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INVASIVE ALIEN SPECIES IN URBANISED MARINE ENVIRONMENTS: MANAGEMENT APPLICATIONS OF NATURAL AND MANIPULATED SALINITY STRESS



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Abstract

The development of the world's oceans is not only changing the physical appearance of our coastlines, but also the marine communities that inhabit them. Increasing human activity in the seas has contributed to the spread of a vast number of marine invasive non-native species (NNS), which can have serious environmental and economic impacts in their introduced regions. In order to reduce the spread of these species, research into ways to cost-effectively monitor the arrival of new species and control or eradicate harmful species is needed. NNS are especially prevalent as fouling species on artificial structures within urbanised environments, such as harbours and marinas, where the high level of disturbance and changes in environmental conditions may facilitate their establishment. This thesis aims to investigate the effects of natural and manipulated changes in salinity on NNS within these environments, to address knowledge gaps in the management of NNS in Scotland.

In order to assess the long-term patterns of individual marine NNS establishment and spread, this thesis begins by updating data on the baseline distribution of marine and brackish NNS in Scotland. In 2016, rapid assessment surveys (RAS) were carried out in eighteen of the largest marinas in mainland Scotland, finding eighteen fouling NNS, triple the number of species detected in 2008 when marinas were last surveyed. The average number of species per site has also increased, rising by 3.2 species over the ten year period from 2006 to 2016. Since the last compilation of marine NNS records in 2012, increased survey efforts across the country have seen an additional 1006 presence records added to a synthesised list of marine and brackish NNS. These records show that there have been a number of new species introduced to Scotland in this period, with the number of recorded marine NNS now totalling 32 species, 22 of which are considered established or likely to be established. Sites identified in this study as having high abundances of NNS should be incorporated into strategic monitoring to allow for early detection of NNS new to Scotland.

The influence of salinity variation and other abiotic factors on variation in marina fouling communities was then evaluated. Patterns of NNS diversity were investigated among marinas found on a spectrum of high to low salinity. Field surveys were conducted to assess how native and non-species diversity contributed to fouling community composition

on floating pontoons, with primary and secondary data collected on temperature and salinity, site location, marina features and river parameters. Fouling communities are dominated by native species, although NNS occupied more than 50% cover in one site, and over 10% cover in another three sites. NNS and native species diversity were influenced by salinity conditions within a site, with sites further away from rivers having significantly more diverse fouling assemblages and higher numbers of NNS. This data could be used to develop a profile of sites with higher susceptibility to NNS colonisation, allowing for more cost-efficient assignment of monitoring effort.

Finally, two experiments were conducted to assess the effectiveness of treatment with hypo-saline waters as a tool for managing biofouling and controlling fouling NNS. Immersion in water with a salinity lower than 10 was shown to reduce biofouling load, resulting in loss of 20 - 50% of biofouling cover. Variation in species' tolerance to osmotic change resulted in a shift in community composition in the eight weeks following treatment. Susceptibility to low salinity treatment also varied between NNS, such that growth of some NNS actually increased following treatment with hypo-saline water, potentially due to reduced competition from dominant native species. Effectiveness of treatment with freshwater was lessened in sites where communities were exposed to low salinity periods, with no effect of treatment on communities in the site with high freshwater input. These results support previous research indicating the potential of osmotic shock as a method for reducing biofouling, but highlight the potential for unintended consequences if community and NNS tolerance are not given due consideration.

These findings highlight opportunities to use the natural salinity variation in Scottish urbanised waters to guide development of NNS monitoring and management strategies. Increased attention is being given to marine NNS management in Scotland, as opportunities for biosecurity planning emerge in the development of regional marine plans, and as marine industries are increasingly becoming aware of their responsibilities to follow best practice guidance in NNS management. The results of this study should be used to inform biosecurity plan development, and to help develop a strategic framework for national monitoring of NNS.

Lay summary

The development of the world's oceans is not only changing the physical appearance of our coastlines but also the marine communities that inhabit them. Increasing human activity in the seas has contributed to the spread of a vast number of marine invasive non-native species (NNS). NNS are organisms which are moved between regions by human action, and which can go on to cause serious harm to the environment and the economy. NNS are especially common in marinas and harbours where they grow on structures such as pontoons and pilings. In order to reduce the spread of these species, research into ways to cost-effectively monitor the arrival of new species and control or eradicate harmful species is needed. This thesis aims to identify ways in which natural variation in salinity, which affects all aquatic species, can be used to manage NNS.

This thesis begins by updating information on where marine NNS are found within Scotland. In 2016, a survey was carried out in eighteen of the largest marinas in mainland Scotland, finding eighteen NNS across all sites, triple the number of species detected in 2008 when marinas were last surveyed. The average number of species per site has also increased, rising by 3.2 species over the ten year period from 2006 to 2016. Since records of marine NNS were last compiled in 2012, an additional 1006 records of NNS have been added to a synthesised list of marine and brackish NNS as a result of increased survey effort across the country. These records show that there have been a number of new species introduced to Scotland in this period, bringing the total number of marine NNS to 32, 22 of which are considered as likely to have established reproducing populations. Sites identified in this study as having high abundances of NNS should be incorporated into strategic monitoring to allow for early detection of NNS new to Scotland.

The following chapter investigates patterns in the number of NNS in communities growing on floating pontoons in Scottish marinas. Field surveys were conducted to assess how the presence of NNS and the composition of biological communities was influenced by environmental conditions and the location and design of marinas. The results show that these fouling communities are dominated by native species, although NNS occupied more than 50% of space on pontoons in one site, and over 10% in another three sites. The number of NNS and

native species was influenced by the amount of freshwater entering a marina, with sites further away from rivers having significantly more native and non-native species. This data could be used to develop a profile of sites with higher susceptibility to colonisation by NNS, and help develop cost-efficient monitoring programmes.

Finally, two experiments were conducted to assess the effectiveness of treatment with low salinity water as a tool for managing biofouling and controlling fouling non-native species. Immersion in low salinity water was shown to reduce biofouling load, resulting in loss of 20 - 50% of biofouling cover. Variation in the tolerance of different species to changes in salinity resulted in different species dominating communities in the eight weeks following treatment. Susceptibility to low salinity treatment also varied between NNS, such that growth of some NNS actually increased following treatment with low salinity water, potentially due to reduced competition from dominant native species. Effectiveness of treatment with freshwater was lessened in sites where communities were naturally exposed to periods of low salinity, with no effect of treatment on communities in a site with high freshwater input. These results support previous research indicating the potential of low salinity treatment as a method for reducing biofouling, but highlight the potential for unintended consequences if the natural environmental conditions and tolerance of NNS are not considered.

These findings highlight opportunities to use the natural salinity variation in Scottish urbanised waters to guide development of NNS monitoring and management strategies. The results of this study should be used to inform the development of biosecurity plans during the process of regional marine planning, and to help develop a strategic framework for national monitoring of NNS.

Author's Declaration

I declare that this thesis has been composed solely by myself and that it has not been submitted, either in whole or in part, in any previous application for a degree. Except where otherwise acknowledged, the work presented is entirely my own.



Rebecca Jane Giesler

31 October 2019

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Carcinus maenas *and other aliens*

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List of Abbreviations

CBD	Convention on Biological Diversity
CPCE	Coral Point Count Extension
CTD	Conductivity, Temperature and Depth device
DAISIE	Delivering Alien Invasive Species Inventories for Europe
eDNA	Environmental Deoxyribonucleic Acid
GB NNSS	Great Britain Non-Native Species Secretariat
HACCP	Hazard analysis and critical control points
HELCOM	Baltic Marine Environment Protection Commission
IAS	Invasive Alien Species
IMO	International Maritime Organisation
INNS	Invasive Non-Native Species
IUCN	International Union for the Conservation of Nature
MPA	Marine Protected Area
MSFD	Marine Strategy Framework Directive
NBN	National Biodiversity Network
nMDS	non-metric Multi-Dimensional Scaling
NMP	National Marine Plan for Scotland
NMPi	National Marine Plan interactive
NNS	Non-Native Species
OSPAR	Commission for the Protection of the Marine Environment of the North-East Atlantic
RAS	Rapid Assessment Survey
RAFTS	Rivers and Fisheries Trusts of Scotland
RAPID	Reducing and Preventing Invasive Alien Species Dispersal
RBMP	River Basin Management Plan
RYA	Royal Yachting Association
SAC	Special Area of Conservation
SEPA	Scottish Environmental Protection Agency
SNH	Scottish Natural Heritage
SSSI	Site of Special Scientific Interest
WANE Act	Wildlife and Natural Environment (Scotland) Act
WFD	Water Framework Directive

CHAPTER 1

General Introduction

1.1 An introduction to marine non-native species

The introduction of novel organisms is an important factor contributing to global change in marine systems (Carlton, 1996a; Occhipinti-Ambrogi, 2007). Plant, animals, and microorganisms have been accompanying humans in their global movements for millenia, either as intentional introductions for food or trade, or as accidental hitchhikers on transports or equipment (Manchester and Bullock, 2000; Hulme, 2009). Human movement has enabled species to cross bio-geographic boundaries into new environments, where they occasionally find conditions suitable to their establishment and spread (IUCN, 2000). Once established in their new habitat, these non-native species (hereafter NNS) can cause changes in marine communities, impacting resident species through mechanisms such as predation or competition, or by causing structural changes to habitat.

Globalisation, and the resulting growth of trans-oceanic trade and maritime activities, has resulted in increasing numbers of species introductions into marine environments (Streftaris et al., 2005; Meyerson and Mooney, 2007; Seebens et al., 2013, 2017). Advances in international shipping have reduced transport times, increased the volume of water transported around the world, and increased the potential for NNS transport (Levine and D'Antonio, 2003; Hulme, 2009; Clarke Murray et al., 2012). Accompanying this growth in transport efficiency, is a rising demand for the oceans to provide solutions to problems of food and energy supply and support growing coastal populations (Bulleri and Chapman, 2010). The development of maritime industries to meet these demands has resulted in the construction of wide range of supporting infrastructure, from shoreline modifications to offshore installations, in the form of harbours, ports, marinas, coastal defence structures, aquaculture farms, artificial reefs, renewable energy devices, and oil and gas platforms

(Sheehy and Vik, 2010; Mineur et al., 2012; Dafforn et al., 2015b). These artificial structures may facilitate the establishment and spread of NNS, by providing hard substrate for colonisation, increasing connectivity and creating corridors for the movement of species (Bulleri and Chapman, 2010; Mineur et al., 2012; Johnston et al., 2017).

In Scotland, marine industries have long made up a substantial sector of the economy, with the development of aquaculture, offshore renewable energy, and coastal tourism contributing to the growth of the marine economy in recent decades (Baxter et al., 2011; Marine Scotland, 2017). The sustainable development of marine industries offers a number of benefits, but also brings challenges in the form of impacts to natural habitat, changes to marine connectivity and ecological and economic impacts arising from the spread of marine NNS (Baxter et al., 2011; Mineur et al., 2012).

1.1.1 Impacts of invasive species

1.1.1.1 Ecological impacts

The introduction of NNS is contributing to changes in species assemblages in both terrestrial and aquatic environments (Occhipinti-Ambrogi and Savini, 2003). The introduction of novel species to a community can have no impact, or positive or negative effects on the system to which it is introduced (Ruiz et al., 1999; Gribben et al., 2013). Assessing impact in novel regions can be difficult, as it is often hard to gather information on NNS distribution. Experimental studies assessing impact of NNS on other species are even more limited. In Scotland, there has been limited assessment of the geographical extent and ecological impact of NNS.

Of the 12,000 terrestrial and aquatic NNS in Europe, 10-15% are estimated to be invasive (Sundseth, 2014). In a study of the aquatic NNS in Chesapeake Bay, Ruiz et al. (1999) determined that approximately 20% of the known 196 NNS were thought to be having an ecological impact. However, the study authors highlighted that at the time quantitative data was likely available for approximately 5% of NNS present in other bays and estuaries. NNS introductions may interact with other anthropogenic stresses on the marine environment, making quantifying the level of impact of invasive species difficult, further exacerbated by a lack of baseline data (Ruiz et al., 1999; Manchester and Bullock, 2000). Despite these restrictions, invasive species are thought to be one of the main contributors to global biodiversity loss (Bax et al., 2003; Vilà et al., 2010).

