, P.E. (2015). Invasion pathways at a crossroad: policy and research challenges for managing alien species introductions. *Journal of Applied Ecology*, 52, 1418-1424.

ICES (2005). ICES Code of Practice on the Introductions and Transfers of Marine Organisms 2005. 1-30.

Katsanevakis, S., Tempera, F. & Teixeira, H. (2016). Mapping the impact of alien species on marine ecosystems: the Mediterranean Sea case study. *Diversity and Distributions*, 22, 694-707.

Katsanevakis, S., Tsirintanis, K., Tsaparis, D., Doukas, D., Sini, M., Athanassopoulou, F., Kolygas, M.N., Tontis, D., Koutsoubas, D. & Bakopoulos, V. (2019). The cryptogenic parasite *Haplosporidium pinnae* invades the Aegean Sea and causes the collapse of *Pinna nobilis* populations. *Aquatic Invasions*, 14, 150-164.

Kelly, D.W., Paterson, R.A., Townsend, C.R., Poulin, R. & Tompkins, D.M. (2009). Parasite Spillback: A Neglected Concept in Invasion Ecology? *Ecology*, 90, 2047-2056.

Kerr, R., Ward, G.M., Stentiford, G.D., Alfjorden, A., Mortensen, S., Bignell, J.P., Feist, S.W., Villalba, A., Carballal, M.J., Cao, A., Arzul, I., Ryder, D. & Bass, D. (2018). *Marteilia refringens* and *Marteilia pararefringens* sp. nov. are distinct parasites of bivalves and have different European distributions. *Parasitology*, 145, 1483-1492.

Krakau, M., Thieltges, D.W. & Reise, K. (2006). Native parasites adopt introduced bivalves of the North Sea. *Biological Invasions*, 8, 919-925.

Laeseke, P., Schiller, J., Letschert, J. & Doolittle Llanos, S. (2020). "Theories, Vectors, and Computer Models: Marine Invasion Science in the Anthropocene" in YOUMARES 9 - The Oceans: Our Research, Our Future, eds. S. Jungblut, V. Liebich and M. Bode-Dalby (Springer, Cham), 195-209.

Lafferty, K.D. & Kuris, A.M. (1999). How environmental stress affects the impacts of parasites. *Limnology and Oceanography*, 44, 925-931.

Lafferty, K.D. & Hofmann, E.E. (2016). Marine disease impacts, diagnosis, forecasting, management and policy. *Philosophical Transactions of the Royal Society B*, 371(1689), 20150200.

Lagrue, C. (2017). Impacts of crustacean invasions on parasite dynamics in aquatic ecosystems: A plea for parasite-focused studies. *International Journal for Parasitology: Parasites and Wildlife*, 6, 364-374.

Lattos, A., Giantsis, I.A., Karagiannis, D. & Michaelidis, B. (2020) First detection of the invasive Haplosporidian and Mycobacteria parasites hosting the endangered bivalve *Pinna nobilis* in Thermaikos Gulf, North Greece. *Marine Environmental Research*, 155, 104889.

Le Roux, F., Wegner, K.M., Baker-Austin, C., Vezzulli, L., Osorio, C.R., Amaro, C., Ritchie, J.M., Defoirdt, T., Destoumieux-Garzón, D., Blokesch, M., Mazel, D., Jacq, A., Cava, F., Gram, L., Wendling, C.C., Strauch, E., Kirschner, A. & Huehn, S. (2015). The emergence of *Vibrio* pathogens in Europe: ecology, evolution, and pathogenesis (Paris, 11–12th March 2015). *Frontiers in Microbiology*, doi: 10.3389/fmicb.2015.00830.

Lodge, D.M., Williams, S., MacIsaac, H.J., Hayes, K.R., Leung, B., Reichard, S., Mack, R.N., Moyle, P.B., Smith, M., Andow, D.A., Carlton, J.T. & McMichael, A. (2006). Biological Invasions: Recommendations for U.S. Policy and Management. *Ecological Applications*, 16, 2035-2054.

Lõhmus, M., & Björklund, M. (2015). Climate change: what will it do to fish – parasite interactions? *Biological Journal of the Linnean Society*, 116, 397-411.

Lucy, F.E., Davis, E., Anderson, R., Booy, O., Bradley, K., Britton, J.R., Byrne, C., Caffrey, J.M., Coughlan, N.E., Crane, K., Cuthbert, R.N., Dick, J.T.A., Fisher, J., Gallagher, C., Harrison, S., Jebb, M., Johnson, M., Lawton, C., Lyons, D., Mackie, T., Maggs, C., Marnell, F., McLoughlin, T., Minchin, D., Monahan, O., Montgomery, I., Moore, N., Morrison, L., Muir, R., Nelson, B., Niven, A., O'Flynn, C., Osborne, B., O'Riordan, R.M., Reid, N., Roy, H., Sheehan, R., Stewart, D., Sullivan, M., Tierney, P., Treacy, P., Tricario, E. & Trodd, W. (2020). Horizon scan of invasive alien species for the island of Ireland. *Management of Biological Invasions*, 11(2), 155-177.

Lynch, S.A., Armitage, D.V., Coughlan, J., Mulcahy, M.F. & Culloty, S.C. (2007). Investigating the possible role of benthic macroinvertebrates and zooplankton in the life cycle of the haplosporidian *Bonamia ostreae*. *Experimental Parasitology*, 115, 359-368.

Lynch, S.A., Coghlan, A., O'Leary, B., Morgan, E. & Culloty, S. (2020). Northward establishment of the mediterranean mussel *Mytilus galloprovincialis* limited by changing climate. *Biological Invasions*, 22, 2725-2736.

Macey, B.M., Achilihu, I.O., Burnett, K.G. & Burnett, L.E. (2008). Effects of Hypercapnic Hypoxia on Inactivation and Elimination of *Vibrio campbellii* in the Eastern Oyster, *Crassostrea virginica*. *Applied Environmental Microbiology*, 74, 6077-6084.

Mackenzie, C.L., Lynch, S.A., Culloty, S.C. & Malham, S.K. (2014). Future Oceanic Warming and Acidification Alter Immune Response and Disease Status in a Commercial Shellfish Species, *Mytilus edulis* L. *PLos ONE*, 9(6), e99712, 1-12.

MacLeod, C.D. & Poulin, R. (2015). Differential tolerances to ocean acidification by parasites that share the same host. *International Journal for Parasitology*, 45, 485-493.

Marcogliese, D.J. (2001). Implications of climate change for parasitism of animals in the aquatic environment. *Canadian Journal of Zoology*, 79, 1331-1352.

Marcogliese, D.J. (2008). The impact of climate change on the parasites and infectious diseases of aquatic animals. Revue Scientifique et Technique (International Office of Epizootics). 27(2), 467–484.

Maynard, J., van Hooidonk, R., Harvell, C.D., Eakin, C.M., Liu, G., Willis, B.L., Williams, G.J., Groner, M.L., Dobson, A., Heron, S.F., Glenn, R., Reardon, K. & Shields, J.D. (2016). Improving marine disease surveillance through sea temperature monitoring, outlooks and projections. *Philosophical Transactions of the Royal Society B*. 379(1689), 20150208.

McKindsey, C.W., Landry, T., O'Beirn, F.X. & Davies, I.M. (2007). Bivalve Aquaculture and Exotic Species: A Review of Ecological Considerations and Management Issues. *Journal of Shellfish Research*, 26, 281-294.

Mellin, C., Lurgi, M., Matthews, S., MacNeil, M.A., Caley, M.J., Bax, N., Przeslawski, R. & Fordham, D.A. (2016). Forecasting marine invasions under climate change: Biotic interactions and demographic processes matter. *Biological Conservation*, 204, 459-467.

Miller, A., Inglis, G.J. & Poulin, R. (2008). Use of the introduced bivalve, *Musculista senhousia*, by generalist parasites of native New Zealand bivalves. *New Zealand Journal of Marine and Freshwater Research*, 42, 143-151.

Minchin, D., Gollasch, S., Cohen, A.N., Hewitt, C.L. & Olenin, S. (2009). "Characterizing Vectors of Marine Invasion" in Biological Invasions in Marine Ecosystems. Ecological Studies (Analysis and Synthesis), eds. Gil Rilov and Jeffrey A. Crooks (Springer, Berlin, Heidelberg), Vol. 204, 109-116.

Morley, N.J. (2010). Interactive effects of infectious diseases and pollution in aquatic molluscs. *Aquatic Toxicology*, 96, 27-36.

Mortensen, S., Strand, Å., Bodvin, T., Alfjorden, A., Skår, C.K., Jelmert, A., Aspán, A., Sælemyr, L., Naustvoll, L.-J. & Albretsen, J. (2016). Summer mortalities and detection of ostreid herpesvirus microvariant in Pacific oyster *Crassostrea gigas* in Sweden and Norway. *Diseases of Aquatic Organisms*, 117, 171-176.

Muehlbauer, F., Fraser, D., Brenner, M., Van Nieuwenhove, K., Buck, B.H., Strand, O., Mazurié, J., Thorarinsdottir, G., Dolmer, P., O'Beirn, F., Sanchez-Mata, A., Flimlin, G. & Kamermans, P. (2014). Bivalve aquaculture transfers in Atlantic Europe. Part A: Transfer activities and legal framework. *Ocean & Coastal Management*, 89, 127-138.

Nawrot, R., Albano, P.G., Chattopadhyay, D. & Zuschin, M. (2017). Climate change and body size shift in Mediterranean bivalve assemblages: unexpected role of biological invasions. *Proceedings of the Royal Society B*, 284, 20170357.

Naylor, R.L., Williams, S.L. & Strong, D.R. (2001). Aquaculture – A Gateway for Exotic Species. *Science*, 294(5547), 1655-1656.

NOAA (2017). https://coastalscience.noaa.gov/project/mussel-watch-program-assessment-chesapeake-bay-charleston-harbor/. Accessed 10/01/2021.

O'Reilly, A.J., Laide, C., Maloy, A., Hutton, S., Bookelaar, B., O'Sullivan, K., Lynch, S.A. & Culloty, S.C. (2018). The role of the mussel *Mytilus* spp. in the transmission of ostreid herpesvirus-1 microVar. *Parasitology*, 145, 1095-1104.

Pagenkopp Lohan, K.M., Ruiz, G.M. & Torchin, M.E. (2020). "Invasions can drive marine disease dynamics" in Marine Disease Ecology, eds. Donald C. Behringer, Brian R. Silliman and Kevin D. Lafferty (Oxford University Press), 115-138.

Paillard, C. (2004). A short-review of brown ring disease, a vibriosis affecting clams, *Ruditapes philippinarum* and *Ruditapes decussatus*. Aquatic Living Resources, 17, 467-475.

Park, K-I., Paillard, C., le Chevalier, P. & Choi, K-S. (2006). Report on the occurrence of brown ring disease (BRD) in Manila clam, *Ruditapes philippinarum*, on the west coast of Korea. *Aquaculture*, 255, 610-613.

Peeler, E.J., Oidtmann, B.C., Midtlyng, P.J., Miossec, L. & Gozlan, R.E. (2011). Non-native aquatic animals introductions have driven disease emergence in Europe. *Biological Invasions*, 13, 1291-1303.

Pernet, F., Lupo, C., Bacher, C. & Whittington, R.J. (2016). Infectious diseases in oyster aquaculture require a new integrated approach. *Philosophical Transactions of the Royal Society B*, 371(1689), 20150213.

Pierce, M.L. & Ward, J.E. (2018). Microbial Ecology of the Bivalvia, with an Emphasis on the Family Ostreidae. *Journal of Shellfish Research*, 37, 793-806.

Pörtner, H.-O, Roberts, D.C., Masson-Delmotte, V., Zhai, P., Tignor, M., Poloczanska, E., Mintenbeck, K., Alegría, A., Nicolai, M., Okem, A., Petzold, J., Rama, B. & Weyer, N.M. (eds.) (2019). IPCC 2019: Summary for Policymakers. In: IPCC Special Report on the Ocean and Cryosphere in a Changing Climate. 1-36.

Poulin, R., Paterson, R.A., Townsend, C.R., Tompkins, D.M. & Kelly, D.W. (2011). Biological invasions and the dynamics of endemic diseases in freshwater ecosystems. *Freshwater Biology*, 56, 676-688.

Poulin, R. (2017). Invasion ecology meets parasitology: Advances and challenges. *International Journal for Parasitology: Parasites and Wildlife*, 6, 361-363.

Rech, S., Salmina, S., Borrell Pichs, Y.J. & García-Vazquez, E**.** (2018). Dispersal of alien invasive species on anthropogenic litter from European mariculture areas. *Marine Pollution Bulletin*, 131, 10-16.

Rowley, A.F., Cross, M.E., Culloty, S.C., Lynch, S.A., Mackenzie, C.L., Morgan, E., O'Riordan, R.M., Robins, P.E., Smith, A.L., Thrupp, T.J., Vogan, C.L., Wootton, E.C. & Malham, S.K. (2014). The potential impact of climate change on the infectious diseases of commercially important shellfish populations in the Irish Sea - A review. *ICES Journal of Marine Science*, 71, 741-759.

Ruesink, J.L., Lenihan, H.S., Trimble, A.C., Heiman, K.W., Micheli, F., Byers, J.E. & Kay, M.C. (2005). Introduction of Non-Native Oysters: Ecosystem Effects and Restoration Implications. *Annual Review of Ecology, Evolution, and Systematics*, 36, 643-689.

Schade, F.M., Raupach, M.J. & Wegner, K.M. (2016). Seasonal variation in parasite infection patterns of marine fish species from the Northern Wadden Sea in relation to interannual temperature fluctuations. *Journal of Sea Research*, 113, 73-84.

Shannon, C., Quinn, C.H., Dunn, A.M. & Stebbing, P.D. (2020). Coherence of marine alien species biosecurity legislation: A study of England and Wales. *Marine Pollution Bulletin*, 161, 111796.

Smolowitz, R. (2013). A Review of Current State of Knowledge Concerning *Perkinsus marinus* Effects on *Crassostrea virginica* (Gmelin) (the Eastern Oyster). *Veterinary Pathology*, 50, 404-411.

Solomieu, V.B., Renault, T. & Travers, M.-A. (2015). Mass mortality in bivalves and the intricate case of the Pacific oyster, *Crassostrea gigas*. *Journal of Invertebrate Pathology*, 131, 2-10.

Soon, T.K. & Zheng, H. (2019). Climate Change and Bivalve Mass Mortality in Temperate Regions. *Reviews of Environmental Contamination and Toxicology*, 251, 109-129.

Sorte, C.J.B., Ibáñez, I., Blumenthal, D.M., Molinari, N.A., Miller, L.P., Grosholz, E.D., Diez, J.M., D'Antonio, C.M., Olden, J.D., Jones, S.J. & Dukes, J.S. (2013). Poised to prosper? A cross-system comparison of climate change effects on native and non-native species performance. *Ecology Letters*, 16, 261-270.

Steeves, L.E., Filgueira, R., Guyondet, T., Chassé, J. & Comeau, L. (2018). Past, Present, and Future: Performance of Two Bivalve Species Under Changing Environmental Conditions. *Frontiers in Marine Science*, 5(184), 1-14.

Telfer, S. & Bowen, K. (2012). The effects of invasion on parasite dynamics and communities. *Functional Ecology*, 26, 1288-1299.

Thieltges, D.W., Reise, K., Prinze, K. & Jensen, K.T. (2009). Invaders interfere with native parasite–host interactions. *Biological Invasions*, 11, 1421-1429.

Thieltges, D.W., Engelsma, M.Y., Wendling, C.C. & Wegner, K.M. (2013). Parasites in the Wadden Sea food web. *Journal of Sea Research*, 82, 122-133.

Toews, S., Beverley-Burton, M. & Lawrimore, T. (1993). Helminth and protist parasites of zebra mussels, *Dreissena polymorpha* (Pallas, 1771), in the Great Lakes region of southwestern Ontario, with comments on associated bacteria. *Canadian Journal of Zoology*, 71, 1763-1766.

Torchin, M.E., Lafferty, K.D. & Kuris, A.M. (2001). Release from parasites as natural enemies: increased performance of a globally introduced marine crab. *Biological Invasions*. 3, 333-345.

Torchin, M.E., Lafferty, K.D. & Kuris, A.M. (2002). Parasites and marine invasions, *Parasitology*, 124, 137-151.

Vázquez, N., Frizzera, A. & Cremonte, F. (2020). Diseases and parasites of wild and cultivated mussels along the Patagonian coast of Argentina, southwest Atlantic Ocean. *Diseases of Aquatic Organisms*, 139, 139-152.

Vezzulli, L., Colwell, R.R. & Pruzzo, C. (2013). Ocean Warming and Spread of Pathogenic Vibrios in the Aquatic Environment. *Microbial Ecology*, 65, 817-825.

Vignon, M. & Sasal, P. (2010). Fish introduction and parasites in marine ecosystems: a need for information. *Environmental Biology of Fishes*, 87, 1-8.

Walther, G.R., Roques, A., Hulme, P.E., Sykes, M.T., Pyšek, P., Kühn, I., Zobel, M., Bacher, S., Botta-Dukát, Z., Bugmann, H., Czúcz, B., Dauber, J., Hickler, T., Jarosík, V., Kenis, M., Klotz, S., Minchin, D., Moora, M., Nentwig, W., Ott, J., Panov, V.E., Reineking, B., Robinet, C., Semenchenko, V., Solarz, W., Thuiller, W., Vilà, M., Vohland, K. & Settele, J. (2009). Alien species in a warmer world: risks and opportunities. *Trends in Ecology & Evolution*, 24, 686-693.

Watts, J.C., Carroll, J.M., Munroe, D.M. & Finelli, C.M. (2018). Examination of the potential relationship between boring sponges and pea crabs and their effects on eastern oyster condition. *Diseases of Aquatic Organisms*, 130, 25-36.

Wijsman, J.W.M., Troost, K., Fang, J. & Roncarati, A. (2019). "Global Production of Marine Bivalves. Trends and Challenges" in Goods and Services of Marine Bivalves, eds. A.C. Smaal, J.G. Ferreira, J. Grant, J.K. Petersen and Ø. Strand (Springer), 7-26.

Williams, S., Davidson, I.C., Pasari, J.R., Ashton, G.V., Carlton, J.V., Crafton, R.E., Fontana, R.E., Grosholz, E.D., Miller, A.W., Ruiz, G.M. & Zabin, C.J. (2013). Managing Multiple Vectors for Marine Invasions in an Increasingly Connected World. *BioScience*, 63, 952-966.

Xu, X., Huang, L., Su, Y. & Yan, Q. (2018). The complete genome sequence of *Vibrio aestuarianus* W-40 reveals virulence factor genes. *MicrobiologyOpen*, 7(3), 1-10.

Yanagida, T., Shirakashi, S., Iwaki, T., Ikushima, N. & Ogawa, K. (2009). Gymnophallid digenean *Parvatrema duboisi* uses Manila clam as the first and second intermediate host. *Parasitology International*, 58, 303-310.

Yang, C.-C., Yu, Y.-C., Valles, S.M., Oi, D.H., Chen, Y.-C., Shoemaker, D., Wu, W.-J. & Shih, C.-J. (2010). Loss of microbial (pathogen) infections associated with recent

invasions of the red imported fire ant *Solenopsis invicta*. *Biological Invasions*, 12, 3307-3318.

Zannella, C., Mosca, F., Mariani, F., Franci, G., Folliero, F., Galdiero, M., Tiscar, P.G. & Galdiero, M. (2017). Microbial Diseases of Bivalve Mollusks: Infections, Immunology and Antimicrobial Defense. *Marine Drugs*, 15(182), 1-36.

Chapter 3: The Role of Invasive Tunicates as Reservoirs of Molluscan Pathogens

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Abstract

Ascidian tunicates frequently display rapid expansion when introduced beyond their native range and are considered successful invaders. This invasive potential may be exacerbated by a warming climate, allowing for the occupation of environmental niches previously held by native species. Research into tunicate invasion ecology is prevalent, but less is known about their role in pathogen maintenance. This study investigated the impact of invasive tunicates on the maintenance of pathogens that affect commercial bivalves, including the cultured species *Ostrea edulis* (European flat oyster) and *Crassostrea gigas* (Pacific cupped oyster), and the fished species *Cerastoderma edule* (common cockle). Focal pathogens included ostreid herpesvirus OsHV-1 µVar, *Vibrio aestuarianus*, *Bonamia ostreae* and *Minchinia* spp. The range of pathogens in their natural molluscan hosts was determined and the tunicates *Botrylloides violaceus*, *Didemnum vexillum* and *Styela clava* were then screened for these same pathogens, using both field samples from oyster culture sites and marinas and a series of laboratory cohabitation trials. Sample sites reflected areas close to and further away from known pathogen sources. PCR, Sanger sequencing and histology confirmed the presence of *B. ostreae* and *Minchinia mercenariae*-like in *S. clava*, and *V. aestuarianus* was confirmed by qPCR in *B. violaceus* and *D. vexillum*. Furthermore, histology confirmed *Minchinia mercenariae*-like sporonts in *S. clava* suggesting that the tunicate can facilitate replication of this species. *S. clava* also maintained *B. ostreae* in tanks with no oysters present. The results indicate that tunicates can act as reservoirs of infection in areas where disease occurs, thus maintaining the disease within the environment, and potentially transport diseases to uninfected sites.

Keywords: Invasive, Tunicates, Oysters, Aquaculture, Pathogens

Introduction

The Subphylum Tunicata is a diverse group of invertebrates belonging to the Phylum Chordata, and members of this group are globally recognised as significant contributors to biofouling communities, particularly species within the Class Ascidiacea; the sea squirts (Rosa *et al*., 2013; Comeau *et al*., 2015; Zhan *et al*., 2015). A number of tunicate species, both colonial and solitary, have become high profile invasive species worldwide due to their significant impacts on the aquaculture sector, including costs associated with their control and removal when fouling occurs, and their ability to compete with commercial shellfish for food resources (Hillock and Costello, 2013; Casso *et al*., 2018).

Botrylloides violaceus (orange sheath tunicate) is a colonial tunicate native to the northwest Pacific but now established in temperate regions around the globe (Simkanin *et al*., 2013) and known for its invasive tendencies. This species can become competitively dominant over commercial species, thus impeding settlement (Cordell *et al*., 2012) and fouling cultured stock (Paetzold *et al*., 2012). *Didemnum vexillum* (carpet sea squirt) is another high-profile colonial tunicate that has steadily increased its global range from Japan (thought to be its native range) to New Zealand, North America and Europe. Similar to *B. violaceus*, *D. vexillum* can impact commercial aquaculture practices, for example heavy growth results in shellfish valve openings or siphons becoming obstructed (Ferguson *et al*., 2017).

Styela clava (leathery sea squirt/club tunicate) is a solitary ascidian native to the Northwest Pacific and its high tolerance of fluctuating environmental conditions has seen it become a successful invader, expanding its range from the Pacific coasts of Asia and Russia to extend throughout the northern and southern hemispheres (Goldstien *et al*., 2010). This tunicate attaches to hard substrates such as rocks and bivalve shells, and when natural substrates are unavailable it acts as a biofouling organism on artificial structures and aquaculture gear. Mature adults are hermaphroditic and oviparous, and densities can reach 500-1000 individuals/m^2 (Wong *et al*., 2011), as such the cleaning of culture gear has been known to make shellfish production more labour intensive (Bourque *et al*., 2007).

Interactions between invasive species, pathogens and native communities are complex, as invaders that have not lost their associated parasites along the invasion pathway may transmit novel parasites to native hosts (Dunn & Hatcher, 2015) or become infected by native pathogens in the invaded range. Furthermore, invasive species may also inhibit or promote the transmission of pathogens by native species (Goedknegt *et al*., 2016).

While many studies have focused on tunicates as model organisms for understanding invasion success, less is known about their role in the maintenance of parasites and pathogens and their subsequent interactions with commercial bivalves. Franchi & Ballarin (2017) noted that tunicate physiology results in natural defences against parasites and pathogens, as the tunic acts as a physical barrier and the vascularised oral siphon and pharynx have circulating haemocytes that trigger immune responses that can lead to inflammation and phagocytosis of foreign material. Despite these defences, tunicates have been shown to be susceptible to specific pathogens, for example *Styela clava* has been established as a potential carrier of the flagellated protozoan *Azumiobodo hoyamushi* - the causative agent of Soft Tunic Syndrome (Kumagai *et al*., 2014). This disease widely affects cultured tunicates such as the edible ascidian *Halocynthia roretzi* and has caused economic losses in the aquaculture sector in Korea and Japan.

Rosa *et al*. (2013) tested the ability of biofouling ascidians including *Styela clava* and *Botrylloides violaceus* to distribute potentially harmful algal cells to new geographic ranges by exposing them to cultured strains of algae capable of forming harmful algal blooms. Algal cells were found to pass through the ascidian digestive system and remain capable of re-establishing bloom populations. Rueckert *et al*. (2015) also detected a number of apicomplexan parasites within the genus *Lankesteria* infecting Pacific ascidians and Lynch *et al*. (2016) found four parasite groups including ciliates and trematodes in the European sea squirt *Ascidiella aspersa*.

Determining the ability of bacteria, protists and viruses to maintain themselves in the environment can inform how they contribute to disease cycling, particularly in commercially important bivalves. However, this can prove difficult due to free-living life stages, the potential ability to persist short-term outside a host and the use of alternative hosts. Haplosporidia belonging to the genus *Minchinia* have traditionally been detected

in a range of bivalves including clams, cockles and mussels (Ramilo *et al*., 2018). Other haplosporidian species, including *Bonamia ostreae* and *Haplosporidium nelsoni*, are well studied within their hosts but knowledge of their life-history, diversity and mechanisms of transmission is limited (Ward *et al*., 2019). *B. ostreae* is an intra-haemocytic parasite of the European flat oyster *Ostrea edulis* that induces physiological disorder and mortality. All oyster life-stages are susceptible, although individuals over two years are more adversely affected, and the pathogen is present throughout the year but infections appear to increase from autumn and peak in late winter/early spring. Laboratory experiments suggest that an intermediate host is not required, and the pathogen is capable of surviving in seawater for up to one week (OIE, 2019). It is also known that *B. ostreae* is capable of utilising alternative hosts, for example in a laboratory setting the brittle star *Ophiothrix fragilis* caused transmission of *B. ostreae* to naive *O. edulis* (Lynch *et al*., 2007).

The bacterium *Vibrio aestuarianus* is associated with mortality events in populations of the Pacific cupped oyster *Crassostrea gigas* in Europe, with adult oysters of marketable size primarily affected and mortalities mainly occurring in summer (Lupo *et al*., 2019). The ostreid herpesvirus OsHV-1 µVar is another pathogen associated with *C. gigas* mortality events, with larval, spat and juvenile life-stages the most susceptible. Transmission is primarily direct although the virus is also hypothesised to travel via vector particles in the water column. Mortalities occur more frequently in summer water temperatures although temperature effects differ between geographic locations, for example Europe and Australia (OIE, 2019). The virus has also been detected in other phyla, for example the shore crab *Carcinus maenas* can transmit OsHV-1 µVar from crabs exposed to the virus in the wild to naive crabs in a laboratory setting (Bookelaar *et al*., 2018).

Comprehensive information on the entire host range for pathogens remains difficult to achieve, and this can have implications for monitoring programmes, as these tend to focus on known susceptible hosts and may therefore miss other potential hosts. Accordingly, applied research of potential alternative host species could provide more robust

conclusions about the host range of pathogens and also potential carriers, reservoirs and vectors (Carnegie *et al*., 2016).

Climate change is predicted to increase introductions of problematic species, and affect the range, abundance and impacts of invasive species (Beaury *et al*., 2019). This study selected tunicates as a model taxon that is heavily represented by species with invasive tendencies, which may expand their range under shifting climate conditions. Three species of tunicate that are considered invasive outside of their native range, *Botrylloides violaceus*, *Didemnum vexillum* and *Styela clava*, were screened for parasites and pathogens that are known to affect commercial bivalve species. These pathogens included the herpesvirus OsHV-1 µVar, a virus that can cause mass mortalities in *C. gigas* (Prado-Alvarez *et al*., 2016), and *Vibrio aestuarianus*, a bacterium also linked to *C. gigas* mortality events (McCleary & Henshilwood, 2015). Screening was also conducted for the Phylum Haplosporidia, in particular *Bonamia ostreae*, the intra-haemocytic parasite that affects *O. edulis* and is the causative agent of bonamiosis (Lynch *et al*., 2010, Prado-Alvarez *et al*., 2015), and *Minchinia* spp. as the haplosporidia *Minchinia tapetis* and *Minchinia mercenariae*-like (as per Ramilo *et al*., 2018) have also been detected in common cockles (*Cerastoderma edule*) in Cork Harbour and are known to cause losses for the cockle sector (Albuixech-Martí *et al*., 2020). Screening was also conducted for a broader range of haplosporidian species, as previous research conducted in similar sample sites to this study has indicated the presence of *Haplosporidium nelsoni* in *C. gigas* and *O. edulis*, and a further *Haplosporidium* sp., most likely *Haplosporidium armoricanum*, in *O. edulis* (Lynch *et al*., 2013a).

The aim of this study was to focus on the potential impact of invasive tunicates on the maintenance of pathogens known to be problematic within the aquaculture and shellfishery sectors. This was investigated by determining the current status of pathogens known to cause significant mortalities in *Ostrea edulis* and *Crassostrea gigas* sampled from aquaculture sites in Ireland, and also by reviewing the pathogens currently affecting the fished species *Cerastoderma edule*; then establishing if these same pathogens are present in invasive tunicates cohabiting with the oysters at the aquaculture sites. The study also aimed to determine whether pathogens can be detected in invasive tunicates sampled

from an area with no aquaculture but potentially other sources of introduction such as heavy recreational shipping traffic. The final aim was to establish the potential role of tunicates as reservoirs of infection using laboratory cohabitation trials.

Methods

Study Sites

Sampling for tunicates and oysters was conducted under licence from Ireland's National Parks and Wildlife Service and the Marine Institute. Sampling in 2018 was conducted in summer (July) and late autumn/winter (October-November) to ensure that pathogen screening encompassed both cold winter and elevated summer water temperatures. Sampling was conducted in two locations within Cork Harbour and sea temperatures were sourced from Copernicus (Figure 1 and Table 1). The first sample site was an oyster culture site in Rossmore, Co. Cork where both the oysters *Ostrea edulis* (n = 60) and *Crassostrea gigas* (n = 60) and tunicate *Styela clava* (n = 90) were collected.

Sampling of the colonial tunicate *Botrylloides violaceus* in 2018 was conducted in the second location in Co. Cork, a marina in Crosshaven, to ascertain whether growth in an area with heavy recreational shipping would result in the presence of pathogens due to incidental transport.

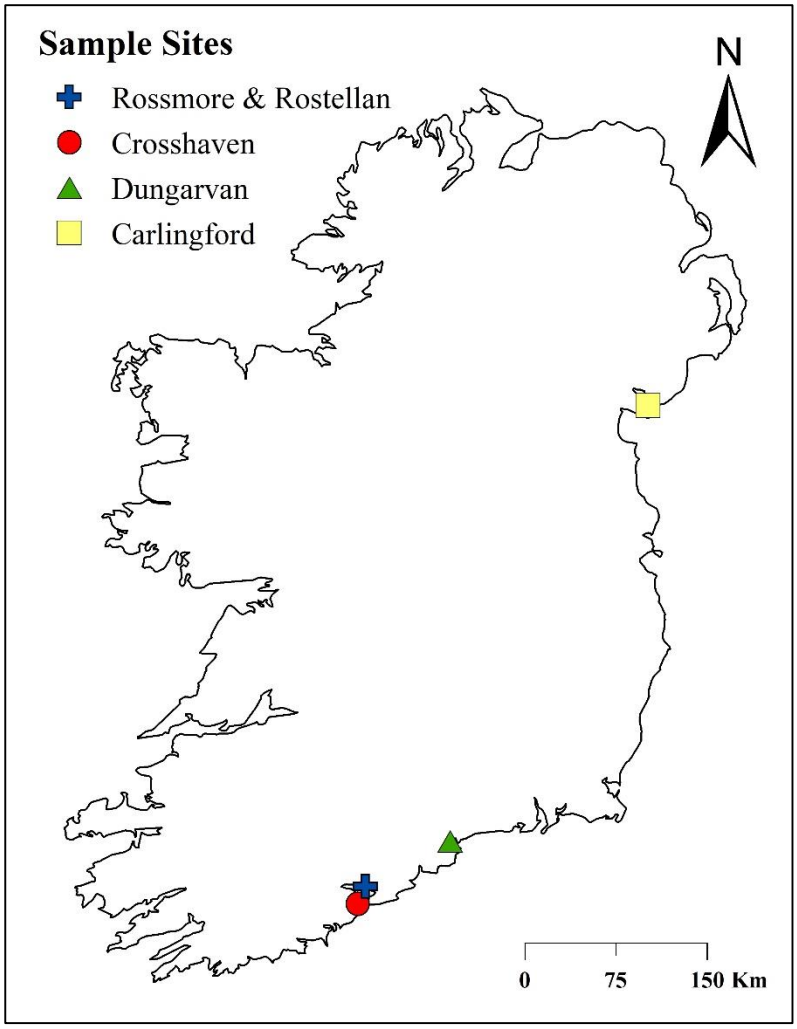

Figure 1: Map of sample sites for 2018 and 2019 samples.

Summary of Pathogen Screening

The oysters, as natural hosts of the pathogens of interest, were screened to determine presence/absence of the pathogens for which they are the primary host and prevalence if present, before screening the tunicates to determine if proximity to the oysters resulted in a positive detection. In cases where the number of tissue samples is higher than the number of individuals (Table 1) it is because multiple tissue types were collected per individual (see *Sample Processing – 2018 and 2019 Samples* for a detailed description).

Ostrea edulis was screened using specific primers for *Bonamia ostreae* and *Haplosporidium nelsoni* (causative agent of multinucleate sphere X disease (MSX)). General haplosporidian primers were then also used to screen *O. edulis* and ensure that

any further haplosporidian species (e.g. *Haplosporidium* spp., *Minchinia* spp.) were detected if present. *Crassostrea gigas* was screened for *Vibrio aestuarianus* and OsHV-1 µVar, as these are both pathogenic to *C. gigas* but not *O. edulis*. *C. gigas* were also screened with general primers for haplosporidian spp., to ensure that any pathogens within this phylum were detected. *Styela clava* was then screened for *B. ostreae*, OsHV-1 µVar, general haplosporidian spp. and *V. aestuarianus*. *Botrylloides violaceus* was screened for *V. aestuarianus*, OsHV-1 µVar and haplosporidian spp. using general primers (Table 2) (see *Molecular Screening* for a detailed description and Table 4 for details of all primers used).

Table 1: Summary of oyster and tunicate samples collected from Cork Harbour in 2018.

Species	Site	Date Collected	Avg. Monthly Sea Temperature (°C)	Individuals	n Tissue Samples*
Cork Harbour					
Crassostrea gigas	Rossmore	11-07-2018	16.47	30	30
Crassostrea gigas	Rossmore	2-11-2018	11.93	30	30
Ostrea edulis	Rossmore	11-07-2018	16.47	30	30
Ostrea edulis	Rossmore	23-11-2018	11.93	30	60
Styela clava (Intertidal)	Rossmore	4-07-2018	16.47	30	76
Styela clava (Subtidal)	Rossmore	11-07-2018	16.47	30	144
Styela clava (Subtidal)	Rossmore	2-11-2018	11.93	30	162
Crosshaven Marina					
Botrylloides violaceus	Crosshaven	16-07-2018	16.57	30**	90
Botrylloides violaceus	Crosshaven	25-10-2018	14.38	10**	30

* See '*Sample Processing – 2018 and 2019 Samples*' for detailed explanation of column 'n Tissue Samples'

***Botrylloides violaceus* is a colonial organism, therefore 'individuals' refers to the different lobes of three large colonies that extended along the underside of marina pontoons

Table 2: Summary of the pathogens screened in each oyster and tunicate species in 2018.

Species	***Crassostrea gigas*** **n = 60**	***Ostrea edulis*** **n = 60**	***Botrylloides violaceus*** **n = 40***	***Styela clava*** **n = 90**
Bonamia ostreae	X	✓	X	✓
Haplosporidium nelsoni	X	✓	X	X
Haplosporidian spp.	✓	✓	✓	✓
OsHV-1 µVar	✓	X	✓	✓
Vibrio aestuarianus	✓	X	✓	✓

**Botrylloides violaceus* is a colonial organism, therefore 'individuals' refers to the different lobes of three large colonies that extended along the underside of marina pontoons

Laboratory Trials:2019

Samples for the subsequent laboratory cohabitation trials in 2019 were obtained from both Co. Cork (Rossmore & Rostellan), Dungarvan, Co. Waterford and Carlingford, Co. Louth (Figure 1 and Table 3). All sampling locations were oyster aquaculture sites and tunicate species were collected from the trestles.

Trial 1: Cohabitation trial between *Bonamia ostreae* infected *Ostrea edulis* and *Styela clava* to determine if *S. clava* is a reservoir or carrier of infection

150 *Ostrea edulis* were randomly sampled from Rossmore, Cork Harbour and of these an initial sample (n = 30) was screened to assess the prevalence of *Bonamia ostreae*. The remaining oysters were divided into three experimental 50L tanks, each containing 40 oysters, and held in a constant temperature room at 14°C, a salinity of 33-35 and a 12:12 hour light/dark regime.

Ostrea edulis were held for 75 days to allow the intensity and prevalence of infection with *Bonamia ostreae* to increase. After 75 days, 32 *Styela clava* of all sizes were randomly sampled from Rostellan, Cork Harbour. Tunicates growing on algae rather than directly on the oyster trestles were collected, and a piece of the algal substrate was taken too, as this ensured the tunicates were not damaged in the collection process and could survive the transfer to the tanks. An initial sample of the tunicates (n = 8) was screened for *B. ostreae* and the remaining 24 placed in three 50L tanks, with eight tunicates per tank; this resulted in one control tank with eight *S. clava* only, and two experimental tanks each containing the *B. ostreae*-infected oysters and tunicates (8 *S. clava* and 35 *O. edulis* X 2 tanks). The final control tank contained 35 *O. edulis* only. The trial ran for 60 days and had seven oyster mortalities and no tunicate mortalities.

As both *Minchinia tapetis* and *Minchinia mercenariae*-like have been recorded in *Cerastoderma edule* from Cork Harbour (Albuixech-Martí *et al*., 2020), a subsample of eight *Styela clava* was also screened for both *Minchinia tapetis* and *Minchinia mercenariae*-like at the end of the trial, using PCR with primers specific to each species, histology as per Ford *et al*. (2009) and Sanger sequencing. This subsample encompassed

two tunicates from the initial baseline screening, three from the oyster cohabitation tanks and three from the control tank with tunicates only.

Trial 2: Cohabitation trial between *Vibrio aestuarianus* infected *Crassostrea gigas* and *Didemnum vexillum*

150 *Crassostrea gigas* were randomly sampled from Dungarvan, Waterford (selected because OsHV-1 µVar and *Vibrio aestuarianus* are both present at this location (Bookelaar, 2018)) and of these an initial sample (n = 30) was screened to assess the prevalence of *V. aestuarianus*. The remaining oysters were divided into three experimental 50L tanks, each containing 40 oysters, and held in a constant temperature room at 16°C and a salinity of 33-35. After 34 days there were 42 oyster mortalities and a further 10 were removed and screened for *V. aestuarianus*. The remaining oysters (n = 68) were subsequently divided into two tanks, 34 per tank.

28 colonies of *Didemnum vexillum*/colonial spp. were collected from Carlingford, Co. Louth. The target species was *D. vexillum* as this is a prolific global invader, and ID guides were used to identify this species in the field, however given the morphological similarities between colonial tunicate species, and the heterogeneous nature of colonies fouling the oyster trestles, other species were also collected and included in the trials. A tissue sample from all 28 colonies were sequenced at the end of the trial using the primer pair F16–R497 (Price *et al.*, 2005) to amplify the 18s RNA gene and confirm the community composition. An initial sample (n = 7 colonies) was removed to assess the baseline prevalence of *V. aestuarianus*. The remaining 21 colonies were placed in three 50L tanks with seven colonies per tank; this resulted in one control tank with seven *D. vexillum*/colonial tunicate spp. colonies only, and two experimental tanks each containing the *V. aestuarianus*-infected oysters and tunicates (7 *D. vexillum*/colonial tunicate spp. colonies and 34 *C. gigas* X 2 tanks). The trial ran for 11 days.

In all laboratory experiments tanks were checked twice daily and any moribund or dead oysters and tunicates removed and processed. Tanks were constantly aerated using airlines with airstones and oysters and tunicates were fed standard marine invertebrate food 3-4 times per week.

Table 3: Summary of samples for laboratory trials 2019.

Species	Site	Date Collected	Avg. Monthly Sea Temperature (°C)	n individuals	n tissue samples*
Ostrea edulis	Rossmore	5-04-2019	10.08	150	300
Styela clava (Intertidal)	Rostellan	18-06-2019	12.07	32	248
Crassostrea gigas	Dungarvan	4-07-2019	15.37	150	150
Didemnum vexillum	Carlingford	6-08-2019	14.72	28**	140

* See '*Sample Processing – 2018 and 2019 Samples*' for detailed explanation of column 'n Tissue Samples'

***Didemnum vexillum* is a colonial organism with an amorphous shape, therefore 'individuals' refers to different (and often heterogeneous) colonies that extended along the underside of oyster trestles

Sample Processing – 2018 and 2019 Samples

Oyster species were processed by carefully opening the shell without disrupting the tissue within and draining the excess cavity fluid and seawater onto clean tissue. In *Ostrea edulis*, heart and gill smears were prepared as per *Bonamia ostreae* screening (Flannery *et al*., 2014). Smears were screened for *B. ostreae* using a Leica DM500 microscope at the 40x lens. Each slide was observed for five minutes and the results categorised using the following staging criteria; Class 0: no cells visible after five minutes, Class 1: 1 – 10 cells, Class 2: 11-100 cells and Class 3: heavy infection throughout the slide. Gill smears were used as a second reference on occasions where cells were difficult to distinguish within the heart smears.

The *Ostrea edulis* heart and piece of gill tissue were then used for DNA extraction (after smearing) using 10% Chelex ® 100 resin and subsequent PCR analysis. In *Crassostrea gigas* a piece of gill tissue only was removed for DNA extraction. Oyster sampling in 2018 consisted of 60 *O. edulis* individuals with 90 tissue samples screened (gill only in July 2018, heart and gill for all future screening), and 60 *C. gigas* individuals with 60 tissue samples screened (Table 1). Oyster sampling in 2019 trials consisted of 150 *O. edulis* individuals with 300 tissue samples screened, and 150 *C. gigas* individuals with 150 tissue samples screened (Table 3). In both oyster species a transverse section

consisting of gill, gonad, digestive tract and mantle was then preserved in Davidson's fixative as per the OIE (2019) and transferred to 70% ethanol for long-term storage.

Solitary tunicate samples (*Styela clava*) were prepared for molecular work by measuring the length, wet weight and drained weight then removing the tunic and dissecting the soft tissue. Tunicates ranged from 2.7 cm to 11.8 cm in length, therefore the number of tissue samples was scaled to the size of the individual (minimum 2 tissue samples and maximum 12) and taken from base to oral siphon to ensure all tissue types were preserved. DNA extraction was performed in the same manner as the oyster tissue. *S. clava* sampling in 2018 consisted of 90 individuals with 382 tissue samples screened (Table 1). *S. clava* sampling in 2019 trials consisted of 32 individuals with 248 tissue samples screened (Table 3). Samples also had a histological section consisting of one piece from base to oral siphon fixed in Davidson's solution and prepared as per Flannery *et al*. (2014), and samples from 2019 also had a sample (again base to oral siphon) prepared for *in situ* hybridisation (ISH) as per Lynch *et al*. (2008). Images were captured on a Leica DM500 microscope using LAS 4.12.0 software.

Colonial samples were also preserved for molecular work and long-term storage for histology. In the case of *Botrylloides violaceus* each small lobe had 3 tissue sections removed for DNA extraction (120 tissue samples in total) and 1 section per lobe fixed in Davidson's solution (40 in total). In the case of *Didemnum vexillum* each colony had 5 tissue sections removed for DNA extraction (140 tissue samples in total) and 5 sections fixed in Davidson's solution (140 in total).

Molecular Screening

Prior to molecular screening the quality and concentration of DNA was tested using a NanoDrop™ 1000 Spectrophotometer. Conventional PCR assays were initially conducted on oysters, for pathogens of which they are the natural hosts (see Table 2). If positive results were obtained from the oysters, the tunicates were then screened for those pathogens. Screening for *Bonamia ostreae* consisted of the BO-BOAS primer pair to amplify the small subunit (SSU) ribosomal DNA gene (Cochennec *et al*., 2000), and amplifications were carried out in a total volume of 50 µl, including 1 µl DNA, 25.75 µl ddH_20, 10 µl 5x Green GoTaq® Reaction Buffer (Promega),10 µl dNTPs (1.25 mM stock

solution), 2 µl $MgCl_2$ (25mM), 0.25 µl GoTaq® DNA Polymerase (Promega) and 0.5 µl each of forward and reverse primers. Reactions were carried out on a thermocycler at 94°C for five minutes (one cycle), then one minute each at 94°C, 55°C and 72°C for 35 cycles and finally 72°C for ten minutes.

Haplosporidian primer pairs consisted of MSXA-MSXB (to amplify the SSU rRNA gene) for *Haplosporidium nelsoni*, and the HapF1-HapR3 pair (SSU rDNA) for generic haplosporidian species. Haplosporidian amplifications were carried out in a total volume of 20 µl, including 1 µl DNA, 10 µl ddH_20, 4 µl 5x Green GoTaq® Reaction Buffer (Promega), 4 µl dNTPs (1.25 mM stock solution), 0.2 µl GoTaq® DNA Polymerase (Promega) and 0.4 µl of forward and reverse primers. Reactions were carried out on a thermocycler at 95°C for three minutes, then 95°C (30 seconds), 59°C(MSXA-MSXB)/48°C(HapF1-HapR3) (30 seconds) and 72°C (30 seconds HapF1-HapR3/one minute MSXA-MSXB) for 35 cycles and finally 72°C for five minutes.

The primer pair OHVA-OHVB, amplifying the C region of the ORF4 gene, was used to detect the presence of OsHV-1 µVar as per Lynch *et al*. (2013b), with the addition of 1.5 µl dMSO to the mix. The primer pair DnaJf420-DnaJr456 along with the probe DnaJp441 were used to detect a 58bp fragment of the *Vibrio aestuarianus* DnaJ gene using qPCR with a total volume of 25 µl, including 5 µl DNA, 5.62 µl ddH_20, 12.5 µl TaqMan®, 0.38 µl probe and 0.75 µl each of forward and reverse primers. The qPCR thermal profile was as per McCleary & Henshilwood (2015).

The primer pairs TAP-For/Rev and MER-For/Rev (both amplifying the SSU rDNA gene) were used for *Minchinia tapetis* and *Minchinia mercenariae*-like respectively (on a subset of *Styela clava* from 2019) as per Albuixech-Martí *et al*. (2020). PCR negative controls consisted of double distilled H_2O (ddH_2O) and two positive controls for each screen. Positive controls for *V. aestuarianus* were obtained from the Marine Institute in the form of a stock solution and dilutions had Ct (cycle threshold) values ranging from 27-34, a mean Ct below 37 was considered positive. In the case of *B. ostreae*, positive controls were not used in the initial PCR on *Ostrea edulis* samples but DNA that was isolated in those samples was used as a positive control in all subsequent screenings. Results were

visualised using gel electrophoresis on a 2% agarose gel. All primer pairs are listed in Table 4 and on occasions where Sanger sequencing was utilised, samples were sent to Source BioScience, Co. Waterford.

Table 4: List of forward and reverse primers used for PCR/qPCR in this study.

Primer	Sequence 5'-3'	Amplicon
BO-BOAS (Cochennec *et al*., 2000)	CATTTAATTGGTCGGGCCGC CTGATCGTCTTCGATCCCCC	300 bp
HapF1-HapR3 (Renault *et al.*, 2000)	GTTCTTTCWTGATTCTATGMA AKRHRTTCCTWGTTCAAGAYGA	190 bp
OHVA-OHVB (Lynch *et al*., 2013b)	TGCTGGCTGATGTGATGGCTTTGG GGATATGGAGCTGCGGCGCT	385 bp
MSXA-MSXB (Renault *et al*., 2000)	CGACTTTGGCATTAGGTTTCAGACC ATGTGTTGGTGACGCTAACCG	573 bp
TAP-For/Rev (Albuixech-Martí *et al*., 2020)	ATCTAACTAGCTGTCGCTAACTCGT CTTTCAAGATTACCCGGCTCTGC	165 bp
MER-For/Rev (Albuixech-Martí *et al*., 2020)	ATCTAACTAGCTGTCACTATGGAAAA ACGCACATTAAAGATTGCCCAGCTCTTT	170 bp
DnaJp441 (probe) DnaJf420-DnaJr456 (McCleary & Henshilwood, 2015)	FAM-AGG GCA CGT CGG C-MGB GTGAAGGGACGGGTGCTAAG CCATGACAAGTGCCACAAGTCT	N/A

Results

2018 Field Samples (Cork Harbour)

Table 5 outlines the screening for pathogens in the two oyster species over the summer and winter samples. In Cork Harbour *Crassostrea gigas* samples were negative for OsHV-1 µVar in both July 2018 and November 2018, but positive for *Vibrio aestuarianus*, with a higher prevalence in July (10%) than November (3.3%). Haplosporidian spp. were detected in *C. gigas* samples using general haplosporidian primers, with 3.3% and 10%

prevalence of infection for July and November respectively. Subsequently, one sample from July and three samples from November were sequenced but were unsuccessful in identifying the haplosporidian species present in *C. gigas*. Pacific oysters were not screened for *Bonamia ostreae* as they are not the primary host for the pathogen.

26.6% of *Ostrea edulis* from the November samples were positive for haplosporidian spp. using general primers (HapF1-HapR3), and Sanger sequencing of one sample (after purification using a Qiagen QIAquick® Gel Extraction Kit) identified it as *Bonamia ostreae* (100% BLASTn). *Bonamia ostreae*-specific primers then detected this pathogen in *O. edulis* samples from both months, with a higher prevalence in November (46%) compared to July (3.3%). *O. edulis* samples were all negative for *Haplosporidium nelsoni* (MSX). Flat oysters were not screened for OsHV-1 µVar or *Vibrio aestuarianus*.

Microscopic examination of *Ostrea edulis* heart smears determined that 56.6% of oysters had no visible *Bonamia ostreae* cells, with 70% infections Class 0 in July and 43.3% Class 0 in November. For oysters with a visible infection, Class 1 infections were the most prevalent, again in both months. A single Class 3 infection was detected in November (Figure 2).

Table 5: PCR (Haplosporidian spp., *H. nelsoni*, *Bonamia ostreae* and OsHV-1 µVar), qPCR (*Vibrio aestuarianus*) and *B. ostreae*/*Ostrea edulis* heart smear results for the detection of pathogens in *Crassostrea gigas* (n = 60) and *O. edulis* (n = 60). (% = percentage of positive samples, NS = Not Screened and NA = Not Applicable).

Species	**Date**	**Generic Haplosporidian spp. PCR (%)**	***B. ostreae* PCR (%)**	**Heart Smears (%)**	***H. nelsoni* (MSX) PCR (%)**	**OsHV-1 µVar PCR (%)**	***V. aestuarianus* qPCR (%)**
C. gigas	11-07-2018	3.3	NS	NA	NS	0	10.0
C. gigas	2-11-2018	10.0	NS	NA	NS	0	3.3
O. edulis	11-07-2018	0	3.3	30.0	0	NS	NS
O. edulis	23-11-2018	26.6	46.6	56.6	0	NS	NS

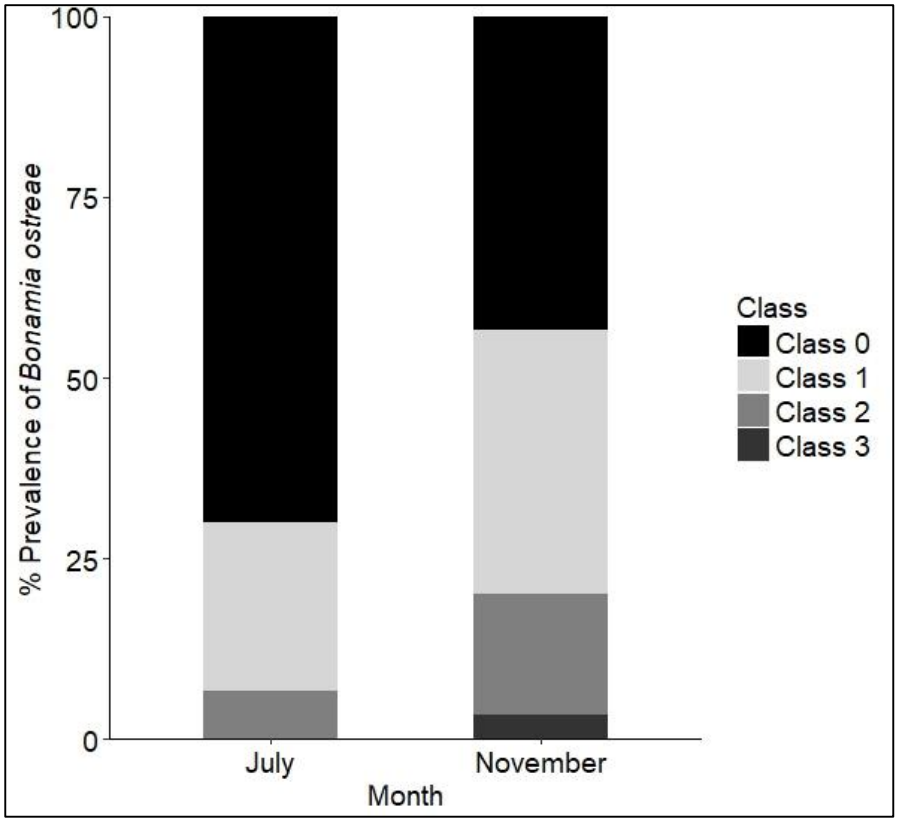

Figure 2: Different classes of *Bonamia ostreae* infection in *Ostrea edulis* heart smears with a sample size of 30 in each month (see text for each class category).

Styela clava were screened from Cork Harbour and were negative for both haplosporidian spp. and OsHV-1 µVar (Table 6). Samples were not screened for *H. nelsoni* (MSX) as it had not been detected in the oyster samples. *Vibrio aestuarianus* was detected in *S. clava* in the July samples (6.6%, lowest cycle threshold 35.5). *Bonamia ostreae* was also detected in *S. clava* in July (5%) and November (10%), and four samples were sequenced with one confirmed as *B. ostreae* (90.6% match, BLASTn). This study also calculated the wet weight and drained weight of *S. clava* to ascertain the potential for tunicates to transport water containing *B. ostreae* cells, with 27% of tunicate biomass from 62 samples (from field trials and subsequent laboratory cohabitation trials) comprised of water contained within the tunic.

Botrylloides violaceus sampled from the marina in Crosshaven were negative for haplosporidian spp. and OsHV-1 µVar. However, *Vibrio aestuarianus* was detected in *B.*

violaceus samples from July (33.3%, lowest Ct 35.5). *B. violaceus* samples were not screened for *H. nelsoni* (MSX).

Table 6: Molecular results for the detection of pathogens in *Botrylloides violaceus* (n = 40) and *Styela clava* (n = 90). (% = percentage of positive samples, NS = Not Screened).

Species	**Date**	**Haplosporidian spp. PCR (%)**	***Bonamia ostreae* PCR (%)**	***H. nelsoni* (MSX) PCR (%)**	**OsHV-1 µVar PCR (%)**	***Vibrio aestuarianus* qPCR(%)**
B. violaceus	16-07-2018	0	NS	NS	0	33.3
B. violaceus	25-10-2018	0	NS	NS	0	0
S. clava	4-07-2018	0	6.6	NS	0	0
S. clava	11-07-2018	0	3.3	NS	0	6.6
S. clava	2-11-2018	0	10	NS	0	0

2019 Laboratory Cohabitation Trials

Trial 1: Cohabitation trial between *Bonamia ostreae* infected *Ostrea edulis* and *Styela clava*

Of the 150 *Ostrea edulis* collected on 5th April 2019, 30 were instantly removed and screened for *Bonamia ostreae* using PCR, with the prevalence of infection recorded at 13.3%. Oysters were then maintained in the laboratory for 75 days to allow a higher prevalence and intensity of infection to develop before cohabitation trials began. 15 live and moribund animals were screened before the addition of tunicates. *B. ostreae* prevalence was 100%, and 46.6% of these were animals with Class 3 infections as evidenced with the heart smears. This 100% infection was considered the baseline oyster infection when adding *S. clava*. The prevalence of *B. ostreae* in *O. edulis* decreased from the start to the end of the trial, from 100% to 84.3%, and the prevalence of infection in the control *O. edulis* tank (oysters only) was 60% (Figure 3).

Styela clava screened at the start of the study showed a 25% prevalence of *Bonamia ostreae* and this was considered the baseline tunicate infection. At the end of the trial, after cohabiting with infected oysters for two months, this prevalence was 18.75%. The

prevalence of infection in the *Styela clava* in the control tank, i.e. tunicates only, was 37.5%.

All *S. clava* screened for *Minchinia* spp. (samples that were positive for *Bonamia ostreae*, n = 8) had 100% prevalence of infection of *M. mercenariae*-like using PCR, with six samples sequenced for confirmation (100% BLASTn: *Minchinia* sp. ex *Cerastoderma edule* clone 3 small subunit ribosomal RNA gene, partial sequence; 96.34% BLASTn: *Minchinia mercenariae* small subunit ribosomal RNA gene, partial sequence). These positive samples encompassed individual tunicates from the trial baseline sample, experimental tanks and control tank, and these same samples were all negative for *M. tapetis*. Histology also revealed sporonts and spore-like structures belonging to *Minchinia mercenariae*-like (Figure 4).

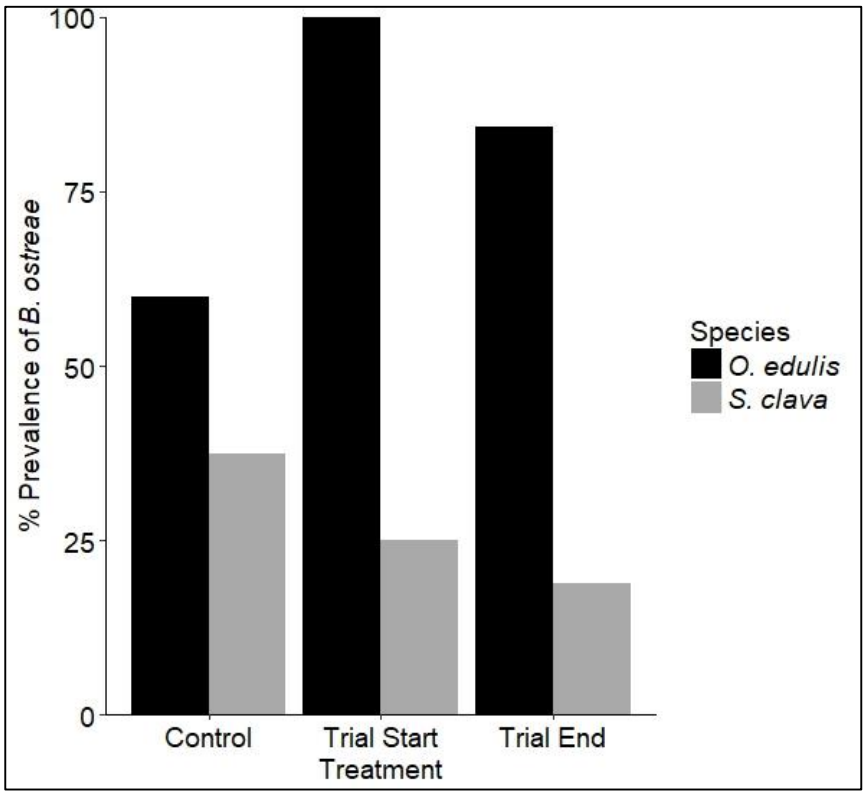

Figure 3: % Prevalence of *Bonamia ostreae* in *Ostrea edulis* and *Styela clava* over a 60-day trial, screened using PCR (Control screened at end of cohabitation trial).

Haplosporidian cells were visualised in a subsample of *Styela clava* (n = 5) from the trials using *in situ* hybridisation and histology. *Bonamia ostreae*-specific *in situ* hybridisation revealed cells that fit the profile of *B. ostreae*, but there was also the potential for them to be considered aspecific 'loose' content due to deterioration of the tunicate tissue during the hybridisation process. However histological examination of the coinfected tunicate samples revealed uninucleate haplosporidian cells consistent with either *B. ostreae* or *Minchinia mercenariae*-like in addition to the sporonts and spore-like structures belonging to the *M. mercenariae*-like species (Figure 4).

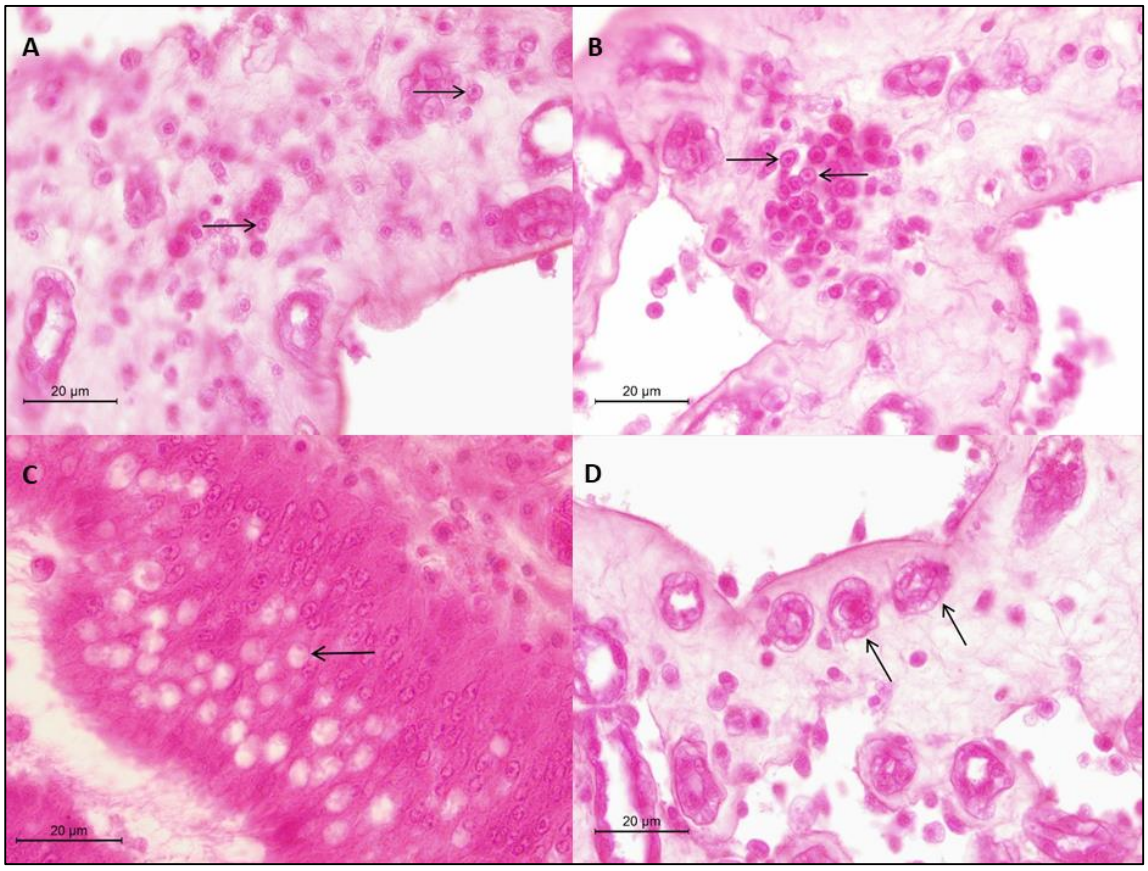

Figure 4: (A) and (B) Uninucleate haplosporidian 'fried egg' cells consistent with *Bonamia ostreae* or *Minchinia mercenariae*-like in the connective tissue of *Styela clava* (C) Vacuoles in *Styela clava* epithelial tissue arising from *Minchinia mercenariae*-like spore-like stage with polar nuclei visible (D) *Minchinia mercenariae*-like sporonts in *Styela clava* connective tissue.

Trial 2: Cohabitation trial between *Vibrio aestuarianus* infected *Crassostrea gigas* and *Didemnum vexillum*

Of the 150 *Crassostrea gigas* collected on 4th July 2019, 30 were instantly removed and screened for *Vibrio aestuarianus* using qPCR, with the prevalence of infection recorded at 93.3%. In the 32 days prior to the addition of the tunicates there were 42 disease-induced oyster mortalities, all of which were screened and had 100% *V. aestuarianus* infection with high levels of *V. aestuarianus* in each oyster. Tunicates were added to tanks on 6th August and prior to addition of tunicates a further ten oysters were screened, with 50% oysters infected with *V. aestuarianus*. This 50% was considered the baseline infection of oysters at the start of the cohabitation trial. Due to the limited number of oysters as a result of the early high mortality rate there was no tank of oysters only as a control. There were no further oyster mortalities while the cohabitation trial was running and at the end of the trial all remaining oysters were screened (n = 68), with the prevalence of V. *aestuarianus* just 0.03%.

The total prevalence of infection for the baseline tunicate sample (7 heterogeneous colonies) was 71.4% and the total prevalence of infection in the experimental sample (14 heterogeneous colonies) was 50%. No *Vibrio aestuarianus* was detected in the control tunicates (7 heterogeneous colonies) that were held alone in a tank without any oysters being present. Given the heterogeneous nature of the tunicate colonies in Carlingford, and the morphological similarities between species, a sample from each of the 28 colonies was sequenced at the end of the trial to determine the full species composition of the 28 colonies. Sanger sequencing confirmed 11 *Didemnum vexillum* (invasive) colonies and 9 *Aplidium glabrum* (native) colonies. There was also one sample each of the tunicate genera *Botryllus/Botrylloides* sp., *Styela* sp. and *Ascidiella* sp. The remaining five sequences failed but were visually identified (by comparing morphology to other sequenced individuals) as *D. vexillum* (x4) and *A. glabrum* (x1). Infection was higher in *D. vexillum* than *A. glabrum*/other at both the baseline sample (100% in *D. vexillum*, 33.3% in *A. glabrum*/other species) and at the end of the cohabitation trial with mortalities included (55.5% in *D. vexillum*, 40% in *A. glabrum*/other). In the control tank with no

oysters present there was no infection detected in any tunicate species either from mortalities or at completion of the trial (Figure 5).

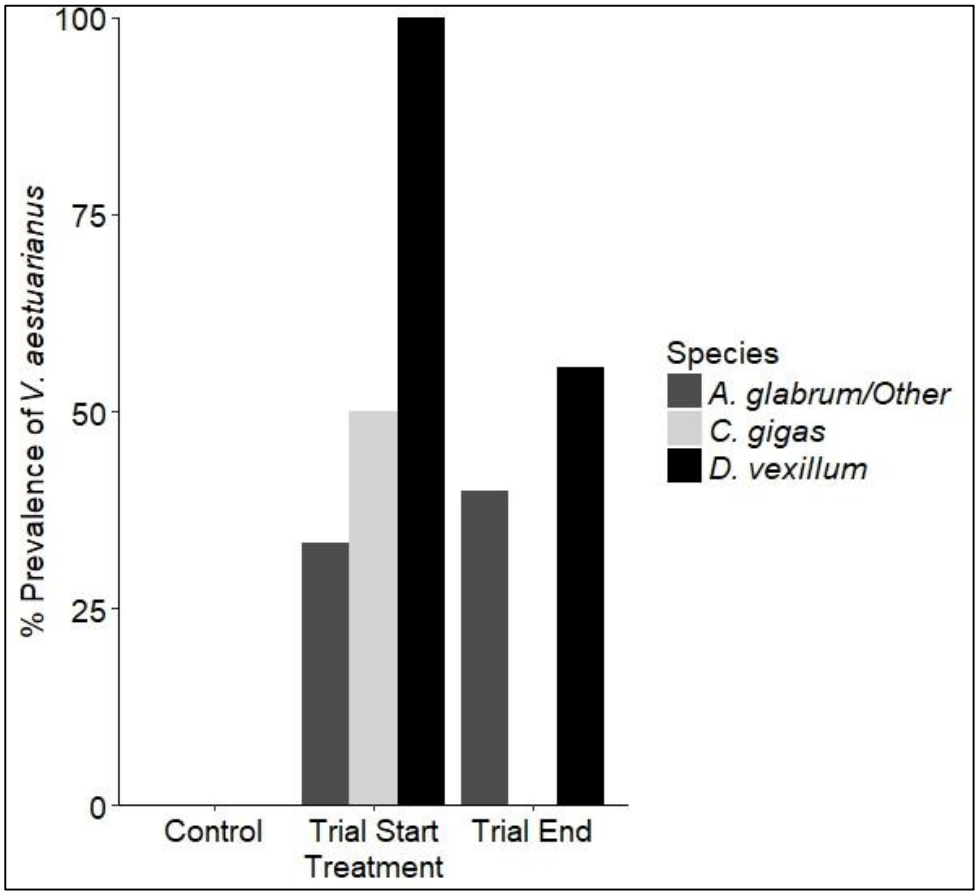

Figure 5: % Prevalence of *Vibrio aestuarianus* in *Crassostrea gigas*, *Didemnum vexillum* and *Aplidium glabrum*/Other species.

Discussion

This study used OIE (World Organisation for Animal Health)-recommended diagnostic techniques and current literature to demonstrate that of a number of molluscan pathogens recorded in Cork Harbour, *Bonamia ostreae*, *Minchinia mercenariae*-like and *Vibrio aestuarianus* are currently present in commercial bivalves. Furthermore, field surveys and laboratory trials indicated that these same pathogens are present in invasive tunicates cohabiting with oysters at the aquaculture sites. Field surveys also demonstrated that *V. aestuarianus* can occur in invasive tunicates present in marinas removed from aquaculture sites, raising the possibility that transport of pathogens may occur from aquaculture sites

and remain in reservoirs outside the zone of infection, for example via currents or recreational shipping. Additionally, the haplosporidian *M. mercenariae*-like was capable of replicating in *Styela clava*, suggesting that this tunicate is not only a carrier of the pathogen but potentially a viable host. *S. clava* were also capable of maintaining a second haplosporidian pathogen, *B. ostreae*, without the presence of the primary host *Ostrea edulis*. The presence of both *B. ostreae* and *M. mercenariae*-like in *S. clava* individuals also demonstrates that the tunicates are susceptible to coinfection.

For both *Styela clava*/*Bonamia ostreae* and *Didemnum vexillum*/*Vibrio aestuarianus* the prevalence of the respective pathogens was higher at the start of the trial, in the baseline samples, than at the end of the trial in the experimental samples. The filter feeding mechanism of the tunicates may explain how they take in the disease and it is possible that in a larger aquaculture area the tunicates are filtering more so that although the geographic spread is significantly larger than in an enclosed tank the increased filtering could result in a greater intake of pathogenic cells and a higher infection.

In the case of *Vibrio aestuarianus* it is possible that *Didemnum vexillum* and other colonial spp. may need to be in contact with oysters to maintain the disease, as both the baseline sample and the experimental treatments proved positive despite a low prevalence of *V. aestuarianus* in the cohabitating *Crassostrea gigas* at the end of the trial which suggested that the remaining oysters were 'survivors' that had not suffered *Vibrio*-induced mortality unlike those at the start of the trial. Conversely the control treatment of tunicates only was negative for *V. aestuarianus*.