Introduced species may negatively impact native biodiversity through a variety of

mechanisms (Thompson et al., 2002; Molnar et al., 2008). These include competition, predation, spread of parasites and disease, hybridisation with native species, and habitat modification (Ruiz et al., 1999; Grosholz, 2002; Cook et al., 2016). The Indo-Pacific lionfish *Pterois volitans* (Linnaeus, 1758), thought to have been introduced to the Florida coast as an accidental aquarium release, has now spread across much of the Caribbean (Côté and Maljković, 2010). As a voracious predator, the lionfish has had significant impact on coral reef fish populations, and predation of this species on herbivorous fish is thought to have resulted in a phase shift from coral to algal dominated systems along some reefs (Côté and Maljković, 2010; Lesser and Slattery, 2011). Invasive macroalgae species, such as *Caulerpa taxifolia* (M.Vahl) C.Agardh 1817, and *Undaria pinnatifida* (Harvey) Suringar, 1873 which are both included on the list of the world's 100 worst invasive species (Lowe et al., 2000), are capable of extensive spread within their introduced ranges and may compete with native macroalgae and seagrass species (Schaffelke et al., 2006; Anderson, 2005; Maggi et al., 2015; Epstein and Smale, 2017a). The effects of invasive macroalgae on higher trophic levels are more variable due to their role in forming habitat for both epiphytic and mobile species (Maggi et al., 2015; Arnold et al., 2016; Katsanevakis et al., 2016).

NNS which fundamentally alter the ecological and/or physical characteristics of habitats into which they are introduced are termed 'ecosystem engineers' (Jones et al., 1994; Ruiz et al., 1997; Guy-Haim et al., 2018). The impact of these species may be extensive as they may alter the availability of resources, for example, by providing additional hard substrate or altering light, nutrient, or space availability (Crooks, 2002). For example, the dispersal of the Pacific oyster *Magallana gigas* following its introduction for shellfish aquaculture, has resulted in the transformation of muddy intertidal areas to dense oyster reefs (Cook et al., 2016). Habitat formation can have positive or negative effects for native biota, for instance, by either reducing habitat suitability for native species, or facilitating growth through provision of additional habitat complexity (Bruno, 2005; Rodriguez, 2006; Gribben et al., 2013). The establishment of NNS has also been shown to facilitate further establishment of other NNS, termed 'invasional meltdown' (Simberloff et al., 1999; Ricciardi and Cohen, 2007).

1.1.1.2 Socio-economic impacts

The economic impacts of marine invasive species stem from impacts on human health, productivity of marine industries, and costs associated with removal of unwanted or fouling species (Bax et al., 2003). The impact of invasive species on native biodiversity may reduce

fishing catches, such as in the case of the now famous example of the invasive ctenophore, *Mnemiospis leidyi* Agassiz, 1865, into the Black Sea. Introduced in the 1980s, to a system already suffering from the impacts of eutrophication and overfishing, huge blooms of *M. leidyi* competed with, and predated on fish larvae, causing collapse of the anchovy fishery by the early 1990s (Shiganova, 1998; Mee et al., 2005). Thus systems which are already degraded as a result of anthropogenic impact, may be especially vulnerable to impacts of invasive species.

Much of the marine aquaculture around the world is increasingly reliant on using specific non-natives as stock, unwanted pest species can cause problems by introducing diseases or impacting the growth of cultivated stock and reducing yield (Watson et al., 2009; Cottier-Cook et al., 2016). Fouling of stock and equipment can result in increased cleaning costs and reduced opportunities for sale (Bourque et al., 2005; Watson et al., 2009; Fitridge et al., 2012). Non-native ascidian species in particular have caused problems for shellfish aquaculture companies, species such as *Styela clava* Herdman 1881 and *Didemnum vexillum* Kott 2002 are capable of settling in high densities on aquaculture structures and smothering mussel species (Figure 1.1) (Dafforn et al., 2012; Ferguson et al., 2017). Larvae of the bay scallop *Argopectin irradians irradians* were even shown to avoid settlement on colonies *D. vexillum*, potentially affecting commercial sea scallop fisheries in the USA (Morris et al., 2009).

NNS may even detrimentally impact public health. For example, large swarms of the non-native tropical scyphozoan, *Rhopilema nomadica* Galil, 1990, in the Levantine Sea have blocked intake pipes of power plant cooling systems, interfered with fishing, and their severe stings represent a health risk, driving tourists away from beaches (Yahia et al., 2013; Galil et al., 2015).

The costs associated with NNS can thus be significant, and include economic losses as well as money spent removing or attempting to control species. In 2005, Pimentel et al. (2005) identified that the environmental damage and loss associated with invasive NNS in the USA totalled around $120 billion annually. The combined costs of control and biosecurity of terrestrial and aquatic species to the UK economy was calculated at £1.7 billion in 2010, and to the Scottish economy at over £244 million (Williams et al., 2010). Research to determine the ecological processes which influence the establishment and spread of NNS in thus crucial in developing appropriate management measures.



Figure 1.1: *Example of* Didemnum vexillum *Kott, 2002 fouling a rope in a marina. Fouling of* D. vexillum *can result in increased cleaning costs for marine industries.*

1.2 The ecology of marine non-native species

1.2.1 Definition of terms

The field of biological invasions has a variety of interchangeable terms for non-native species, including 'non-indigenous species', 'alien species', 'exotic species', and 'introduced species'. The terminology used and the definition of what constitutes a NNS (or introduced, alien species etc.) can vary between regions, institutes and policy documents (Occhipinti-Ambrogi and Galil, 2004; Galil et al., 2014). This study uses the term non-native species (NNS), and defines NNS as *any species or subspecies introduced as a consequence of human action outside its natural range, which includes any part, gamete or propagule of such species which might survive and subsequently reproduce* (IUCN, 2000).

Species for which the introduction status is uncertain are deemed to be of 'cryptogenic origin' (Carlton, 1996b). As species introductions have been occurring for centuries, determining if a species was introduced as a result of human activity and is thus considered 'non-native' can be challenging, especially if species have a widespread or cosmopolitan distribution (Carlton, 1996b). The status of cryptogenic species may change as further research is generated (Kakkonen et al., 2019). Determining the natural range of species may be further complicated by species range shifts occurring as a result of anthropogenic influenced climate change (Harley et al., 2006; Occhipinti-Ambrogi, 2007; Burrows et al., 2011). While the argument could be made that species shifting as a result of modern climate change are arriving as a result of indirect human mechanisms, further consideration of these species is beyond the scope of this thesis.

The human driven mechanisms by which NNS are introduced are referred to as 'vectors' of species introduction (Gollasch, 2002). It is important to emphasise that species which are introduced into a region by human vectors, but which then spread further via natural dispersal mechanisms are still considered NNS in all areas of their introduced range. The intra-regional spread of NNS (via natural dispersal or onward human-mediated transfer) is termed 'secondary spread' and can play an important role in connecting urbanised areas of primary introduction with more remote areas containing potentially vulnerable ecosystems (Clarke Murray et al., 2011; Ashton et al., 2014; Nall et al., 2015).

The majority of NNS introduced to a region do not form self-sustaining populations, those that do are considered 'established' (Williamson and Fitter, 1996; Nall et al., 2015). In a

study of marine and brackish NNS in British waters, 40% of those introduced were thought to have not become established (Minchin et al., 2013). Of those that are established, the majority have no discernible impact on their environment (Eno, 1997; Blackburn et al., 2014); however, a minority of species go on to spread widely within their introduced regions and may cause harm to native biodiversity, the economy, human health, or the cultural value of the environment (Williams et al., 2010; Cook et al., 2016). The term 'invasive' is used in this thesis to describe NNS *which have the ability to spread, and which have an adverse impact of native biodiversity, the environment or social or economic interests* (Scottish Government, 2012; GBNNSS, 2015; Lehtiniemi et al., 2015a). This definition is the most frequently used in the UK (GBNNSS, 2015). However, consensus on when it is appropriate to characterise a species as 'invasive' is largely missing from the scientific and policy literature on biological invasions (Galil et al., 2014).

Occhipinti-Ambrogi and Galil (2004) argue that only the scale of NNS spread should be considered when defining an species as invasive, as establishing the degree of impact on native ecosystems and the economy is challenging and often beyond the scope of individual studies (Ricciardi and Cohen, 2007; Galil et al., 2014). Other definitions restrict the definition of invasive to species which threaten native biodiversity, but do not include consideration of human impacts (IUCN, 2000). This thesis uses the definition most frequently used in the UK (GBNNSS, 2015); however, the variation in the field is worth bearing in mind when considering referenced studies (Ricciardi and Cohen, 2007). The variation in the field is worth bearing in mind when considering referenced studies (Ricciardi and Cohen, 2007).

1.2.2 Spread of marine NNS

The main pathways of marine species transfer around the globe are via mobile vectors, principally commercial shipping and movement of species for marine aquaculture (Gollasch, 2006; Minchin et al., 2013; Seebens et al., 2013). The transport of species for use in aquariums, or as part of the live food or bait trade are other examples of how species may be intentionally moved across the globe, but which are then intentionally or unintentionally released into the 'wild' (Padilla and Williams, 2004; Katsanevakis et al., 2013; Yeomans and Clark, 2016; Passarelli and Pernet, 2019). These mechanisms account for fewer introductions by volume, but are thought to be responsible for the establishment of highly invasive species. For example, the invasive macroalgae *Caulerpa taxifolia* is suspected to have been accidentally released from the Monaco aquarium in the late 1980s, and has since colonised large areas

of the Mediterranean (Meinesz et al., 2001). In the UK, there have been few records of species introduced via these mechanisms, with the primary pathways of marine NNS introduction into British waters shown to be via the movement of vessels (47% of known species introduction into the UK) and from activities associated with aquaculture (30%) (Figure 1.2) (Minchin et al., 2013).

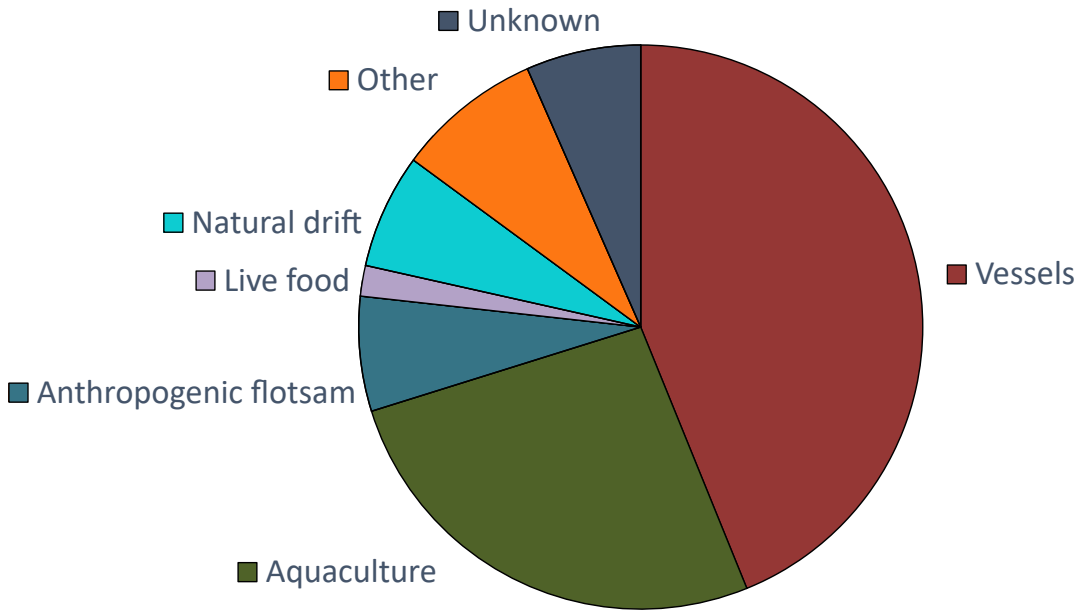


Figure 1.2: *Figure showing the proportion of marine NNS introduced into the UK via different pathways. Data for this figure is from Minchin et al. (2013).*

The aquaculture industry has expanded rapidly in recent decades, but some of the earliest documented NNS introductions are thought to be a result of aquaculture activities. Species may be intentionally transferred for use as stock, as with the widespread culture of the Pacific oyster *Magallana gigas*. Alternatively species may be transferred unintentionally as epibionts or parasites of aquaculture stock species (Minchin, 2007a; Williams and Smith, 2007). Species have also been accidentally released through their use as packing material to help during the transportation of cultured organisms. This is thought to be the mechanism by which the invasive wireweed *Sargassum muticum* was introduced to Europe from the Pacific coast of North America (Critchley, 1983). Salmon and shellfish aquaculture is an important industry in rural Scotland. Biosecurity practices in the industry have improved, primarily as a result of fears over disease, but the potential for accidental transfer of NNS remains.