In the case of *Bonamia ostreae*, *Styela clava* in control tanks maintained the disease for two months without the presence of oysters. Furthermore, the percentage prevalence of infection was higher in the control than the baseline and experimental samples, which indicates that replication in the system may be possible and the pathogen may have been transmitting. This would again suggest that *S. clava* is potentially a host, not just a carrier or reservoir. The fact that *B. ostreae* prevalence was higher in control tunicates than tunicates cohabiting with oysters may also be because the oysters were filtering more quickly and therefore picking up *B. ostreae* cells so the infection was reduced in tunicates.

Another key point is that the percentage prevalence of *B. ostreae* was higher in *O. edulis* cohabiting with *S. clava*, rather than in the *O. edulis* control tank, meaning a cumulative effect of *S. clava* and *O. edulis* on *B. ostreae* percentage prevalence cannot be ruled out. *O. edulis* in this study came from a site where a selective breeding programme commenced in 1988 for over 30 years (Lynch *et al*., 2014) and the slight reduction in *B. ostreae* in the oysters from the baseline to the end of the trial may support that they have been bred to be resistant to *B. ostreae* and can maintain the disease at a sub-lethal level.

This study focused on *Didemnum vexillum* as the target species for laboratory trials, however this species was growing in heterogeneous colonies and it is therefore possible that the interwoven nature of colonial tunicates means they may circle pathogens both from zooid to zooid within the colony, but also from one species to another thus heightening the potential for disease transmission. Indeed, the combination of *Aplidium glabrum*/other saw a slight increase in *V. aestuarianus* at the end of the trial (but again no disease was detected in the control tank with no oysters present). Furthermore, given the detection of *Vibrio aestuarianus* it is highly likely that other *Vibrio* spp. that are potentially pathogenic to commercial bivalves could also be carried by tunicates.

Although the impact of these pathogens on tunicate health was not investigated in this study, the combined results across the solitary and colonial forms indicate that invasive tunicates can potentially act as reservoirs of pathogens. Other studies have indicated that in many cases such reservoirs can transmit the infection back to susceptible bivalves, for example when *Crassostrea gigas* infected with *Bonamia ostreae* were held with naive *Ostrea edulis*, the *O. edulis* cavity fluid then tested positive for the pathogen (Lynch *et al*., 2010). *B. ostreae* has also been detected in a number of benthic macroinvertebrates, including the native European sea squirt *Ascidiella aspersa* and other phyla including Phylum Crustacea (*Carcinus maenas*) and Phylum Cnidaria (*Actinia equina)* (Lynch *et al*., 2007). This circling of the disease through diverse invertebrate groups could suggest that there are multiple avenues for reservoirs to come in contact with the disease.

Small and Pagenkopp (2011), noted that the environment itself can act as a reservoir and this is demonstrated by *Bonamia ostreae*, as the OIE states that it can live for up to one week in the water column. This also means that *Styela clava* could potentially act as a

mechanical vector and carry water with the protist present to different sites if transferred on improperly cleaned aquaculture gear or non-biosecure stock shipments.

Tunicates were used as a model taxon in this study, however it is important to note that invasive species across different phyla may be also able to move significant pathogens. Davies *et al*., (2019) investigated the globally invasive shore crab (*Carcinus maenas*) in Wales and found it infected with *Hematodinium* sp. and *Hematodinium perezi.* The disease was also present in seawater eDNA samples, possibly due to the release of infectious stage dinospores from moribund individuals. Shore crabs in the study site were cohabiting with the commercially valuable edible brown crab, and this demonstrates that ability of disease reservoirs to enable pathogens to persist in habitats utilised by commercial shellfish.

The presence of disease in invasive tunicates is of significant interest to the aquaculture sector, particularly in the context of climate change, as species potentially expand their invaded range (Hellmann *et al*., 2008). When coupling the mechanical impacts of the tunicates themselves, for example competition for space, with their potential to interact with pathogens affecting commercial bivalve species, they emerge as a threat that warrants serious consideration and enhanced biosecurity. The Food and Agriculture Organization of the United Nations recognised the risk of invasive species in the 2030 Agenda for Sustainable Development, and noted the need to introduce measures to eradicate or control such species (FAO, 2017). A number of aspects need to be addressed to lessen invasive impacts; reproductive strategies need to be identified, transport via shipping or other pathways such as aquaculture needs to be minimised and mechanisms identified to remove and inactivate fouling organisms. Examples of these mechanisms include controls such as those described in Hillock and Costello (2013) with a study on desiccation methods for *Styela clava*, or Turrell *et al*. (2018) with a study of different bath treatments that could be applied to commercial bivalve consignments to induce full mortality in *Didemnum vexillum.*

It is necessary to develop a global approach to ensure aquaculture transfer takes management of both invasive species and disease into account, thereby developing legislation and codes of practice. In 2016 shelled molluscs constituted 58.8% of the

combined production of marine and coastal aquaculture (FAO, 2018) and growth projections for the future are positive, meaning that molluscan aquaculture will play an essential role in global food resources (Rodgers *et al*., 2015; Arzul *et al*., 2017). However, this sector is intrinsically linked to the movement of invasive species and disease cycling is a further constraint on growth. Guidelines and legislation are only as good as the knowledge that informs them, and it is not feasible to protect against species on which there is little knowledge about health impacts or potential roles in disease cycling. Accordingly, it is necessary to broaden the understanding of the how pathogens utilise invasive species, and the subsequent impact on the commercial sector.

This study raises questions relating to the viability of tunicates as sources of infection and suggests that the taxon should be taken into account in risk assessments and disease management, particularly as pathogens can endure in species that are not their true host (Lynch *et al*., 2010). Screening of the tunicates focused specifically on pathogens that had previously been detected in the natural hosts, however further work could expand on the diversity and seasonality of pathogen species, as if tunicates are reservoirs then a range of pathogens might be present in them at times of the year when they are not detected in oysters. It also demonstrates that it is important to have good monitoring networks and collaborative efforts so that information on invasive species with potential impacts is widely shared and available within the aquaculture sector (Brenner *et al*., 2014).

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References

Albuixech-Martí, S., Lynch, S.A. & Culloty, S.C. (2020). Biotic and abiotic factors influencing haplosporidian species distribution in the cockle *Cerastoderma edule* in Ireland. *Journal of Invertebrate Pathology*, 174:1-12.

Arzul, I., Corbeil, S., Morga, B. & Renault, T. (2017). Viruses infecting marine molluscs. *Journal of Invertebrate Pathology*, 147:118-135.

Beaury, E.M., Fusco, E.J., Jackson, M.R., Laginhas, B.B., Morelli, T.L., Allen, J.M., Pasquarella, V.J. & Bradley, B.A. (2019). Incorporating climate change into invasive species management: insights from managers. *Biological Invasions*, https://doi.org/10.1007/s10530-019-02087-6.

Bookelaar, B., O'Reilly, A.J., Lynch, S.A. & Culloty, S.C. (2018). Role of the intertidal predatory shore crab *Carcinus maenas* in transmission dynamics of ostreid herpesvirus-1 microvariant. *Diseases of Aquatic Organisms*, 130:221-233.

Bookelaar, B. (2018). Understanding and minimizing the impacts of host-pathogen-environment interactions in the Pacific oyster *Crassostrea gigas*. PhD Thesis, University College Cork.

Bourque, D., Davidson, J., MacNair, N.G., Arsenault, G., LeBlanc, A.R., Landry, T. & Mirron, G. (2007). Reproduction and early life history of an invasive ascidian *Styela clava* Herdman, in Prince Edward Island, Canada. *Journal of Experimental Marine Biology and Ecology*, 342:78-84.

Brenner, M., Fraser, D., Van Nieuwenhove, K., O'Brien, F., Buck, B.H., Mazurié, J., Thorarinsdottir, G., Dolmer, P., Sanchez-Mata, A., Strand, O., Flimlin, G., Miossec, L. & Kamermans, P. (2014). Bivalve aquaculture transfers in Atlantic Europe. Part B: Environmental impacts of transfer activities. *Ocean & Coastal Management*, 89:139-146.

Carnegie, R.B., Arzul, I. & Bushek, D. (2016). Managing marine mollusc diseases in the context of regional and international commerce: policy issues and emerging concerns. *Philosophical Transactions of the Royal Society B*, 371:20150215.

Casso, M., Navarro, M., Ordóñez, V., Fernández-Tejedor, M., Pascual, M. & Turon, X. (2018). Seasonal patterns of settlement and growth of introduced and native ascidians in bivalve cultures in the Ebro Delta (NE Iberian Peninsula). *Regional Studies in Marine Science*, 23:12-22.

Cochennec, N., Le Roux, F., Berthe, F. & Gérard, A. (2000). Detection of *Bonamia ostreae* Based on Small Subunit Ribosomal Probe. *Journal of Invertebrate Pathology*, 76:26-32.

Comeau, L.A., Filgueira, R., Guyondet, T. & Sonier, R. (2015). The impact of invasive tunicates on the demand for phytoplankton in longline mussel farms. *Aquaculture*, 441:96-105.

Cordell, J.R., Levy, C. & Toft, J.D. (2012). Ecological implications of invasive tunicates associated with artificial structures in Puget Sound, Washington, USA. *Biological Invasions*, 15:1303-1318.

Davies, C.E., Batista, F.M., Malkin, S.H., Thomas, J.E., Bryan, C.C., Crocombe, P., Coates, C.J. & Rowley, A.F. (2019). Spatial and temporal disease dynamics of the parasite *Hematodinium* sp. in shore crabs, *Carcinus maenas*. *Parasites & Vectors*, 12:472. https://doi.org/10.1186/s13071-019-3727-x.

Dunn, A.M. & Hatcher, M.J. (2015). Parasites and biological invasions: parallels, interactions, and control. *Trends in Parasitology*, 31(5):189-199.

FAO (2017). The 2030 Agenda and the Sustainable Development Goals: The Challenge for Aquaculture Development and Management. *FAO Fisheries and Aquaculture Circular*, No. 1141 Rome, Italy

FAO (2018). The State of World Fisheries and Aquaculture 2018 - Meeting the sustainable development goals. Rome, Licence: CC BY-NC-SA 3.0 IGO.

Ferguson, L.F., Davidson, J.D.P., Landry, T., Clements, J.C., Therriault, T.W. (2017). *Didemnum vexillum*: invasion potential via harvesting and processing of the Pacific oyster (*Crassostrea gigas*) in British Columbia, Canada. *Management of Biological Invasions*, 8(4):553-558.

Flannery, G., Lynch, S.A., Longshaw, M., Stone, D., Martin, P., Ramilo, A., Villalba, A. & Culloty, S. (2014). Interlaboratory variability in screening for *Bonamia ostreae*, a protistan parasite of the European flat oyster *Ostrea edulis*. *Diseases of Aquatic Organisms*, 110:93-99.

Ford, S.E., Stokes, N.A., Burreson, E.M., Scarpa, E., Carnegie, R.B., Kraeuter, J.N. & Bushek, D. (2009). *Minchinia mercenariae* n. sp. (Haplosporidia) in the Hard Clam *Mercenaria mercenaria*: Implications of a Rare Parasite in a Commercially Important Host. *Journal of Eukaryotic Microbiology*, 56(6):542-551.

Franchi, N. & Ballarin, L. (2017). Immunity in Protochordates: The Tunicate Perspective. *Frontiers in Immunology*, 8(674):1-16.

Goedknegt, M.A., Feis, M.E., Wegner, K.M., Luttikhuizen, P.C., Buschbaum, C., Camphuysen, K.(C.J.), van der Meer, J. & Thieltges, D.W. (2016). Parasites and marine invasions: Ecological and evolutionary perspectives. *Journal of Sea Research*, 113:11-27.

Goldstien, S.J., Schiel, D.R. & Gemmell, N.J. (2010). Regional connectivity and coastal expansion: differentiating pre-border and post-border vectors for the invasive tunicate *Styela clava*. *Molecular Ecology*, 19:874-885.

Hellmann, J.J., Byers, J.E., Bierwagen, B.G. & Dukes, J.S. (2008). Five Potential Consequences of Climate Change for Invasive Species. *Conservation Biology*, 22(3):534-543.

Hillock, K.A. & Costello, M.J. (2013). Tolerance of the invasive tunicate *Styela clava* to air exposure. *Biofouling*, 29(10):1181-1187.

Kumagai, A., Sakai, K., Miwa, S. (2014). The Sea Squirt *Styela clava* is a Potential Carrier of the Kinetoplastid *Azumiobodo hoyamushi*, the Causative Agent of Soft Tunicate Syndrome in the Edible Ascidian *Halocynthia roretzi*. *Fish Pathology*, 49(4):206-209.

Lupo, C., Travers, M.-A., Tourbiez, D., Barthélémy, C.F., Beaunée, G. & Ezanno, P. (2019). Modeling the Transmission of *Vibrio aestuarianus* in Pacific Oysters Using Experimental Infection Data. *Frontiers in Veterinary Science*, 6(142):1-15.

Lynch, S.A., Armitage, D.V., Coughlan, J., Mulcahy, M.F. & Culloty, S.C. (2007). Investigating the possible role of benthic macroinvertebrates and zooplankton in the life cycle of the haplosporidian *Bonamia ostreae*. *Experimental Parasitology*, 115:359-368.

Lynch, S.A., Mulcahy, M.F. & Culloty, S.C. (2008). Efficiency of diagnostic techniques for the parasite, *Bonamia ostreae*, in the flat oyster, *Ostrea edulis*. *Aquaculture*, 281:17-21.

Lynch, S.A., Abollo, E., Ramilo, A., Cao, A., Culloty, S.C. & Villalba, A. (2010). Observations raise the question if the Pacific oyster, *Crassostrea gigas*, can act as either a carrier or a reservoir for *Bonamia ostreae* or *Bonamia exitiosa*. *Parasitology*, 137:1515-1526.

Lynch, S.A., Villalba, A., Abollo, E., Engelsma, M., Stokes, N.A. & Culloty, S.C. (2013a). The occurrence of haplosporidian parasites, *Haplosporidium nelsoni* and *Haplosporidium* sp., in oysters in Ireland. *Journal of Invertebrate Pathology*, 112:208-212.

Lynch, S., Dillane, E., Carlsson, J. & Culloty, S.C. (2013b). Development and Assessment of a Sensitive and Cost-Effective Polymerase Chain Reaction to Detect Ostreid Herpesvirus 1 and Variants. *Journal of Shellfish Research*, 32(3):657-664.

Lynch, S.A., Flannery, G., Hugh-Jones, T., Hugh-Jones, D. & Culloty, S.C. (2014). Thirty-year history of Irish (Rossmore) *Ostrea edulis* selectively bred for disease resistance to *Bonamia ostreae*. *Diseases of Aquatic Organisms*, 110:113-121.

Lynch, S.A., Darmody, G., O'Dwyer, K., Gallagher, M.C., Nolan, S., McAllen, R. & Culloty, S.C. (2016). Biology of the invasive ascidian *Ascidiella aspersa* in its native habitat: Reproductive patterns and parasite load. *Estuarine, Coastal and Shelf Science*, 181:249-255.

McCleary, S. & Henshilwood, K. (2015). Novel quantitative TaqMan® MGB real-time PCR for sensitive detection of *Vibrio aestuarianus* in *Crassostrea gigas*. *Diseases of Aquatic Organisms*, 114:239-248.

OIE Manual of Diagnostic tests for Aquatic Animals (2019). https://www.oie.int/en/international-standard-setting/aquatic-manual/access-online. Accessed 26/06/2020.

Paetzold, S.C., Hill, J. & Davidson, J. (2012). Efficacy of high-pressure seawater spray against colonial tunicate fouling in mussel aquaculture: inter-annual variation. *Aquatic Invasions*, 7(4):555-566.

Prado-Alvarez, M., Couraleau, Y., Chollet, B., Tourbiez, D. & Arzul, I. (2015). Whole-genome amplification: a useful approach to characterize new genes in unculturable protozoan parasites such as *Bonamia exitiosa*. *Parasitology*, 142:1523-1534.

Prado-Alvarez, M., Darmody, G., Hutton, S., O'Reilly, A., Lynch, S.A. & Culloty, S.C. (2016). Occurrence of OsHV1 in *Crassostrea gigas* Cultured in Ireland during an Exceptionally Warm Summer. *Frontiers in Physiology*, 7(492):1-14.

Price, A., Collie, J. & Smith, D. (2005). 18S ribosomal RNA and cytochrome oxidase gene sequences of *Didemnum* sp., an invasive colonial tunicate. *SURFO Technical Reports*, No.2006-01.

Ramilo, A., Abollo, E., Villalba, A. & Carballal, M.J. (2018). A *Minchinia mercenariae*-like parasite infects cockles *Cerastoderma edule* in Galicia (NW Spain). *Journal of Fish Disease*, 41:41-48.

Renault, T., Stokes, N.A., Chollet, B., Cochennec, N., Berthe, F., Gérard, A. & Burreson, E.M. (2000). Haplosporidiosis in the Pacific oyster *Crassostrea gigas* from the French Atlantic coast. *Diseases of Aquatic Organisms*, 42, 207-214.

Rodgers, C.J., Carnegie, R.B., Chávez-Sánchez, M.C., Martínez-Sánchez, C.C., Furones Nozal, M.D. & Hine, P.M. (2015). Legislative and regulatory aspects of molluscan health management. *Journal of Invertebrate Pathology*, 131:242-255.

Rosa, M., Holohan, B.A., Shumway, S.E., Bullard, S.G., Wikfors, G.H., Morton, S. & Getchis, T. (2013). Biofouling ascidians on aquaculture gear as potential vectors of harmful algal introductions. *Harmful Algae* 23:1-7.

Rueckert, S., Wakeman, K.C., Jenke-Kodama, H. & Leander, B.S. (2015). Molecular systematics of marine gregarine apicomplexans from Pacific tunicates, with descriptions of five novel species of *Lankesteria*. *International Journal of Systematic and Evolutionary Microbiology*, 65:2598-2614.

Simkanin, C., Dower, J.F., Filip, N., Jamieson, G. & Therriault, T.W. (2013). Biotic resistance to the infiltration of natural benthic habitats: Examining the role of predation in the distribution of the invasive ascidian *Botrylloides violaceus*. *Journal of Experimental Marine Biology and Ecology*, 439:76-83.

Small, H.J. & Pagenkopp, K.M. (2011). Reservoirs and alternate hosts for pathogens of commercially important crustaceans: A review. *Journal of Invertebrate Pathology*, 106:153-164.

Turrell, W.R., Brown, L., Graham, J., Gubbins, M.J., Hermann, G., Matejusova, I. & Robinson, C. (2018). Selecting a Bath Treatment for the Marine Carpet Sea Squirt *Didemnum vexillum*, Kott 2002 in Scottish Shellfish Aquaculture. *Scottish Marine and Freshwater Science Report* 9(12):1-94.

Ward, G.M., Feist, S.W., Noguera, P., Marcos-López, M., Ross, S., Green, M., Urrutia, A., Bignell, J.P. & Bass, D. (2019). Detection and characterisation of haplosporidian parasites of the blue mussel *Mytilus edulis*, including description of the novel parasite *Minchinia mytili* n. sp. *Diseases of Aquatic Organisms*, 133:57-68.

Wong, N.A., McClary, D. & Sewell, M.A. (2011). The reproductive ecology of the invasive ascidian, *Styela clava*, in Auckland Harbour, New Zealand. *Marine Biology*, 158:2775-2785.

Zhan, A., Briski, E., Bock, D.G., Ghabooli, S. & MacIsaac, H.J. (2015). Ascidians as models for studying invasion success. *Marine Biology*, 162:2449-2470.

Chapter 4: Regional Differences in Zooplankton-Associated Bacterial Communities and Aquaculture Pathogens Across Two Shelf Seas

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Abstract

Microbial pathogens can persist in the marine environment in a number of ways, for example during a free-living life stage or colonising the surface of plankton. These pathogens include viruses, protistans and bacteria, which may adversely impact the aquaculture and shellfishery sectors. Microbial diversity is well documented in nearshore bivalve culture environments, however, less is known about microbial communities in offshore environments. Oceanic fronts and currents may facilitate (or inhibit) planktonic dispersal, which can then allow for the movement of associated microbes across otherwise restrictive barriers. This study used zooplankton samples collected from the Irish and Celtic Seas to assess the zooplankton community structure and that of its associated microbial community. Zooplankton samples were identified using microscopy and screened using polymerase chain reaction (PCR) to identify pathogens with a potential aquaculture impact. Samples from four subareas representing the Irish Sea, Celtic Front, Eastern Celtic and Southern Celtic also underwent sequencing of the bacterial V3-V4 region to define the bacteriome within these areas. All samples were dominated by crustaceans, and high molluscan numbers were evident around the Celtic Front. There were distinct bacterial profiles, dominated by cyanobacteria and proteobacteria, and a positive relationship between proteobacteria and copepods, chaetognaths and molluscs. A degree of connectivity was observed between the Easter Celtic and Celtic Front, but the front acted as a barrier to the Irish Sea. The study also detected the bacterium *Vibrio splendidus*, potentially pathogenic to bivalves, and a low prevalence of a haplosporidian. The haplosporidian species, detected in just one previous study to date, was of interest as haplosporidia are one of the main pathogen groups in bivalves. Identifying the regions in which aquaculture-pathogens are found can lead to an understanding of infection pathways and also be used to plan mitigation efforts, for example when choosing offshore locations to culture bivalves.

Keywords: Hydrodynamics, Microbes, Sequencing, Haplosporidia, Vibrios, Plankton

Introduction

Seawater is a favourable medium for the transport of pathogens including viruses, bacteria, spores and other parasites and, depending on physico-chemical characteristics of the water such as temperature, salinity and pH, pathogens can often survive outside the host. Connectivity is then dependent on the rate of reproduction/replication, dispersal, host contact and host infection (Cantrell *et al*., 2020). Pathogens can persist in the marine environment in a number of ways, for example during a transmissible free-living life stage or adhering to the surface of inorganic particles or live plankton, thus creating a more stable and transportable medium (Evans *et al*., 2014). Marine bacterial plankton are typically classified as free-living or attached, with attached prokaryotes developing dense communities of cells (Mestre *et al*., 2017). Zooplankton provide a habitat that is rich in both organic and inorganic matter and nutrients, which allow for bacterial colonies to thrive (De Corte *et al*., 2018).

Routine horizon scanning is essential to detect potential threats before they become an established problem (Sutherland & Woodroof, 2009). In recent years, planktonic research has been utilised to shed light on the offshore distribution of microbial organisms, both free-living and hosted within the planktonic community. This potential for microbial dispersal is often contextualised by studying the barriers and facilitators to movement, for example oceanic fronts and currents (McCallum *et al*., 2003; Hernando-Morales *et al*., 2017). Planktonic organisms are strong indicators of biological change and respond to temperature and currents by expanding and contracting their range (Hays *et al*., 2005), thereby influencing the dispersal of associated microbes. From an ecological and commercial perspective, the dispersal of microorganisms is particularly important when considering the distribution of those species that are pathogenic to the aquaculture sector and in understanding some potential infection pathways. Although there is wide variation in the degree of connectivity between marine regions, highly connected regions can allow rapid spread of pathogenic organisms (McCallum *et al*., 2003).

One bacterial genus that is frequently associated with marine zooplankton is *Vibrio*, a gram-negative bacterium that can be suspended in water or attached to zooplankton (Harriague *et al*., 2008). Species within the genus are known to attach to chitinous

organisms such as copepods (Constantin de Magny *et al*., 2011), the most abundant zooplankton component in terms of biomass, abundance and diversity (de Oliveira Soares *et al*., 2018). The *Vibrio* spp. adhere to crustacean carapaces, attaching to the moults and exoskeletons and then growing in the form of a biofilm, and are frequently the causative agents of shell diseases (Small & Pagenkopp 2011). *Vibrio splendidus* and *V. alginolyticus* have been detected also adhering to gelatinous zooplankton in the Classes Hydrozoa and Scyphozoa (Clinton *et al*., 2020).

Vibrio populations are strongly influenced by environmental conditions, including temperature, salinity, pH and nutrient availability. In one Galician study, *V. splendidus* was dominant during the warm months and *V. aestuarianus* was predominant throughout the cold season. The study also demonstrated that *V. splendidus* required higher salinity than what is typically required by *V. aestuarianus* and *Vibrio splendidus* was more prevalent than *V. aestuarianus* in the environment and animals (Romero *et al*., 2010). This was similar to the findings from the EU BIVALIFE project which suggested that the *V. splendidus* clade bacteria are in general more capable of persisting in the aquatic environment than *V. aestuarianus* (BIVALIFE, 2014). *Vibrio* seasonality is linked to plankton composition, with seasonal shifts in plankton leading to changes in *Vibrio* numbers, and there is evidence that climate change is influencing outbreaks of *Vibrio* globally (Vezzulli *et al*., 2016).

Vibrio species are also problematic to bivalve aquaculture and shellfisheries, as they have been linked to mortalities in multiple bivalve species including oysters and clams (Solomieu *et al*., 2015). The Irish Sea and Celtic Sea regions are areas of aquaculture industry, where bivalve culture and fisheries provide substantial income to local economies in Ireland and Wales (Rowley *et al*., 2014). Shellfish culture in these regions is primarily focused on the Pacific cupped oyster *Crassostrea gigas* and the blue mussel *Mytilus edulis*. A number of *Vibrio* species have been detected in *C. gigas* mortality events in Irish culture sites. These include *Vibrio aestuarianus*, *V. splendidus*, *V. splendidus*-like, and *V. gallicus* (Bookelaar, 2018). Out of these species, *V. aestuarianus* and *V. splendidus* in particular have been highlighted as problematic by the EU BIVALIFE project (BIVALIFE, 2014). It has also been suggested that co-infection with *V. splendidus* and *V.*

aestuarianus could lead to synergistic virulence effects (Saulnier *et al*., 2010). Given the importance of the aquaculture sector in this region, an understanding of offshore pathogens is necessary, as currents may facilitate pathogen dispersal and influence the extent to which *Vibrio* spp. spread between culture sites. Indeed, *V. aestuarianus*, has been detected in wild mussels (*Mytilus* spp.) up to 30 km removed from infection hotspots, those areas with high infection rates resulting in *Vibrio*-induced oyster mortality, at Pacific cupped oyster culture sites located on the south coast of Ireland (Bookelaar, 2018).

Viruses are another group pathogenic to aquaculture that display a zooplankton association. Arzul *et al*. (2017) reviewed marine molluscs and disease and noted that the ostreid herpesvirus microvariant OsHV-1µVar has caused significant mortalities for the oyster culture sector, particularly *Crassostrea gigas*. Herpesvirus may be present in seawater in a free viral form, attached to particles or flocculated but there is little information available on how it is spread over geographic distance in seawater or how it is detected in seawater and particles in seawater (Evans *et al*., 2014). However, herpesviruses may also be attached to, or infect, planktonic hosts thus gaining a degree of protection in the stage of their lifecycle outside of molluscan hosts (Arzul *et al*., 2017). Maximum survival time outside the host is unknown, but seawater assays seeded with the virus had viral DNA detected after 22 days at 4°C, and 12 days at 20°C. These results suggested that detection of OsHV-1 DNA decreased as temperatures increased, however, the degree of infectivity arising from DNA detection was unknown (Vigneron *et al*., 2004).

Mortality of spat and juveniles associated with ostreid herpesvirus OsHV-1µVar was first recorded in Dungarvan on the south coast of Ireland in 2008 and has been recorded almost annually since that time. However, from 2012 an increase in oyster mortality beginning in May and continuing through until October has been recorded, with cumulative mortality for the summer period generally reaching between 30 and 50%. *Vibrio aestuarianus* has been isolated regularly during these mortality episodes with studies demonstrating a link between rising mortality and increasing levels of *V. aestuarianus* in the oysters (VIVALDI, 2020).

The Phylum Haplosporidia is another pathogenic group that is considered of major concern to shellfish industries worldwide and has caused the most significant losses (Arzul & Carnegie, 2015). This phylum contains the genera *Bonamia*, *Haplosporidium*, *Minchinia* and *Urosporidium*, all of which affect molluscan hosts and a wider variety of taxa too, although it is the genus *Haplosporidium* that is associated with the widest host range (Arzul & Carnegie, 2015). Ward *et al*. (2019) detected haplosporidian sequences in water and sediment samples, suggesting the possibility of either free-living stages or associations with planktonic fauna. The haplosporidian *Bonamia ostreae*, the causative agent of the oyster disease bonamiosis, has been detected in grouped zooplankton samples (Lynch *et al*., 2007) and is also capable of surviving in seabed borewater for up to one week at 15°C (Arzul *et al*., 2009).

Molecular techniques are increasingly utilised to disentangle the relationships between different planktonic groupings, namely bacteria, viruses, prokaryotes, microbial eukaryotes, zooplankton and phytoplankton. Previous studies have compared the bacterial communities associated with copepods to free-living bacteria in the surrounding seawater in the North Atlantic Ocean and found some shared bacterial Operational Taxonomic Units (OTUs), suggesting that there was exchange between these two habitats. However, the microhabitats of attached bacteria were orders of magnitude higher than those of free-living bacteria. Furthermore, zooplankton perform diel vertical migration and this allows bacteria to cross barriers that otherwise would restrict movement, for example pycnoclines (De Corte *et al*., 2014).

Sequencing technologies, including amplicon sequencing, have been used to characterise planktonic community profiles in multiple ways. Illumina amplicon sequencing is a targeted approach that enables analysis of specific genomic regions of interest and has been used to identify distinct geographic breaks in zooplankton community structure (Pitz *et al*., 2020), and to demonstrate that bacterial diversity peaks at mid latitudes rather than at the Equator (Milici *et al*., 2015). The 16S rRNA gene is commonly used in bacterioplankton studies, for example to shed light on community composition, distribution and spatial-temporal changes (Cottrell *et al*., 2000; Shan *et al*., 2015; Ma *et al*., 2016). Analysis of the 16S rRNA gene of bacterioplankton has also demonstrated a

strong shift in the community composition across frontal zones in New Zealand, indicating that these areas act as transition zones and boundaries for bacterioplankton distribution (Baltar *et al*., 2016).

The V3-V4 region of the 16S rRNA gene has been used to assess the bacterial community associated with commercially important whiteleg shrimp *Litopenaeus vannamei*, with selection of this hypervariable region producing high diversity and richness, and enhancing efforts to monitor how shrimp may be susceptible to pathogens (García-López *et al*. 2020). Developing tools for disease surveillance and mitigation in the marine environment is an ongoing process and molecular techniques such as these may be used to overcome issues with strategic sampling and traditional surveys, and to develop tools with a greater degree of detection sensitivity for problematic species (Brown *et al*., 2016).

The Celtic Sea acts as a transition zone between the Atlantic waters at the edge of the northwest European continental shelf and the coastal waters of the Bristol Channel and the Irish Sea (Brown *et al*., 2003). Waters on the Celtic Shelf have a relatively long residence time, for example an Argos buoy drogued at 20m and deployed south of the Scilly Isles in 1992 measured 284 days on the slope before battery failure (Pingree *et al*., 1999). The highest salinities in the region are along the Cornish Coast, suggesting the intrusion of more saline ocean water, and the salinity then transitions from the more saline Celtic Sea to salinities <35.0 in the Irish Sea. Waters from the Celtic Sea travel northwards along the Cornish Coast and westward along St. George's Channel, before continuing along the Irish coastline and around the southwest tip of Ireland (Figure 1).

Thermal stratification in summer within the Celtic Shelf region reduces exchange between the Celtic and Irish Seas. Furthermore, tidal variability within this region determines the water-column structure which is dominated by thermal stratification from May-November. A distinct density-driven front builds up between the stratified Celtic Sea and the tidally mixed waters of the southern Irish Sea and Bristol Channel and this is known as the Celtic Sea Front (Figure 2). The main driver of the front is the balance between thermal stratification and a dense core of cold, saline water carried over from winter. The tidal energy funnels around the core of cold water thus creating mixed water at the fringes of the front and in the Irish Sea. Flow from St. George's Channel into the Celtic Sea often

presents as a meander in the Celtic Sea Front in satellite imagery, and this meander moves the cooler Irish Sea water into the Celtic Sea and is an enduring feature in the summertime. At the southward extension of this meander, a clockwise circulation of 40 km diameter is formed before water then joins the westward flow around the southwest tip of Ireland (Brown *et al*., 2003). The primary hypothesis addressed here was that regional hydrography, particularly surround the Celtic Sea Front, would result in distinct zooplankton-associated microbial communities, with potential aquaculture pathogens also present.

Figure 1: Overview of summer thermohaline circulation on the northwest European continental shelf. Frontal jets (red arrows) are associated with bottom fronts at the boundaries of dense cold and saline pools. In the English Channel movement is eastwards upon entry and westwards upon exit, with some movement also occurring northwards across the channel (adapted from Hill *et al*., 2008)

Aims

This study aimed to identify the diversity and abundance of zooplankton across the Irish and Celtic Seas, and to determine if any pathogens with an aquaculture association were present and assess the zooplankton as a source of potential infection. Lastly, the study aimed to assess whether there were regional differences in bacteria adhering to the zooplankton community, with sample groupings selected from the Irish Sea (above the Celtic Front), the Front itself, the Eastern Celtic Sea and lastly the Southern Celtic Sea (Figure 2). These sample areas were selected to ascertain the level of connectivity within the study regions, and to understand how bacteria and other microbes may be dispersing.

Methods

Sample Collection

The research cruise to collect the plankton was conducted aboard the RV *Celtic Voyager* between 17th-26th May 2018. This time-period was selected due to the build-up of the Celtic Sea Front and to correspond with the abundance of spring-spawning zooplankton. The cruise consisted of 65 stations. A multinet was deployed as a single sampler with a 300 µm mesh size and a maximum sample depth of 50 m at each station and one sample was taken per station. The multinet had an associated flow meter to record the volume of water filtered for each sample. Sampling at stations 1-44 was conducted as oblique V-shaped tows, with each sample split in half and preserved in ethanol for molecular work and formalin for visual identification. From 23rd May mechanical issues with the multinet and its associated real-time data collection unit meant that samples 45-65 were conducted as two vertical dips per station both conducted in the same spot (rather than one oblique tow), and again half preserved in ethanol and half in formalin. The necessary conversion from V-shaped tows to vertical dips meant that lower volumes of water were filtered in the dips, however there was still an average of 43.7 m^3 filtered in these samples, resulting in dense plankton samples. Furthermore, to ensure consistency in data analysis all results were standardised to one cubic meter. Prior to preservation the net was washed down and all samples were then visually inspected using a light box in the vessel wet lab, with fish larvae then removed and preserved for separate analyses (see Ratcliffe *et al*., 2020). Accordingly, fish larvae were not included in this present study.

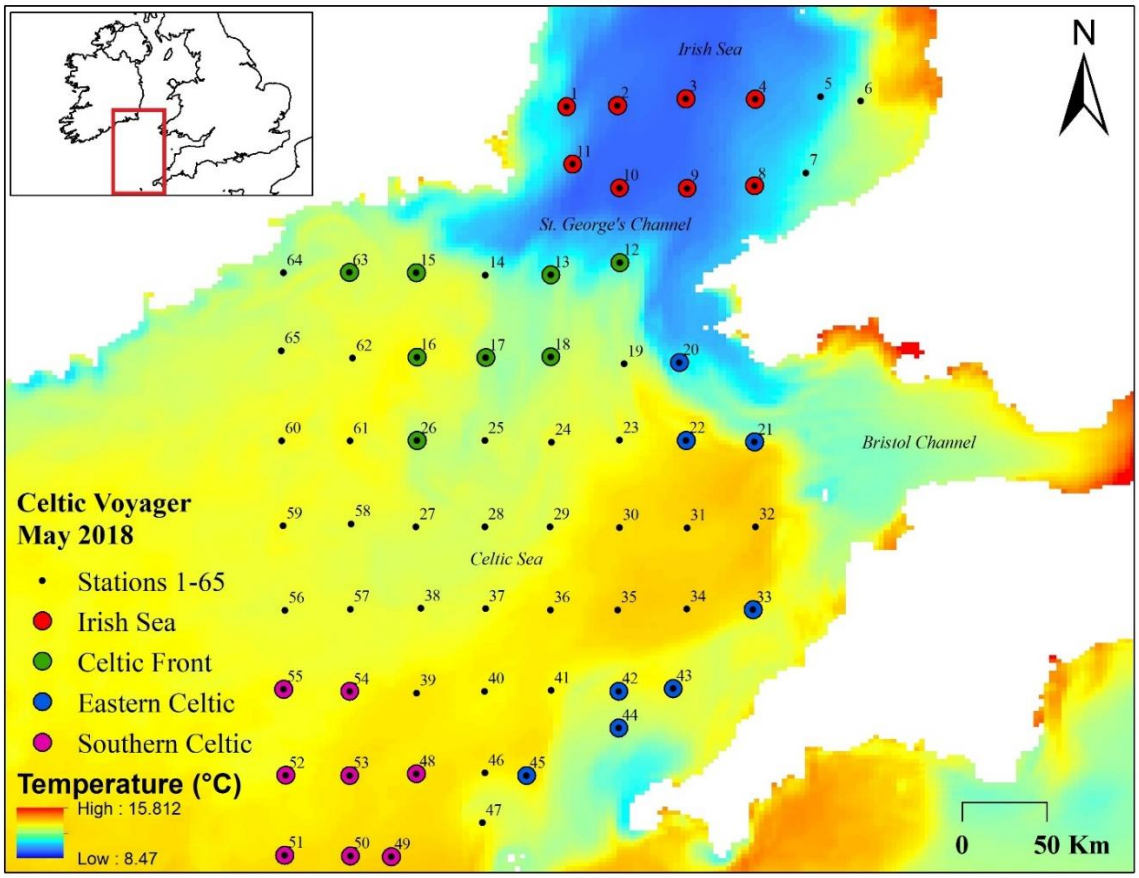


Figure 2: 65 multinet sample stations from the Irish and Celtic Seas (17th-26th May, 2018) displaying the location of the Celtic Sea Front along St. George's Channel, with sea temperatures sourced from Copernicus. The plankton community in all 65 samples was identified and enumerated, and each sample screened for haplosporidia and ostreid herpesvirus OsHV-1 µVar using PCR. A subset of 32 samples (in coloured circles - red, green, blue and violet) were sent for amplicon-based metagenomics to sequence their associated bacterial communities.

Identification of Zooplankton (Formalin-Preserved Samples)

Sixty-four samples representing the total number of formalin samples (sample 5 was compromised) were first prepared by decanting the formalin using a 180 µm sieve (Endecotts) and washing the sample in fresh tap-water. The sample remained in freshwater for the duration of the identification process and was then returned to formalin. Samples were analysed using a Zeiss Stemi 305 darkfield stereomicroscope and

MOCapture software was used for imaging. Macroscopic gelatinous taxa belonging to the Phylum Cnidaria were removed and enumerated separately at the start of the sample analysis to facilitate an even split. Each sample was then split using a Folsom splitter to determine quantitative data for the other dominant taxa. Samples were identified as per Baxter *et al*. (2011), Conway (2012) and Haberlin *et al*. (2016).

DNA Extraction for PCR and Illumina Sequencing (Ethanol-Preserved Samples)

Sixty-five ethanol samples (total number of samples) were processed by spinning the sample gently by hand so that plankton was homogenously distributed throughout the ethanol, then removing 1 ml using a P1000 micropipette with the tip cut off so as not to be clogged with clumped or gelatinous samples. DNA was extracted using a QIAGEN DNeasy® Blood & Tissue Kit. DNA extractions were performed alongside negative controls consisting of kit components only with no plankton added. The quality and concentration of DNA was then tested using a NanoDrop™ 1000 Spectrophotometer, as was each control to ensure that there had been no external contamination. Polymerase Chain Reaction (PCR) assays were used to screen all 65 samples for haplosporidia using the primer pairs HapF1-HapR3 to amplify the small subunit (SSU) rDNA gene (Renault *et al*., 2000). Haplosporidian amplifications were carried out in a total volume of 20 µl, including 1 µl DNA, 10 µl ddH_20, 4 µl 5x Green GoTaq® Reaction Buffer (Promega), 4 µl dNTPs (1.25 mM stock solution), 0.2 µl GoTaq® DNA Polymerase (Promega) and 0.4 µl of forward and reverse primers. Reactions were carried out on a thermocycler at 95°C for three minutes, then 95°C (30 seconds), 48°C (30 seconds) and 72°C (30 seconds) for 35 cycles and finally 72°C for five minutes. All 65 samples were also screened for ostreid herpesvirus OsHV-1 µVar using the primer pair OHVA-OHVB to amplify the C region of the ORF4 gene (Lynch *et al*., 2013). OsHV-1 µVar screening was conducted as per Lynch *et al*. (2013), with the addition of 1.5 µl dMSO to the mix. Results were visualised using gel electrophoresis on a 2% agarose gel.

A subset of 32 ethanol-preserved samples representing the Irish Sea, Celtic Front, Eastern Celtic and Southern Celtic (see Figure 2) was selected for bacteriome sequencing. Samples were selected based on sea temperature data from Copernicus, to encompass the cooler Irish Sea water, the Celtic Front and meander, the Eastern Celtic (including a

coastal thermocline) and the warmer waters of the Southern Celtic Sea. Sequencing was conducted using the services of Novogene, Cambridge for Illumina 16S amplicon based metagenomic sequencing of the 16S rRNA gene coupled with their standard bioinformatics package. DNA was again extracted using a QIAGEN DNeasy® Blood & Tissue Kit coupled with negative controls, then packaged in freezer-blocks and shipped to Novogene for quality control and 16S rDNA amplicon sequencing. Quality control of the DNA was performed using a Qubit fluorimeter and agarose gel to confirm there was no degradation or protein contamination within the DNA. In the sequencing process the V3-V4 (466bp) region was amplified using the primer pair 341F (CCTAYGGGRBGCASCAG) and 806R (GGACTACNNGGGTATCTAAT). Amplicons were sequenced on the Illumina NovaSeq 6000 paired-end platform to generate 250 bp paired-end raw reads (Raw PE) with a sequencing depth of 100,000 tags, and then merged and pre-treated to obtain Clean Tags. Merging of reads was conducted using FLASH (Fast Length Adjustment of SHort reads). During data quality control the chimeric sequences in Clean Tags were detected using QIIME (Quantitative Insights Into Microbial Ecology) and removed to obtain the Effective Tags. Effective tags were grouped by 97% similarity into OTUs which were then used for subsequent analyses. OTU clustering and species annotation were performed using UPARSE, PyNAST (Python Nearest Alignment Space Termination) and Mothur, while subsequent species abundance and distribution analyses were performed using RDP (Ribosomal Database Project) and BLAST. Alpha and beta diversity analyses were conducted using QIIME and R.

Statistical analysis

Statistical modelling was conducted to determine if selected zooplankton taxa had an effect on prevalent bacterial phyla. Two linear mixed effect regressions (*LMER*) were used to explore the relationship between *i*) cyanobacteria and species groupings and *ii*) proteobacteria and species groupings. ‘Area’ (Irish Sea, Celtic Front, Eastern Celtic and Southern Celtic) was used as a random effect to account for repeated samples within regions. All statistical models underwent appropriate model checks and were conducted

in RStudio version 1.3. Models were selected through a step-wise selection comparing AIC values (Symonds & Moussalli, 2011).

Results

Zooplankton Community Structure

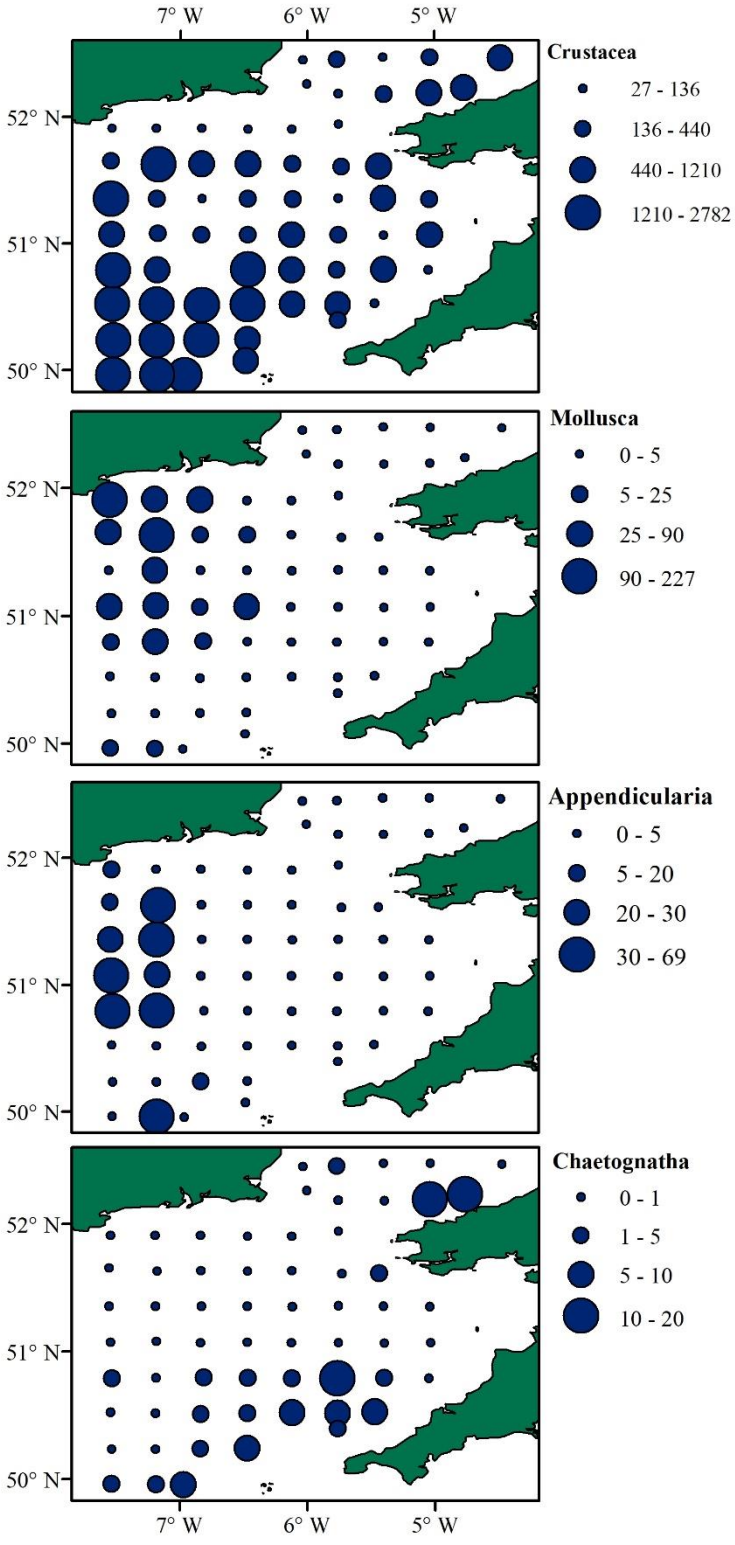
All 64 samples were dominated by crustaceans, particularly calanoid copepods and pleocyemata, both of which were present in all samples. Pleocyemata were not quantified to species level but were primarily composed of true crabs, porcelain crabs and squat lobster. Multiple crustacean life-stages were observed, for example copepod nauplii and adults, and euphausiid eggs, nauplii, calyptopis (early larval stage) and furcilia (late larval stages). Molluscan larvae had limited numbers of sea angels *Cliona* sp., (present in 10.94% of samples), more frequent sightings of bivalve larvae (31.25%) and high numbers of predatory sea snails (90.63%). There was also a single recording of a larval curled octopus, *Eledone cirrhosa*. Gelatinous communities were primarily composed of hydrozoa and ctenophores, with just three records of scyphozoa (4.69%) and three of anthozoa (4.69%). Other common records included appendicularians, chaetognaths, fish eggs and polychaetes. There were also high, albeit patchy, numbers of the dinoflagellate *Noctiluca scintillans*, so this was included despite not being a zooplankton taxon to encompass any microbiota potentially attached to the *N. scintillans* cells. Less common sightings included bryozoa (cyphonaute larvae), echinoderms and three recordings of a rarer taxon, phoronid larvae (horseshoe worms). See Table 1 for the presence (%) of all taxa.

Table 1: Percentage of formalin samples in which zooplankton taxa were present (n = 64).

Taxon	% Prevalence
Phylum Mollusca	
Class Gastropoda (Sea snails)	90.625
Class Bivalvia	31.250
Class Gastropoda (Sea angels)	10.938
Class Cephalopoda	1.563
Unidentified Molluscan sp.	1.563
Subphylum Crustacea	
Subclass Copepoda	100
Suborder Pleocyemata	100
Order Euphausiacea	98.438
Order Amphipoda	70.313
Suborder Cladocera	67.188
Euphausiid Eggs	20.313
Suborder Macrura Reptantia	6.250
Order Cumacea	6.250
Phylum Cnidaria	
Order Siphonophorae	90.625
Order Anthomedusae	81.250
Order Leptomedusae	62.500
Order Trachymedusae	54.688
Actinula larvae	9.375
Unidentified Cnidarian sp.	6.250
Other Taxa	
Class Appendicularia	92.188
Phylum Chaetognatha	90.625
Fish Eggs	76.563
Class Polychaeta	70.313

Phylum Ctenophora	51.563
Phylum Echinodermata	14.063
Phylum Bryozoa	12.500
Class Anthozoa	4.688
Phylum Phoronida	4.688
Class Scyphozoa	4.688
Phylum Nematoda	3.125
Phylum Arthropoda	1.563
Phylum Platyhelminthes	1.563
Noctiluca scintillans	15.625

Densities of the dominant zooplankton taxa (excluding fish eggs) are displayed in Figure 3, with crustaceans and molluscs displaying the highest numbers. *Noctiluca scintillans* was also included, despite not being a zooplankton taxon, as it contributed high numbers to the biomass of samples in which it was present.


7° W
6° W
5° W
52° N
51° N
50° N
Crustacea
27 - 136
136 - 440
440 - 1210
1210 - 2782
Mollusca
0 - 5
5 - 25
25 - 90
90 - 227
Appendicularia
0 - 5
5 - 20
20 - 30
30 - 69
Chaetognatha
0 - 1
1 - 5
5 - 10
10 - 20

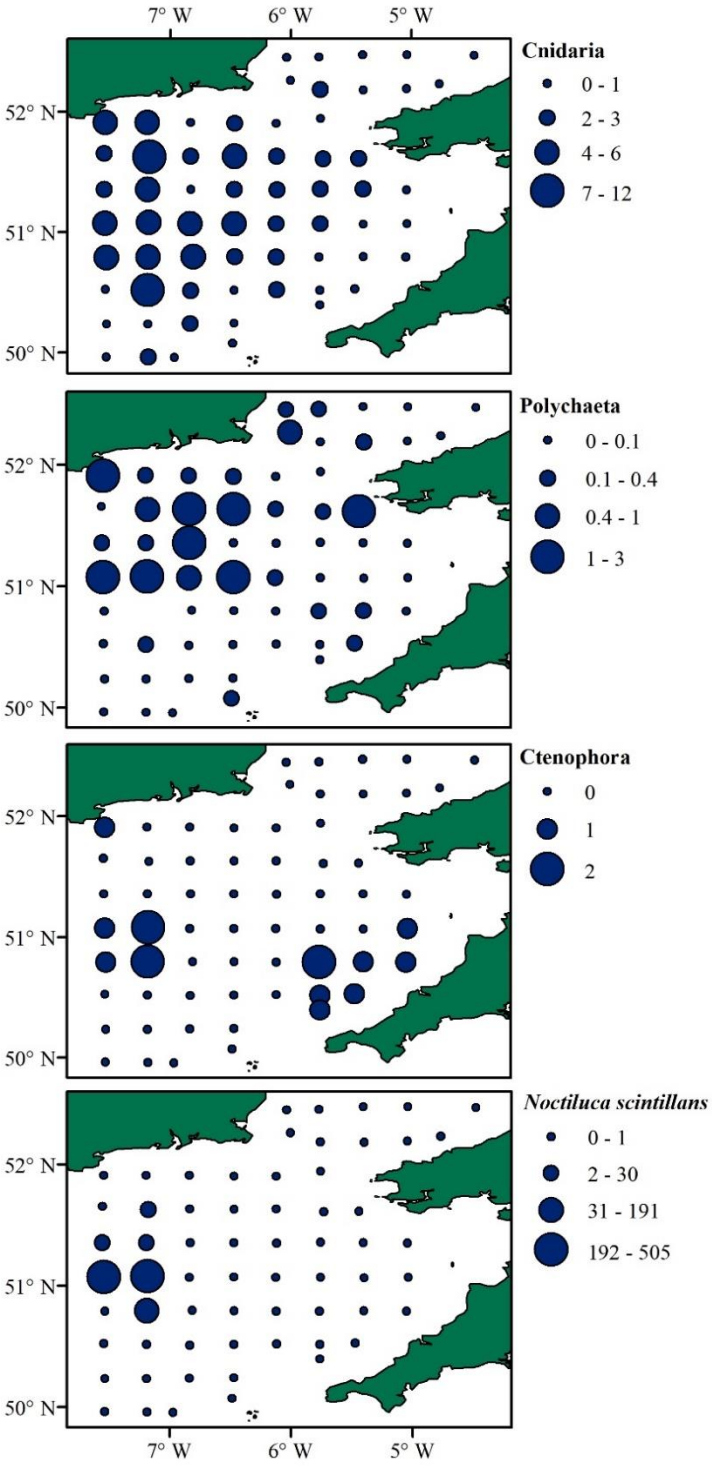


Figure 3: Density (per m^3) and distribution of the dominant species across the entire study area (n = 63 (two samples excluded - one compromised sample and one where the associated water volume was incorrectly recorded)). Note, the scale for each taxon is different.

Zooplankton Shannon diversity indices were conducted on plankton densities for the four subareas composed of the Irish Sea (n = 8), Celtic Front (n = 8), Eastern Celtic (n = 7) and Southern Celtic (n = 7), corresponding to samples that underwent amplicon based metagenomics. One sample from the Eastern Celtic was excluded from all analyses requiring zooplankton density as the volume of water filtered was incorrectly recorded. One sample was also excluded from the Southern Celtic as amplicon sequencing corresponding to that sample failed. Analyses were therefore standardised to density/m^3 and the Celtic Front had the highest diversity. The remaining three areas had similar indices, although the Southern Celtic was the lowest, however it is possible the slightly lower Southern Celtic index may be attributed to the vertical dip sampling protocol meaning less water was filtered (Table 2).

Table 2: Shannon diversity indices for zooplankton within the four subareas (standardised to density/m^3).

Area	**Shannon**
Celtic Front	0.912
Eastern Celtic	0.252
Irish Sea	0.199
Southern Celtic	0.104

Copepods were the most dominant zooplankton taxon in every area (by orders of magnitude), however when copepods were removed crustaceans remained the dominant taxa (Figure 4). The density and relative abundance of organisms per m^3 differed between areas, with the Irish Sea having the lowest density/abundance and the Celtic Front the highest.

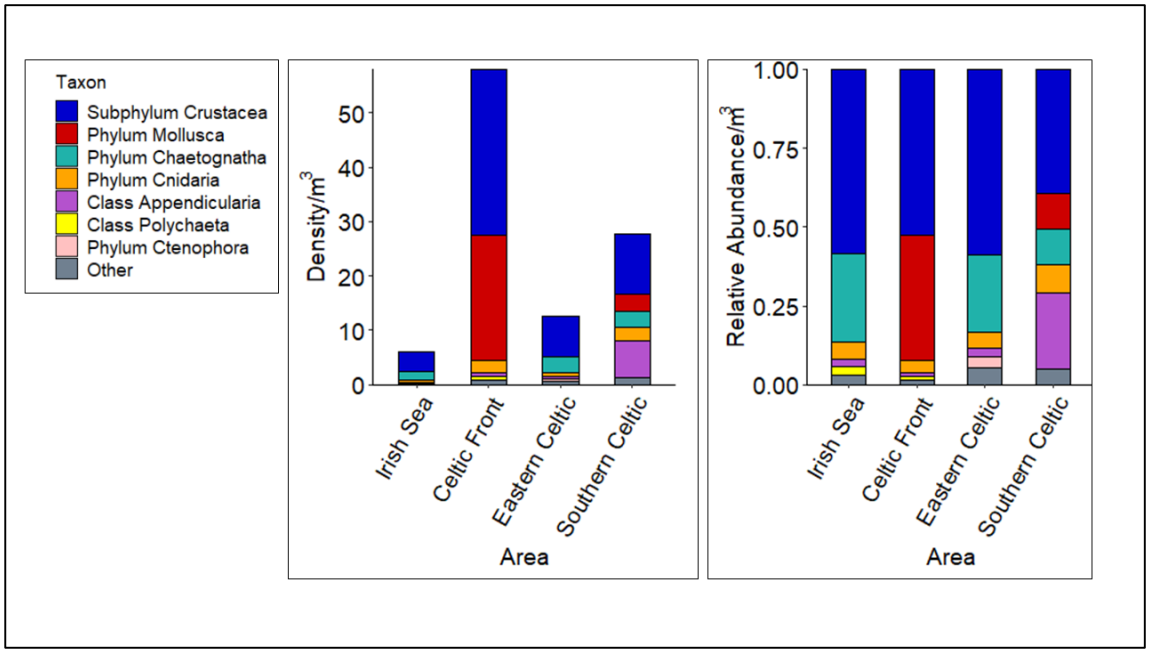


Figure 4: (A) Density/m^3 and (B) relative abundance/m^3 of the dominant zooplankton taxa in the Irish Sea, Celtic Front, Eastern Celtic and Southern Celtic collected in May 2018 (excluding copepods).

Screening of Potential Aquaculture Pathogens: Polymerase Chain Reaction

All 65 ethanol-preserved zooplankton samples (total number of samples) were screened by PCR for pathogens associated with aquaculture bivalve species. There was no detection of OsHV-1 µVar in any of the samples, however a single haplosporidian positive (1.54% prevalence) was detected in a sample south of Wexford in the meander area of the Celtic Sea Front region (sample 26 – see Figure 2). Sanger sequencing of this haplosporidian confirmed it to be an uncultured haplosporidian clone (BLASTn 99.47% match to 18_Haplo_BMVA_WEY, 65% Query Coverage) with GenBank Accession number KF208557.1.

Of the 32 samples sent for sequencing of the V3-V4 region; 31 (97%) were successfully sequenced (sample 55 being the exception). The resulting sample numbers for the four subareas were as follows: Irish Sea (n = 8), Celtic Front (n = 8), Eastern Celtic (n = 8) and Southern Celtic (n = 7). The Southern Celtic group had seven samples whereas all other groups had eight, however Rarefaction Curves demonstrated that the sample still provided an accurate representation of Operational Taxonomic Unit richness. See *Appendix A* for

further information about the OTUs in individual samples and rarefaction curves, plus details on Alpha and Beta diversity.

Screening of Potential Aquaculture Pathogens: Amplicon Based Metagenomics

The ten dominant bacterial families were assessed to determine their association to specific regions (Figure 5) and to ascertain if any families with potential impacts on bivalves were present (Table 3). The family Vibrionaceae was detected and further investigated, as it is known to contain species potentially pathogenic to aquaculture. Vibrionaceae had no association with the Irish Sea area, however it did have an association with the Celtic Front, Eastern Celtic and Southern Celtic Sea (Figure 5). There was also a single detection of the genus *Piscirickettsia* sp., which although too small to register statistically is potentially pathogenic to salmonid species.

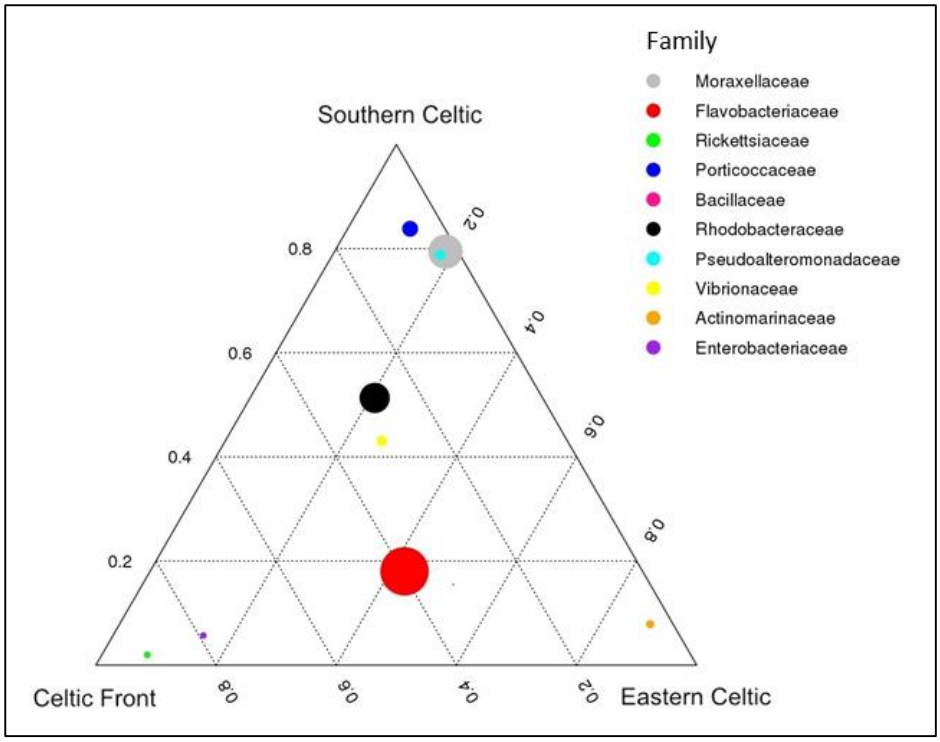


Figure 5: Ternary plot of the ten most abundant bacterial families associated with the Celtic Front, Eastern Celtic and Southern Celtic. The plot graphically depicts the proportion of bacterial abundance within each area, with values summing to one. Proximity to an axis corner shows higher relative abundance in that area compared to other areas, and larger circles indicate more dominant families.

Table 3: A brief description of the ten most abundant bacterial families and their potential pathogenicity to bivalves.

Family	Habitat	Pathogenic to Bivalves	References
Moraxellaceae	Terrestrial and aquatic systems	No	Rossau *et al*. (1991)
Flavobacteriaceae	Ubiquitous in terrestrial and aquatic systems	No	Gavriilidou *et al*. (2020)
Rickettsiaceae	Endosymbionts of primarily terrestrial arthropods, less dominant in aquatic systems	Rickettsiales-like organism (RLOs) are pathogenic	Klinges *et al*. (2019); Cruz-Flores *et al*. (2020)
Porticoccaceae	Mainly inhabit marine environments	No	Spring *et al*. (2015)
Bacillaceae	Ubiquitous in all ecosystems	No	Mandic-Mulec *et al*. (2015)
Rhodobacteraceae	Primarily marine and saline environments	No	Simon *et al*. (2017)
Pseudoalteromonadaceae	Primarily marine environments	No	WoRMS (2021)
Vibrionaceae	Ubiquitous and abundant in aquatic systems with a wide host-range	Yes	Thompson *et al*. (2004)
Actinomarinaceae	Terrestrial and aquatic systems	No	Ghai *et al*. (2013)
Enterobacteriaceae	Terrestrial and aquatic systems (frequently within host intestines)	No (bivalves may act as indicator-species for their presence)	Grevskott *et al*. (2017)

The Vibrionaceae family was composed of six *Vibrio* species plus a bioreactor metagenome sequence, with *V. splendidus* having the highest prevalence within the family (Table 4). The highest *V. splendidus* detection occurred in the Southern Celtic (comprising 3.05% of all bacterial species present), followed by the Eastern Celtic (comprising 1.78% of all bacterial species present). There were also trace amounts of *Photobacterium damselae* subs. *damselae*, pathogenic to cultured finfish and selected species of shark, turtle, cephalopod, shrimp and cetacean.

The two *Vibrio splendidus* OTU sequences arising from screening were input in BLASTn to identify any previous studies which had detected the same, and the associated environmental parameters. There was a Query Cover of 100% and Percentage Identities of 99.77% and 97.45% for a number of strains from a variety of sources on BLASTn (Table 5), displaying an association with oysters, phytoplankton cultures from bivalve hatcheries and marine sponges native to this present study region.

Table 4: The total percentage of Vibrionaceae species in each area, with samples from each group pooled. Percentages were calculated from the relative taxa abundance. The total percentage of non-Vibrionaceae families are included as ('Other') to clearly indicate the abundance of Vibrionaceae relative to other bacterial families.

Species	**Irish Sea (%)**	**Celtic Front (%)**	**Eastern Celtic (%)**	**Southern Celtic (%)**
Vibrio splendidus	0.0621	0.5528	1.7802	3.0477
Aliivibrio logei	0.0235	2.2656	0.4477	0.4086
Vibrio gallaecicus	0.3073	0.0156	0.013	0.0505
Photobacterium phosphoreum	0.003	0.0025	0.0149	0.0924
Photobacterium leiognathi subsp. *mandapamensis*	0.0005	0.0125	0.0003	0
Photobacterium damselae subsp. Damselae	0	0.0091	0	0
Bioreactor metagenome	0.0006	0.0017	0	0.0002
Other (Non-Vibrionaceae)	99.603	97.1402	97.7439	96.4006

Table 5: Sequencing of *Vibrio splendidus* strains in BLASTn. Results from the first five independent studies are included and in cases where a study had entered multiple sequences/accession numbers the first one is displayed.

OTU_145	**% Query Cover**	**% Identity**	**Accession Number**	**Year Entered to BLASTn**	**Sample Type**	**Geographic Location**	**Further Information**
Vibrio sp. strain 12e 16S ribosomal RNA gene, partial sequence	100	99.77	MT484171.1	2020	Genomic DNA	Nord-Pas-de-Calais, France	Crumb-of-Bread Sponge *Hymeniacidon perlevis* from Wimereux beach.
Vibrio splendidus strain FS1 16S ribosomal RNA gene, partial sequence	100	99.77	MT445179.1	2020	Genomic DNA	Not provided	Olive flounder *Paralichthys olivaceus* (North-western Pacific).
Vibrio sp. strain 915 16S ribosomal RNA gene, partial sequence	100	99.77	MN974047.1	2020	Genomic DNA	Basque Country	Coastal water samples, 5m, 500m, 1000m in depth.
Vibrio splendidus strain 6-2 16S ribosomal RNA gene, partial sequence	100	99.77	MN945385.1	2020	Genomic DNA	Not provided	Not provided
Vibrio sp. strain F2_24 16S ribosomal RNA gene, partial sequence	100	99.77	MN481029.1	2019	Genomic DNA	Not provided	Not provided
OTU_5840							
Uncultured bacterium clone SanDiego_a2897 16S ribosomal RNA gene, partial sequence	100	97.45	KF799449.1	2014	Genomic DNA	San Diego, USA and Naples, Italy	Pooled gut samples collected from the invasive Vase tunicate *Ciona intestinalis* in all seasons.
Vibrio sp. CG6 gene for 16S ribosomal RNA, partial sequence	100	97.45	AB819699.1	2013	Genomic DNA	Japan	Immersion seawater of shocked oyster.
Vibrio sp. 2197 partial 16S rRNA gene, isolate 2197	100	97.45	HF549237.1	2013	Genomic DNA	Galicia, Spain	Phytoplankton cultures in a bivalve hatchery. Primarily *splendidus* and *harvey*i clades.
Uncultured gamma proteobacterium clone FII-OX002 16S ribosomal RNA gene, partial sequence	100	97.45	JQ579651.1	2013	Genomic DNA	Galicia, Spain	Sediment samples from Figueiras Beach.
Uncultured *Vibrio* sp. clone H02C48-43 16S ribosomal RNA gene, partial sequence	100	97.45	HQ161442.1	2011	Genomic DNA	Eastern Mediterranean Sea (35.75 N 21.0 E)	Culture with chemotactic enrichments and carbon substrates.

Regional Differences in Bacterial Communities (V3-V4 Bacteriome Sequencing)

The number of Operational Taxonomic Units (OTUs) in each area was enumerated and OTUs common to ≥ two areas were visualised (Figure 6). The highest number of OTUs was found in the Eastern Celtic (n = 4,685) and the lowest in the Celtic Front (n = 3,378). In total there were 1,384 OTUs shared between all four areas.

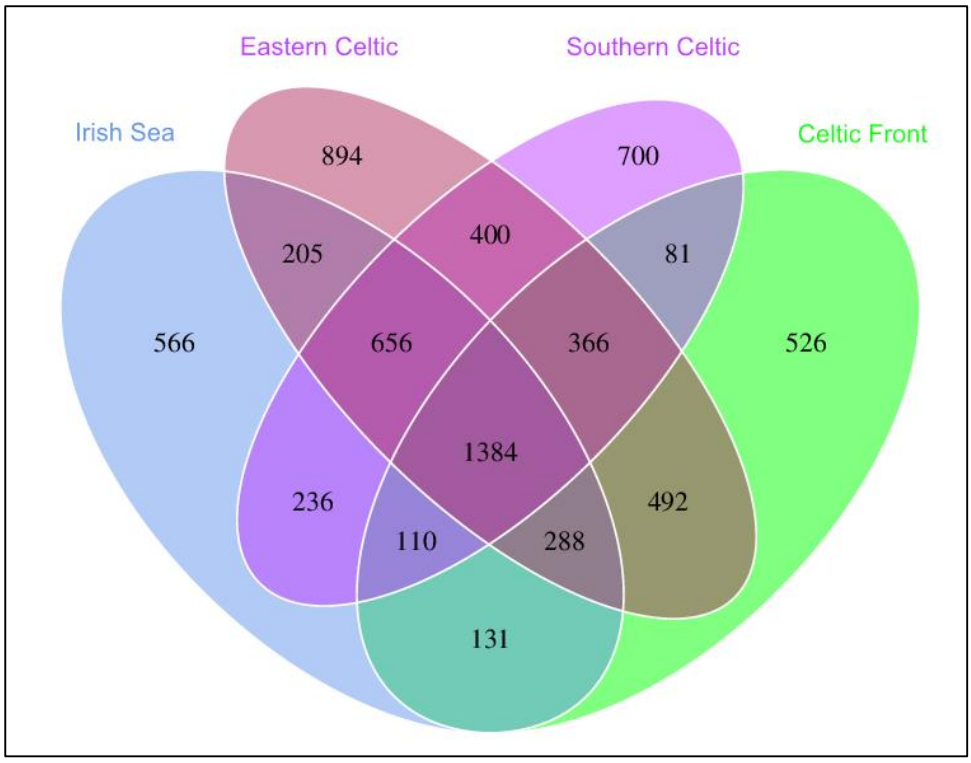


Figure 6: Zooplankton-associated bacterial OTUs depicting the OTUs unique to the Irish Sea, Celtic Front, Eastern Celtic and Celtic Sea and the OTUs that were shared between areas.

The two dominant bacterial phyla in the four areas were cyanobacteria and proteobacteria, with bacteroidetes also present. The abundance of cyanobacteria decreased moving south from the Irish Sea to the Celtic Front, then Eastern Celtic and lastly the Southern Celtic Sea, while the abundance of proteobacteria increased (Figure 7). Cyanobacteria were primarily composed of the Class Oxyphotobacteria, while Proteobacteria was composed of the Orders Pseudomonadales (28.12%), Rhodobacterales (24.83%), Cellvibrionales (19.5%), Alteromonadales (14.17%), Vibrionales (8.10%) and Rickettsiales (5.27%).