Vectors associated with shipping include entrainment of organisms within ballast water or within sea chests, and the attachment of fouling species on vessel hulls (Gollasch, 2002;

Seebens et al., 2013). The discharge of ballast water from large commercial vessels has been implicated in the global transfer of a large number of aquatic species, ranging from unicellular organisms to fish (David and Gollasch, 2018). Ballast water transfer is not limited to solely marine species, as many global ports are located in estuaries, thus euryhaline species are frequently introduced through ballast water exchange (Leppäkoski and Olenin, 2000; Paavola et al., 2005). Estuaries containing large ports are among the most invaded global systems, with the frequency and size of commercial shipping visits contributing to high rates of species introductions. In Europe, ballast water transfer is thought to be responsible for the introduction of over 1000 marine and brackish NNS (Gollasch et al., 2007).

The majority of marine NNS introduced to temperate waters, including in the UK, are fouling species. Fouling species are organisms which are capable of attaching to immersed hard substrates. Fouling communities quickly develop on any hard substrate immersed into the marine environment, whether natural or organic in origin. The process starts with biochemical conditioning of the surface, colonization by bacteria and unicellular organisms such as algae and diatoms to form an initial biofilm, followed by settlement of multicellular organisms (Chambers et al., 2006; Nall et al., 2015).The establishment of foundation species may facilitate settlement of other fouling species or provide substrate for epibionts. The majority of known NNS in the UK are organisms which could be classed as macro-fouling, comprising macroalgae and sessile and 'clinging' invertebrates (Bishop et al., 2015; Nall et al., 2015). Common fouling invertebrates are barnacles, mussels, bryozoans, and sea-squirts.

Fouling organisms are likely to be frequently introduced as NNS as they are capable of being transported within biofouling communities on vessel hulls, or to have high propagule supply in port areas where ballast water may be taken up (Gollasch, 2002). In a study of large vessels (mainly container ships) visiting German ports in the 1990s, NNS were detected in 96% of hull samples (Gollasch, 2002). The role of hull-fouling on large ocean-going vessels acting as a pathway for a NNS has thus been known for over two decades. Less well studied are the fouling communities on slower moving barges and supply vessels, although their role in connecting offshore infrastructure with heavily invaded port areas could be an important pathway for the secondary spread of NNS (Godwin, 2003; Mineur et al., 2012).

In the last decade, the role of small private and commercial vessels in transporting NNS has become increasingly recognised (Minchin et al., 2006; Clarke Murray et al., 2011; Ashton et al., 2014). Studies of the hulls on recreational yachts have shown that these vessels may have high levels of biofouling, including of NNS, especially if anti-fouling paint is not maintained

(Ashton et al., 2006b). Fouling on recreational or slow-moving vessels may also be exacerbated by their slower speeds and longer periods spent stationary (Mineur et al., 2012; Roche et al., 2015; Foster et al., 2016) Niche areas, where application of anti-fouling paint is difficult such as propeller shafts or keels, may be especially susceptible to colonisation by NNS (Ashton et al., 2006b; Clarke Murray et al., 2011; Peters et al., 2017).

The frequent movement of smaller vessels between larger ports and marinas in more remote areas may be an important vector of intraregional spread, especially for organisms with restricted natural dispersal ability (Acosta and Forrest, 2009; Davidson et al., 2010; Zabin et al., 2014). Zabin et al. (2014) demonstrated the importance of smaller fishing and recreational vessels in connecting highly invaded ports with smaller marinas by analysing connectivity in terms of vessel movement and biofouling communities between San Francisco bay (the site of a large international port) and smaller marinas on the adjacent coast. Apte et al. (2000) speculated that these artificial environments could act as 'stepping stones' for species dispersal by providing suitable habitat for enough time for organisms to colonise another vector, even if the species does not ultimately establish in the harbour environment. NNS propagules released in ports and harbours can inoculate visiting vessels which go on to spread organisms further afield (Floerl and Inglis, 2005; Floerl et al., 2009). Environmental conditions in ports and harbours can trigger spawning of species arriving on vessels, enabling transfer of species between vessels (Apte et al., 2000; Minchin and Gollasch, 2003). Marinas may thus act as entry-points for the initial introduction of NNS, supporting their establishment in new regions (Floerl and Inglis, 2005; López-Legentil et al., 2015; Ferrario et al., 2017).

Once an introduced species is established in an area, it can also spread naturally via the movement of reproductive individuals, propagules, or vegetative fragments by the wind, ocean currents, or other species (Harries et al., 2007). However, species will be limited in their ability to naturally disperse either by environmental barriers (e.g. landmasses, current patterns, temperature, habitat boundaries) or by their dispersal potential (time they remain in planktonic phase). The creation of artificial structures in the sea can alter these dispersal limitations, for instance by providing a corridor for species movement or providing stepping stones to increase the dispersal range of a species, thus affecting levels of ecological connectivity (Gollasch et al., 2006; Strain et al., 2018). Increased connectivity may prove beneficial for some species, for instance in facilitating adaptive range shifts of species affected by rising sea temperatures, but could also accelerate or facilitate the spread of NNS

Artificial structures can act as corridors by connecting two previously isolated areas of

suitable habitat (Bulleri and Airoldi, 2005). This could take the form of canals connecting two waterbodies (e.g. the Suez canal), or as a result of artificial structures (e.g. seawalls or breakwaters) providing corridors of hard substrate. This could enable movement of species with limited dispersal distances, or facilitate movement across areas of unsuitable habitat such as soft sediment seafloor (Bulleri and Airoldi, 2005). In offshore areas, NNS have been shown to colonise oil and gas platforms and renewable energy devices (Page et al., 2006; Sheehy and Vik, 2010; De Mesel et al., 2015). Modelling studies have shown the installation of wind farms could enable species with pelagic larvae to spread across the Irish Sea (Adams et al., 2014). NNS have also been recorded on floating offshore monitoring buoys and floating wave devices in Scotland, suggesting novel renewable energy infrastructure such as floating wind turbines might also be colonised by NNS (Macleod, 2013; Nall, 2015). Surveying these structures and testing management methods can be difficult due to their offshore nature, thus lessons learned from studies in ports and marinas can act as a useful trial.

Ports, harbours and marinas introduce large amounts of artificial structures to the marine environment providing large amounts of uncolonised hard substrate for settlement by marine fouling organisms (Bulleri and Chapman, 2010). The transport of species via shipping is likely to pre-select organisms which are capable of establishing on artificial substrates and which are more tolerant of exposure to pollutants or anti-fouling paints (Floerl and Inglis, 2005). Ruiz et al. (2009), in a survey of NNS in North America, found that 71% of recorded NNS were found on hard substrata, with the majority colonising artificial structures in docks and marinas.

Floating docks and pontoons present a similar habitat to vessel hulls in terms of surface and orientation, and NNS may have a competitive advantage over other species in these environments. Leclerc et al. (2019) examined colonisation of settlement plates deployed on fixed and floating structures along 100 km of Chilean coastline and found that floating structures were far more susceptible to invasion by NNS compared with non-floating structures in both 3 and 13 month old communities. Fouling communities within marinas, and especially on pontoons and other floating structures, thus make useful sites to detect novel NNS introductions as NNS may be more likely to establish on these structures than nearby fixed structures such as pillars or seawalls (Neves et al., 2007; Dafforn et al., 2009b). This thesis focuses on marinas for this reason, and will also use the fouling communities found on marina pontoons to investigate the factors which affect the likelihood of establishment. High levels of fouling on marinas structures can represent a significant reservoir of non-

native species, thus identification of factors affecting establishment could be used to guide monitoring and management practices and prevent further transfer and spread of NNS into natural environments (Ashton et al., 2006a; Glasby et al., 2007; Ruiz et al., 2009).

1.2.3 NNS establishment in urbanised environments

Invasion success is thought to be determined by three main factors: the frequency and volume of propagules introduced (propagule pressure), the characteristics of the invading species, and the susceptibility of the receiving environment to invasion (Lonsdale, 1999; Davis et al., 2000). The susceptibility of the receiving environment will be determined by the availability of resources and the suitability of physical and environmental conditions for NNS settlement, growth and reproduction (Figure 1.3).

Probability of species establishment will be increased in areas where the propagule pressure is high (Von Holle and Simberloff, 2005; Britton-Simmons and Abbott, 2008; Clark and Johnston, 2009; Johnston et al., 2009; Lockwood et al., 2009). Thus ports with high shipping traffic are some of the most highly invaded systems, as are bays with a long history of aquaculture activities (Minchin, 2007a). The degree of connectivity to the sites of primary introduction may be an important factor in determining the risk of introduction to smaller sites such as marinas (Zabin et al., 2014; Choi et al., 2016). The concentration of high vector activity in areas with high volumes of connected marine infrastructure may be a significant contributor to the predominance of NNS on artificial structures (Ruiz et al., 2009). Artificial structures may also alter circulation dynamics, resulting in retainment of propagules as a result of reduced flushing within enclosed ports and harbours (Floerl and Inglis, 2003; Rivero et al., 2013; Dafforn et al., 2015a). However, experimental studies have shown that propagule pressure alone does not maximise invasion success, instead the interaction between propagule pressure and disturbance regulates success of marine invasive species establishment (Britton-Simmons and Abbott, 2008; Clark and Johnston, 2009).

1.2.3.1 Resource availability and disturbance

Novel fouling species require adequate resources to enable settlement and future growth. The availability of space is a major limiting resource in intertidal epifaunal communities (Shea and Chesson, 2002; Britton-Simmons and Abbott, 2008). In space limited systems such as fouling communities, processes which provide new substrate for colonisation, decrease the diversity of resident species, or shift competitive interactions have been shown to facilitate

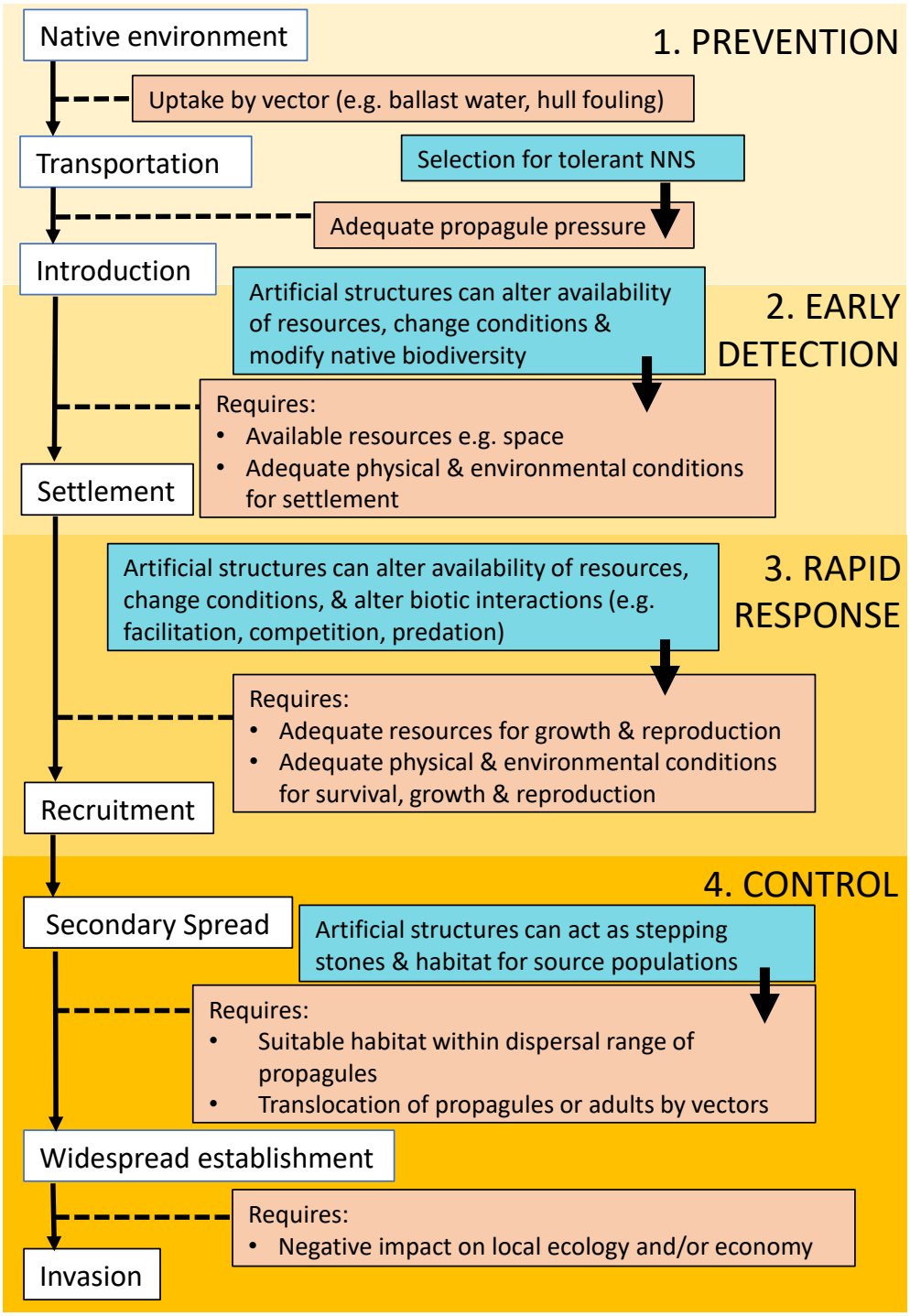


Figure 1.3: *Figure showing the process of fouling species introduction and establishment in novel environments, adapted from (Macleod, 2013; Johnston et al., 2017; Collin et al., 2015a). Stages of the invasion process are shown in white boxes, with requirements for each step shown in red boxes. Potential ways in which artificial structures alter success are shown in blue. Stages of possible management interventions are numbered on the right.*

establishment of a number of marine NNS (Byers, 2002; Stachowicz et al., 2002; Britton-Simmons and Abbott, 2008; Janiak et al., 2013). High levels of disturbance are associated with non-native species establishment in both terrestrial and marine systems (Davis et al., 2000; Britton-Simmons and Abbott, 2008), and experiments have shown that disturbed assemblages are more likely to be invaded than non-disturbed ones (Altman and Whitlatch, 2007; Britton-Simmons and Abbott, 2008; Bugnot et al., 2016).