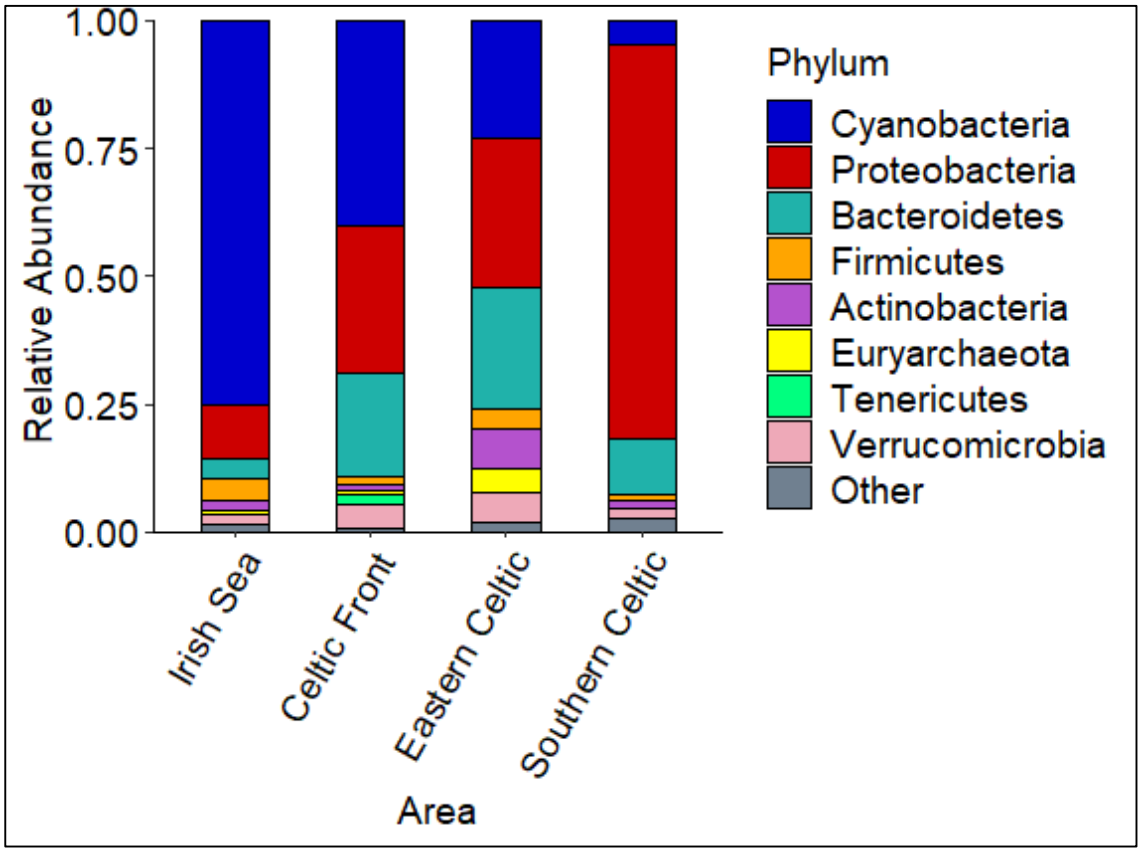


Figure 7: Relative abundance of the dominant zooplankton-associated bacterial phyla for the Irish Sea, Celtic Front, Eastern Celtic and Southern Celtic collected in May 2018. These phyla were detected in all samples, and samples within each group were pooled to provide the relative abundance as depicted here.

A cluster-tree was created using the Unweighted Pair-Group Method with Arithmetic Mean (UPGMA) and to demonstrate the connectivity of each individual sample (Figure 8). Samples with the closest distance, i.e. those that are most similar, are clustered together to form a new node on the tree. Samples from the Southern Celtic were clustered closely together, whereas samples from the Celtic Front were not clustered as closely.

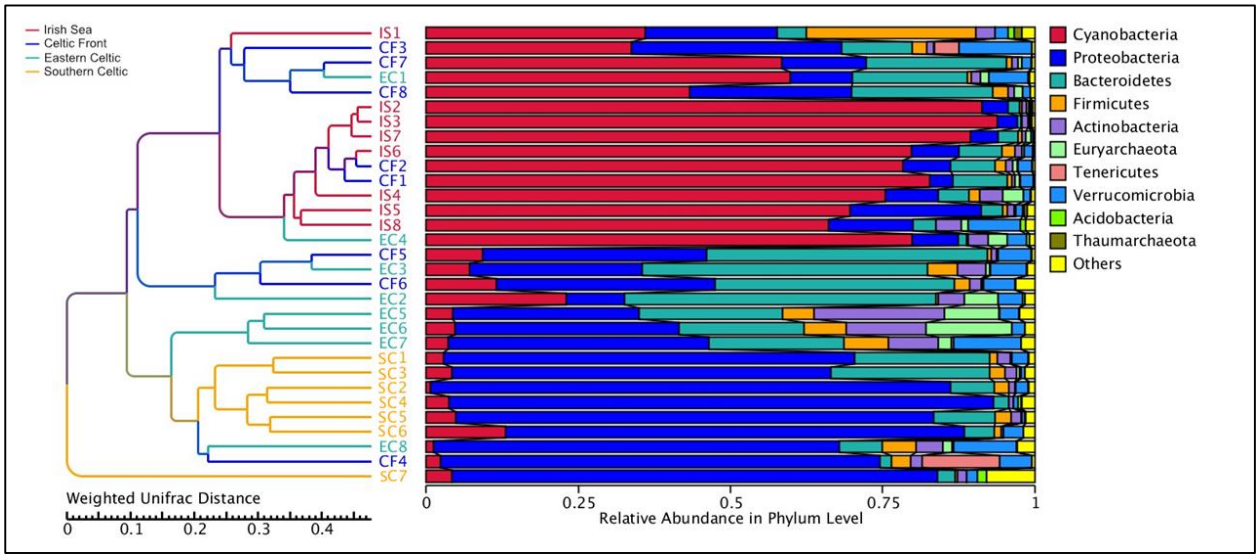


Figure 8: UPGMA tree based on weighted Unifrac distance demonstrating sample clustering and relative abundance at phylum level for samples that underwent bacteriome sequencing using amplicon based metagenomics (n = 31).

Analysis of Molecular Variance (AMOVA) was conducted on the OTUs to determine whether the difference of bacterial community structure between areas was significant. All area comparisons, with the exception of the Celtic Front-Eastern Celtic, demonstrated significant differences. The two groupings with the most significant differences ($p < 0.001$) were between the Irish Sea-Southern Celtic and a comparison of all four groups: Irish Sea-Celtic Front-Eastern Celtic-Southern Celtic (Table 6).

Table 6: AMOVA table showing groups where the difference in bacterial community structure was significant. (SS = sums of squares of deviations, df = degree of freedom, MS = SS/df, Fs = F-test value. Values in parentheses stand for Residual Error). F-test values were used to determine whether the variability between group means was larger than the variability of the observations within the groups i.e. there was differentiation between the populations. Significance was informed by the p-values.

Group	SS	df	MS	Fs	p-value
CF-EC	0.297(3.076)	1(14)	0.297(0.219)	1.351	0.249
IS-CF	0.686(1.680)	1(14)	0.686(0.120)	5.7198	**0.005**
IS-SC	2.765(1.106)	1(13)	2.765(0.085)	32.509	**<0.001**
IS-EC	1.339(2.037)	1(14)	1.339(0.145)	9.205	**0.001**
EC-SC	1.175(2.502)	1(13)	1.175(0.192)	6.104	**0.002**
CF-SC	1.413(2.145)	1(13)	1.413(0.165)	8.564	**0.001**
IS-CF-EC-SC	3.789(4.182)	3(27)	1.263(0.155)	8.153	**<0.001**

Zooplankton as Predictors of Bacteria

The six most abundant zooplankton taxa (Figure 4) were modelled against the two dominant bacterial phyla, cyanobacteria and proteobacteria (Figure 7), to determine if zooplankton community composition predicted bacterial composition. There was no discernibly robust statistical relationship between zooplankton taxa and cyanobacteria. A model predicting proteobacteria showed positive relationships with copepods ($F_{3.49}$ = 5.90, p<0.01), chaetognaths ($F_{25.3}$ = 5.05, p=0.070), and molluscs ($F_{25.8}$ = 13.78. p<0.01). This model had a marginal R^2= 0.548 and a conditional R^2= 0.642. The marginal R^2 represents the variation in the data explained by the fixed effects alone, while the conditional R^2 represents the variation in the data explained by the fixed effects and random effects. This shows support for a true relationship between copepods, chaetognaths and molluscs with proteobacteria with a moderate effect of geographic area in the random effects.

Discussion

This study investigated the associations between zooplankton communities and potential aquaculture pathogens in the Irish and Celtic Seas. Molecular screening revealed the presence of a haplosporidian sequence in one sample and low instances of the bacterium *Vibrio splendidus*, strains of which may be pathogenic to bivalves. These associations suggest that zooplankton may act as a transport mechanism for potentially pathogenic organisms or invasive pathogens, albeit to a limited extent. Overall, there was a regional influence in bacterial distribution and community structure, with significant differences between the Irish Sea, Celtic Front and Southern Celtic areas. A degree of connectivity was observed between the Eastern Celtic and Celtic Front, suggesting prevailing currents may be facilitating some bacterial mixing. Positive relationships were observed between selected zooplankton groups and particular bacterial communities, and zooplankton diversity was consistent with long-term plankton records from Plymouth Marine Laboratory (Conway, 2012).

The presence of multiple aquaculture sites within the Irish and Celtic Sea regions may contribute to the presence of potentially pathogenic microbes, as high density culture sites may facilitate disease transmission between bivalves present at those sites and release pathogens that disperse to other sites. Hydrodynamic movement will influence this dispersal, as zooplankton travel via prevailing currents along with their associated microbial communities. It is also possible that the potentially pathogenic microbes did not originate from aquaculture sites, but instead were drawn up with the prevailing flow from the Southern Celtic Sea, along the Eastern Celtic and across the Celtic Front.

The haplosporidian detected here was identified as a novel sequence first described in seawater samples collected off Weymouth, United Kingdom between 2011-2012 (along with further novel haplosporidian lineages) and the authors suggested that the haplosporidia detected were either parasites of planktonic hosts or possessed planktonic dispersal stages. The study also noted the sequence was closely aligned to the genus *Haplosporidium*, rather than *Bonamia*, *Minchinia* or *Urosporidium* (Hartikainen *et al*., 2013). This present detection occurred in a sample from the Celtic Front region, which differs from the original detection in that it was offshore rather than nearshore. This

detection indicates that the haplosporidian does have an association with plankton and/or seawater and its initial detection was not incidental. The zooplankton community composition in which the positive detection occurred was standard, being dominated by crustacea, hydrozoa and polychaetes. Characterising the diversity and distribution of haplosporidians is challenging and this present study builds on the work of Hartikainen *et al*. (2013) which aimed to reveal distinct lineages of previously undescribed haplosporidia. This genus contains important pathogens within bivalve aquaculture and it is necessary to develop an understanding of their diversity and potential impacts, as climate change may impact the distribution and life-history of hosts, pathogens and/or vectors potentially resulting in disease outbreaks (Lynch *et al*., 2020).

The proetobacterium *Vibrio splendidus* detected throughout this study is a species that is potentially pathogenic to bivalve aquaculture. The highest prevalence of *V. splendidus* in this study was detected in the Southern Celtic Sea area, with only trace amounts detected in the Irish Sea. It is possible that this is due to thermal differences, as warmer temperatures correspond to higher *Vibrio* abundance (Vezzulli *et al*., 2016). It may also be attributed to the prevailing currents moving up from the Celtic Sea and across St. George's Channel westwards, rather than into the Irish Sea.

Strains of *Vibrio splendidus* have been confirmed as the causative agents of disease in laboratory experiments utilising blue mussel larvae *Mytilus edulis* (Charles *et al*., 2019), carpet shell clams *Ruditapes decussatus (*Gómez-León *et al*., 2005), great scallops *Pecten maximus* (Sandlund *et al*., 2006) and Pacific cupped oyster spat *Crassostrea gigas* (Gay *et al*., 2004). The *V. splendidus* sequences detected in this study corresponded with sequences previously detected in phytoplankton cultures used as food sources for bivalve hatcheries in Galicia. The authors noted that hatchery production is highly susceptible to disease outbreaks arising from *Vibrio* infections, and monitoring routes of entry is a first step to preventing outbreaks (Dubert *et al*., 2015). Similar sequences were also found in the crumb-of bread sponge *Hymeniacidon perlevis* (Rodriguez Jimenez *et al*., 2021), a cosmopolitan species in European waters and the Vase tunicate *Ciona intestinalis*, a cryptogenic species in the North Atlantic that displays invasive tendencies. The combination of current-assisted movement and potential associations with native

invertebrates and invasive tunicates as seen in the literature suggests that *Vibrio splendidus* is a species with high dispersal capabilities.

Microbial biogeography in water columns is influenced by barriers such as fronts, density gradients and shelf bathymetry, and vectors such as eddies, currents and diapycnal mixing (Hernando-Morales *et al*., 2017). In this present study, distinct bacterial groupings were observed in the four areas comprising the Irish Sea, Celtic Front, Eastern Celtic and Southern Celtic, although different areas displayed different levels of OTU mixing. The UPGMA cluster tree revealed that samples from the Irish Sea, Celtic Front and Eastern Celtic were relatively well-mixed in their relationships, with a degree of connectivity between them as evidenced by shared OTUs, however samples from the Southern Celtic Sea were more contained within their group. The movement of bacteria is inherently linked to the life-history, physiology and behavior of the hosts. In oceanic environments zooplankton perform diel, seasonal or ontogenetic vertical and horizontal migration and this can span large distances which can then affect transport of the bacteria kilometres away. This migration can also allow bacteria to pass across otherwise impenetrable density gradients (Grossart *et al*. 2010). Both zooplankton and phytoplankton can aggregate along pycnoclines, sometimes for osmotic reasons, but also because at times pycnoclines can be a place of high nutrient flux. It is therefore likely that zooplankton migrations, coupled with aggregations in productive areas, are facilitating this bacterial mixing.

Despite the bacterial mixing and the presence of shared OTUs, nearly all inter-group comparisons resulted in significant differences between areas, the only exception being the Celtic Front-Eastern Celtic. It is perhaps unsurprising that there is no significant difference between these two areas, as the seasonal prevailing flow travels from the Eastern Celtic samples along the Celtic Front thereby demonstrating high levels of connectivity. The front may in turn inhibit movement to and from the Irish Sea. This inhibitory effect has been described previously, for example distinct zooplankton assemblages were observed dividing along the frontal boundary thereby producing sharp ecotones with no evidence of transitional communities (McGinty *et al*., 2014). It is possible that the distinct bacterial communities presented here are seasonal, and that the

microbial composition becomes more homogenous in winter months due to general flow of oceanic water onto the shelf and the breakdown of the Celtic Sea Front.

The two dominant phyla that contributed to the bacterial profiles were cyanobacteria and proteobacteria. The high prevalence of cyanobacteria within the zooplankton community is expected, as cyanobacterial cells contain proteins, lipids, minerals, carbohydrates and vitamins making them a known food-source for copepods and other zooplankton. Furthermore, cyanobacteria can themselves be a substrate for bacterial growth (Wilk-Woźniak, 2020). The prevalence of proteobacteria in the Celtic Sea is in-line with previous studies, as alphaproteobacteria have been confirmed to dominate bacterioplankton biomass in early summer in that region (Zubkov *et al*., 2001).

A positive relationship between proteobacteria and copepods, chaetognaths (arrow worms) (though not significant) and molluscs was detected in this study. The association between proteobacteria and multiple taxa suggests that the bacteria can adhere to different organic surface compositions – chitinous copepods, the calcium carbonate ($CaCO_3$) of the molluscs and the cuticle of chaetognaths. Bacteria are also known to adhere to taurine, and organo-sulfanate found in marine invertebrate tissues (De Corte *et al*., 2018). Chaetognath individuals also possess a large surface area, with many macroscopic individuals of ~1 cm in length observed in this study. This surface area could result in large biomass, meaning bacteria may encounter chaetognaths to colonise on a regular basis. Additionally, chaetognaths are primarily holoplanktonic predators and the fact that they do not settle out of the water column enhances the potential for bacterial dispersal facilitated by chaetognath movement. Copepods are similarly holoplanktonic, which may again facilitate bacterial dispersal, and represent a major reservoir for *Vibrio* spp. (contained within the Gammaprotebacteria) (Vezzulli *et al*., 2016).

Marine aquaculture is expanding into deeper offshore environments, primarily as a response to consumer demand. However, this expansion brings a series of challenges, including technological constraints, environmental concerns and the spread of disease (Gentry *et al*., 2017). Spatial distribution of aquaculture sites can play an important role in modifying this risk (Murray & Gubbins, 2016). Determining where aquaculture-associated pathogens are present and to where they can be potentially transported can be

used to plan mitigation efforts, for example siting molluscan aquaculture farms in low risk sites. This present study covered a wide area, however localised screening of zooplankton could be considered in proposed aquaculture sites, in conjunction with current profiles and oceanic dynamics, to better understand the potential for disease transmission. While this study covered a further offshore environment than would be utilised by aquaculture facilities, it can shed light on how pathogens may be maintained and transported to a more coastal environment. Intensive screening of the Southern Celtic Sea may be beneficial, to further describe the presence of *V. splendidus*. Further sampling may also detect other pathogenic species including *Vibrio aestuarianus*, known to persist in sediments thus enabling it to overwinter and emerge when temperatures are favourable (Azandégbé *et al*., 2010), or different haplosporidian species. Seasonal screening at proposed culture locations would also be valuable, to determine if temporal variation exists and if the seasonality of pathogen communities differs offshore compared to nearshore. Overall, determining the zooplankton-associated microbial communities will contribute to an understanding of regional hydrography and pathogen dispersal.

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References

Azandégbé, A., Garnier, M., Andrieux-Loyer, F., Kérouel, R., Philippon, X. & Nicolas, J-.L. (2010). Occurrence and seasonality of *Vibrio aestuarianus* in sediment and Crassostrea gigas haemolymph at two oyster farms in France. *Diseases of Aquatic Organisms*, 91, 213-221.

Arzul, I. & Carnegie, R. (2017). New perspective on the haplosporidian parasites of molluscs. *Journal of Invertebrate Pathology*, 131, 32-42.

Arzul, I., Gagnaire, B., Bond, C., Chollet, B., Morga, B., Ferrand, S., Robert, M. & Renault, T. (2009). Effects of temperature and salinity on the survival of *Bonamia ostreae*, a parasite infecting flat oysters *Ostrea edulis*. *Diseases of Aquatic Organisms*, 85, 67-75.

Arzul, I., Corbeil, S., Morga, B. & Renault, T. (2015). Viruses infecting marine molluscs. *Journal of Invertebrate Pathology*, 147, 118-135.

Baltar, F., Currie, K., Stuck, E., Roosa, S. & Morales, S.E. (2016). Oceanic fronts: transition zones for bacterioplankton community composition. *Environmental Microbiology Reports*, 8(1), 132-138.

Baxter, E.J., Rodger, H.D., McAllen, R. & Doyle, T.K. (2011). Gill disorders in marine-farmed salmon: investigating the role of hydrozoan jellyfish. *Aquaculture Environment Interactions*, 1, 245-257.

BIVALIFE (2014). Controlling Infectious Diseases in Oysters and Mussels in Europe. https://cordis.europa.eu/project/id/266157/reporting. Accessed 24/07/2021.

Bookelaar, B.E. (2018). Chapter 4: The spread of the oyster pathogen *Vibrio aestuarianus* from a "hotspot" of infection in the marine environment. PhD Thesis 'Understanding and minimizing the impacts of host-pathogen environment interactions in the Pacific oyster *Crassostrea gigas'*, 103-137.

Brown, J., Carrillo, L., Fernand, L., Horsburgh, K.J., Hill, A.E., Young, E.F. & Medler, K.J. (2003). Observations of the physical structure and seasonal jet-like circulation of the Celtic Sea and St. George's Channel of the Irish Sea. *Continental Shelf Research*, 23, 533-561.

Brown, E.A., Chain, F.J.J., Zhan, A., MacIsaac, H.J. & Cristescu, M.E. (2016). Early detection of aquatic invaders using metabarcoding reveals a high number of non-indigenous species in Canadian ports. *Diversity and Distributions*, 22, 1045-1059.

Cantrell, D.L., Groner, M.L., Ben-Horin, T., Grant, J. & Revie, C.W. (2020). Modeling Pathogen Dispersal in Marine Fish and Shellfish. *Trends in Parasitology*, 36(3), 239-249.

Charles, M., Trancart, S., Oden, E. & Houssin, M. (2019). Experimental infection of *Mytilus edulis* by two *Vibrio splendidus*-related strains: Determination of pathogenicity level of strains and influence of the origin and annual cycle of mussels on their sensitivity. *Journal of Fish Diseases*, 43, 9-21.

Clinton, M., Kintner, A.H., Delannoy, C.M.J., Brierley, A.S. & Ferrier, D.E.K. (2020). Molecular identification of potential aquaculture pathogens adherent to cnidarian zooplankton. *Aquaculture*, 518, 734801.

Conway, D.V.P. (2012). Marine zooplankton of southern Britain, Parts 1, 2 & 3. A.W.G. John (ed.). Occasional Publications. Marine Biological Association of the United Kingdom, Numbers 25, 26 & 27, Plymouth, United Kingdom.

Cottrell, M.T. & Kirchman, D.L. (2000). Community Composition of Marine Bacterioplankton Determined by 16S rRNA Gene Clone Libraries and Fluorescence In Situ Hybridization. *Applied and Environmental Microbiology*, 66, 5116-5122.

Cruz-Flores, R. & Cáceres-Martínez, J. (2020). Rickettsiales-like organisms in bivalves and marine gastropods: a review. *Reviews in Aquaculture*, 12, 2010-2026.

De Corte, D., Lekunberri, I., Sintes, E., Garcia, J.A.L., Gonzales, S. & Herndl, G.J. (2014). Linkage between copepods and bacteria in the North Atlantic Ocean. *Aquatic Microbial Ecology*, 72, 215-225.

De Corte, D., Srivasta, A., Koski, M., Garcia, J.A.L., Takaki, Y., Yokokawa, T., Nunoura, T., Elisabeth, N.H., Sintes, E. & Herndl, G.J. (2018). Metagenomic insights into zooplankton-associated bacterial communities. *Environmental Microbiology*, 20(2), 492-505.

De Magny, G., Mozumder, P.K., Grim, C.J., Hasan, N.A., Niamul Naser, M., Alam, M., Sack, R.B., Huq, A. & Colwell, R.R. (2011). Role of Zooplankton Diversity in *Vibrio cholerae* Population Dynamics and in the Incidence of Cholera in the Bangladesh Sundarbans. *Applied and Environmental Microbiology*, 77, 6125-6132.

De Oliveira Soares, M., Coelho Campos, C., Oliveira Santos, N.M., de Sousa Barroso, H., Targino Mota, E.M., Bezerra de Menezes, M.O., Rossi, S. & Martins Garcia, T. (2018). Marine bioinvasions: Differences in tropical copepod communities between inside and outside a port. *Journal of Sea Research*, 134, 42-48.

Dubert, J., Fernández-Pardo, A., Nóvoa, S., Barja, J.L. & Prado, S. (2015). Phytoplankton production systems in a shellfish hatchery: variations of the bacterial load and diversity of vibrios. *Journal of Applied Microbiology*, 118, 1264-1275.

Evans, O., Paul-Pont, I., Hick, P. & Whittington, R.J. (2014). A simple centrifugation method for improving the detection of Ostreid herpesvirus-1 (OsHV-1) in natural seawater samples with an assessment of the potential for particulate attachment. *Journal of Virological Methods*, 210, 59-66.

García-López, R., Cornejo-Granados, F., Lopez-Zavala, A.A., Sánchez-López, F., Cota-Huízar, A., Sotelo-Mundo, R.R., Guerrero, A., Mendoza-Vargas, A., Gómez-Gil, B. & Ochoa-Leyva, A. (2020). Doing More with Less: A Comparison of 16S Hypervariable Regions in Search of Defining the Shrimp Microbiota. *Microorganisms*, 1-28 doi:10.3390/microorganisms8010134 www.

Gavriilidou, A., Gutleben, J., Versluis, D., Forgiarini, F., van Passel, M.W.J., Ingham, C.J., Smidt, H. & Sipkema, D. (2020). Comparative genomic analysis of Flavobacteriaceae: insights into carbohydrate metabolism, gliding motility and secondary metabolite biosynthesis. *BMC Genomics*, 21(569), 1-21.

Gay, M., Renault, T., Pons, A.-M. & Le Roux, F. (2004). Two *Vibrio splendidus* related strains collaborate to kill *Crassostrea gigas*: taxonomy and host alterations. *Diseases of Aquatic Organisms*, 62, 65-74.

Gentry, R.R., Lester, S.E., Kappel, C.V., White, C., Bell, T.W., Stevens, J. & Gaines, S.D. (2017). Offshore aquaculture: Spatial planning principles for sustainable development. *Ecology and Evolution*, 7, 733-743.

Ghai, R., Megumi Mizuno, C., Picazo, A., Camacho, A. & Rodriquez-Valera, F. (2013). Metagenomics uncovers a new group of low GC and ultra-small marine Actinobacteria. *Scientific Reports*, 3(2471), doi: 10.1038/srep02471.

Gómez-León, J., Villamil, L., Lemos, M.L., Novoa, B. & Figueras, A. (2005). Isolation of *Vibrio alginolyticus* and *Vibrio splendidus* from Aquacultured Carpet Shell Clam (*Ruditapes decussatus*) Larvae Associated with Mass Mortalities. *Applied and Environmental Microbiology*, 71, 98-104.

Grevskott, D.H., Svanevik, C.S., Sunde, M., Wester, A.L. & Lunestad, B.T. (2017). Marine Bivalve Mollusks As Possible Indicators of Multidrug-Resistant *Escherichia coli* and Other Species of the Enterobacteriaceae Family. *Frontiers in Microbiology*, 8(24), doi: 10.3389/fmicb.2017.00024, 1-11.

Grossart, H.-P., Dziallas, C., Leunert, F. & Tang, K.W. (2010). Bacteria dispersal by hitchhiking on zooplankton. *Proceedings of the National Academy of Sciences*, 107(26), 11959-11964.

Haberlin, D., Mapstone, G., McAllen, R., McEvoy, A.J. & Doyle, T.K. (2016). Diversity and occurrence of siphonophores in Irish coastal waters. *Biology and Environment: Proceedings of the Royal Irish Academy*, 116B(2), 1-11.

Harriague, A.C., Di Brino, M., Zampini, M., Albertelli, G., Pruzzo, C. & Misic, C. (2008). Vibrios in association with sedimentary crustaceans in three beaches of the northern Adriatic Sea (Italy). *Marine Pollution Bulletin*, 56, 574-579.

Hartikainen, H., Ashford, O.S., Berney, C., Okamura, B., Feist, S.W., Baker-Austin, C., Stentiford, G.D. & Bass, D. (2013). Lineage-specific molecular probing reveals novel diversity and ecological partitioning of haplosporidians. *The ISME Journal*, 8, 177-186.

Hays, G.C., Richardson, A.J. & Robinson, C. (2005) Climate Change and Marine Plankton. *Trends in Ecology and Evolution*, 26, 337-344.

Hernando-Morales, V., Ameneiro, J. & Teira, E. (2017). Water mass mixing shapes bacterial biogeography in a highly hydrodynamic region of the Southern Ocean. *Environmental Microbiology*, 19(3), 1017-1029.

Hill, A.E., Brown, J., Fernand, L., Holt, J., Horsburgh, K.J., Proctor, R., Raine, R. & Turrell, W.R. (2008). Thermohaline circulation of shallow tidal seas. *Geophysical Research Letters*, 35 (L11605), 1-4.

Klinges, J.G., Rosales, S.M., McMinds, R., Shaver, E.C., Shantz, A.A., Peters, E.C., Eitel, M., Wörheide, G., Sharp, K.H., Burkepile, D.E., Silliman, B.R. & Vega Thurber, R.L. (2019). Phylogenetic, genomic, and biogeographic characterization of a novel and ubiquitous marine invertebrate-associated Rickettsiales parasite, *Candidatus* Aquarickettsia rohweri, gen. nov., sp. nov. The *ISME Journal*, 13, 2938-2953.

Lynch, S.A., Armitage, D.V., Coughlan, J., Mulcahy, M.F. & Culloty, S.C. (2007). Investigating the possible role of benthic macroinvertebrates and zooplankton in the life cycle of the haplosporidian *Bonamia ostreae*. *Experimental Parasitology*, 115, 359-368.

Lynch, S., Dillane, E., Carlsson, J. & Culloty S.C. (2013). Development and Assessment of a Sensitive and Cost-Effective Polymerase Chain Reaction to Detect Ostreid Herpesvirus 1 and Variants. *Journal of Shellfish Research*, 32(3), 657-664.

Lynch, S.C., Lepée-Rivero, S., Kelly, R, Quinn, E., Coghlan, A., Bookelaar, B., Morgan, E., Finarelli, J.A., Carlsson, J. & Culloty, S.C. (2020). Detection of haplosporidian protistan parasites supports an increase to their known diversity, geographic range and bivalve host specificity. *Parasitology*, 147, 584-592.

Ma, L., Mao, G., Liu, J., Gao, G., Zou, C. Bartlam, M.G. & Wang, T. (2016). Spatial-Temporal Changes of Bacterioplankton Community along an Exhorheic River. *Frontiers in Microbiology*, doi: 10.3389/fmicb.2016.00250.

Mandic-Mulec, I., Stefanic, P. & van Elsas, J.D. (2015). Ecology of Bacillaceae. *Microbial Spectrum*, 3(2), doi:10.1128/microbiolspec.TBS-0017-2013.

McCallum, H., Harvell, D. & Dobson, A. (2003). Rates of Spread of Marine Pathogens. *Ecology Letters*, 6, 1062-1067.

McGinty, N., Johnson, M.P. & Power, A.M. (2014). Spatial mismatch between phytoplankton and zooplankton biomass at the Celtic Boundary Front. *Journal of Plankton Research*, 36, 1446-1460.

Mestre, M., Borrull, E., Montserrat Sala, M. & Gasol, J.M. (2017). Patterns of bacterial diversity in the marine planktonic particulate matter continuum. *The ISME Journal*, 11, 999-1010.

Milici, M., Tomasch., J., Wos-Oxley, M.L., Wang, H., Jáuregui, R., Camarinha-Silva, A., Deng, Z-L., Plumeier, I., Giebel, H-A., Wurst, M., Pieper, D.H., Simon, M. & Wagner-Döbler, I. (2015). Low diversity of planktonic bacteria in the tropical ocean. *Scientific Reports*, 6, 19054.

Murray, A.G. & Gubbins, M. (2016). Spatial management measures for disease mitigation as practiced in Scottish aquaculture. *Marine Policy*, 70, 93-100.

Pingree, R.D., Sinha, B. & Griffiths, C.R. (1999). Seasonality of the European slope current (Goban Spur) and ocean margin exchange. *Continental Shelf Research*, 19, 929-975.

Pitz, K.J., Guo, J., Johnson, S.B., Campbell, T.L., Zhang, H., Vrijenhoek, R.C., Chavez, F.P. & Geller, J. (2020). Zooplankton biogeographic boundaries in the California Current System as determined from metabarcoding. *PLoS ONE*, 15(6), e0235159, 1-20.

Ratcliffe, F.C., Uren Webster, T.M., Garcia de Leaniz, C. & Consuegra, S. (2020). A drop in the ocean: Monitoring fish communities in spawning areas using environmental DNA. *Environmental DNA, Special Issue*, 1-12, doi: 10.1002/edn3.87.

Renault, T., Stokes, N.A., Chollet, B., Cochennec, N., Berthe, F., Gérard, A. & Burreson, E.M. (2000). Haplosporidiosis in the Pacific oyster *Crassostrea gigas* from the French Atlantic coast. *Diseases of Aquatic Organisms*, 42, 207-214.

Rossau, R., Van Landschoot, A., Gillis, M. & De Ley, J. (1991). Taxonomy of Moraxellaceae fam. nov., a New Bacterial Family To Accommodate the Genera Moraxella, Acinetobacter, and Psychrobacter and Related Organisms. *International Journal of Systematic and Evolutionary Bacteriology*, 41(2), 310-319.

Rowley, A.F., Cross, M.E., Culloty, S.C., Lynch, S.A., Mackenzie, C.L., Morgan, E., O'Riordan, R.M., Robins, P.E., Smith, A.L., Thrupp, T.J., Vogan, C.L., Wootton, E.C. & Malham, S.K. (2014). The potential impact of climate change on the infectious diseases of commercially important shellfish populations in the Irish Sea - A review. *ICES Journal of Marine Science*, 71, 741-759.

Rodriguez Jimenez, A., Dechamps, E., Giaux, A., Goetghebuer, L., Bauwens, M., Willenz, P., Flahaut, S., Laport, M.S. & George, I.F. (2020). The sponges *Hymeniacidon perlevis* and *Halichondria panicea* are reservoirs of antibiotic-producing bacteria against multi-drug resistant *Staphylococcus aureus*. *Journal of Applied Microbiology*, doi:10.1111/jam.14999.

Romero, A., del mar Costa, M., Forn-Cuni, G., Balseiro, P., Chamorro, R., Dios, S., Figueras, A. & Novoa, B. (2014). Occurrence, seasonality and infectivity of *Vibrio* strains in natural populations of mussels *Mytilus galloprovincialis*. *Diseases of Aquatic Organisms*, 108, 149-163.

Sandlund, N., Torkildsen, L., Magnesen, T., Mortensen, S. & Bergh, Ø. (2006). Immunohistochemistry of great scallop *Pecten maximus* larvae experimentally challenged with pathogenic bacteria. *Diseases of Aquatic Organisms*, 69, 163-173.

Saulnier, D., De Decker, S., Haffner, P., Cobret, L., Robert, M. & Garcia, C. (2010). A Large-Scale Epidemiological Study to Identify Bacteria Pathogenic to Pacific Oyster *Crassostrea gigas* and Correlation Between Virulence and Metalloprotease-like Activity. *Microbial Ecology*, 59, 787-798.

Shan, D., Wei, G., Li, M., Wang, W., Li, X., Gao, Z. & Shao, Z. (2015). Distribution and diversity of bacterioplankton communities in subtropical seawater around Xiamen Island, China. *Microbiological Research*, 175, 16-23.

Simon, M., Scheuner, C., Meier-Kolthoff, J.P., Brinkhoff, T., Wagner-Döbler, I., Ulbrich, M., Klenk, H.-P., Schomburg, D., Petersen, J. & Göker, M. (2017). Phylogenomics of Rhodobacteraceae reveals evolutionary adaptation to marine and non-marine habitats. *The ISME Journal*, 11, 1483-1499.

Small, H.J. & Pagenkopp, K.M. (2011). Reservoirs and alternate hosts for pathogens of commercially important crustaceans: A review. *Journal of Invertebrate Pathology*, 106, 153-164.

Solomieu, V.B., Renault, T. and Travers, M.-A. (2015). Mass mortality in bivalves and the intricate case of the Pacific oyster, *Crassostrea gigas*. *Journal of Invertebrate Pathology*, 131, 2-10.

Spring, S., Scheuer, C., Göker, M. & Klenk, H.-P. (2015). A taxonomic framework for emerging groups of ecologically important marine gammaproteobacteria based on the reconstruction of evolutionary relationships using genome-scale data. *Frontiers in Microbiology*, 6(281). doi: 10.3389/fmicb.2015.00281.

Sutherland, W.J. & Woodroof, H.J. (2009). The need for environmental horizon scanning. *Trends in Ecology and Evolution*, 24, 523-527.

Symonds, M.R.E. & Moussalli, A. (2011). A brief guide to model selection, multimodel inference and model averaging in behavioural ecology using Akaike's information criterion. *Behavioral Ecology and Sociobiology*, 65(1), 13-21.

Thompson, F.L., Iida, T. & Swings, J. (2004). Biodiversity of Vibrios. *Microbiology and Molecular Biology Reviews*, 68(3), 403-431.

Troussellier, M., Escalas, A, Bouvier, T. & Mouillot, D. (2017). Sustaining Rare Marine Microorganisms: Macroorganisms As Repositories and Dispersal Agents of Microbial Diversity. *Frontiers in Microbiology*, 8:947. doi:10.3389/fmicb.2017.00947.

Vezzulli, L., Grande, C., Reid, P.C., Hélaouët, P., Edwards, M., Höfle, M.G., Brettar, I., Colwell, R.R. & Pruzzo, C. (2016). Climate influence on *Vibrio* and associated human diseases during the past half-century in the coastal North Atlantic. *Proceedings of the National Academy of Sciences*, 113, 1-10.

Vigneron, V., Solliec, G., Montanié, H. & Renault, T. (2004). Detection of Ostreid Herpesvirus 1 (OsHV-1) DNA in seawater by PCR: influence of water parameters in bioassays. *Diseases of Aquatic Organisms*, 62, 35-44.

VIVALDI (2020). Preventing and Mitigating Farmed Bivalve Diseases. https://www.vivaldi-project.eu/Activities/Our-key-study-sites/Dungarvan-Bay-Marine-Institute-Ireland. Accessed 23/07/2021.

Ward, G.M., Feist, S.W., Noguera, P., Marcos-López, M., Ross, S., Green, M., Urrutia, A., Bignell, J.P. & Bass, D. (2019). Detection and characterisation of haplosporidian parasites of the blue mussel *Mytilus edulis*, including description of the novel parasite *Minchinia mytili* n. sp. *Diseases of Aquatic Organisms*, 133, 57-68.

Wilk-Woźniak, E. (2020). An introduction to the 'micronet' of cyanobacterial harmful algal blooms (CyanoHABs): cyanobacteria, zooplankton and microorganisms: a review. *Marine and Freshwater Research*, 71, 636-643.

WoRMS - World Register of Marine Species (2021). http://www.marinespecies.org/aphia.php?p=taxdetails&id=570323. Accessed 27/03/2021.

Zubkov, M.V., Fuchs, B.M., Burkill, P.H. & Amann, R. (2001). Comparison of Cellular and Biomass Specific Activities of Dominant Bacterioplankton Groups in Stratified Waters of the Celtic Sea. *Applied and Environmental Microbiology*, 67(11), 5210-5218.

Chapter 5: Assessing the Potential for Invasive Species Introductions and Secondary Spread Using Vessel Movements in Maritime Ports

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Abstract

Ship-mediated transport can facilitate the introduction of invasive species and pathogens via ballast water and hull fouling, with deleterious impacts on native ecosystems. Regional management of invasive species may be strengthened by identifying the routes in a shipping network, to allow for targeted ship inspections and surveys. As an island nation, Ireland is heavily dependent on the shipping industry for trade and transport, leading to a heightened risk of species introductions. This study used cargo shipping records over a two-year timeframe to establish the connectivity of shipping routes between international ports and four ports in Ireland, selected due to high shipping traffic. Potential pathways of invasion were ranked by establishing the connections to ports where multiple voyages originate. A horizon scanning exercise for species likely to invade in Ireland (and with potential aquaculture impacts) was carried out, with a focus on the following species: American razor clam *Ensis leei* (*directus*), Asian shore crab *Hemigrapsus sanguineus*, Brush clawed shore crab *Hemigrapsus takanoi*, Chinese mitten crab *Eriocheir sinensis* and the American slipper limpet *Crepidula fornicata* which is already established in Northern Ireland. These species are all invasive along European coastlines and can utilise vessels as vectors of invasion. Furthermore, each species may interact with parasites thus facilitating co-invasions of their associated micro/macroparasitic communities. A total of 9,291 cargo ship journeys were analysed, to investigate vessel residence times and journey times and to determine the relative importance of hull fouling and ballast water. On average, vessels spent up to five days in port and less than five days at sea also. However, there was strong variation, for example general cargo ships (representing 13.7% of all journeys) recorded times of up to 13 days in port. Seventeen ports contributed over 50 journeys to ports in Ireland, and the presence of the focal invasives (as listed above) in these ports suggested that the potential for invasion is a strong possibility in the near future, with further potential for parasitic co-invasions. High connectivity across the island of Ireland means that secondary spread is also likely.

Keywords: Ballast Water, Hull fouling, Cargo Shipping, Horizon Scanning,

Introduction

The global oceanic shipping network accounts for over 80% of the world's trade (Sardain *et al*., 2019) and is projected to continue to expand in the future (Valdor *et al*., 2020). The extent of this global network results in high connectivity between coastal regions worldwide, with the movement of ships facilitating the transport of organisms across large spatial scales and across otherwise restrictive biogeographic barriers. This ship-mediated transport can lead to the introduction of invasive species and pathogens, disrupting coastal ecosystem functioning and contributing to the spread of disease (de Castro *et al*., 2017).

Invasive species richness in the marine environment is high, for example de Castro *et al*. (2017) reviewed literature relating to the shipping trade and invasive species in the Atlantic Ocean and collated records of 44 high-impact species associated with the northeast Atlantic and 15 high-impact species associated with the southwest Atlantic, with ballast water and biofouling identified as the most likely vectors of introduction. The blue crab (*Callinectes sapidus*) is one such invasive species, native to the western Atlantic but introduced via ballast water to Asia and Europe (particularly the Mediterranean) where it poses a threat to cultivation of bivalves such as *Mytilus galloprovincialis* (Prado *et al*., 2020). Until recently the blue crab had never been sighted in Ireland, however a single sighting (of a dead individual) was reported from Dollymount Strand, Dublin to the National Biodiversity Centre Ireland (Biodiversity Ireland, 2021) in February 2021, followed by a crab claw sighting, thought to be from the same individual, in March. The method of introduction remains unknown but ship-mediated transport was suggested, as was the theory that it was imported for the culinary trade and subsequently released.

Ship tracking has provided a mechanism by which to identify likely routes of invasion, particularly after the introduction of the Automatic Identification System (AIS) in 2004 (O'Brien *et al*., 2017). This is an important aspect of regional management, allowing for both horizon scanning exercises and targeted surveys to identify and remove species before they have the chance to establish and proliferate (Seebens *et al*., 2013). Horizon scanning is the systematic review of prospective threats and opportunities and is applied to invasive species management by prioritising the threats posed by potential invasives (Roy *et al*., 2014, Lucy *et al*., 2020). Crucially, most introduced species remain scarce in

the new environment and distinguishing between those that will cause serious harm and those whose effects will be minimal ensures that management efforts for control can be appropriately deployed (Colautti *et al*., 2014). Targeted 'next pest' surveys are increasingly being utilised to design monitoring surveys for species that are identified in horizon scanning exercises as those are most likely to establish in the near future and cause deleterious impacts (Bishop & Hutchings, 2011). These exercises assess invasion patterns relative to biotic and abiotic factors, identify vectors that are prevalent in the jurisdiction, and focus on species that display invasive tendencies elsewhere. Scanning is also undertaken with the proviso that it is important not to become too focused and potentially miss other species (Bishop & Hutchings, 2011).

The two primary shipping vectors by which invasive species are transported currently in the marine environment are ballast water and hull fouling. Throughout the transit, potentially invasive species are subject to stressors associated with the invasion pathway, including fluctuating oceanic and coastal conditions, water washing over the hull and variable oxygen concentrations in the ballast water. Regardless, recipient habitats are colonised even at long distances from the donor site provided that the environmental conditions are suitable, although longer distances mean lower survival (Bouda *et al*., 2018). Although it is difficult to assign propagule pressures to ships, decreased transit times can increase invasion risk (Seebens *et al*., 2013; O'Brien *et al*., 2017).

Ballast water tanks can contain a diverse array of taxa and life-history stages. Sampling studies primarily reveal larval organisms and individuals measuring just millimeters in length, however there have been instances of juvenile decapod crustaceans and fish being recorded (Gollasch & David, 2019). Given the presence of mesh coverings protecting ballast water intake systems it is likely that these larger organisms were either taken in as eggs/larvae or indeed that reproduction occurred inside the tank. Sediment sampling inside the ballast tanks has also revealed the presence of eggs, cysts, phytoplankton spores and invertebrate juvenile and adult stages. Furthermore, biofouling on internal walls can facilitate the spread of species that reproduce in between tank cleaning cycles which range from 2-5 years (Gollasch & David, 2019). To date, ballast water invasions are better represented by invertebrates and harmful algae, but there is strong evidence that it can

also be a vector for bacterial pathogens including faecal-indicator species and *Vibrio* spp. (Lymperopoulou & Dobbs, 2017).

As a result of the high species diversity contained within, ballast water has taken precedence over biofouling as a vector of concern in recent years, and the International Maritime Organisation (IMO) has therefore focused on ballast water controls (Bouda *et al*., 2018) with the International Convention for the Control and Management of Ships' Ballast Water and Sediments (2004) entering into force in September 2017. The IMO Convention aims to prevent the introduction and proliferation of invasive species and has been adopted by 81 state parties, representing 80.76% of the gross tonnage of the global merchant fleet (UNCTAD, 2019).

The two dominant measures within the Convention are termed D-1 and D-2, with D-1 enforcing exchange and release of 95% of ballast volume away at least 200 nautical miles from the coast in water at least 200 m in depth. When these requirements cannot be met, areas may be designated where ships can conduct Ballast Water Exchange (BWE) (GloBallast, 2017). Three distinct groupings form the basis of D-2 regulatory standards, namely specified maximum concentrations of indicator bacteria including *Escherichia coli*, *Vibrio cholerae* and intestinal *Enterococci*, organisms >50 μm and organisms >10 μm but <50 μm (Tamburri *et al*., 2020). However, there is a focus on organisms >50 μm, meaning regulations may have little to no effect on microbiota (Pagenkopp Lohan *et al*., 2020).

Ballast water exchange as a method of control provides limited biosecurity, as rough seas and stability concerns can inhibit ballast water exchange (Hess-Erga *et al*., 2019). Furthermore, ships that transit within the same territorial waters, typically 200 nautical miles from shore, do not have to conduct BWE, which may then facilitate dispersal of introduced organisms within a country (Brinkmeyer, 2016). In many cases BWE is considered the 'Best Available Technology' (Carney *et al*., 2017) and is considered an interim measure only, indeed ballast water samples have revealed geographic affiliation of bacterial, phytoplankton and zooplankton assemblages that is inconsistent with complete ocean exchange (Lymperopoulou & Dobbs, 2017). As a result, open sea BWE is due to be phased out entirely by 2024 (Gollasch & David, 2019).

Given the fallible nature of BWE, compliance with D-2 regulations is a core requirement of the Convention. Compliance with measure D-2 necessitates that new ships need to be fitted with Ballast Water Management (BWM) systems, for example combination treatments using UV, ozonation, deoxygenation, biocides, electrochemical systems, ultrasound and heat (Hess-Erga *et al*., 2019), and older ships will need to transition to this. At present, uptake of the systems differs by ship types, but the total global fleet (bulk carriers, chemical tankers, container ships, ferries and passenger ships, general cargo ships, liquefied natural gas carriers, offshore supply vessels, oil tankers, other/not available) is only 7.66% fitted as of 2019 (UNCTAD, 2019).

Despite increased biosecurity measures, issues with ballast water biosecurity still arise when taking into consideration the prospect of 'invisible invaders'- microbial species such as viruses and bacteria <10 μm in size. Microorganisms are difficult to control for a number of reasons, including difficulties treating water with high particle concentrations, the robust nature of particle-associated bacteria, spore formation and the occurrence of bacterial growth post-treatment (Hess-Erga *et al*., 2019). Indeed, analysis of ballast water sampled from 2015-2017 used Illumina sequencing of the V3-V4 regions and identified ballast and harbour water samples that exceeded D-2 thresholds for *Escherichia coli* and *Enterococcus* (Gerhard & Gunsch, 2019). The authors suggested that there can be ports that are hubs of microbial activity and may be hotspots for microbial translocation. From a management perspective, screening of incoming high-risk vessels from hubs such as these will minimise the propagation of invasive species and pathogens (Ng *et al*., 2018).

Understanding vessel movements can lead to enhanced management practices, for example Bouda *et al*. (2018) noted that prolonged durations in port can result in increased biofouling. A further study suggested that this could influence potential management strategies, as it is not feasible to inspect all ships but there is potential to inspect long-term duration stays (Sing Lim *et al*., 2017). New introductions are inevitable even with excellent management practices, resulting in a need to facilitate intervention before the species spreads, and preventing internal dispersal from the ports is a major aspect of control given that eradication after establishment is almost impossible.

There are difficulties in combining invasive species research with management requirements and policy. One study analysed 347 journal articles relating to invasive flora and found that over half of them referred to the same four invasive species and a number of other high profile invaders were absent (Matzek *et al*., 2014). The study also noted long lag times before publications and small temporal and spatial scales which could make the transition from research to policy difficult, indeed it was mentioned that scientific research does not necessarily translate into management practice. It is also possible that there may be a lag time between when managers actually become concerned about a species and scientists obtaining funding and resources to study it. To counteract these issues, the aforementioned practice of horizon scanning is systematically used to identify and mitigate against potential invaders.

As an island nation, Ireland is heavily dependent upon the maritime transport industry with over 90% of traded goods and 10% of passengers being transported by sea (IMDO, 2020). The Irish shipping sector is managed at governmental level by the Irish Maritime Development Office (IMDO) which places heavy emphasis upon the economic importance of the sector. In addition to shipping trade, coastal communities within Ireland rely heavily on the aquaculture industry. For example, as of June 2020 there were 323 aquaculture licenses issued for the east and south-west coasts, boundaried by Louth and West-Cork, primarily for the Pacific cupped oyster *Crassostrea gigas* and the blue mussel *Mytilus edulis*. This region of aquaculture overlaps with the locations of the Ports of Dublin, Cork and Rosslare, recipients of the highest vessel arrival numbers in Ireland (CSO, 2020), and as a result could be adversely impacted if shipping industry within these ports facilitates the introduction of invasive species with aquaculture impacts.

Aims

This study used cargo vessel shipping records to establish the connectivity between international ports (encompassing the continents of Europe, Africa, Asia, North America and South America) and four ports in the Republic of Ireland, namely Dublin, Cork (city quays and Tivoli container terminal), Rosslare (County Wexford) and New Ross (County Wexford). These ports were selected to provide spatial coverage across the Irish and Celtic Sea regions and also for port-specific characteristics. Specifically, Dublin accounts

for two-thirds of all containerised trade, New Ross is Ireland's only inland port, Rosslare (also known as Rosslare Europort) is the closest point from the southern coast to the United Kingdom and continental Europe (and may see enhanced activity if direct routes to the continent are increasingly utilised due to Brexit), and Cork is situated at the shipping terminus of a large natural harbour.

The study aimed to rank routes of importance by focusing on links to ports where multiple voyages originate and ports with confirmed invasive species. It follows that ships originating from these ports would be optimum candidates for sample analysis as it is not feasible to inspect every ship, and sample protocols must be aware of the time-sensitive nature of the shipping industry. Inspections may be conducted to verify ballast water plans and management, reveal potential breaches of procedure and to analyse ballast water samples if necessary (Gollasch & David, 2019). Further aims were to assess temporal trends and consistencies in pathways and analyse journey times (to establish which routes could feasibly have invasive organisms survive in ballast water) and residence times (to establish whether different categories of cargo vessel may be more likely to be colonised by hull-fouling organisms).

The potential pathways available to invasive species with impacts on aquaculture were a priority in this study, and as such a horizon scanning exercise was incorporated to identify any connections to international ports with species that may have impacts on aquaculture and fisheries, both due to their direct ecological impacts but also the parasites and pathogens with which they may interact (Table 1).

Methods

The data utilised in this study was sourced from Marine Traffic and comprised of 2018 and 2019 shipping records (n = 9,291) for cargo vessels that entered Dublin (53.3455°/-6.206939°), Cork (51.9004°/8.43568°), Rosslare (52.25507°/-6.337719°) and New Ross (52.39417°/-6.948834°) ports. Cargo ships were selected because vessels in this shipping category often sail empty which means exchanging high volumes of ballast water (Kaluza *et al*., 2010). The parameters selected included the specific cargo vessel type, the previous port (prior to reaching port in Ireland), the journey time between previous port calls and Ireland, the length of stay in port in Ireland upon arrival and the next destination.

Anchorage records were excluded from the Previous/Next port parameters as otherwise all port calls to Dublin would have 'Dublin Anchorage' as their previous port call. The geographic locations of ports were used to identify pathways of invasion and the potential for secondary spread. Vessel journey times and residence times were used to assess the relative importance of hull-fouling and ballast water. Lastly, ports from which the highest number of voyages originated were isolated to determine the likelihood of an invasion.

Focal invasive species were selected based on a recently published horizon scanning exercise conducted for the island of Ireland, creating a list of the top 40, and subsequently top 10, most likely invaders across freshwater, marine and terrestrial realms (Lucy *et al*., 2020). The species selected were: The American razor clam *Ensis leei* (*directus*), the Asian shore crab *Hemigrapsus sanguineus* (which may already be present), the Brush clawed shore crab *Hemigrapsus takanoi*, the Chinese mitten crab *Eriocheir sinensis* (sighted on four occasions in the Waterford Estuary, in 2006, 2009, late 2020 and early 2021. The 2021 sighting was of a berried female, so there is a suggestion the crab may now be considered established (Biodiversity Ireland, 2021)) and the American slipper limpet *Crepidula fornicata* which is already present in Belfast Lough, Northern Ireland with sporadic sightings in the Republic of Ireland (Guy *et al*., 2013; Biodiversity Ireland, 2021*).* These species are all invasive along European coastlines and use vessels, along with aquaculture gear, as vectors of invasion. They can also potentially impact commercialised native species, for example predation upon blue mussels *Mytilus edulis* (Table 1).

Statistical analysis was conducted in RStudio version 1.3 and maps were created in ArcMap version 10.5.1. Any incomplete records, for example incoming ships at the end of 2019 that did not have a next destination record (due to leaving in 2020) were excluded from the analysis. Information on the focal species was gathered using a literature search that included peer-review papers and survey and management reports on invasive species from local and national authorities.

Table 1: Species with the potential to become invasive in Ireland and their associations with parasites.

Species	Time in Plankton	Lifespan	Fecundity	Impacts	Interactions with Parasites	References
Crepidula fornicata American slipper limpet	Egg capsules: 3-4 weeks; Larvae: 2-4 weeks	10 years; Females ovigerous at 2cm	10-15,000 eggs per brood with 2-4 broods per year	Biofouling, densities >4700/m^2 recorded	Dilution effect: Limpet presence decreases transmission of the trematode *Himasthla elongata* to native blue mussel *Mytilus edulis*	CABI 108234, 2020; Bohn *et al*., 2015; Richard *et al*., 2006; Thieltges *et al*., 2004; Thieltges *et al*., 2009
Ensis leei (directus) American razor clam	10 days-4 weeks; Secondary dispersal 6-8 weeks	20 years; Sexual maturity at 1 year	Unconfirmed	Biofouling, densities of 150 adults/m^2 and 1914 juveniles/m^2 recorded	Clam acquired native trematode *Renicola roscovita* in invaded range, which also infects native cockle *Cerastoderma edule*	Armonies, 2001; Dannheim & Rumohr, 2012; Gollasch *et al*., 2015; Krakau *et al*., 2006
Eriocheir sinensis Chinese mitten crab	3-5 months	5 years; Sexual maturity at ≥3 years	250,000-1 million eggs; Adults die after breeding	Damage to netted fish; Potential predation on fish eggs	Intermediate host for human lung fluke *Paragonimus westermani*; Epizootic barnacle *Polyascus gregarius* hosted in native range; Mites from 8 genera in native range; Microsporidian sp. detected in native and invaded range. Transmits crayfish plague *Aphanomyces astaci*	Anger, 1991; CABI 84120, 2020; Herborg *et al*., 2005; Kelly *et al*., 2009; Li *et al*., 2003 Normant *et al*., 2013 Stentiford *et al*., 2011 Webster *et al*., 2015 Schrimpf *et al*., 2014
Hemigrapsus sanguineus Asian shore crab	16-55 days	3 years, Sexual maturity at 1 year	>50,000; Females store sperm for several broods per year	Predation on intertidal blue mussel *Mytilus edulis*	Native range: Rhizocephalan barnacles, trematodes and microsporan protozoans; Invaded European range: Acquired acanthocephalan *Profilicollis botulus*; Invaded USA range: Unidentified larval nematodes, microphallic trematodes and acanthocephalans	CABI 107738, 2020; Epifanio, 1998; Epifanio, 2013; Goedknegt *et al*., 2017
Hemigrapsus takanoi Brush clawed shore crab	Temperature-dependent: 86 days at 12°C	2-3 years; Sexual maturity at 1 year	>56,000, 5-6 broods per year	Predation on small blue mussel *Mytilus edulis*	Limited information but known to host *Profilicollis botulus*	Nour *et al*., 2020; CABI 109143, 2020; van den Brink *et al*., 2013; Gothland *et al*., 2014; McDermott, 2011

Results

A total of 9,291 incoming voyages were recorded over two years; 4,644 in 2018 and 4,647 in 2019. The primary destination was Dublin Port at 82.85%, followed by Cork (13.03%), New Ross (2.61%) and Rosslare (1.51%). Ships arriving into these four ports originated from 232 incoming ports, and outgoing vessels had 283 destination ports. Twenty-seven of the incoming ports originated from either Northern Ireland or the Republic of Ireland, i.e. on the island, and 205 of the incoming ports originated elsewhere. The 205 off-island ports were considered the potential routes of invasion, and the 27 on-island ports were considered from the perspective of secondary spread. Figure 1 displays the connectivity of the Island to other ports globally. Europe was the most heavily represented continent, with high connectivity between Ireland and Western Europe, the United Kingdom and Scandinavia. There was also connectivity to the shores surrounding the Black Sea and Mediterranean Sea. There was a lesser degree of connectivity to the North of Africa and Gulf of Mexico.

Figure 2 demonstrates the connectivity within Ireland, thereby highlighting the potential for secondary spread and colonisation of an introduced invasive species. A high degree of connectivity was evident, particularly when centered around the ports of Dublin and Cork. The east and south coasts, bordering the Irish and Celtic Seas respectively, were more heavily utilised than the north and west.

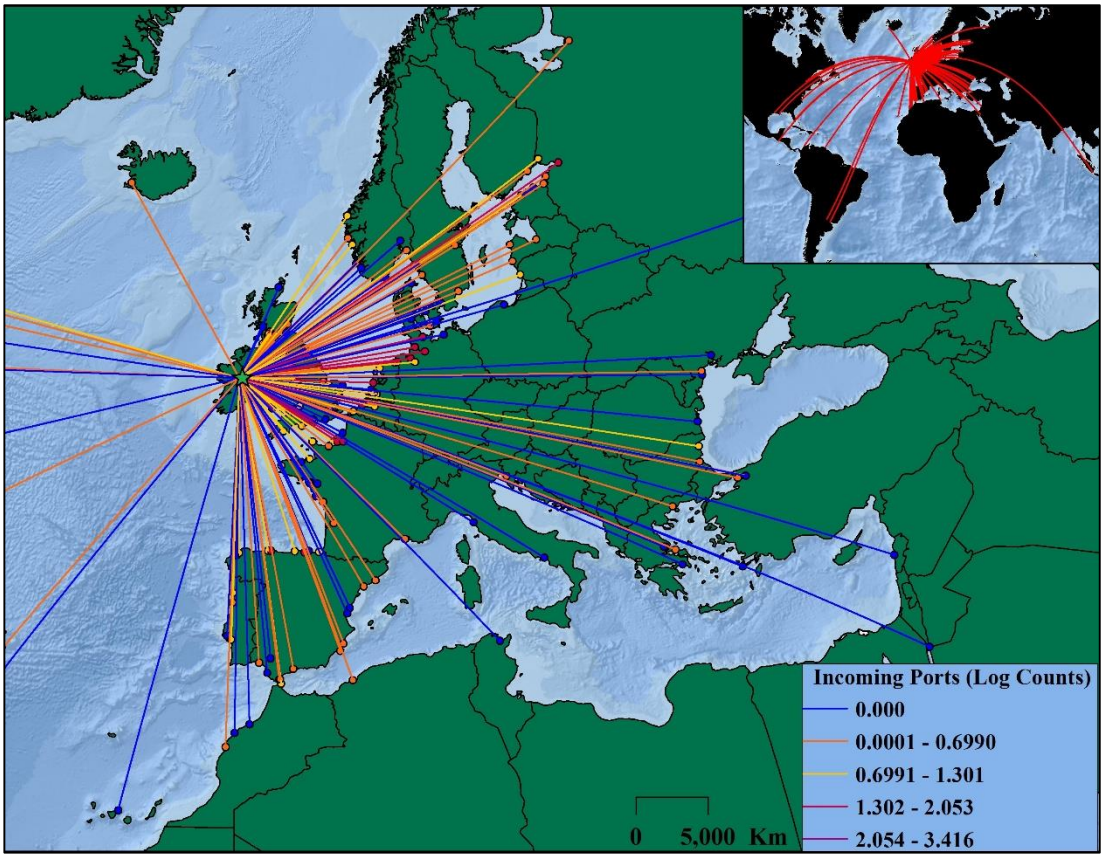

Figure 1: Graduated map demonstrating the density of journeys from incoming ports, and the potential routes of introduction for invasive species. Counts are logged to allow for clearer visualisation of the data. Vessels entering Dublin, Cork, New Ross and Rosslare from ports located in either the Republic of Ireland or Northern Ireland are not included.

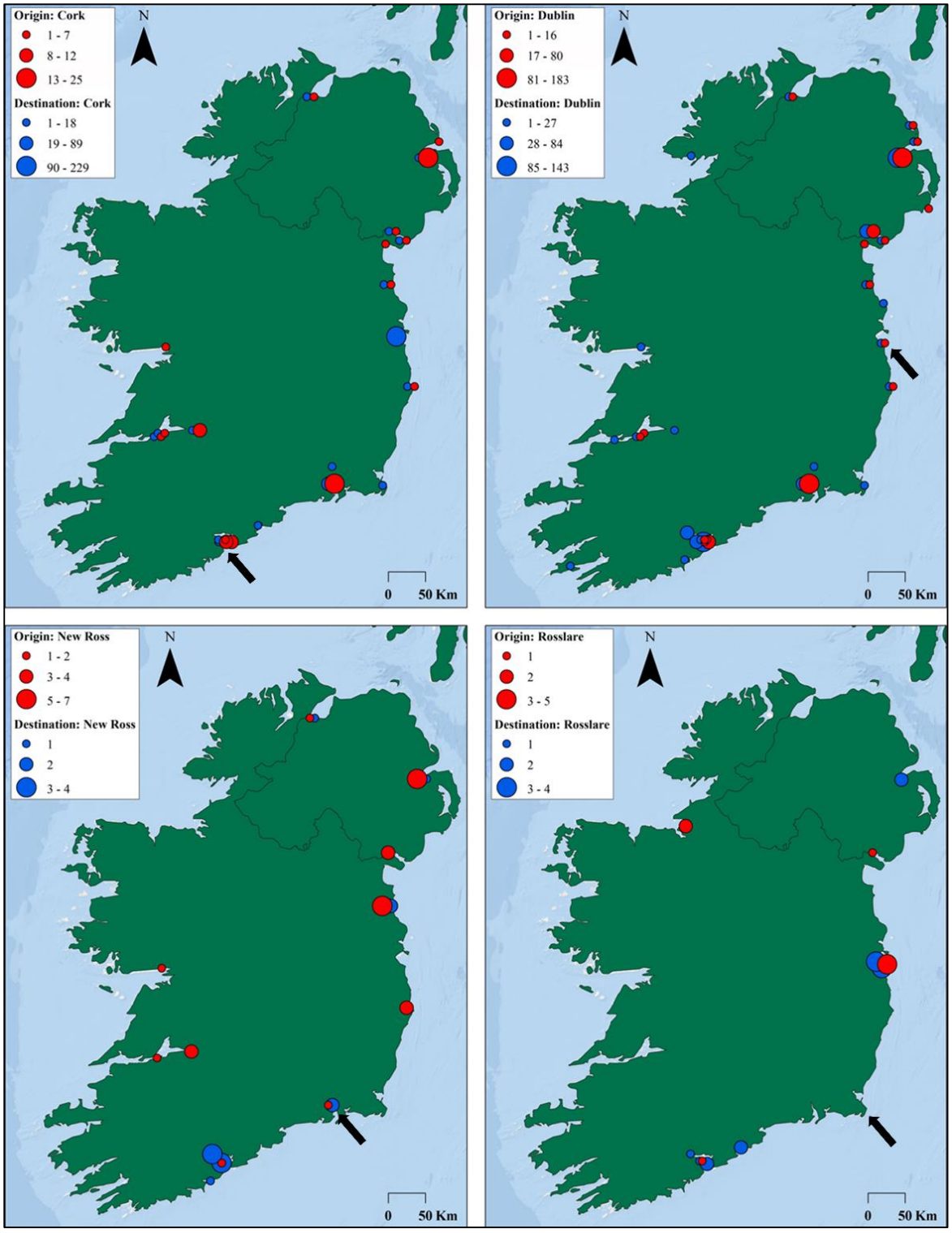

Figure 2: Maps demonstrating the connectivity of the four focal ports (black arrows) to other ports on the island. Clockwise from top left: Cork, Dublin, Rosslare and New Ross. Red circles indicate when the journey originated from a focal port, blue circles indicate when a focal port is the destination. Overlapping points have been offset slightly to allow for visualisation. Note different keys per port.

Twenty-two cargo vessel types were recorded, but of these vessels ten particular types accounted for 99.1% of journeys. Box plots were created to interpret vessel residence time in ports (Figure 3) and vessel travel time while at sea (Figure 4), in an effort to determine the relative risk of hull fouling versus ballast water. On average vessels spent less than five days in port, however there was a strong degree of variation, particularly within Container Ships, General Cargo and Oil/Chemical Tankers.

Vessel journey times were generally under five days also, however again there was strong variation with Bulk Carriers measuring up to 37.7 days at sea and General Cargo and Oil/Chemical Tankers again displaying large variance (Figure 4).

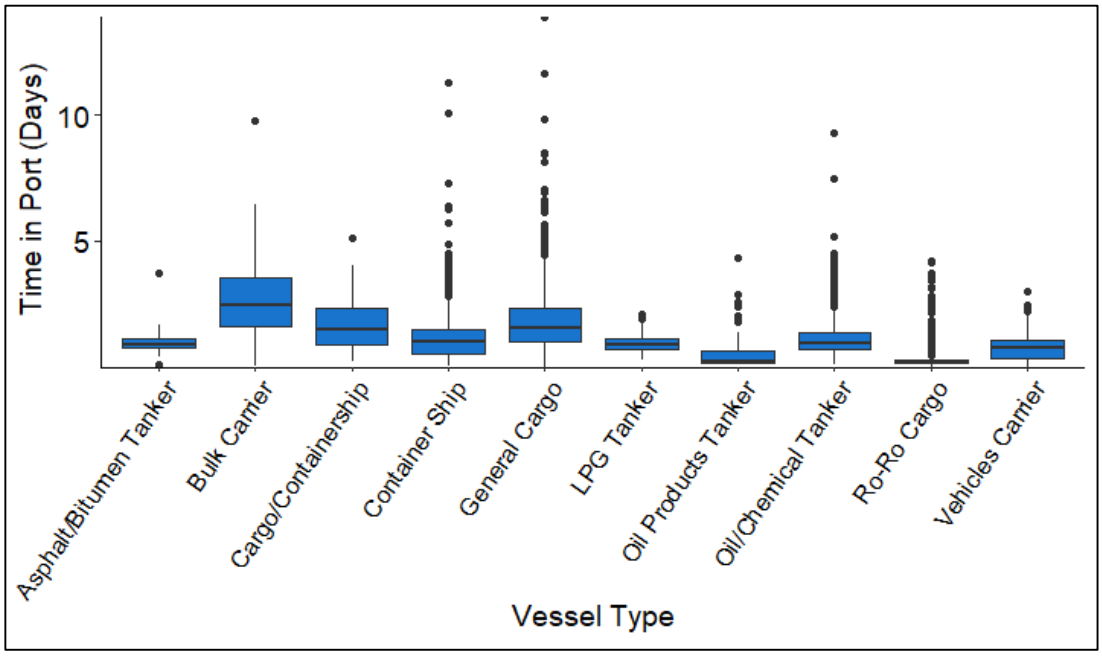

Figure 3: Time in port (days) for the ten dominant cargo vessel types based on 2018-2019 vessel arrivals to Dublin, Cork, Rosslare and New Ross.

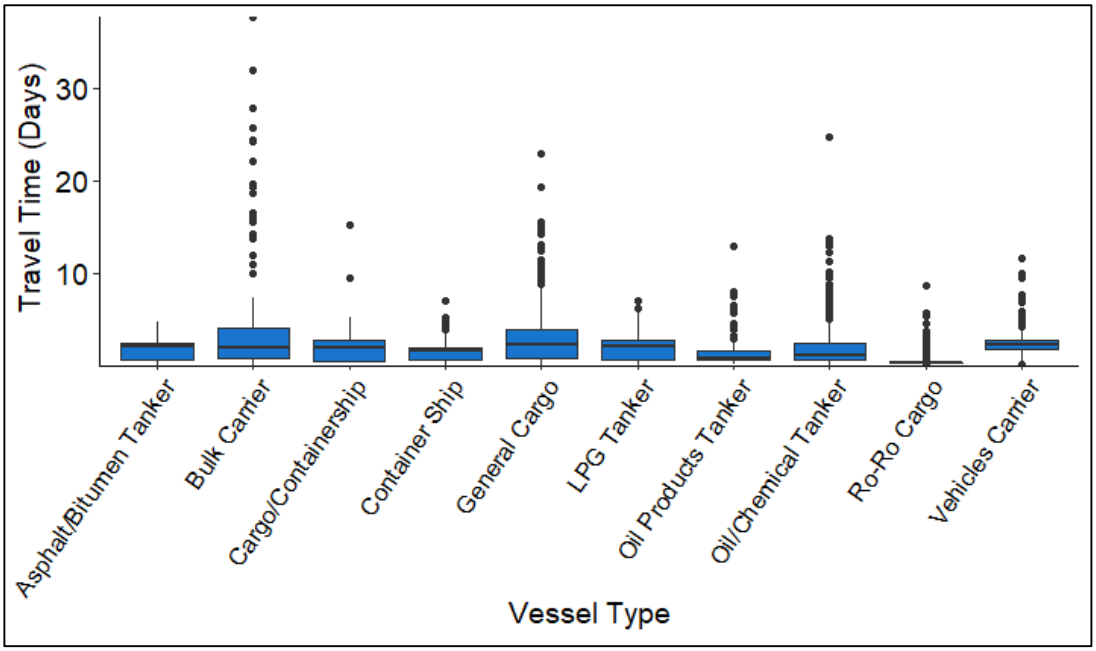

Figure 4: Time spent travelling (days) between the preceding port and arriving at the destination in Dublin, Cork, New Ross or Rosslare.

International ports (off-island) that accounted for over 50 incoming voyages (2018/2019 combined) were considered ports with high propagule pressure. Seventeen ports in total had over 50 journeys and were graphed to identify any temporal trends in patterns, however incoming journeys from all ports were evenly split between the seasons (Figure 5).

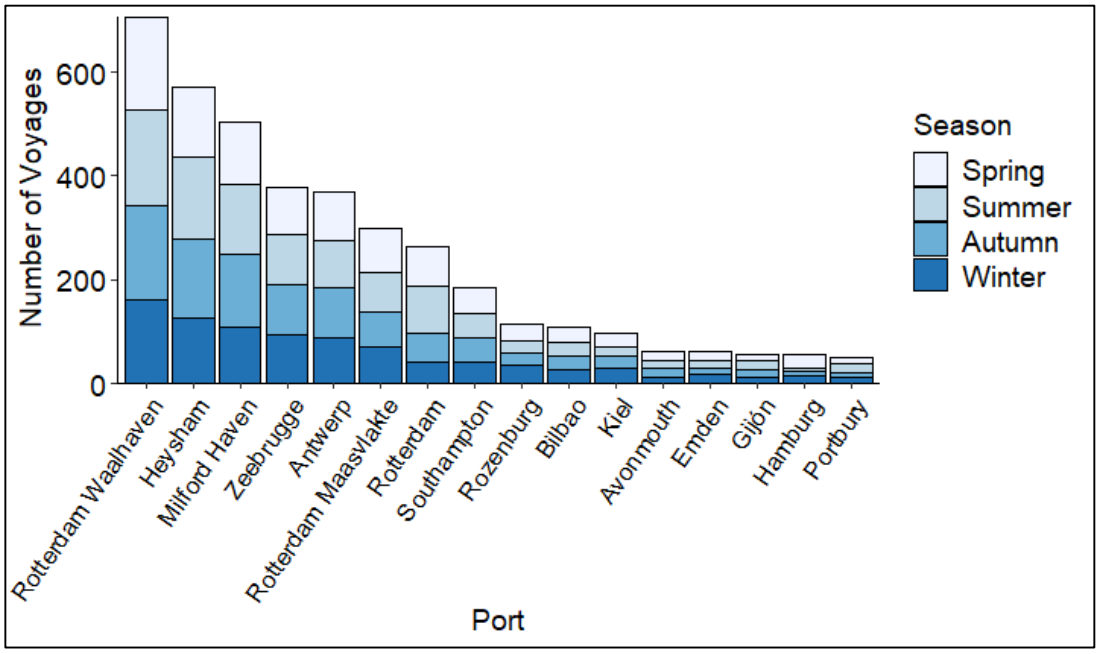

Figure 5: The ports from which the most voyages originated, and the breakdown of seasonal travel. The highest number of journeys (2,608) originated from Liverpool, however this port is excluded from the figure due to the difference in scale (after determining that Liverpool also displayed an even seasonal distribution), the next highest being Rotterdam Waalhaven at 706.