Communities associated with marine artificial environments may be subject to increased disturbance as a result of maintenance activities, frequent movement of vessels, eutrophication and chemical pollution from run-off and toxic paints, and the construction work involved in building (or decommissioning) artificial structures (Turner et al., 1997; Bulleri and Airoldi, 2005; Piola and Johnston, 2008; Crooks et al., 2010; Airoldi and Bulleri, 2011). These disturbance events may increase resource availability either directly (e.g. by scraping off resident fouling communities) or indirectly by creating conditions which fewer native species can tolerate.

Byers (2002) proposed that artificial structures may present a novel habitat to which both native and non-native species are not adapted, due to the differences in substrate and orientation between natural and artificial habitats (Glasby, 2000; Tyrrell and Byers, 2007; Chase et al., 2016; Megina et al., 2016). This may reduce the competitive advantage of established native species and reduce biotic resistance to colonisation of fouling NNS (Shea and Chesson, 2002; Tyrrell and Byers, 2007). In addition (or alternatively), NNS may have a competitive advantage over native species in urbanised marine environments such as ports or marinas, as a result of pre-selection of organisms capable of tolerating chemical and environmental stress and high levels of disturbance during transfer (Altman and Whitlatch, 2007; Lenz et al., 2011; Clarke Murray et al., 2012). NNS have been shown to be more tolerant of metal pollution and the presence of anti-fouling chemicals than native species, potentially as a result of exposure during transfer by shipping vectors (Piola and Johnston, 2008; Dafforn et al., 2009a; Crooks et al., 2010; Canning-Clode et al., 2011; Osborne et al., 2018). Further competitive advantage for NNS may be due to release from predators and parasites in novel regions (Blakeslee et al., 2012).

The interaction between NNS and resident communities can influence invasion success not just through competition, but also through, predation or facilitation. Resident communities with high species diversity have been hypothesised to have increased biotic resistance to invasion as a result of reduced availability of resources for newly introduced

species. However, species may also facilitate settlement or growth of NNS, for example, by providing substrate for colonisation.

Artificial structures may also alter the biotic resistance by removing species which control or prevent NNS establishment in natural communities. Artificial structures may create refuges for non-native species due to their positioning in the water column or distance from shore (Dumont et al., 2011b; Johnston et al., 2017). This may explain why floating structures, such as pontoons, have been observed to have greater abundances of NNS than in benthic habitats or static columns which are accessible to benthic predators (Connell, 2000; Boos et al., 2011; Dumont et al., 2011a; Forrest et al., 2013). Forrest et al. (2013) used predator exclusion experiments to demonstrate that native benthic predators were capable of controlling growth of *Didemnum vexillum* when they grew on substrates accessible from the seafloor, but not on floating structures.

The concentration of large numbers of transport vectors in areas with high densities of marine infrastructure and anthropogenic disturbance may create conditions with elevated rates of NNS introduction and establishment (Cohen and Carlton, 1998; Grosholz, 2002). The interaction between these factors may explain why estuaries and bays represent some of the most invaded systems in the world.

1.2.3.2 Environmental conditions

If NNS introduction is to succeed, adequate physical and environmental conditions for settlement, and then for growth and spread are required (Figure 1.3). Physical requirements include an adequate substrate for settlement. The introduction of hard structures to the marine environment provides large amounts of uncolonised substrate for settlement by marine fouling organisms (Bulleri and Chapman, 2010). Differences in the type of artificial substrate have been shown to affect native and NNS colonisation (Glasby et al., 2007; Tyrrell and Byers, 2007), with fibreglass structures more susceptible to NNS colonisation than concrete or wood (Glasby, 2000; Vaz-Pinto et al., 2014; Chase et al., 2016).

Environmental parameters affect the functioning of vital physiological processes in aquatic organisms, and thus temperature, salinity, pH, and current dynamics will influence the initial establishment of marine NNS, and determine the range limits of future spread (Ashton et al., 2007; Pineda et al., 2012; Gröner et al., 2011; Epelbaum et al., 2009). As such environmental variables, particularly salinity and temperature, have been used to predict the range limits of individual NNS in invaded regions based on their thermal and osmotic

tolerances (Kimball et al., 2004; Herborg et al., 2009; Harries et al., 2007; Ashton et al., 2007).

Species are more likely to establish in their recipient waters if the environmental conditions in the new area match the environment conditions of the place from which they originated. Environmental matching has been incorporated into risk assessment models to determine the likelihood of species introduction via ballast water exchange between linked ports (David et al., 2013). The development of these risk assessments has been driven by the International Maritime Organisation's (IMO) Ballast Water Management (BWM) convention, which requires vessels to carry out ballast water exchange mid-voyage, unless the specific route between two ports they are traveling has been granted an exemption (David et al., 2013). Exemptions are granted where the risk of NNS introduction is assessed as being low based on a mis-match between areas of ballast water origin and discharge.

There have been a number of different approaches to development of these risk assessment models, incorporating different environmental variables (Keller et al., 2011; Chan et al., 2013; David et al., 2013). Gollasch (2002) used a scheme based on matching climatic conditions (temperature) in the recipient and donor area to establish likelihood of species establishment to the North Sea. Salinity and temperature are the two most frequently used variables, with salinity the only parameter common to all risk assessments reviewed by David et al. (2013). Salinity was frequently found to be the most influential variable in environmental similarity models (Keller et al., 2011). As such, salinity was the only parameter selected by David et al. (2013) to be used in the development of a risk assessment as a standard for IMO BWM exemptions in the Baltic Sea. David et al. (2013) classified transfer of ballast water between freshwater conditions (<0.5 PSU) and fully marine conditions (>30 PSU) as being the lowest risk, with a slightly higher risk for species moved between sites with <0.5 PSU and brackish conditions higher than 18 PSU. These divisions were based on the Venice Salinity system (Table 1.1) and the work of Remane (1934) (David et al., 2013). As a result of many global ports being located in estuaries, euryhaline species may be the most frequently picked up in ballast water and transferred to ports on the other side of the world which nevertheless have similar salinities, explaining why brackish ports are some of the most heavily invaded marine regions (Paavola et al., 2005; Leppäkoski and Olenin, 2000).

These risk assessment studies have focused on ballast water as a vector for species introductions, but fewer studies have investigated the influence of transfer between marine and low salinity conditions for hull-fouling NNS. The principles of environmental matching should also apply for hull-fouling species. Furthermore, hull-fouling species on vessels

Table 1.1: *The Venice Salinity system (Venice System, 1959) reproduced from David et al. (2013).*

Zone	Salinity (PSU)
Hyperhaline	>±40
Euhaline	±40 to ±30
Mixohaline	(±40) ±30 to ±0.5
Mixoeuhaline	>±30 but < euhaline sea
(Mixo-) polyhaline	±30 to ±18
(Mixo-) mesohaline	±18 to ±5
(Mixo-) oligohaline	±5 to ±0.5
Limnetic	<±0.5

entering low salinity marinas will be more exposed than those entrained in ballast water to a large change in salinity conditions from marine to possibly near freshwater. This could act as a barrier to the introduction of most marine NNS, and indeed freshwater ports are known to have a role in purging ships of some marine biota (Minchin and Gollasch, 2003). Scottish guidance on developing biosecurity plans thus identifies sites with high freshwater input from rivers as likely to have a lower risk of NNS introduction (Payne et al., 2014). This is supported by research from Foster et al. (2016) which analysed existing records of NNS numbers in UK marinas, finding that the probability of NNS presence in a marina was found to be significantly higher in marinas more than 2000 m from a river (Figure 1.4). However, many of the marinas suspected to have lower salinities in Scotland have never been surveyed for NNS. There is also little known about how the presence of freshwater sources in or near marinas will affect the salinity conditions within the marina and the resulting effect on the composition of fouling communities.

Ports and marinas located in river mouths or estuaries may also experience large changes in environmental conditions as a result of the mixing of freshwater and saline waters. NNS establishment may also be influenced by the temporal variation in salinity (Sorte et al., 2010a; Pardo et al., 2011). As some NNS have been shown to have a greater tolerance to salinity changes than related native species (Gröner et al., 2011), they may be more able to recover from salinity shock. Rapid changes in salinity can act as major disturbance events, flushing bays and estuaries and either removing invasive species populations or opening up novel space for colonisation (Mineur et al., 2012; Jimenez et al., 2017). Variation in winter rainfall and resultant low and high salinity events have been suggested as the driving force behind turnover of fouling species communities in the San Francisco Bay, increasing dominance of NNS (Chase et al., 2016; Chang et al., 2018). Climate change is predicted to increase rainfall

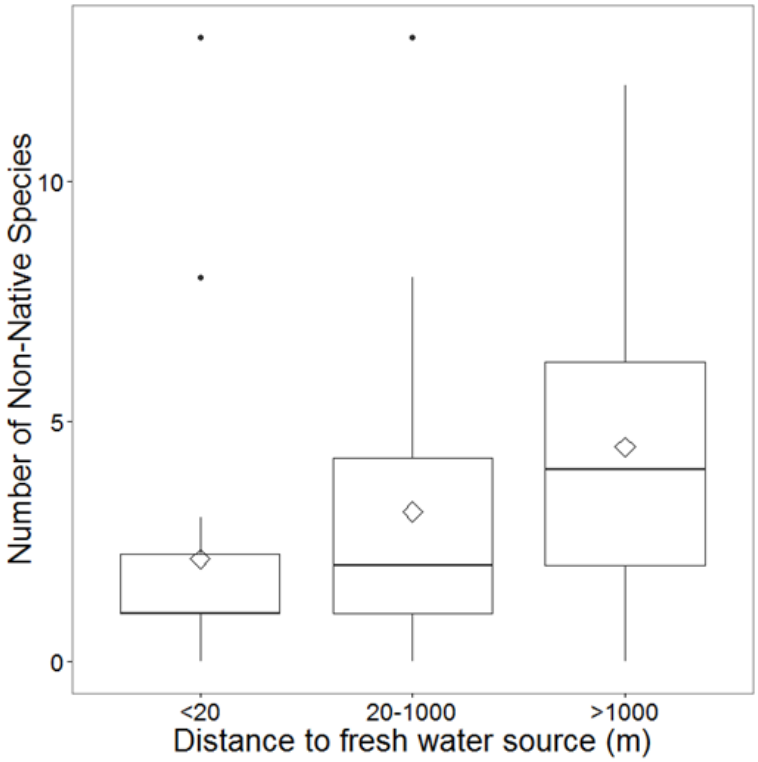


Figure 1.4: *Figure from Foster et al. (2016, see Appendix D). A boxplot showing the influence of a marina's distance (m) to the nearest freshwater source on the number of NNS found in UK marinas. The boxplot includes median values (solid horizontal line) and the mean (open diamond).*

and frequency of storms, potentially leading to increased frequencies of hyposaline events in coastal areas (Pardo et al., 2011). Research to understand the relationship between salinity and the establishment of marine NNS in urbanised environments could thus help identify the potential effects of climate change on invasion of marine coastal communities (Harley et al., 2006; Sorte et al., 2010a; Bradley et al., 2012).

1.3 Marine biosecurity

In recognition of the profound economic and environmental impacts IAS may have in their introduced regions, a number of policies and regulations have addressed the need to reduce the number of species introductions and control existing invasive populations (Genovesi et al., 2014; Hulme, 2015). The management of the risks posed by introduction of NNS is referred to as 'biosecurity' (biological security) Hewitt et al. (2004). Policy on the control of invasive alien species has been part of international agenda for over 60 years, starting with the International Plant Protection Convention in 1951 (The Law Commission, 2014). In 1992, Article 8(h) of the Convention on Biological Diversity (CBD) required parties to "prevent the introduction of, control or eradicate those alien species which threaten ecosystems, habitats or species" (CBD, 1992). Other large international conventions have introduced similarly broad requirements for member states to reduce the spread and impact of invasive species.

Within the European Union (EU), the first EU regulation which dealt specifically with invasive species, the 'regulation on the prevention and management of the introduction and spread of invasive alien species' (the IAS Regulation) was introduced in 2014 Council Regulation (EU) No 1143/2014. More holistic EU policies introduced in the last two decades have aimed to improve the status of the environment as opposed to mitigating impact of individual sectors on the environment. These include the Water Framework Directive (WFD) (Council Directive (EC) 2000/60/EC, 2000), and the Marine Strategy Framework Directive (MSFD) (Council Directive (EC) 2008/56/EC, 2008). While the WFD does not directly reference NNS it requires that pressures that may affect the ecological status of aquatic environments are addressed, and NNS are considered to fall under the umbrella of 'anthropogenic pressures' (SEPA, 2013; UK TAG on the WFD, 2013). The MSFD was adopted in 2008, and includes as one of its descriptors of good environmental status (GES), the provision that "non-indigenous species introduced by human activities are at levels that do not adversely alter the ecosystems" (Council Directive (EC) 2008/56/EC, 2008, Descriptor 2). Following an initial assessment of the status of marine waters, Member states must produce a programme of measures that set out how GES will be achieved, as well as developing a monitoring programme to gather information and assess progress towards GES (Stebbing et al., 2014).

The EU Marine Strategy Framework Directive (Council Directive (EC) 2008/56/EC) and the Invasive Alien Species Regulation (Council Regulation (EU) No 1143/2014) both impose

surveillance requirements for NNS, which include monitoring of high risk pathways and sites of entry (GBNNSS, 2015). To meet these international objectives, implementation and development of NNS policy in the UK is facilitated by the Great Britain Non-Native Species Secretariat (GB NNSS). The GB NNSS was created as part of the development of the 'Invasive Non-native Species Framework Strategy for Great Britain', which was published in 2008 and then reviewed in 2015 (GBNNSS, 2015). In the UK, the environment is a devolved policy competence, meaning that environmental law differs between Scotland, Northern Ireland, and England and Wales. Thus while the GB NNSS coordinates between UK nations, development and implementation of NNS policy differs between nations (DEFRA, 2014; Stebbing et al., 2014).

Monitoring of NNS may fulfil a number of objectives including; detecting the arrival of new NNS to enable rapid response, detecting changes in the distribution of established NNS to assess impact and guide control, detecting spread into vulnerable habitats such as protected areas, or into important economic regions (Inglis, 2001). Despite the requirement for monitoring of marine NNS to meet UK policy obligations, there is no UK national monitoring scheme for marine NNS. Instead, monitoring of NNS in the UK and Scotland is currently reliant on surveys carried out by researchers as part of larger research projects (Nall et al., 2015; Ashton et al., 2006a; Want et al., 2017; Cook et al., 2013b), or when commissioned by industry or regulatory bodies (Beveridge et al., 2011; Cook et al., 2014a). These are frequently prompted by the arrival of a new species, but are not set up to detect arrival in the first place. There is thus an absence of routine survey work in high risk areas such as ports and marinas in much of Scotland, with no guarantee of funding for future sampling (Stebbing et al., 2014). The exception to this is in the Orkney and Shetland Isles, where regular monitoring for NNS has been carried out since 2012 (Collin et al., 2015a; Kakkonen et al., 2019).

The monitoring scheme in Shetland adopted a framework developed in New Zealand, to identify sites where NNS surveillance should be prioritised to enable early detection of high risk marine NNS, forming the basis of a national surveillance programme (Leewis and Gittenberger, 2011; Shucksmith and Shelmerdine, 2015; Woods et al., 2017). The Marine High Risk Site Surveillance (MHRSS) surveys are conducted at major ports and marinas on a bi-annual basis, in addition to baseline surveys in areas vulnerable to NNS establishment. In Shetland, an assessment of pathways and stepping stones of NNS introduction was integrated with a categorisation of areas based on their economic or environmental value and sensitivity to NNS impacts (Shucksmith and Shelmerdine, 2015). This assessment was combined with

results from an initial rapid assessment survey in eighteen sites to identify priority sites for further NNS monitoring (Collin et al., 2015b). The framework used in New Zealand and Shetland could be replicated for the whole of Scotland, using a similar methodology to establish vulnerability to invasion based both on the likelihood of species being introduced (pathway analysis), and vulnerability of economic and environmental features to invasion. However, this requires information on the current distribution of marine NNS to identify areas which have a high rate of species introductions. Surveys of some of Scotland's largest marinas have not been carried out since 2008, and the last compilation of data on marine NNS was in 2012 (Ashton et al., 2006a; Nall et al., 2015). Part of the work of this thesis will be to address these knowledge gaps.

In Scotland, to encourage implementation of biosecurity measures and enable management activities to be carried out, legislation was introduced in 2014 in the form of an amendment to the Wildlife and Countryside Act (1981), as amended by the Wildlife and Natural Environment (Scotland) Act (2011) (WANE Act). Section 14 of the WANE Act states that it is an offence to release, allow to escape, or cause any animal or plant to grow or be at a place outwith its native range. The Act specifies that an offence can be avoided if it can be shown that all "all reasonable steps and… all due diligence to avoid committing the offence" were exercised. This thus places the legal obligation on those who might intentionally or unintentionally transfer a NNS to carry out best practice by implementing the appropriate biosecurity policies (Scottish Government, 2012; Tartre, 2018).

To support individuals, land owners, and institutions in their responsibilities, the Scottish Government published a 'Code of Practice on Non-Native Species' in 2012 (Scottish Government, 2012). The code promotes adoption of a precautionary approach, the use of risk assessments, following good practice guidance, and reporting the presence of NNS (Payne et al., 2014). This follows the internationally recognised hierarchical approach of prevention first, followed by rapid detection, eradication and control (Hulme, 2006; Scottish Government, 2012; The Law Commission, 2014). These principles of NNS management aim to address key stages in the introduction process, as illustrated in Figure 1.3 by Collin et al. (2015a). The development of management measures to address NNS can thus be split into interventions at the pre-border and border stage (preventing species from being transported and introduced), and post-arrival measures (rapid detection of new arrivals, risk assessment to determine the appropriate response, and the application of eradication and control measures) (Forrest et al., 2009; Piola et al., 2009a).

1.3.1 Prevention

Preventing species transfer has been identified as a priority for invasive species management, due to difficulties in eradicating and controlling species following arrival and establishment (Genovesi, 2005; Hewitt and Campbell, 2007; Molnar et al., 2008). However, predicting which NNS will go on to spread and potentially cause harm to the environment is challenging, as many species display novel characteristics within their introduced range (Hulme, 2006). Thus while most NNS will have no impact following introduction, following a precautionary approach is recommended to achieve effective management of NNS (Grosholz, 2002; Ojaveer et al., 2015).

Effective prevention relies on research identifying the most common pathways for NNS introduction, and development of ways in which transfer of organisms by these vectors can be reduced (Molnar et al., 2008). Preventative measures may include quarantine precautions, risk assessments, and tools developed to minimise transfer by specific pathways (Hewitt and Campbell, 2007). For example, in acknowledgement of the issues of species transfer in ships' ballast water, the International Maritime Organisation (IMO) adopted the International Convention for the Control and Management of Ships' Ballast Water and Sediment (BWM Convention) in 2004 (David and Gollasch, 2018). The BWM convention was ratified in 2018, and requires ballast water management measures to be implemented such as mid-ocean exchange and installation of ballast water treatment systems (Gollasch et al., 2007).

The IMO has also developed hull fouling guidelines, which set out measures for ports, shipping companies and member states to reduce risk of transfer on hulls (IMO, 2011). These include recording biofouling management practices, good anti-fouling maintenance, and recommendations for inspection procedures (IMO, 2011). Individual countries may have their own prevention policies. New Zealand and Australia have implemented strict requirements for arriving vessels to have minimal hull fouling meeting strict criteria (NZ Government, 2014). Despite these measures, hull fouling as a pathway for NNS spread remains largely unregulated, especially for recreational vessels where anti-fouling practices may be less well maintained (Roberts and Tsamenyi, 2008; Clarke Murray et al., 2011; Roche et al., 2015; Foster et al., 2016).

Even once pathways have been identified, reducing transport of NNS can be difficult, especially if it requires changes in stakeholder behaviour or significant economic cost (Williams et al., 2013). Marine users may be unwilling to invest resources into preventing the

arrival of unknown species, where there is significant uncertainty over which species may be introduced and their resulting impact if they were (Finnoff et al., 2007). Thus though preventative options may be the preferred method for reducing NNS impact (Williams and Grosholz, 2008), they are unlikely to be implemented to the desired level, especially as marine borders are particularly difficult to manage (Hewitt et al., 2004). As complete implementation of preventative measures is therefore unlikely, there is a continued need to develop effective post-border management methods to deal with NNS upon arrival.

1.3.2 Post-arrival measures

The predominance of NNS in communities on artificial structures has led to research examining ways to prevent establishment and further spread in these environments, building on principles from the field of ecological engineering (Dafforn et al., 2016; Johnston et al., 2017; Bugnot et al., 2018). For example, eco-engineering projects have trialled strategies to increase the biotic resistance of resident communities to invasion by promoting the growth of native species, or by increasing predation pressure (Bulleri and Chapman 2010; Mayer-Pinto et al., 2017). Methods of promoting native biodiversity have included 'seeding' structures with habitat forming species such as kelp, or altering the design of structures to increase colonisation by native species (Mayer-Pinto et al., 2017). Other suggestions include reducing the volume of floating structures or incorporating quarantine and treatment facilities (Bax et al., 2002; Holt and Cordingley 2011; Mayer-Pinto et al., 2017). Identification of management actions such as these relies on research to determine the role of different aspects of anthropogenic activity in facilitating the success of NNS establishment (Johnston et al., 2017).

More traditional post-arrival measures for NNS management include increased surveillance of high risk sites to rapidly detect the arrival of new species either before they have spread to the environment or soon after introduction. Rapid detection is important as successful control and eradication has primarily been achieved when applied to small contained populations, before a species has a chance to become widespread (Bax et al., 2002; Williams and Grosholz, 2008; Holt and Cordingley, 2011). For example, arriving vessels known to have high levels of hull fouling with potential NNS may be required to undergo cleaning before onward travel.

Existing mechanisms for removing biofouling mostly involve either in-water cleaning or vessels being removed and cleaned in dry-docks (Minchin and Gollasch, 2003; Inglis et al.,

2012). In-water cleaning of arriving vessels presents a biosecurity risk as organisms are released into the wider environment, and many fouling species are capable of regrowing from small fragments (Bullard et al., 2007; Jute and Dunphy, 2017). However, removal of a vessel from the water risks dislodging species, and some dry-dock facilities do not always prevent species from being washed back into the environment (Coutts et al., 2010). Once a novel species has been introduced to the marine environment, the feasibility of successfully eradicating it is low (Thresher and Kuris, 2004; Booy et al., 2017). There is thus a need for tools which can be used to remove fouling species that are effective, practical to apply, cost-effective and which have minimal impact on the surrounding environment (Thresher and Kuris, 2004).

Where established NNS cannot be eradicated, the control of populations may reduce their further spread and impact on native biodiversity (Genovesi, 2005; Epstein and Smale, 2017a). Control techniques use similar tools as eradication, but with different objectives. Methods trialled to control and eradicate marine invasive NNS include manual removal of individuals (Buhle et al., 2005; Guy and Roberts, 2010), air drying (Forrest and Blakemore, 2006; Hopkins et al., 2016) and wrapping in plastic to encourage deoxygenation. In California, quick discovery of a small patch of the invasive *Caulerpa taxifolia* was contained through wrapping individual patches (Anderson, 2005). Research on the efficacy of biocontrol, using other species to control NNS growth and spread, has also been trialled in New Zealand (Atalah et al., 2015), and reviewed in Atalah et al. (2014) who concluded that while there are some examples of indigenous species effectively controlling NNS spread, its applicability at large spatial scales was limited.