A literature search was conducted to identify ports in which horizon scanned species for the island of Ireland (Lucy *et al*., 2020) are already present. *Eriocheir sinensis* was confirmed at the highest number of ports (n = 7), the two *Hemigrapsus* species were confirmed at the same number of ports (n = 6) but did not always overlap and *Ensis leei* (*directus*) was confirmed in the lowest number (n = 5). However, *E. leei* (*directus*) was also considered abundant in the region at a further seven ports so it is highly likely to be present in multiple ports (Table 2).

Table 2: Invasive species in ports from which over 50 journeys originate. Ports are arranged by country. Dark blue indicates that the species has been confirmed in the port, the lighter shade when it is abundant in the region and the lightest shade indicates that there have been occasional recordings in the region. White is used when the species has not been recorded in the port or region. References for this table may be found in *Appendix B*.

Port	**Country**	***E. leei* (*directus*)**	***E. sinensis***	***H. sanguineus***	***H. takanoi***
Liverpool	England				
Heysham	England				
Southampton	England				
Avonmouth	England				
Portbury	England				
Rotterdam Waalhaven	Netherlands				
Rotterdam Maasvlakte	Netherlands				
Rotterdam	Netherlands				
Rozenburg	Netherlands				
Zeebrugge	Belgium				
Antwerp	Belgium				
Milford Haven	Wales				
Kiel	Germany				
Emden	Germany				
Hamburg	Germany				
Bilbao	Spain				
Gijón	Spain				

Discussion

This study confirmed that four ports in the Republic of Ireland demonstrate high connectivity with maritime ports globally, providing multiple routes of entry for potentially invasive species. Furthermore, many of the connections are to ports in which invasive species are confirmed to be present, suggesting the potential for invasion is not abstract, but a strong possibility in the near future. The focal species produce multiple broods per year, suggesting that there is a wide temporal window for larval stages to be taken up by vessels and transported. This exercise identified ships arriving from the Netherlands and Belgium as being potentially high-risk, due to heavy shipping volumes and the confirmed presence of invasives in the region. Ballast water compliance monitoring devices (CMDs) are kits designed to measure ballast water standards, and prioritisation of ships originating from these high-risk ports for monitoring is potentially a strong management strategy.

The horizon scanning approach adopted here also provides an opportunity to investigate the co-invasion of any associated parasites that may arrive with the focal invasive species, as short journey times may facilitate the survival of potentially pathogenic microorganisms. Screening of invasive species upon arrival, along with any cohabiting native species, will clarify the interactions between invasive hosts, parasites and native species. *Eriocheir sinensis*, *Hemigrapsus sanguineus* and *Ensis leei* (*directus*) have known associations with trematodes, and trematodes have mostly complex lifecycles, meaning they require secondary hosts to successfully complete their reproductive cycle (Zemmer *et al*., 2020). This may delay co-invasion with invasive hosts if the absence of a required secondary host prohibits reproduction. It may be more likely that microparasites with simple lifecycles are more likely to co-invade in a shorter timeframe.

Profilicollis botulus is a prevalent acanthocephalan (thorny/spiny headed worm) in the northeast Atlantic that uses crabs as intermediate hosts and avifauna, for example ducks and herring gulls, as definitive hosts (McDermott *et al*., 2010; McDermott, 2011. Whether this species is native to Europe or introduced from North America or native to both regions is unclear (Goedknegt *et al*., 2017), but given that the *Hemigrapsus* spp. are intermediate hosts it follows that transport and transmission of

this species may be facilitated if *Hemigrapsus* spp. invade, as all required hosts are present in Ireland.

Hull fouling has the potential to release macroalgae, invertebrates and microbiota into the surrounding environment (Pagenkopp Lohan *et al*., 2020). In this study different ship types demonstrated variable times in port, perhaps due to adverse weather or port scheduling. For general cargo ships in particular, residence times of over ten-day residence were recorded, providing ample time to release fouling organisms into the surrounding environment. It is also possible that species native to Ireland but invasive in other regions could colonise available hull surfaces and thereby be transported and introduced elsewhere. It is likely that this variability in residence times is repeated in international ports, suggesting that there is time for ships to be continuously colonised by further species. Although it is difficult to quantify the delivery and release of hull fouling biota (Pagenkopp Lohan *et al*., 2020), a potential management technique within ports may be to identify ship types that demonstrate longer residence times in port and prioritise these for inspection.

When considering potential routes of invasion, it is important to balance management techniques between informed concentrated monitoring of high-risk areas, while not disregarding other areas which, although at lower-risk, may still be subject to invasion. Tidbury *et al*. (2014) identified a 50x50 km area south of Waterford as a high-risk area for potential introductions due to interactions between commercial and recreational shipping, aquaculture stock imports and natural dispersal. The results presented here identify the same area as being susceptible to invasion, but also highlight other regions including Cork Harbour, Carlingford Lough and Dublin Bay.

The degree of connectivity within each area is of note, for example this current study focused on vessels arriving into the city in Cork, however the overall Cork Harbour region has multiple berths available including the ports of Ringaskiddy and Cobh (primarily used by ferries), both of which possess deep-water berths, and the local ports of Whitegate and Passage West. Ringaskiddy in particular is a port with high cargo shipping volumes and is undergoing development to make it more so. Similarly, port facilities are being developed in nearby Belvelly for dry cargo vessels (Port of Cork, 2021). The overall effect of current volumes and predicted expansion mean that

regional management in Cork Harbour could benefit from studies focused specifically on this area.

High connectivity around the island of Ireland means that secondary spread is also likely, particularly when taking into account the fact that smaller ports may also be used by recreational and leisure craft. When establishing connectivity, it is crucial to recognise ports as hubs of activity where multiple vectors may overlap (Minchin & Gollasch, 2005). In coastal regions of Ireland, vessel movement often overlaps with areas of aquaculture, and this confluence of vectors may also facilitate the spread of a species that is already established in Northern Ireland; the American slipper limpet *Crepidula fornicata*. The current distribution of two established invasive species, the leathery sea squirt/clubbed tunicate *Styela clava* and the carpet sea squirt *Didemnum vexillum*, may suggest the potential regions that *C. fornicata* could colonise, as their distribution also suggests an association with aquaculture and vessel movement due to their presence in marinas and mariculture sites. *S. clava* has been recorded throughout Cork Harbour and also in Dingle, Tralee and Dublin Bay (Minchin & Duggan, 1988; Minchin *et al*., 2006). *D. vexillum* has been recorded in Strangford Lough (Northern Ireland), Clew Bay, Galway Bay, Cork Harbour, Carlingford Lough and Malahide Marina (Minchin & Sides, 2006; Minchin & Nunn, 2013;).

Horizon scanning for invasive species allows for a proactive response to a species introduction, rather than a response that is entirely reactive. Monitoring programmes can be passive, which involves scanning for the species while conducting other activities in suitable habitat, or active - namely conducting targeted surveys. Establishing effective monitoring and management systems requires strong links between port authorities, the scientific community and state control institutions. These systems could include surveys which may also incorporate citizen science, robust implementation of the Ballast Water Management Convention (ICES, 2018) and eradication attempts if feasible. Molecular tools, for example eDNA metabarcoding, may also be increasingly used to detect problematic species and also test the efficacy of ballast water treatments (Rey *et al*., 2019). In this study incoming voyages were relatively even temporally, with similar vessel arrivals throughout the seasons. This does make it difficult to prioritise vessels for screening but there is the possibility that inspections for larvae or microbial pathogens may be prioritised in warmer months, depending on the thermal niche of the species.

It is highly likely that one or more of the focal species will arrive in Irish waters within a relatively short time-frame, and environmental conditions in Irish regions fall within the thermal/salinity tolerances of these species. The primary question therefore is how to respond if a species is sighted. Several European invasions of *E. sinensis* have been characterised by a lag phase whereby only a few crabs per year were sighted, followed by a sudden population increase under favourable conditions (Veilleux & de Lafontaine, 2007). It follows therefore that an attempt at eradication must be conducted early to have a viable chance of success (Giakoumi *et al*., 2019). If eradication is not successful managers may attempt to minimise the spread, for example physical controls such as traps or barriers to migration in the case of *Eriocheir sinensis* (Eberhardt *et al*., 2016).

A plausible method of control is to create a fishery for the detected species if it reaches a high threshold. *Ensis leei* (*directus*) is one species in particular that may be suitable for a fishery, as it is already harvested in Dutch waters with the primary markets being Spanish and Italian (Marine Stewardship Council, 2021). While the creation of a commercial fishery is in essence the simplest form of control, it is necessary to formalise the fishing method so as not to harm native species and habitat (Addison *et al*., 2006). For example, razor clam fishing is in the form of hydraulic dredges so may impact natural benthic communities.

On a global scale, the level of connectivity among ports on the global shipping network is poorly resolved, however resolving this connectivity will facilitate targeted management for ships that pose the greatest risk of species transport and release (Keller *et al*. 2016). Transoceanic shipping can also lead to cryptic dispersal in connectivity patterns, whereby shipping of propagules may result in species crossing previously restrictive phylogeographic breaks. This can then lead to inaccurate identification of genetic patterns, because the gene flow is due to vessel-assisted movement rather than wide dispersal capabilities (David & Loveday, 2017). Identifying the routes of travel may therefore shed light on the native and invaded range of introduced species. Moving forward, there are uncertainties associated with how climate change will influence shipping and invasive species movement, however it has been postulated that climate change will lead to lower invasion probabilities in the tropics but higher probabilities in temperate regions (Seebens *et al*., 2016). Ware

et al. (2014) also suggested that primary threats will change from hull fouling on research and fishing vessels to ballast water by 2100 (after 2050).

This study focused on five potentially invasive species but there will always be a 'next-pest' that has the potential to arrive and establish. This methodology provides a framework for understanding the likely paths of introduction; it suggests that target species must be identified and their current distribution mapped. It is necessary to then identify ports and regions in which the focal species is present and establish the levels of connectivity, along with the presence of overlapping vectors. If a species does arrive it is important not to consider it in a vacuum but consider it a potential host for parasites or pathogens and screen for these also, to elucidate all potential risks. Targeted surveys, management and prioritisation of high risk vessels will facilitate regional management in either preventing or mitigating the impacts of invasive species.

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References

Addison, J., Palmer, D., Lart, W., Misson, T. & Swarbick, J. (2006). Development of a suitable dredge for exploitation of razorfish (*Ensis directus*) in The Wash. The Centre for Environment, Fisheries & Aquaculture Science (Cefas) & Sea Fish Industry Authority (Seafish). Final Report, 1-77.

Anger, K. (1991). Effects of temperature and salinity on the larval development of the Chinese mitten crab *Eriocheir sinensis* (Decapoda: Grapsidae). *Marine Ecology Progress Series*, 72, 103-110.

Arias, A. & Anadón, N. (2012). First Record of *Mercenaria mercenaria* (Bivalvia: Veneridae) and *Ensis directus* (Bivalvia: Pharidae) on Bay of Biscay, Iberian Peninsula. *Journal of Shellfish Research*, 31, 57-60.

Armonies, W. (2001). What an introduced species can tell us about the spatial extension of benthic populations. *Marine Ecology Progress Series*, 209, 289-294.

Biodiversity Ireland (2021). https://species.biodiversityireland.ie/profile.php?taxonId=22443. Accessed 12/03/2021.

Bishop, M.J. & Hutchings, P.A. (2011). How useful are port surveys focused on target pest identification for exotic species management? *Marine Pollution Bulletin*, 62, 36-42.

Boets, P., Lock, K. & Goethals, P.L.M. (2012). Assessing the importance of alien macro-Crustacea (Malacostraca) within macroinvertebrate assemblages in Belgian coastal harbours. *Helgoland Marine Research*, 66, 175-187.

Bohn, K., Richardson, C.A. & Jenkins S.R. (2015). The distribution of the invasive non-native gastropod *Crepidula fornicata* in the Milford Haven Waterway, its northernmost population along the west coast of Britain. *Helgoland Marine Research*, 69, 313-325.

Bouda, A., el I.B., N., Nacef, L. & Bensari, B. (2018). Risk Analysis of Invasive Species Introduction in the Port of Arzew, by Calculation of Biofouling Surface on Ships' Hulls. *Environmental Modelling & Assessment*, 23, 185-192.

Brinkmeyer, R. (2016). Diversity of bacteria in ships ballast water as revealed by next generation DNA sequencing. *Marine Pollution Bulletin*, 107, 277-285.

CABI *Crepidula Fornicata* (2020). https://www.cabi.org/isc/datasheet/108234. Accessed 07/06/2020.

CABI *Eriocheir sinensis* (2020). https://www.cabi.org/isc/datasheet/84120. Accessed 07/06/2020.

CABI *Hemigrapsus sanguineus* (2020). https://www.cabi.org/isc/datasheet/107738. Accessed 07/06/2020.

CABI *Hemigrapsus takanoi* (2020). https://www.cabi.org/isc/datasheet/109143. Accessed 07/06/2020.

Carney, K.J., Minton, M.S., Holzer, K.K., Miller, A.W., McCann, L.D. & Ruiz, G.M. (2017). Evaluating the combined effects of ballast water management and trade dynamics on transfers of marine organisms by ships. *PLoS ONE*, 12(3), e0172468, 1-20.

Colautti, R.I., Parker, J.D., Cadotte, M.W., Pyšek, P., Brown, C.S., Sax, D.F. & Richardson, D.M. (2014). Quantifying the invasiveness of species. *NeoBiota*, 21, 7-27.

CSO (2020). https://www.cso.ie/en/statistics/transport/statisticsofporttraffic/. Accessed 06/07/2020.

Dannheim, J. & Rumohr, H. (2012). The fate of an immigrant: *Ensis directus* in the eastern German Bight. *Helgoland Marine Research*, 66, 307-317.

Dauvin, J.-C., Rius, A.T. & Ruellet, T. (2009). Recent expansion of two invasive crabs species *Hemigrapsus sanguineus* (de Haan, 1835) and *H. takanoi* Asakura and Watanabe 2005 along the Opal Coast, France. *Aquatic Invasions*, 4, 351-465.

David, A.A. & Loveday, B.R. (2018). The role of cryptic dispersal in shaping connectivity patterns of marine populations in a changing world. *Journal of the Marine Biological Association of the United Kingdom*, 98(4), 647-655.

de Castro, M.C.T., Fileman, T.W. & Hall-Spencer, J.M. (2017). Invasive species in the Northeastern and Southwestern Atlantic Ocean: A review. *Marine Pollution Bulletin*, 41-47.

Drotz, M.K., Berggren, M., Lundberg, K., Lundin, K. & von Proschwitz, T. (2010). Invasion routes, current and historical distribution of the Chinese mitten crab (*Eriocheir sinensis* H. Milne Edwards, 1853) in Sweden. *Aquatic Invasions*, 5, 387-396.

Eberhardt, A., Pederson, J. & Bisson, B. (2016). Rapid Response Plan for Management and Control of the Chinese Mitten Crab - Northeast United States and Atlantic Canada. New Hampshire, MIT and Maine Sea Grant Programs, 1-54.

Epifanio, C.E., Dittel, A.I., Park, S., Schwalm, S. & Fouts, A. (1998). Early life history of *Hemigrapsus sanguineus*, a non-indigenous crab in the Middle Atlantic Bight (USA). *Marine Ecology Progress Series*, 170, 231-238.

Epifanio, C.E. (2013). Invasion biology of the Asian shore crab *Hemigrapsus sanguineus*: A review. *Journal of Experimental Marine Biology and Ecology*, 441, 33-49.

Geburzi, J.C., Graumann, G., Köhnk, S. & Brandis, D. (2015). First record of the Asian crab *Hemigrapsus takanoi* Asakura & Watanabe, 2005 (Decapoda, Brachyura, Varunidae) in the Baltic Sea. *BioInvasions Records*, 4, 103-107.

Geburzi, J.C., Ewers-Saucedo, C., Brandis, D. & Hartl, G.B. (2020). Complex patterns of secondary spread without loss of genetic diversity in invasive populations of the Asian shore crab *Hemigrapsus takanoi* (Decapoda) along European coasts. *Marine Biology*, 167(180) 1-18.

Gerhard, W.A. & Gunsch, C.K. (2019). Metabarcoding and machine learning analysis of environmental DNA in ballast water arriving to hub ports. *Environment International*, 124, 312-319.

Giakoumi, S., Katsanevakis, S., Albano, P.G., Azure, E., Cardoso, A.C., Cambrian, E., Deaden, A., Delist, D., Franc our, P., Jimenez, C., Maci, V., Occhipinti-Ambrogi, A., Rilov, G. & Ramzi Sghaier, Y. (2019). Management priorities for marine invasive species. *Science of the Total Environment*, 976-982.

Gittenberger, A., Rensing, M., Niemantsverdriet, P., Schrieken, N. & Stegenga, H. (2014). Port of Rotterdam survey and monitoring non-native species conform HELCOM/OSPAR protocol. *GiMaRIS 2014_31*, 1-111.

Goedknegt, M.A., Havermans, J., Waser, A.M., Luttikhuizen, P.C., Velilla, E., Kamphuysen, K.C.J., van der Meer, J. & Thieltges, D.W. (2017). Cross-species comparison of parasite richness, prevalence, and intensity in a native compared to two invasive brachyuran crabs. *Aquatic Invasions*, 12(2), 201-212.

Gollasch, S., Kerckhof, F., Craeymeersch, J., Goulletquer, Jensen, K., Jelmert, A. & Minchin, D. (2015). Alien Species Alert: *Ensis directus*. Current status of invasions by the marine bivalve *Ensis directus*. *ICES Cooperative Research Report*, No. 323, 1-32.

Gothland, M., Dauvin, J.C., Denis, L., Dufossé, F., Jobert, S., Ovaert, J., Pezy, J.P., Tous Rius, A. & Spilmont, N. (2014). Biological traits explain the distribution and colonisation ability of the invasive shore crab *Hemigrapsus takanoi*. *Estuarine, Coastal and Shelf Science*, 142, 41-49.

Guy, C., Reid, N. & Roberts, D. (2013). Ageing of Slipper Limpet (*Crepidula fornicata*) shells from Belfast Lough. *Irish Naturalists' Journal*, 32(1), 45-48.

Gollasch, S. & David, M. (2019). Ballast Water: Problems and Management. From *World Seas: an Environmental Evaluation (2nd Edition) Volume III Ecological Issues and Environmental Impacts*, Chapter 13, 237-250.

Herborg, L.-M., Rushton, S.P., Clare, A.S. & Bentley, M.G. (2003). Spread of the Chinese mitten crab (*Eriocheir sinensis* H. Milne Edwards) in Continental Europe: analysis of a historical data set. *Hydrobiologia*, 503, 21-28.

Herborg, L.-M., Rushton, S.P. Clare, A.S. & Bentley, M.G. (2005). The invasion of the Chinese mitten crab (*Eriocheir sinensis*) in the United Kingdom and its comparison to continental Europe, *Biological Invasions*, 7, 959–968.

Hess-Erga, O-K., Moreno-Andrés, J., Enger, Ø. & Vadstein, O. (2019). Microorganisms in ballast water: Disinfection, community dynamics, and implications for management. *Science of the Total Environment*, 657, 704-716.

ICES (2018). Report of the Working Group on Ballast and Other Ship Vectors (WGBOSV), 5–7 March 2018, Madeira, Portugal. ICES CM 2018/HAPISG:12. 1-115.

Irish Maritime Development Office (2020) Irish Maritime Transport Economist: Vol. 17, Dublin: Irish Maritime Development Office.

Kaluza, P., Kölzsch, A., Gastner, M.T. & Blasius, B. (2010). The complex network of global cargo ship movements. *Journal of the Royal Society Interface*, 7, 1093-1103.

Keller, R.P., Drake, J.M., Drew, M.B. & Lodge, D.M. (2016). Linking environmental conditions and ship movements to estimate invasive species transport across the global shipping network. *Diversity and Distributions*, 17, 93-102.

Kelly, J. & Maguire, C.M. (2009). Chinese Mitten Crab (*Eriocheir sinensis*) Invasive Species Action Plan. Prepared for NIEA and NPWS as part of Invasive Species Ireland.

Kerckhof, F., Haelters, J. & Gollasch, S. (2007). Alien species in the marine and brackish ecosystem: the situation in Belgian waters. *Aquatic Invasions*, 2, 243-257.

Krakau, M., Thieltges, D.W. & Reise, K. (2006). Native parasites adopt introduced bivalves of the North Sea. *Biological Invasions*, 8, 919-925.

Landeira, J.M., Cuseta, J.A. & Tanaka, Y. (2019). Larval development of the brush-clawed shore crab *Hemigrapsus takanoi* Asakura & Watanabe, 2005 (Decapoda, Brachyura, Varunidae). *Journal of the Marine Biological Association of the United Kingdom*, 99, 1153-1164.

Landschoff, J., Lackschewitz, D., Kest, K. & Reise, K. (2013). Globalization pressure and habitat change: Pacific rocky shore crabs invade armored shorelines in the Atlantic Wadden Sea. *Aquatic Invasions*, 8, 77-87.

Li, H., Yan, Y., Yu, X., Miao, S. & Wang, Y. (2011). Occurrence and Effects of the Rhizocephalan Parasite, *Polyascus gregarius*, in the Chinese Mitten Crab, *Eriocheir sinensis*, Cultured in a Freshwater Pond, China. *Journal of the World Aquaculture Society*, 42(3), 1-10.

Lim, C.S., Yeong, Y.L. & Tan, K.S. (2017). Managing the risk of non-indigenous marine species transfer in Singapore using a study of vessel movement. *Marine Pollution Bulletin*, 115, 332-344.

Lucy, F.E., Davis, E., Anderson, R., Booy, O., Bradley, K., Britton., J.R., Byrne, C., Caffrey, J.M., Coughlan, N.E., Crane, K., Cuthbert, R.N., Dick, J.T.A., Dickey, J.W.E., Fisher, J., Gallagher, C., Harrison, S., Jebb, M., Johnson, M., Lawton, C., Lyons, D., Mackie, T., Maggs, C., Marnell, F., McLoughlin, T., Minchin, D., Monaghan, O., Montgomery, I., Moore, N., Morrison, L., Muir, R., Nelson, B., Niven, A., O'Flynn, C., Osborne, B., O'Riordan, R.M., Neil, R., Roy, H., Sheehan, R., Stewart, D., Sullivan, M., Tierney, P., Treacy, P., Tricario, E. & Trodd, W. (2020). Horizon scan of invasive alien species for the island of Ireland. *Management of Biological Invasions*, 11(2), 155-177.

Lymperopoulou, D.S. & Dobbs, F.C. (2017). Bacterial Diversity in Ships' Ballast Water, Ballast-Water Exchange, and Implications for Ship-Mediated Dispersal of Microorganisms. *Environmental Science & Technology*, 51, 1962-1972.

Marine Stewardship Council (2021). https://fisheries.msc.org/en/fisheries/dfa-dutch-north-sea-ensis/market-information/. Accessed 28/02/2021.

Matzek, V., Pujalet, M. & Cresci, S. (2015). What Managers Want From Invasive Species Research Versus What They Get. *Conservation Letters*, 8(1), 33-40.

McDermott, J.J., Williams, J.D., Boyko, C.B. (2010). The unwanted guests of hermits: A global review of the diversity and natural history of hermit crab parasites. *Journal of Experimental Marine Biology and Ecology*, 394, 2-44.

McDermott, J.J. (2011). Parasites of shore crabs in the genus *Hemigrapsus* (Decapoda: Brachyura: Varunidae) and their status in crabs geographically displaced: a review. *Journal of Natural History*, 45, 2419-2441.

Minchin, D. & Duggan, D.B. (1988). The Distribution of the Exotic Ascidian, *Styela clava* Herdman, in Cork Harbour. *The Irish Naturalists' Journal*, 22, 388-393.

Minchin, D. & Gollasch, S. (2005). Vector Pathways and the Spread of Exotic Species in the Sea. ICES Cooperative Research Report, No. 271, 1-30.

Minchin, D., Davis, M.H. & Davis, M.E. (2006). Spread of the Asian tunicate *Styela clava* Herdman, 1882 to the east and south-west coasts of Ireland. *Aquatic Invasions*, 2, 91-96.

Minchin, D. & Sides, E. (2006). Appearance of a cryptogenic tunicate, a *Didemnum* sp. fouling marina pontoons and leisure craft in Ireland. *Aquatic Invasions*, 1, 143-147.

Minchin, D.M. & Nunn, J.D. (2013). Rapid assessment of marinas for invasive alien species in Northern Ireland. Northern Ireland Environment Agency Research and Development Series No. 13/06.

Mingkid, W.M., Yokota, M. & Watanabe, S. (2006). Salinity tolerance of larvae in the Penicillate crab *Hemigrapsus takanoi* (Decapoda: Brachyura: Grapsidae). *La Mer*, 44, 17-21.

National Biodiversity Network Atlas (2021). *Eriocheir sinensis*. https://species.nbnatlas.org/species/NHMSYS0001593547. Accessed 27/02/2021.

Nędzarek, A. & Czerniejewski, P. (2021). The edible tissues of the major European population of the invasive Chinese mitten crab (*Eriocheir sinensis*) in the Elbe River, Germany, as a valuable and safe complement in essential elements to the human diet. *Journal of Food Composition and Analysis*, 96(103713), 1-10.

Ng, C., Giek Goh, S., Saeidi, N., Gerhard, W.A., Gunsch, C.K. & Hoong Gin, K.Y. (2018). Occurrence of *Vibrio* species, beta-lactam resistant *Vibrio* species, and indicator bacteria in ballast and port waters of a tropical harbor. *Science of the Total Environment*, 610-11, 651-656.

Normant, M., Zawal, A., Chatterjee, T. & Wójcik, D. (2013). Epibiotic mites associated with the invasive Chinese mitten crab *Eriocheir sinensis* – new records of Halacaridae from Poland. *Oceanologia*, 55(4), 901-915.

North Western Inshore Fisheries and Conservation Authority. (2020). Press Release: Morecambe Bay Chinese Mitten Crab. https://www.nw-ifca.gov.uk/news/press-release-morecambe-bay-chinese-mitten-crab/. Accessed 27/02/2021.

Nour, O.M., Stumpp, M., Morón Lugo, S.C., Barboza, F.R. & Pansch, C. (2020). Population structure of the recent invader *Hemigrapsus takanoi* and prey size selection on Baltic Sea mussels. *Aquatic Invasions*, 15(2), 297-317.

O'Brien, C.E., Johnston, M.W. & Kerstetter, D. (2017). Ports and pests: Assessing the threat of aquatic invasive species introduced by maritime shipping activity in Cuba. *Marine Pollution Bulletin,* 125, 92-102.

Olbert, A.I., Hartnett, M., Dabrowski, T. & Mikolajewicz, U. (2011). Long-term inter-annual variability of a cyclonic gyre in the western Irish Sea. *Continental Shelf Research*, 31, 1343-1356.

Otto, T. & Brandis, D. (2011). First evidence of *Eriocheir sinensis* reproduction from Schleswig—Holstein, Northern Germany, western Baltic Sea. *Aquatic Invasions*, 6, 65-69.

Pagenkopp Lohan, K.M., Ruiz, G.M. & Torchin, M.E. (2020). 'Invasions can drive marine disease dynamics' in Marine Disease Ecology, eds. Donald C. Behringer, Brian R. Silliman & Kevin D. Lafferty (Oxford University Press), 115-138.

Port of Cork (2021). https://www.portofcork.ie/index.cfm/page/about-belvelly-port-facility. Accessed 17/03/2021.

Prado, P., Peñas, A., Ibáñez, C., Cabanes, P., Jornet, L., Álvarez, N. & Caiola, N. (2020). Prey size and species preferences in the invasive blue crab, *Callinectes sapidus*: Potential effects in marine and freshwater ecosystems. *Estuarine, Coastal and Shelf Science*, 245, 106997.

Rey, A., Carney, K.J., Quinones, L.E., Pagenkopp Lohan, K.M., Ruiz, G.M., Basurko, O.C. & Rodríquez-Ezpeleta, N. (2019). Environmental DNA Metabarcoding: A Promising Tool for Ballast Water Monitoring. *Environmental Science & Technology*, 53, 11849-11859.

Richard, J., Huet, M., Thouzeau, G. & Paulet, Y.-M. (2006). Reproduction of the invasive slipper limpet, *Crepidula fornicata*, in the Bay of Brest, France. *Marine Biology*, 149, 789-801.

Roy, H.E., Peyton, J., Aldridge, D.C., Bantock, T., Blackburn, T.M., Britton, R., Clark, P., Cook, E., Dehnen-Schmutz, K., Dines, T., Dobson, M., Edwards, F.,

Harrower, C., Harvey, M.C., Minchin, D., Noble, D.G., Parrott, D., Pocock, M.J.O., Preston, C.D., Roy, S., Salisbury, A., Schönrogge, K., Sewell, J., Shaw, R.H., Stebbing, P., Stewart, A.J.A. & Walker, K.J. (2014). Horizon scanning for invasive alien species with the potential to threaten biodiversity in Great Britain. *Global Change Biology*, 20, 3859-3871.

Schoelynck, J., Van Loon, P., Heirmans, R., Jacobs, S. & Keirsebelik, H. (2020). Design and testing of a trap removing Chinese mitten crabs (*Eriocheir sinensis*, H. Milne Edwards, 1853) from invaded river systems. *River Research and Applications*, doi: 10.1002/rra.3635, 1-11.

Schrimpf, A., Schmidt, T. & Schultz, R. (2014). Invasive Chinese mitten crab (*Eriocheir sinensis*) transmits crayfish plague pathogen (*Aphanomyces astaci*). *Aquatic Invasions*, 9, 203-209.

Sea Pollution (Miscellaneous Provisions) Act: http://www.irishstatutebook.ie/eli/2006/act/29. Accessed 23/06/2020.

Seebens, H., Gastner, M.T. & Blasius, B. (2013). The risk of marine bioinvasions caused by global shipping. *Ecology Letters*, 16, 782-790.

Seebens, H., Schwartz, N., Schupp, P.J. & Blasius, B. (2016) Predicting the spread of marine species introduced by global shipping. *Proceedings of the National Academy of Sciences*, 113(20), 5646-5651.

Seeley, B., Sewell, J. & Clark, P.F. (2015). First GB records of the invasive Asian shore crab, *Hemigrapsus sanguineus* from Glamorgan, Wales and Kent, England. *Marine Biodiversity Records*, 8, 1-4.

Silvestre, F., Dierick, J-F., Dumont, V., Dieu, M., Raes, M. & Devos, P. (2006). Differential protein expression profiles in anterior gills of Eriocheir sinensis during acclimation to cadmium. *Aquatic Toxicology*, 76, 46-58.

Soors, J., Faasse, M., Stevens, M., Verbessem, I., De Regge, N. & Van den Bergh, E. (2010). New crustacean invaders in the Schelde estuary (Belgium). *Belgian Journal of Zoology*, 140, 3-10.

Stentiford, G.D., Bateman, K.S., Dubuffet, A., Chambers, E. & Stone, D.M. (2011). *Hepatospora eriocheir* (Wang and Chen, 2007) gen. et comb. nov. infecting invasive

Chinese mitten crabs (*Eriocheir sinensis*) in Europe. *Journal of Invertebrate Pathology*, 108, 156-166.

Tamburri, M.N., Bailey, S.A., Everett, R.A., First, M.R., Gollasch, S., Outinen, O. & Drake, L.A. (2020). Protocol for the verification of ballast water compliance monitoring devices. *ICES Techniques in Marine Environmental Sciences*, 63, 1-17.

Thieltges, D.W., Strasses, M., van Beusekom, J.E.E. & Reise, K. (2004). Too cold to prosper—winter mortality prevents population increase of the introduced American slipper limpet *Crepidula fornicata* in northern Europe. *Journal of Experimental Marine Biology and Ecology*, 375-391.

Thieltges, D.W., Reise, K., Prinze, K. & Jensen, K.T. (2009). Invaders interfere with native parasite–host interactions. *Biological Invasions*, 11, 1421-1429.

The GloBallast Story: Reflections from a Global Family. (2017). GEF-UNDP-IMO GloBallast Partnerships Programme. *GloBallast Monograph* No. 25.

United Nations Conference on Trade and Development (2019). Review of Maritime Transport. *UNCTAD*, 1-132.

Valdor, P.F., Gómez, A.G., Steinberg, P., Tanner, E., Knights, A.M., Seitz, R.D., Airoldi, L., Firth, L.B., Arvanitidis, C., Ponti, M., Chatzinikolaou, E., Brooks, P.R., Crowe, T.P., Smith, A., Méndez, G., Ovejero, A., Soares-Gomes, A., Burt, J.A., MacLeod, C. & Juanes, J.A. (2020). A global approach to mapping the environmental risk of harbours on aquatic systems. *Marine Policy*, 119, 104051.

Van den Brink, A., Godschalk, M., Smaal, A., Lindeboom, H. & McLay, C. (2013). Some like it hot: the effect of temperature on brood development in the invasive crab *Hemigrapsus takanoi* (Decapoda: Brachyura: Varunidae). *Journal of the Marine Biological Association of the United Kingdom*, 93(1), 189-196.

Veilleux, E. & de Lafontaine, Y. (2007). Biological synopsis of the Chinese mitten crab (*Eriocheir sinensis*). *Canadian Manuscript Report of Fisheries and Aquatic Sciences*, 2812, 1-54.

Ware, C., Berge, J., Sundet, J.H., Kirkpatrick, J.B., Coutts, A.D.M., Jelmert, A., Olsen, S.M., Floerl, O., Wisz, M.S. & Alsos, I.G. (2014). Climate change, non-indigenous

species and shipping: assessing the risk of species introduction to a high-Arctic archipelago. *Diversity and Distributions*, 20, 10-19.

Webster, J.M., Clark, P.F. & Morritt, D. (2015). Laboratory based feeding behaviour of the Chinese mitten crab, *Eriocheir sinensis*, (Crustacea: Decapoda, Brachyura, Varunidae): fish egg consumption. *Aquatic Invasions*, 10(3), 313-326.

Witbaard, R., Bergman, M.J.N., van Weerlee, E. & Duineveld, G.C.A. (2017). An estimation of the effects of *Ensis directus* on the transport and burial of silt in the near-shore Dutch coastal zone of the North Sea. *Journal of Sea Research*, 127, 95-104.

Zemmer, S.A., Detwiler, J.T., Sokol, E.R., Da Silva Neto, J.G., Wyderko, J., Potts, K., Gajewski, Z.J., Sarment, L.V., Benfield, E.F. & Belden, L.K. (2020). Spatial scale and structure of complex life cycle trematode parasite communities in streams. *PLos ONE*, 15(11), e0241973, 1-18.