More aggressive methods include the application of chemicals (LeBlanc et al., 2007; Denny, 2008; Piola et al., 2009b; Roche et al., 2015; Atalah et al., 2016). For example, in Australia, successful eradication of the zebra mussel *Mytilopsis sp.* was achieved through quarantine and treatment of the marinas it had established in with chlorine and copper sulphate (Bax et al., 2002). Other treatments exploit the limitations of an organisms' physiological tolerance, by applying heat treatments (Wotton et al., 2004; Forrest and Blakemore, 2006; Piola and Hopkins, 2012), or subjecting species to salinity shock (McCann et al., 2013; Moreira et al., 2014; de Castro et al., 2018). As most marine species will be unable to survive rapid changes in salinity, low salinity treatment has been proposed as a mechanism for both removing biofouling and as a tool to control or eradicate organisms once a species has been introduced (Moreira et al., 2014). Freshwater immersion has been trialled as a tool for individual invasive NNS but had not been trialled for use on marina fouling communities.

1.4 Thesis objectives

This thesis aims to investigate the distribution and establishment of marine fouling NNS, to address key knowledge gaps hindering the effective management of marine NNS in Scotland. The fouling communities present on artificial structures such as pontoons within harbours and marinas offer a useful case-study for the investigation of community dynamics, factors influencing species establishment, and the efficacy of potential management interventions. The marinas in mainland Scotland present further opportunities as they are located within river mouths, estuaries and sea-lochs with resulting differences in environmental conditions, specifically salinity, within sites. Previous studies have already indicated that low salinity marinas may be less susceptible to invasion than those with more marine conditions, and this has lead to the development of biosecurity guidance based on this premise. However, there is no available data on what salinity conditions can be found in each site, and there are large data gaps on what effect this variation may have on the composition of fouling communities (including native and NNS). Low salinity marinas could also be used to treat contaminated vessels, by mooring vessels in low salinity environments, or to treat equipment and marine infrastructure. Freshwater immersion has been trialled as a tool for individual invasive NNS but no studies have investigated what effects salinity, duration of treatment and community tolerance may have on the outcome.

The principal aims of this study were thus to:

1. Assess the decadal patterns of individual marine non-native species establishment and spread in Scotland.
2. Evaluate the native and NNS diversity in marina fouling communities, and assess the influence of salinity and marina features on community composition.
3. Determine if short-term treatment of fouling communities with hypo-saline water offers an effective management option for reducing biofouling and controlling NNS.
4. Propose management recommendations to improve biosecurity in the Scottish marine environment.

This thesis is structured such that each chapter addresses one of these aims. A summary of the objectives of each chapter is provided:

- *Chapter 2* updates the baseline dataset of fouling non-native species location records by conducting surveys of eighteen Scottish marinas. A subset of these are in sites first

surveyed in 2006 to allow assessment of changes in the establishment of NNS over a decade within high-risk sites. Records of marine and brackish NNS from published data and literature are collated to assess patterns of distribution at a national level, enabling identification of gaps in monitoring effort.

- *Chapter 3* compares the diversity of native and non-native species within fouling community assemblages found on marina pontoons. Trends in non-native and native species diversity are compared. The influence of salinity and other abiotic factors (e.g. location, connectivity, and marina features) on species distribution patterns are evaluated. This information could inform risk-assessment processes and the development of monitoring and biosecurity protocols.
- *Chapter 4* investigates the applicability of hypo-saline treatment as a management tool either for the removal of biofouling or to specifically control fouling non-native species. Two specific research questions are addressed by field experiments using fouling communities grown on settlement panels. The first investigates what salinities and durations of exposure are most effective in reducing cover. The second investigates whether response to freshwater treatment varies between communities expected to have different levels of salinity tolerance. In both experiments response and recovery are compared between native, non-native and cryptogenic species.
- *Chapter 5* synthesises the findings of the previous chapters, and places the results in a global context. Recommendations for marine NNS management in Scotland and opportunities for future research are highlighted.

CHAPTER 2

Characterising non-native species in Scotland

ABSTRACT

This chapter provides an updated account of the distribution of marine non-native species (NNS) around the coast of Scotland. In 2016, a rapid assessment survey (RAS) of 18 of the largest Scottish mainland marinas was undertaken targeting 30 fouling NNS, either known to be in Scotland or likely to soon arrive from elsewhere in the UK or Europe. As part of this study, eight sites which had previously been studied in 2006 to 2008 were resurveyed. In each year between 2006 to 2008, six fouling NNS were recorded in the ten largest Scottish mainland marinas (Ashton et al., 2006a). Eighteen fouling NNS were found in the 2016 RAS, four ascidians, five bryozoans, four other invertebrates and five species of macroalgae. The highest number of NNS recorded at one site was ten (range 2-10 species), and on average 4.36 species were detected per site. This average surpasses the maximum number of four NNS recorded in a marina in 2006 -2008. Of those sites where surveys were repeated, all showed increases in the number of NNS recorded. In order to place these results in a wider context, records of fouling NNS from published literature and publicly available datasets were used to update a complete record of marine NNS presence records in Scotland to 2017. This data was used to map individual species distributions around Scotland. The addition of 1020 records to this synthesis (940 from published data, 80 from survey work in 2016) revealed that there are now location records for 32 marine NNS in Scotland. Twenty-two of these species are considered likely to be established, including two species which have only been recorded in Scotland since 2010. Comprehensive knowledge of the distribution of NNS around Scotland can be used to direct future monitoring and surveillance effort and to inform meaningful management decisions about biosecurity and activities that might facilitate NNS spread.

2.1 Introduction

The rate of marine NNS introductions is increasing, both globally and within the UK, bringing the potential for negative impacts on biodiversity and costs to marine industries (Roy et al., 2014; Stebbing et al., 2014). In 2013, Minchin et al. (2013) identified 90 marine non-native species as having been recorded in British marine or brackish water, 58 of which were considered to be established. The majority of the NNS identified in the UK are fouling species (Bishop et al., 2015; Nall et al., 2015). In the UK, novel species introductions are primarily detected first in the south-west, with secondary spread along coastlines via natural dispersal or with human-mediated vectors (Eno et al., 1997; Minchin et al., 2013). Thus many of the species introduced into Scottish waters were established in England and Wales, or in Ireland first (Ashton et al., 2006a; Minchin et al., 2013; Nall et al., 2015).

The movement of recreational vessels and smaller crafts represents an important vector for the secondary spread of NNS within the UK, connecting areas with higher rates of primary introductions with more remote areas (Wasson et al., 2001; Floerl and Inglis, 2005; Clarke Murray et al., 2011; Zabin et al., 2014). Evidence of recreational craft acting as vectors in Scotland comes from observations of NNS in fouling communities on the hulls of recreational vessels (Ashton et al., 2006a). As NNS can readily spread from vessels to marina infrastructure, marinas may be the first place NNS establish within new regions (Mineur et al., 2012; Airoldi et al., 2015; López-Legentil et al., 2015; Ferrario et al., 2017).

There are over 139 marinas or harbours in Scotland Marine Scotland (2016), and recent growth of the Scottish marine yachting sector has seen marinas expand to match demand (EKOS Ltd., 2016). The number of marina berths in Scotland has increased by 24% from 2009 to 2016, growing from 12,600 to 15,700 (Scottish Tourism Alliance, 2015; EKOS Ltd., 2016). Analysing records from across the UK, Foster et al. (2016) found that over 94% of surveyed marinas contained at least one NNS. The presence of high volumes of NNS in surveyed marinas suggests that they represent high-risk sites for NNS introduction, and may also act as significant reservoir from which NNS may spread into natural habitats may be possible (Ling et al., 2012; DEFRA, 2014).

Monitoring habitats in marinas and harbours has become a recognised method of assessing the presence of NNS within a region, both due to the practicalities of being able to access submerged substrates on floating pontoons at all tidal states, and the high likelihood that NNS will be present in these environments (Mineur et al., 2012; Bishop et al., 2015;

Lehtiniemi et al., 2015b). A frequently used method of carrying out this monitoring, rapid assessment surveys (RAS) offer a cost-effective way to document the presence of NNS over a wide geographic area (Bishop et al., 2015). Rapid assessment surveys involve small teams of experts trained in taxonomic identification of target species, and can be carried out within a couple of hours with minimal equipment (Arenas et al., 2006).

RAS can provide information on which species are established in a site and detect presence of NNS new to a region (Ashton et al., 2006a), and when repeated over a wide geographic area allow patterns of species establishment and spread to be assessed (Bishop et al., 2015). They can also help meet current legislative requirements for the monitoring of NNS in the UK and Scotland (DEFRA, 2012), such as those under the EU Marine Strategy Framework Directive (MSFD) (Council Directive (EC) 2008/56/EC), and Invasive Alien Species regulations (Council Regulation (EU) No 1143/2014). As current statutory monitoring in the UK does not include high risk sites, such as harbours and marinas, RAS and other non-statutory survey programmes provide important baseline data on NNS distributions (DEFRA, 2014; Stebbing et al., 2014; Cottier-Cook et al., 2017).

Regular monitoring of the distribution of NNS is critical in understanding how NNS populations are spreading and persisting in a system. The creation of biosecurity plans at a national and site-based level and the implementation of appropriate management measures, rely on accurate information on the distribution of NNS (Cook et al., 2014b; Inglis, 2001). For instance, management of invasive populations in marinas may help to reduce the likelihood of further spread but will have minimal effectiveness if a species is already widespread (Holt and Cordingley, 2011; Ling et al., 2012; Epstein and Smale, 2017b). Furthermore, differences in the numbers of NNS between sites allows insight into site characteristics such as freshwater input, marina entrance width and seawall length that affect the likelihood of invasion (see study by Foster et al. (2016)). This information can be used to identify sites where more frequent surveillance for new NNS is necessary (Payne et al., 2014; Lehtiniemi et al., 2015b), and to support the development of a national monitoring strategy (Stebbing et al., 2014).

2.1.1 Research aims

In 2006, the first RAS in Scotland surveyed ten of the largest mainland marinas as part of the Marine Aliens programme (Ashton et al., 2006a), with repeat visits conducted in 2007 and 2008 (Cottier-Cook, *unpubl.*). In the last twelve years, surveys to establish baseline site records for NNS have taken place in the north of Scotland (Nall et al., 2015), the Shetlands (Collin et al.,

2015b), and in Orkney (Want et al., 2017; Kakkonen et al., 2019). A number of smaller scale searches targeting specific species have identified that a number of new species have been introduced in this period (Beveridge et al., 2011; Ashton, 2006; Loxton et al., 2017; Bugnot et al., 2018; Cook et al., 2013b; Smith et al., 2015; Cook et al., 2014a; Shelmerdine et al., 2017, Cook et al. *in review*). However, there has been no follow up survey of the original marinas last surveyed in 2008, and no analysis of patterns of species distribution at a national level. This study thus aimed to assess long-term patterns of individual NNS establishment and spread in Scotland.

2.2 Methods

Data on NNS presence/absence in Scottish marinas was collected by the author in 2016, and was supplemented by data collected by researchers from the Scottish Association for Marine Science (SAMS) in 2006 - 2008. Survey methodologies included rapid assessment surveys (RAS), replicated scrape samples, and settlement panels. The methodology for the 2006 -2008 surveys can be found in section 2.2.1, and for the 2016 surveys in section 2.2.2. Table 2.1 shows which survey methods were used for each site, while Table 2.2 presents site details. Sites are coded by location based on Scottish sailing regions (EKOS Ltd., 2016), divided across four regions (Figure 2.1).

Table 2.1: *Overview of survey methodology and the sites surveyed in each year. Survey methodologies include rapid assessment surveys (RAS), replicated scrape samples (Scrape) and settlement panels. Details of settlement panel methodology are provided in Chapter 4.2.*

Year	Survey method	No. Sites	Sites surveyed
2006	RAS, Scrape	10	W1,W2,W4,C7,C8,C9,C10,E1,E3,N3
2007	RAS, Scrape	10	W1,W2,W4,C7,C8,C9,C10,E1,E3,N3
2008	RAS, Scrape	10	W1,W2,W4,C7,C8,C9,C10,E1,E3,N3
2015	Settlement panels	4	W2,W3,W4,C7
2016	RAS (Winter)	1	E3
2016	RAS	18	W1,W2,W3,W4,C1,C2,C3,C4,C5,C6,C7,C8,C9,E1,E2,N1,N2,N3

2.2.1 2006 - 2008 Survey

Ten marinas were surveyed annually in August by researchers from the Scottish Association for Marine Science for three years from 2006-2008 (Ashton et al., 2006a). The sites chosen were the 10 largest marinas in mainland Scotland in 2006. Each year the sites were surveyed for the presence of NNS using two methods, a visual rapid assessment survey (RAS) and scrape sampling. The results of the RAS that took place in 2006 were published in Ashton et al. (2006a), and the procedure used is outlined in that article and was repeated in subsequent years. A similar procedure is used for the 2016 surveys.

Scrape samples of the biofouling community were collected from the side of marina pontoons. Six samples were taken from each marina, divided into inner and outer areas with three samples taken from randomly chosen pontoon floats in each zone. Samples were collected using a modified scraper, fitted with a 15 cm wide fine mesh collection bag. The area scraped on each pontoon float was from 0.5 m depth to the surface, equivalent to a surface

Table 2.2: *Site details for surveys conducted in 2006-2008 and 2016. The number of NIS and cryptogenic species reported is the most recent record for each site.*

Site	Latitude	Longitude	Berths	Depth	2006	2007	2008	2015	2016	NIS	Cryp.
C1	55.86608	-5.41095	200	8					✓	2	0
C2	55.87340	-5.31370	230	12					✓	8	1
C3	55.86140	-5.08610	150	2.4					✓	4	1
C4	55.98350	-4.94780	250	6					✓	2	1
C5	56.01177	-4.77409	210	12					✓	2	0
C6	55.94280	-4.72600	130	5.1					✓	2	0
C7	55.91020	-4.87487	600	3.4	✓	✓	✓	✓	✓	2	0
C8	55.77368	-4.85824	730	3	✓	✓	✓		✓	6	0
C9	55.54543	-4.67639	400	3	✓	✓	✓		✓	5	2
C10	55.64169	-4.82112	280	4.5	✓	✓	✓			5	
E1	55.99466	-3.41238	300	2	✓	✓	✓		✓	7	1
E2	56.22171	-2.69875	102	0					✓	3	1
E3	57.49645	-1.79107	108	2.5	✓	✓	✓		Feb	3	
N1	57.49390	-4.23360	150	3					✓	1	0
N2	57.59050	-3.86180	84	0.5					✓	1	1
N3	57.72389	-3.27957	102	1.5					✓	5	2
W1	56.18248	-5.53081	90	5	✓	✓	✓		✓	4	1
W2	56.21161	-5.55772	250	15	✓	✓	✓	✓	✓	7	1
W3	56.41820	-5.49750	115	12				✓		9	1
W4	56.45068	-5.43363	250	5	✓	✓	✓	✓	✓	3	1

area of 0.06 m^2. Samples were preserved in formalin, then washed using a 250 µm sieve and sorted. Species were identified to their lowest possible taxon in the laboratory using a stereomicroscope.

2.2.2 2016 Survey

In 2016, rapid assessment surveys (RAS) was conducted in 18 marinas by two researchers (one of which was always R. Giesler), between 26 August and 28 October 2016 (see Table 2.2 for site locations). The marinas surveyed were chosen as they are the largest marinas in mainland Scotland (number of berths), excluding Orkney and Shetland which have had independent surveys conducted in recent years (Collin et al., 2015b; Nall et al., 2015). Larger marinas may be expected to have higher vessel traffic and thus an increased risk of NNS introduction. Eight of these 18 sites were initially surveyed in the 2006-2008 survey (see Ashton et al. (2006a)), with two of the original sites (one in the Clyde, and one on the east coast) excluded for logistical reasons. The number of marinas was unevenly divided between regions, with the highest number of marinas located in the west (W1-4) and the Clyde (C1-9), and the rest in the east (E1-2) and north (N1-3) regions (EKOS Ltd., 2016).

In order to collect fouling NNS presence/absence records, RAS were conducted using a

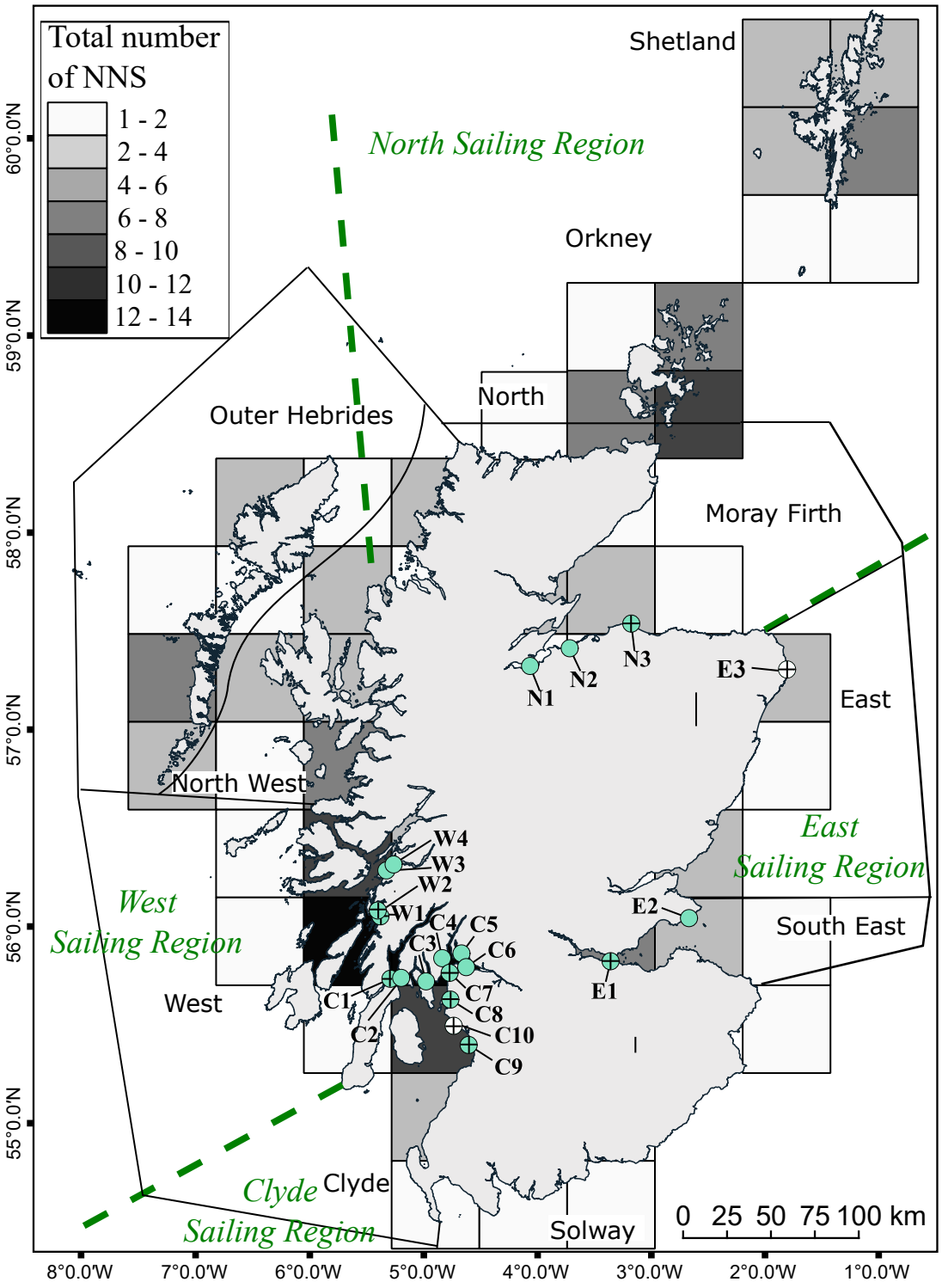


Figure 2.1: *Map showing total number of marine non-native species per 50 x 50 km grid squares (Note: map shows the number of species, not the number of records). The location of sites including in the 2006-2008 survey are indicated by ⊕. Sites in surveyed in 2016 are indicated by green fill. MNCR regions are indicated by black text, and Scottish sailing regions by green text.*

similar methodology to that described by Nall et al. (2015); Ashton et al. (2006a) and Minchin (2007b). A target species list of thirty species was developed based on previous non-native and cryptogenic species records in Scotland and the UK (Minchin et al., 2013; Nall et al., 2015), and in consultation with other researchers (Table 2.3). Prior to commencing the survey, researchers were trained in the identification of target species and native species with similar morphology to increase the probability of a correct sighting. Surveys were conducted for 1-2 hours dependent on the size of the marina, with more time being allocated to those with significantly higher numbers of berths. Accessible pontoons were divided equally between the two researchers. Researchers conducted an independent visual search for the target species on the sides of pontoon floats, concrete columns, trailing ropes and buoys, and other floating structures. Scrapers and hooks were used to allow researchers to access out of reach material. Epibiotic communities attached to kelp growing on the pontoon were also inspected. If a target NNS was identified by either researcher it was recorded as present. On completion of each survey, all equipment and gear was washed with fresh water and sprayed with disinfectant to prevent the transfer of NNS between sites.

To confirm identification, specimens of non-native and suspect species were collected and preserved in 80% ethanol for laboratory analysis. The specimens were then sorted and identified in the laboratory using a stereomicroscope, with confirmation sought from other experts if required. Two suspected invasive carpet sea-squirt *Didemnum vexillum* samples were sent to Marine Scotland Science for genetic analysis (methodology: Cottier-Cook et al. *in review*). A new location record for *D. vexillum* was confirmed by a follow-up survey in August 2017 (Giesler R. *pers. obs.*).

RAS data in 2016 was supplemented by results from other recent surveys. This includes species documented on 15 x 15 cm CoreX settlement panels deployed from June to October 2015 in four marinas, three in the west (W2, W3, W4), and one in the Clyde (C7). Full details of settlement panel methodology are set out in Chapter 4.2. The 2016 data for site E3 was sourced from a separate RAS survey carried out in February 2016 by one of the researchers (Campbell, I. *pers. comm*) using a similar methodology. These results have been included in order to provide as comprehensive a picture as possible of the distribution of NNS in Scotland. A summary of which surveys were conducted in each site is provided in Table 2.1.

2.2.3 Synthesis of Scottish Records

In 2015, a synthesised list of records for marine NNS in Scotland was published, compiling records of known marine NNS from 1836 to 2012 (Nall et al., 2015). To update this list, the methodology published in Nall et al. (2015) was followed. Published literature and the National Biodiversity Network (NBN) Scotland gateway were searched for records, and queries were sent to local researchers known to carry out NNS surveys in the area. The Orkney Harbour Authority provided records collected as part of the Orkney Islands Council Harbour Authority non-native species monitoring programme. For those species included in the original dataset, only records published since 2012 were included, while for new species, a full literature search was conducted. The NBN database was also searched for horizon species likely to arrive in Scotland in the next decade (Table A.1) (Roy et al., 2014).

The establishment status of each species was assessed following the definition used by Nall et al. (2015). A species was considered to be 'established' if it had presence records from at least four years in the same area (Nall et al., 2015; Roy et al., 2012). Species with widespread distributions but few repeat records within an area were assumed to be 'probably established' (Nall et al., 2015).

Duplicate records, published in multiple literature sources and/or on the NBN Scotland gateway were removed. For each record, coordinates, year, Marine Nature Conservation Review (MNCR) region (Figure 2.1), source reference, and if possible observation date were noted. Records have also been further classified by decade. Species names have been updated with the accepted name on the World Register of Marine Species (WoRMS).

2.2.4 Data Analysis

Summary statistics and graphs were produced using the software SigmaPlot. Maps were produced using the software QGIS 2.18, with baseline maps provided via EDINA Digimap. QGIS 3.0 was used to generate 5 km^2 and 50 km^2 grids and produce counts of species within each square. A repeated measures ANOVA was run on log+1 transformed data to test for differences in the number of non-native species in resurveyed sites between years. Analyses were run using the 'lmer' package in the software programme 'R-Studio'.

2.3 Results

2.3.1 NNS decadal trends (2006 and 2016)

In 2006, six NNS (*Styela clava, Tricellaria inopinata, Austrominius modestus, Caprella mutica, Codium fragile ssp. fragile*, and *Sargassum muticum*) were identified across seven Scottish marinas using both RAS and scrape surveys. In 2007 and 2008, these species were recorded in a larger number of the surveyed sites but no new non-native species were found (Figure 2.2). Species were recorded in 2006-2008 either from RAS or from scrape surveys. Across 10 sites and three years, the same NNS were found in both the RAS and scrape survey of a marina on only 6 out of 30 occasions (Figure 2.2). Of the six species identified over the 3 year period, four species were more frequently found using scrape sampling than with the RAS. However, *S. muticum* was not identified in any scrape samples, and observed in three sites in multiple years using the RAS (Figure 2.2).