Chapter 6: Discussion and Concluding Remarks

Despite a growing recognition of the role that invasive species play in the movement of parasites, questions remain outstanding as to how invasive host-parasite complexes interact in the marine environment, and their subsequent impacts on the blue economy. It is likely that coastal ecosystems in Ireland will be increasingly invaded, and parasite-mediated impacts should be assessed when considering the consequences of current and future biological invasions. Cooperation between academia, state bodies and citizen scientists is necessary to facilitate early detection of invasive species, as is cooperation with those employed in the aquaculture sector. Enhanced screening upon detection, coupled with long term monitoring, is also needed to understand invasive parasite-host interactions.

Throughout this study, the intrinsic links between bivalve aquaculture and the movement of invasive species has been examined, whereby movement of shellfish stock may enable invasive species and their associated parasites also to move and subsequently colonise further sites. Empirical evidence has been used to demonstrate how invasive species can act as a reservoir for pathogens that impact commercial bivalves including European flat oysters, Pacific cupped oysters and cockles. The study notes in particular that haplosporidian and bacterial species are present in invasive tunicates and, in the case of the haplosporidian *Minchinia mercenariae*-like, are able to replicate in the tunicate tissue, highlighting the potential for tunicates to act as carriers, reservoirs and hosts of bivalve pathogens, further facilitating their spread.

This research had a particular focus on the Irish Sea and Celtic Sea regions, due to the contribution of bivalve aquaculture to local economies in these areas. The presence of host-associated microbial communities is a characteristic shared by most faunal species, with the interactions that they display ranging from mutualistic to pathogenic (Destoumieux-Garzón *et al*., 2020). Plankton sampling and the subsequent analysis of the large zooplankton dataset revealed distinct microbial communities across the regions, coupled with the presence of microorganisms that are potentially pathogenic to bivalves. The detection of potential pathogens here was in association with current zooplankton compositions, but future screening may be necessary if there is northward migration of (potentially invasive) zooplankton species with new pathogens. Understanding the factors that affect the distribution of diseases, both spatially and

temporally, is crucial for understanding patterns of disease transmission (Filion *et al*., 2020).

The research presented here also looks to the future, identifying the specific routes by which selected invasive species may arrive and subsequently disperse in Ireland. As an island nation, Ireland is provided a degree of protection from marginal dispersal of invasives from other coasts due to its geographical distance. However, high connectivity via the shipping industry may counteract this and lead to species introductions. Analysis of potential corridors of introduction focused on four species; the Chinese mitten crab *Eriocheir sinensis*, the American razor clam *Ensis leei* (*directus*) and the Brush clawed and American shore crabs *Hemigrapsus* spp., which had been identified by horizon scanning to be among the top 40 invasive species likely to arrive in Ireland in the decade 2017-2027 (Lucy *et al*., 2020). High connectivity was observed in shipping routes between Ireland and international ports in which these invasive species are confirmed, suggesting that preparation for their arrival is warranted. *E. sinensis* has been sighted in the Waterford Estuary, specifically one individual in 2006 and 16 individuals in 2009 (Biodiversity Ireland, 2021), although to date the species does not appear to have established. However, this present study noted that previous invasions have demonstrated a pattern of sporadic and isolated sightings followed by a population explosion (Veilleux & de Lafontaine, 2007), so monitoring of this area is advisable. The American slipper limpet *Crepidula fornicata*, currently abundant in Belfast Lough, was also considered and the potential for secondary spread of this species is high. This species is known for its negative impacts on native bivalves, particularly the European flat oyster *Ostrea edulis* (Quinn *et al*., 2020), and curtailing its spread is important for preserving native stocks.

In the course of this research, potential threats to aquaculture arising from invasive species and pathogens have been detected. In the case of the tunicates the threats are already present, however there is the prospect of more host/parasite introductions via natural dispersal facilitated by plankton and currents, and anthropogenic routes enabled by shipping. All locations that receive new invasions subsequently become new potential donor regions (Carlton, 1996). Accordingly, controlling introductions before propagules have the chance to disperse and establish multiple populations is the most effect form of management, as eradication after establishment is nearly impossible. One of the crucial aspects of biosecurity and preventing the spread of

invasive species is early detection and this can take the form of taxonomic surveys, for example visual inspections of aquaculture gear, marina pontoons and other artificial structures, or the use of molecular techniques. Lucy *et al*. (2020) noted that promotion of citizen science and training to identify invasive species is important, as is the establishment of initiatives such as the 'check-clean-cry' programme, designed to prevent the spread of invasive species from one waterbody to another and promoted throughout Ireland.

Recent studies have suggested that invasion biology is entering the 'genomic era' and recommended that employing both DNA-based and traditional technologies will delineate accurate species ranges. DNA metabarcoding has also been identified as an important tool of the future, particularly for species that lack diagnostic features (for example juvenile forms), and may be useful for detecting invasive species in ballast water (Viard *et al*., 2016). Molecular approaches are increasingly used successfully; for example, Borrell *et al*. (2017) sampled eDNA from water samples in eight ports in the Bay of Biscay, selected due to the availability of information about established invasive species, and detected three invasive invertebrates, suggesting that this low-intensity sampling method may be of use in efficient biosecurity protocols. Cottier-Cook *et al*. (2019) acted on unconfirmed results from environmental DNA (eDNA) reports of *Didemnum vexillum* in a Special Area of Conservation in Loch Creran, Scotland to run surveys of a local oyster farm and found confirmed colonies of *Didemnum vexillum*. The site is home to protected serpulids *Serpula vermicularis*, flame shell *Limaria hians* and horse mussel *Modiolus modiolus* and the rapid detection ensured that measures could be put in place with the oyster farmers to ensure the spread was contained.

It is recognised that enhanced pathogen screening and monitoring will lead to increased costs, particularly when utilising molecular techniques. However, accepting these costs will also lead to heightened biosecurity, stronger culture production and potentially the ability to expand the aquaculture sector. These principles have been focused on bivalve culture in the course of this research, but may be applied to finfish culture also, as ultimately monitoring and controlling disease transmission will enhance the environmental and economic viability of the greater aquaculture sector.

The likelihood of future invasions in the Irish and Celtic Sea regions, coupled with the presence of multiple invasive tunicate species and the American slipper limpet, suggests that investigation of all potential forms of control may be beneficial. Chemical and biological controls (biocontrols) are constantly developing research areas in invasion ecology (Thresher & Kuris, 2004), however stakeholder uncertainty about the outcomes of potential control actions is valid. Chemical pesticides have been tested worldwide, however they are indiscriminate and may impact ecosystem health (Altvater *et al*., 2017). By contrast biological controls (biocontrols) display host-specificity but once introduced are likely to persist in the environment and any unforeseen negative effects, particularly associations with native species, may be difficult to prevent or eradicate (Lafferty & Kuris, 1996; Secord, 2003; Bajer *et al*., 2019). Given the caveats associated with chemical and biological controls, other conventional avenues such as heat treatment to aquaculture gear should be studied, in an attempt to limit if not eradicate further spread. Coughlan *et al*. (2019) suggested that direct steam exposure may be a feasible mechanism of control, particularly if the entire object cannot be immersed in hot water, and indeed steaming has been known to induce rapid mortality in highly invasive freshwater Asian clams *Corbicula fluminea*.

Identifying knowledge gaps within risk assessments is an integral part of proposed management plans, for example control of invasive species is a much-studied aspect but control of their associated parasites is less well understood (Lymbery *et al*., 2014). In addition to monitoring invasive species, an understanding of the origin and spread of diseases is therefore crucial to management (Pernet *et al*., 2018) as viruses, bacteria and protozoa have substantial commercial impact and surveillance and control plans are necessary (Zannella *et al*., 2017). The horizon scanning shipping exercise in this research presents a unique opportunity to follow the invasion front of any associated parasites that may arrive with focal invasive species, and investigate what interactions between invasive host, parasite and native species occur. When an invasive species first enters an area there is a lag-time between its arrival and the arrival of any host-specific natural enemies, and instant screening may facilitate an understanding of the timelines. Similar experiments in the terrestrial environment have been undertaken with invasive gypsy moths *Lymantria dispar* in the Midwestern United States and noted that infection levels by parasitic fungus and baculovirus were low in newly

established host populations. However, pathogens were present in host populations behind the expanding front of the invasive moth, meaning that the moths were released from enemies for only a short period of time (Hajek & Tobin, 2011).

Seasonal dynamics can influence many parasite-host interactions and understanding the processes linking seasons and infections is important to better forecast the impacts of climate change (Poulin, 2020). Climate change will interact with different stressors to affect the distribution, spread, abundance and impact of invasive species (Hellmann *et al*., 2008). Increased temperatures can enhance stress and mortality events in native species, thus facilitating the proliferation of invasive species with higher thermal tolerance (Diez *et al*., 2012). Monitoring environmental stressors including temperature, salinity, pH, nutrients and oxygen will aid disease-monitoring, as will increased screening of native and invasive conspecifics.

The research presented here confirms that invasive species and their associated parasites have greater potential to influence disease dynamics in an aquaculture setting than previously recognised. Most importantly, invasives and parasites should not be considered in a vacuum but as interwoven complexes that may pose risks for Irish and Celtic Sea bivalve aquaculture. Integrating the fields of parasitology and invasion ecology will enhance critical understanding of marine diseases, invasive host-parasite dynamics and ultimately potential mitigation strategies.

References

Altvater, L., de Messano, L.V.R., Andrade, M., Apolinário, M. & Coutinho, R. (2017). Use of sodium hypochlorite as a control method for the non-indigenous coral species *Tubastraea coccinea* Lesson, 1829. *Management of Biological Invasions*, 8(2), 197-204.

Bajer, P.G., Ghosal, R., Maselko, M., Smanski, M.J., Lechelt, J.D., Hansen, G. & Kornis, M.S. (2019). Biological control of invasive fish and aquatic invertebrates: a brief review with case studies. *Management of Biological Invasions*, 10(2), 227-254.

Biodiversity Ireland (2021). https://species.biodiversityireland.ie/profile.php?taxonId=22443. Accessed 12/03/2021.

Borrell, Y.J., Miralles, L., Mártinez-Marqués, A., Semeraro, A., Arias, A., Carleos, C.E. & García-Vazquez. (2017). Metabarcoding and post-sampling strategies to discover non-indigenous species: A case study in the estuaries of the central south Bay of Biscay. *Journal for Nature Conservation*, 42, 67-74.

Carlton, J. (1996). Pattern, Process, and Prediction in Marine Invasion Ecology. *Biological Conservation*, 78, 97-106.

Cottier-Cook, E.J., Minchin, D., Giesler, R., Graham, J., Mogg, A.O.M., Sayer, M.D.J., Matejusova, I. (2019). *Management of Biological Invasions*, 10(2), 311-323.

Coughlan, N.E., Cuthbert, R.N., Dickey, J.W.E., Crane, K., Caffrey, J.M., Lucy, F.E., Davis, E. & Dick, J.T.A. (2019). Better biosecurity: spread-prevention of the invasive Asian clam, *Corbicula fluminea* (Müller, 1774). *Management of Biological Invasions*, 10(1). 111-126.

Destoumieux-Garzón, D., Canesi, L., Oyanedel, D., Travers, M.-A., Charrière, G.M., Pruzzo, C. & Vezzulli, L. (2020). *Vibrio*-bivalve interactions in health and disease. *Environmental Microbiology*, 212, 4323-4341.

Diez, J.M., D'Antonio, C.M., Dukes, J.S., Grosholz, E.D., Olden, J.D., Sorte, C.J.B., Blumenthal, D.M., Bradley, B.A., Early, R., Ibáñez, I., Jones, S.J., Lawler, J.J. & Miller, L.P. (2012). Will extreme climatic events facilitate biological invasions*? Frontiers in Ecology and the Environment*, 10, 249-257.

Filion, A., Erikkson, A., Jorge, F., Niebuhr, C.N. & Poulin, R. (2020). Large-scale disease patterns explained by climatic seasonality and host traits. *Oecologia*, 194, 723-733.

Hellmann, J.J., Byers, J.E., Bierwagen, B.G. & Dukes, J.S. (2008). Five Potential Consequences of Climate Change for Invasive Species. *Conservation Biology*, 22(3), 534-543.

Lafferty, K.D. & Kuris, A.M. (1996). *Biological Control of Marine Pests*. *Ecology*, 77(7), 1989-2000.

Lucy, F.E., Davis, E., Anderson, R., Booy, O., Bradley, K., Britton., J.R., Byrne, C., Caffrey, J.M., Coughlan, N.E., Crane, K., Cuthbert, R.N., Dick, J.T.A., Dickey, J.W.E., Fisher, J., Gallagher, C., Harrison, S., Jebb, M., Johnson, M., Lawton, C., Lyons, D., Mackie, T., Maggs, C., Marnell, F., McLoughlin, T., Minchin, D., Monaghan, O., Montgomery, I., Moore, N., Morrison, L., Muir, R., Nelson, B., Niven, A., O'Flynn, C., Osborne, B., O'Riordan, R.M., Neil, R., Roy, H., Sheehan, R., Stewart, D., Sullivan, M., Tierney, P., Treacy, P., Tricario, E. & Trodd, W. (2020). Horizon scan of invasive alien species for the island of Ireland. *Management of Biological Invasions*, 11(2), 155-177.

Lymbery, A.J., Morine, M., Kanani, H.G., Beatty, S.J. & Morgan, D.L. (2014). Co-invaders: The effects of alien parasites on native hosts. *International Journal for Parasitology: Parasites and Wildlife*, 3, 171-177.

Pernet, F., Fuhrmann, M., Petton, B., Mazurié, J., Bouget, J-F., Fleury, E., Daigle, G. & Gernez, P. (2018). Determination of risk factors for herpesvirus outbreak in oysters using a broad-scale spatial epidemiology framework. *Scientific Reports*, 8(10869), 1-11.

Poulin, R. (2020). Meta-analysis of seasonal dynamics of parasite infections in aquatic ecosystems. *International Journal for Parasitology*, 50, 501-510.

Quinn, E.A., Malkin, S.H., Rowley, A.F. & Coates, C.J. (2020). Laccase and catecholoxidase activities contribute to innate immunity in slipper limpets, *Crepidula fornicata*. *Developmental and Comparative Immunology*, 110, 103724, 1-9.

Secord, D. (2003). Biological control of marine invasive species: cautionary tales and land-based lessons. *Biological Invasions*, 5, 117-131.

Thresher, R.E. & Kuris, A.M. (2004). Options for managing invasive marine species. *Biological Invasions*, 6, 295-300.

Viard, F., David, P. & Darling, J.A. (2016). Marine invasions enter the genomic era: three lessons from the past, and the way forward. *Current Zoology*, 62(6), 629-642.

Veilleux, E. & de Lafontaine, Y. (2007). Biological synopsis of the Chinese mitten crab (*Eriocheir sinensis*). *Canadian Manuscript Report of Fisheries and Aquatic Sciences*, 2812, 1-54.

Zannella, C., Mosca, F., Mariani, F., Franci, G., Folliero, V., Galdiero, M., Tiscar, P.G. & Galdiero, M. (2017). Microbial Diseases of Bivalve Mollusks: Infections, Immunology and Antimicrobial Defense. *Marine Drugs*, 15(182), 1-36.

Appendix A

Supplementary Material for Chapter 4: Regional Differences in Zooplankton-Associated Bacterial Communities and Aquaculture Pathogens Across Two Shelf Seas

Alpha Diversity Indices were conducted to analyse the species richness and diversity within samples. Beta Diversity indices were then applied to visualise the variability in microbial community composition between areas.

Alpha Diversity

Figure 1 displays the Rarefaction curve for the four areas (Irish Sea, Celtic Front, Eastern Celtic and Southern Celtic), representing the richness (i.e. the number of OTUs) in a randomly selected subsample of sequences. The curve reflects the richness of microbial community in the whole sample set and was seen here flattening at the top, meaning an adequate number of samples have been taken and only the scarce species remain to be sampled.

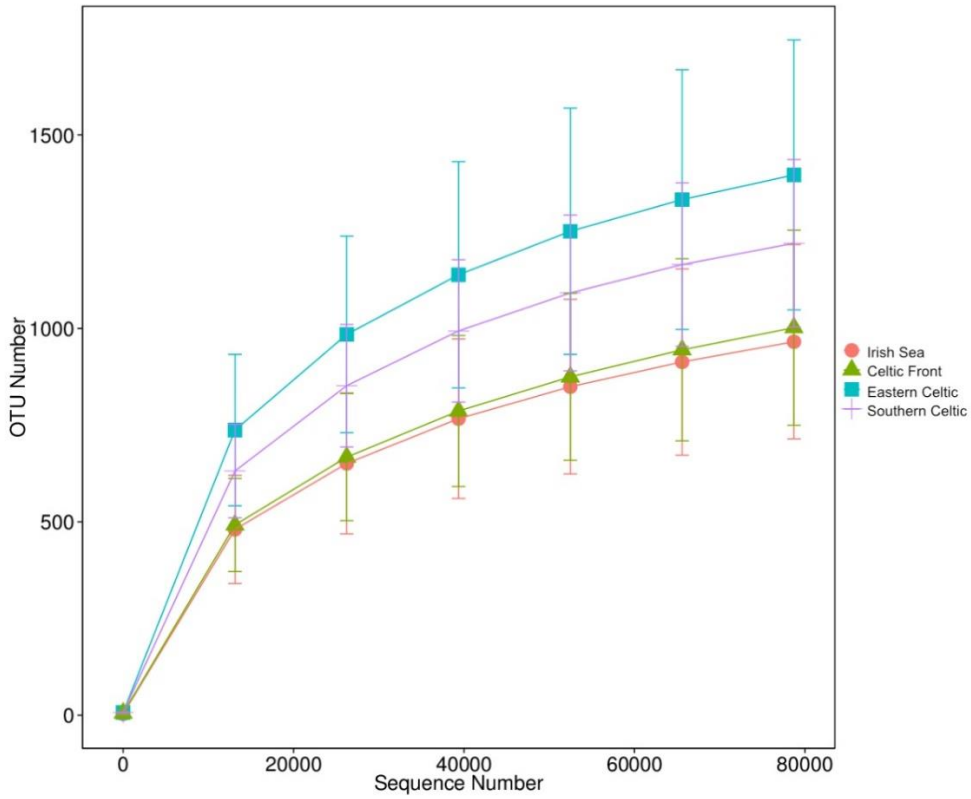

Figure 1: Rarefaction curve for the four areas; Irish Sea, Celtic Front, Eastern Celtic and Southern Celtic, demonstrating that an adequate number of samples has been taken to represent OTU richness.

Figure 2 consists of Flower diagrams that represent the number of OTUs in each individual sample. The core number in the center signifies number of OTUs present in all samples within that area, while numbers in the petals signify unique OTUs in each individual sample. OTUs that were present in some but not all of the individual samples within the group were not included. The Eastern Celtic samples had the highest number of unique OTUs (n = 2,178) followed by the Southern Celtic* (n = 1,953), Irish Sea (n = 1,823) and Celtic Front (n = 1,745).

*The Southern Celtic area had seven samples whereas all other areas had eight, however Rarefaction Curves (Supplementary Material) demonstrated that it still provided an accurate representation of OTU richness.

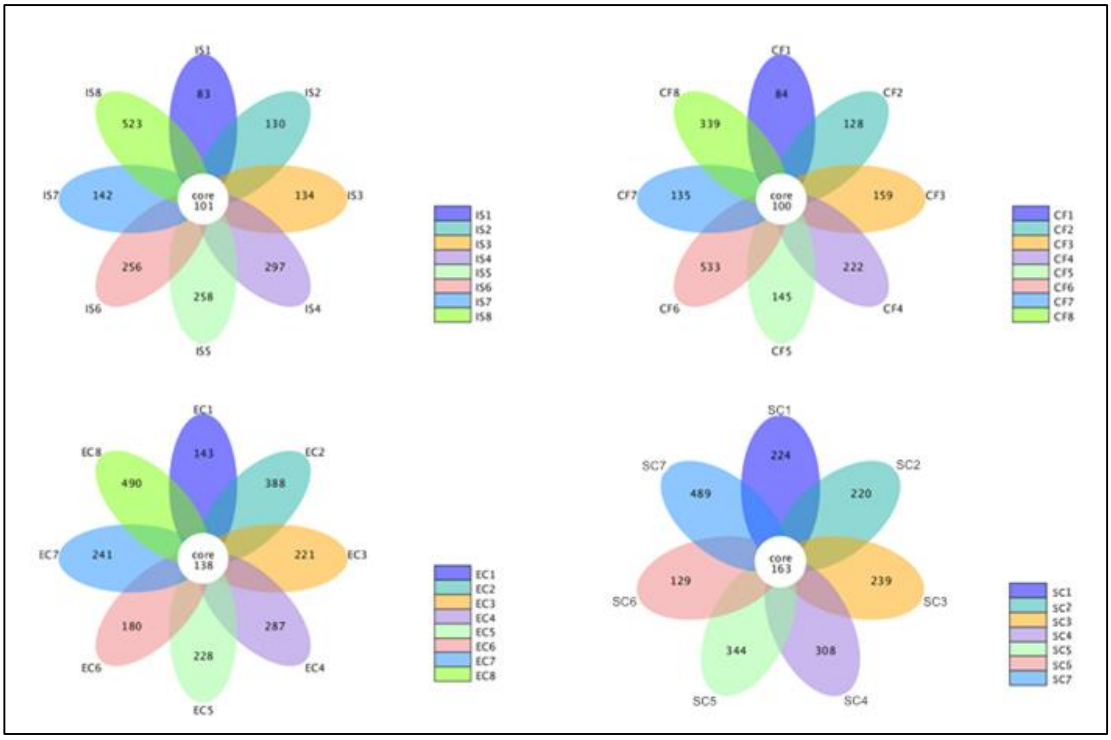

Figure 2: OTU richness in individual samples (petals) plus OTUs that appeared in every sample within an area. Clockwise from top left: Irish Sea, Celtic Front, Southern Celtic and Eastern Celtic

Boxplots were created to reflect the difference in Alpha Diversity indices between areas. Figure 3A depicts the numbers of Observed Species, with the lowest median observed in the Irish Sea (IS) and the highest (n = 1,500) observed in the Eastern Celtic (EC). The Easter Celtic also had the greatest distribution, with the greatest number of species observed between the first quartile and the median. A similar pattern was observed in the Shannon Diversity Index, depicted in Figure 3B.

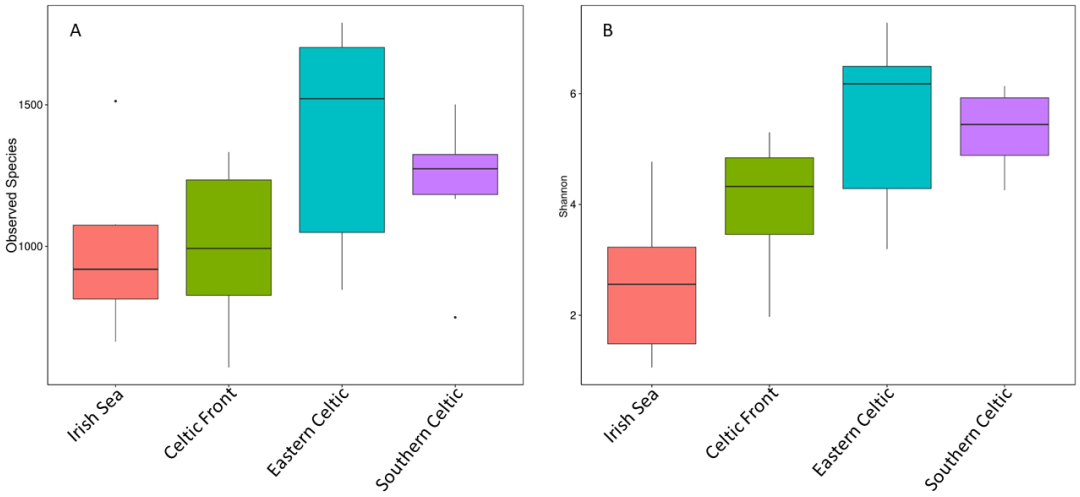

Figure 3: The difference in Observed Species (3A) and Shannon Diversity (3B) between areas**.**

Beta Diversity

Boxplots were created to reflect the difference in Beta Diversity indices between areas. Figure 4A depicts the Unweighted Unifrac results, whereby areas displayed similarities in which species were present/absent and both the Irish Sea and Celtic Front displayed the same median. A different pattern was observed with the weighted unifrac results (abundance data), with the lowest median observed in the Irish Sea and the highest observed in the Eastern Celtic (4B).

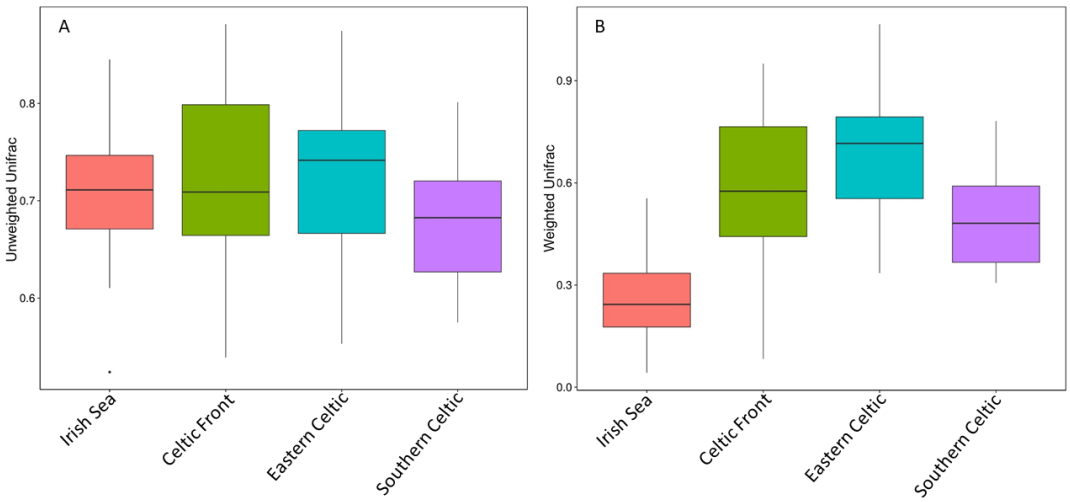

Figure 4: The difference in Unweighted (4A) and Weighted Unifrac (4B) between areas.

Wilcox and Tukey tests were conducted on both the weighted and unweighted data to test whether the differences in Beta Diversity between areas were significant (Table 1). Both Wilcox and Tukey tests conducted on the weighted data showed high levels of significance between areas. This was not the case with unweighted data, as the only significant difference observed was that of the Eastern Celtic-Southern Celtic Wilcox test.

Table 1: Results from weighted and unweighted Tukey and Wilcox tests to test Beta Diversity between areas. (Mean diff. = mean difference, Diff. = difference between groups, LCL = lower confidence interval, UCL = upper confidence interval).

Wilcox				
Weighted	Mean diff.	P-value	LCL	UCL
IS – CF	-40.643	**<0.001**	-52.905	-28.381
IS – EC	-52.464	**<0.001**	-64.727	-40.202
IS – SC	-29.964	**<0.001**	-43.209	-16.719
CF – EC	-11.821	**0.059**	-24.084	0.441
CF – SC	10.679	0.113	-2.566	23.923
EC – SC	22.500	**0.001**	9.255	35.745
Unweighted	Mean diff.	P-value	LCL	UCL
IS – CF	-2.464	0.760	-18.410	13.482
IS – EC	-5.500	0.495	-21.446	10.446
IS – SC	14.012	0.110	-3.212	31.236
CF – EC	-3.036	0.707	-18.982	12.910
CF – SC	16.476	0.061	-0.748	33.700
EC – SC	19.512	**0.027**	2.288	36.736
Tukey				
Weighted	diff	LCL	UCL	P adj
CF – IS	0.314	0.185	0.442	**0.000**
EC – IS	0.407	0.279	0.535	**0.000**
SC – IS	0.225	0.087	0.364	**0.000**
EC – CF	0.093	-0.035	0.221	0.235
SC – CF	-0.088	-0.227	0.050	0.348
SC – EC	-0.181	-0.320	-0.043	**0.005**
Unweighted	diff	LCL	UCL	P adj
CF – IS	0.008	-0.049	0.066	0.981
EC – IS	0.010	-0.047	0.067	0.970
SC – IS	-0.036	-0.098	0.026	0.424
EC – CF	0.002	-0.056	0.059	1.000
SC – CF	-0.044	-0.106	0.017	0.243
SC – EC	-0.046	-0.108	0.016	0.217

Figure 5 displays Unweighted (5A) and Weighted (5B) Principle Coordinates Analysis (PCoA), where samples ordinated closer together are more similar than those ordinated further away. Samples within the areas are more similar in the Weighted analysis, for example the cluster of samples in the Irish Sea is evident.

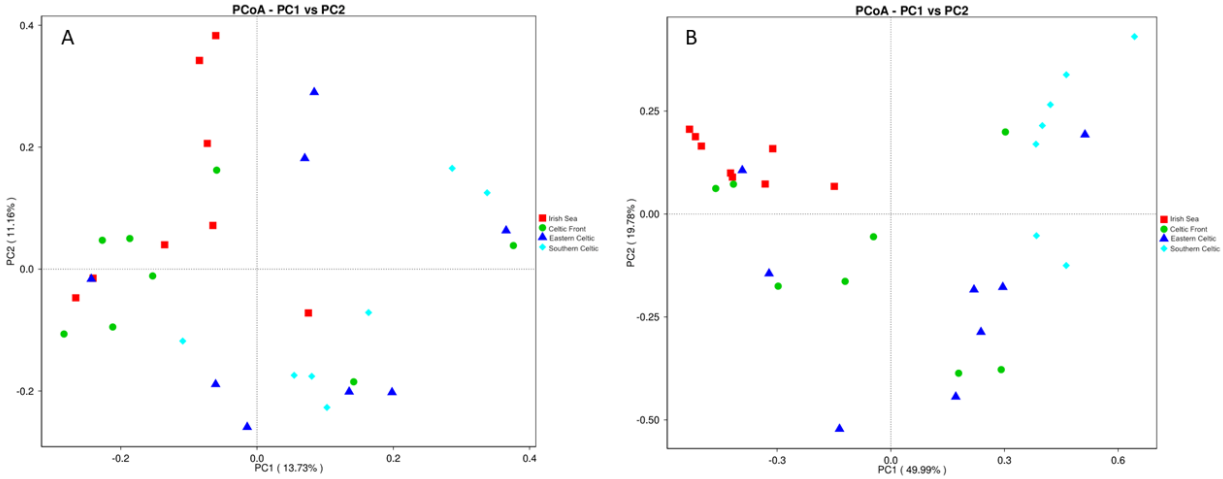

Figure 5: Unweighted (5A) and Weighted (5B) Principle Coordinates Analysis for the areas.

Analysis of Similarity (Anosim) evaluates whether variation between areas is significantly larger than variation within areas. R-values lie between -1 and 1 and a positive R-value means that inter-group variation is considered significant, while a negative R-value suggests that inner-group variation is larger than inter-group variation, (i.e. no significant difference between areas). The confidence degree is represented by P-value, and a value less than 0.05 suggests statistical significance. In all area comparisons the R-value was positive (Table 2), and for all except the Celtic Front-Eastern Celtic the P-value was less than 0.005.

Table 2: Anosim demonstrating that inter-group variation is larger than inner-group variation.

Area	R-value	P-value
IS – SC	1	0.001
EC – SC	0.406	0.003
EC – IS	0.738	0.001
CF – SC	0.786	0.001
CF – IS	0.286	0.004
CF – EC	0.271	0.006

T-tests were performed to determine the Phyla with significant differences (p-value < 0.05) between areas. There were ten Phyla which demonstrated significant differences between at least two areas (Table 3), and of these Phyla, the three that demonstrated significant differences between the most areas were Cyanobacteria, Patescibacteria and Proteobacteria.

Table 3: T-test results for all area combinations demonstrating Phyla with significant differences.

Phylum	Average Group A	Std. dev. Group B	Average Group A	Std. dev. Group B	p-value	q-value	LCL	UCL
Irish Sea *vs* Celtic Front								
Cyanobacteria	0.752	0.188	0.400	0.313	**0.019**	0.079	0.069	0.635
Bacteroidetes	0.038	0.020	0.202	0.158	**0.022**	0.079	-0.297	-0.032
Irish Sea *vs* Eastern Celtic								
Cyanobacteria	0.752	0.188	0.230	0.301	**0.001**	0.041	0.248	0.796
Proteobacteria	0.107	0.075	0.291	0.202	**0.040**	0.156	-0.356	-0.011
Bacteroidetes	0.038	0.020	0.240	0.173	**0.013**	0.127	-0.347	-0.057
Actinobacteria	0.020	0.014	0.076	0.066	**0.047**	0.156	-0.112	-0.001
Verrucomicrobia	0.019	0.028	0.058	0.034	**0.025**	0.156	-0.072	-0.006
Patescibacteria	0.0003	0.0003	0.001	0.0004	**0.013**	0.127	-0.001	-0.0001
Fibrobacteres	6.04E-05	5.34E-05	0.001	0.001	**0.049**	0.156	-0.002	-5.34E-06
Spirochaetes	4.13E-05	5.12E-05	0.0004	0.000	**0.039**	0.156	-0.001	-2.16E-05
Irish Sea *vs* Southern Celtic								
Cyanobacteria	0.752	0.188	0.048	0.039	**8.94E-06**	0.0001	0.545	0.862
Proteobacteria	0.107	0.075	0.769	0.095	**8.75E-09**	1.99E-07	-0.760	-0.564
Patescibacteria	0.0003	0.0003	0.002	0.001	**0.026**	0.200	-0.002	0.000
Celtic Front *vs* Eastern Celtic								
Actinobacteria	0.012	0.005	0.076	0.066	**0.028**	0.140	-0.120	-0.009
Euryarchaeota	0.005	0.005	0.047	0.047	**0.040**	0.140	-0.081	-0.003
Epsilonbacteraeota	0.0002	0.0001	0.001	0.001	**0.050**	0.140	-0.002	-1.12E-06
Patescibacteria	0.0004	0.0004	0.001	0.0004	**0.043**	0.140	-0.001	-1.61E-05
Celtic Front *vs* Southern Celtic								
Cyanobacteria	0.400	0.313	0.048	0.039	**0.015**	0.053	0.089	0.614
Proteobacteria	0.289	0.218	0.769	0.095	**0.0002**	0.002	-0.669	-0.290
Planctomycetes	0.001	0.001	0.003	0.002	**0.035**	0.061	-0.004	-0.0002
Patescibacteria	0.0004	0.0004	0.002	0.001	**0.036**	0.061	-0.002	-0.0001
Eastern Celtic *vs* Southern Celtic								
Proteobacteria	0.291	0.202	0.769	0.095	**0.0001**	0.001	-0.656	-0.300
Actinobacteria	0.076	0.066	0.013	0.007	**0.031**	0.073	0.008	0.118
Euryarchaeota	0.047	0.047	0.002	0.002	**0.029**	0.073	0.006	0.084
Verrucomicrobia	0.058	0.034	0.017	0.011	**0.010**	0.048	0.013	0.070

Appendix B

References for Chapter 5: Assessing the Potential for Invasive Species Introductions and Secondary Spread Using Vessel Movements in Maritime Ports

Table 1: References for Chapter 5, Table 2.

Species	References
Ensis leei (*directus*)	Arias & Anadón (2012); Dannheim & Rumohr (2012); Gollasch *et al*. (2015); Kerckhof *et al*. (2007); Witbaard *et al*. (2017)
Eriocheir sinensis	Drotz *et al*. (2010); Herborg *et al*. (2003); Kerckhof *et al*. (2007); National Biodiversity Network (NBN) Atlas (2021); Nędzarek & Czerniejewski (2021); North Western Inshore Fisheries and Conservation Authority (2020); Otto & Brandis (2011); Schoelynck *et al*. (2020); Silvestre *et al*. (2006)
Hemigrapsus sanguineus	Boets *et al*. (2012); Gittenberger *et al*. (2014); Landschoff *et al*. (2013); Soors *et al*. (2010)
Hemigrapsus takanoi	Boets *et al*. (2012); Dauvin *et al*. (2009); Geburzi *et al*. (2015); Geburzi *et al*. (2020), Gittenberger *et al*. (2014); Landschoff *et al*. (2013); Soors *et al*. (2010)