Eight of these original sites were re-surveyed in 2016. Fourteen of the targeted NNS were found in resurveyed sites, more than doubling the total number of recorded fouling NNS. Using data from all survey methods, the mean (±SD) number of fouling species per marina across all sites was similar in 2006 (2.2 ± 1.135), 2007 (2.3 ± 1.16), and 2008 (2.4 ± 1.27), and had doubled by 2016 (4.368 ± 2.73). Looking only at the eight sites which were resurveyed, 2016 had significantly higher numbers of NNS (5.22 ± 2.28) than in the 2006 - 2008 surveys (2006: 2.0 ± 1.0, 2007: 2.11 ± 1.05, 2008: 2.22 ± 1.20) ($DF=24$, $F=19.002$, $p<0.001$). All nine sites had more NNS in 2016 than in any previous sampling period, with the highest number of non-native species detected in one site rising from 4 in 2006-2008 to 9 in 2016 (Figure 2.3). In 2006-2008, site C10 had the highest consistent number of NNS (four species) over the three year period, with site C8 and E1 also recording four species in 2008, and 2006-2007 respectively. This maximum is now significantly lower than the average number of NNS for all sites in 2016.

Figure 2.2: *Shade plot of six NNS presence/absence records from 2006, 2007, and 2008. Presence is indicated by orange squares, with records for each site over the three years divided by survey technique: rapid assessment survey (R) or scrape sample (S).*

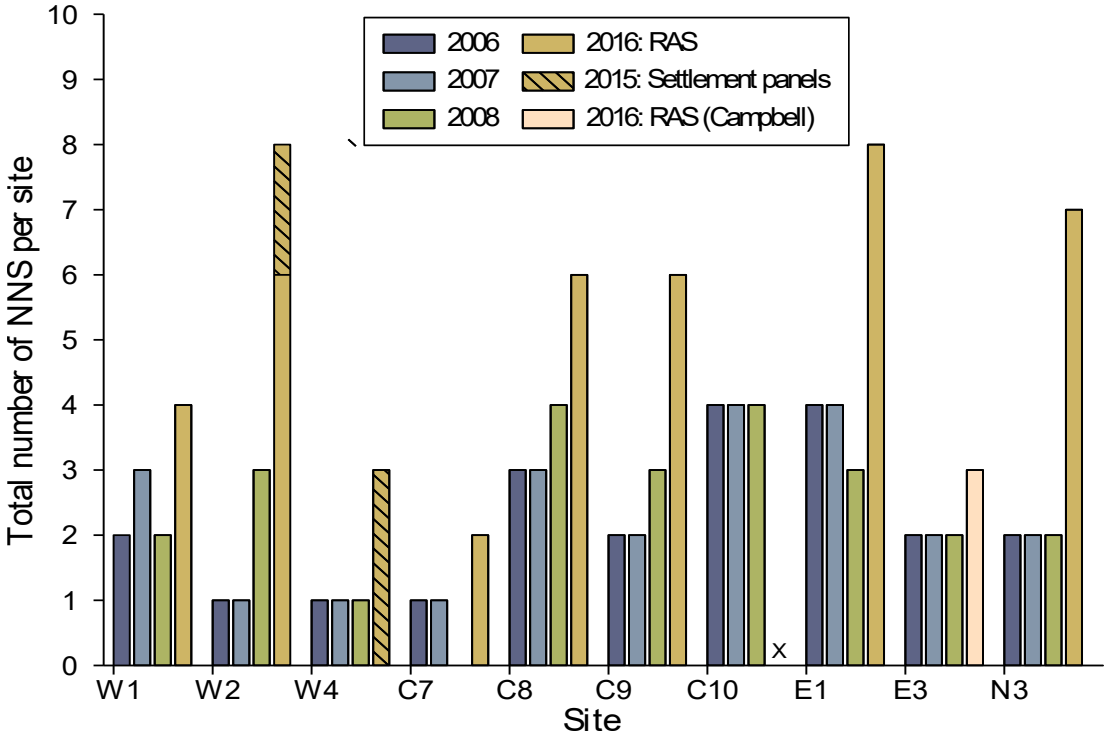
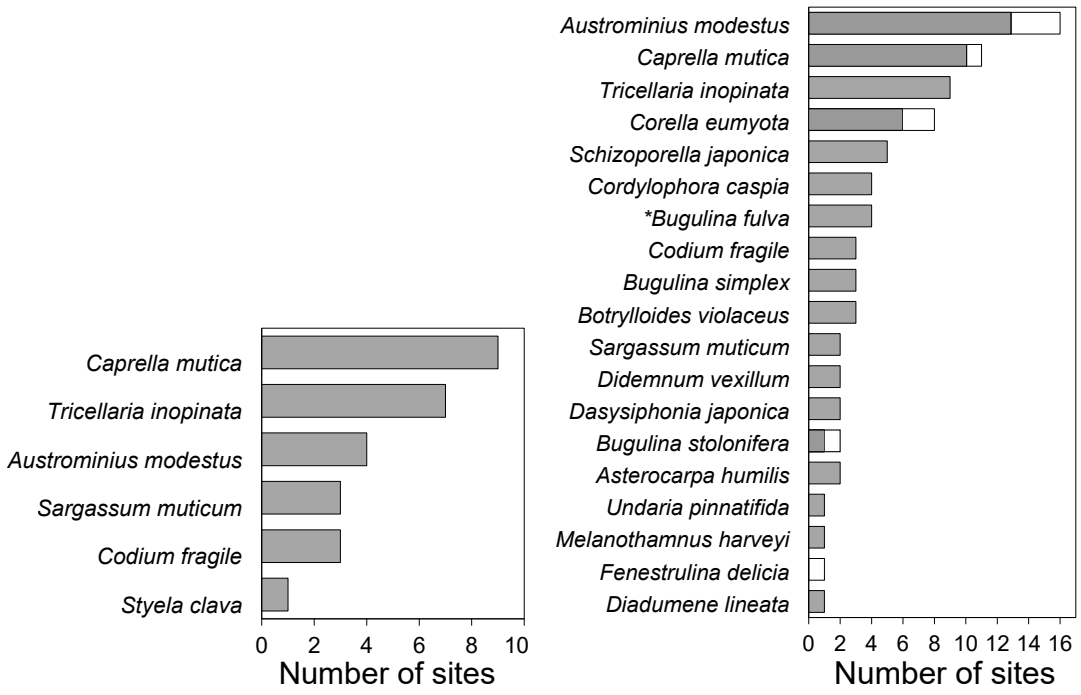


Figure 2.3: *Bar chart showing the total number of NNS recorded in resurveyed sites in 2006, 2007, 2008 and 2016. If a survey was not carried out at a particular site this is indicated with an X. Sites with shaded sections indicate where additional species were recorded on settlement panels in 2015, settlement panels were only set out in sites W2, W4, and C7.*

The most frequently occurring species in 2006 were; *C. mutica, T. inopinata* and *A. modestus*, the same species which remain the top three most commonly found species in 2016 (Figure 2.4a). The non-native barnacle *A. modestus* was found in 12 more sites in 2016 than in 2006, although it has been recorded around Scotland since the 1990s (Figure 2.5). The amphipod *C. mutica* was found in four new sites and the bryozoan *T. inopinata* in an additional two sites in 2016 compared with 2006-2008 (Figure 2.6). The macroalgae *C. fragile ssp. fragile* and *S. muticum* were recorded in new sites in 2016, but were also absent from sites where they had previously been found (Figures 2.8, 2.7).

2.3.2 Current NNS distribution in Scottish marinas

The 2016 rapid assessment survey found 17 of the targeted non-native species and one cryptogenic species, *Bugulina fulva* (Ryland, 1960). One non-native bryozoan which was not targeted in the RAS (*Fenestrulina delicia* Winston, Hayward & Craig, 2000) was also found on settlement panels deployed in 2015 (Table 2.3). Of these, 14 species were invertebrates, mainly bryozoans or ascidians, and five were macroalgae. All species have been identified in Scotland



(a) *2006* **(b)** *2016*

Figure 2.4: *Frequency distribution of non-native species recorded in surveys in 2006 (a) and 2016 (b). Hashed bars indicate the addition of records from settlement panel surveys in 2015 in addition to RAS data. *Cryptogenic species)*

in previous years, except for *Undaria pinnatifida*, which was identified in site E1 in September 2016 (Table 2.3). However, the results show new localities for a number of species. The top three most commonly found fouling NNS in our survey were *A. modestus*, *T. inopinata* and *C. mutica*. These were found in 16, 11, and 9 marinas respectively, when records from settlement panels surveyed in 2015 were included (Figure 2.4b).

Species identification in certain cases was limited by restrictions in identifying diagnostic characteristics. The *Botrylloides spp.* presented are records of specimens suspected to be *Botrylloides violaceus* Oka, 1927. Positive identification of *B. violaceus* is difficult, and in the past it has been confused with the supposed native *Botrylloides leachii* (Savigny, 1816) and the non-native *Botrylloides diegensis* Ritter and Forsyth 1917 (Bishop et al., 2015). Clear morphological distinction of *B. violaceus* depends on examination of seasonally brooded larvae (Bishop et al., 2015), which were not visible in specimens collected during the RAS. However, previous recordings of *B. violaceus* in Troon and Wemyss Bay in the Clyde, and in the Cromarty Firth and Orkney in the north, lends weight to the identification of this species in northern and Clyde sites (Figure A.5) (Nall et al., 2015). The record in site E1 is more suspect, specimens of *B. leachii* were also found at this site. Identification of two bryozoans *F. delicia* and *Bugulina*

Table 2.3: *Presence of targeted NNS recorded in the 2016 RAS of 18 Scottish marinas. Site codes in parentheses are records from alternate surveys conducted by the author in 2015 and 2016 (Chapter 4.3).*

Species	Sites found	Total no of sites
ARTHROPODA		
Austrominius modestus (Darwin, 1854)	W1,C2,C3,C4,C5,C6,C7,C8, C9,E1,E2,N2,N3 (W2,W3,W4)	13(16)
Caprella mutica Schurin 1935	W1,W2,W3,C2,C3,C8,C9,E1, E2,N3 (W4)	10(11)
Eriocheir sinensis H. Milne Edwards, 1853	-	0
ASCIDIANS		
Asterocarpa humilis (Heller, 1878)	W2,W3	2
Botrylloides sp.	C9,E1,N3	3
Corella eumyota Traustedt, 1882	W2,C1,C6,E1,E2,N3,(W3,W4)	6(8)
Didemnum vexillum Kott, 2002	C2,C8	2
Perophora japonica Oka, 1927	-	0
Styela clava Herdman, 1881	-	0
BRYOZOA		
Bugula neritina (Linnaeus, 1758)	-	0
Bugulina fulva (Ryland, 1960)	W2,W3,E1,N3	4
Bugulina simplex (Hincks, 1886)	C2,C8,C9	3
Bugulina stolonifera (Ryland, 1960)	C9 (W2)	1(2)
Fenestrulina delicia Winston, Hayward & Craig, 2000	W3	1
Schizoporella japonica Ortmann, 1890	W2,W3,C2,C3,N3	5
Tricellaria inopinata d'Hondt & Occhipinti Ambrogi, 1985	W1,W2,W3,C1,C2,C8, C9,E1,N3	9
Watersipora subatra (Ortmann, 1890)	-	0
CNIDARIA		
Cordylophora caspia (Pallas,1771)	C4,C7,N1,N2	4
Diadumene lineata (Verrill, 1869)	W1	1
MOLLUSCA		
Magallana gigas (Thunberg, 1793)	-	0
Crepidula fornicata (Linnaeus, 1758)	-	0
POLYCHAETA		
Ficopomatus enigmaticus (Fauvel, 1923)	-	0
ALGAE		
Asparagopsis armata Harvey, 1855	-	0
Bonnemaisonia hamifera Hariot, 1891	-	0
Codium fragile subsp. fragile (Suringar) Hariot, 1889	W3,C2,E1	3
Colpomenia peregrina Sauvageau, 1927	-	0
Dasysiphonia japonica (Yendo) H.-S.Kim, 2012	W3,C3	2
Melanothamnus harveyi (Bailey) Díaz-Tapia & Maggs, 2017	C2	1
Grateloupia turuturu Yamada, 1941	-	0
Sargassum muticum (Yendo) Fensholt, 1955	C5,C8	2
Undaria pinnatifida (Harvey) Suringar, 1873	E1	1

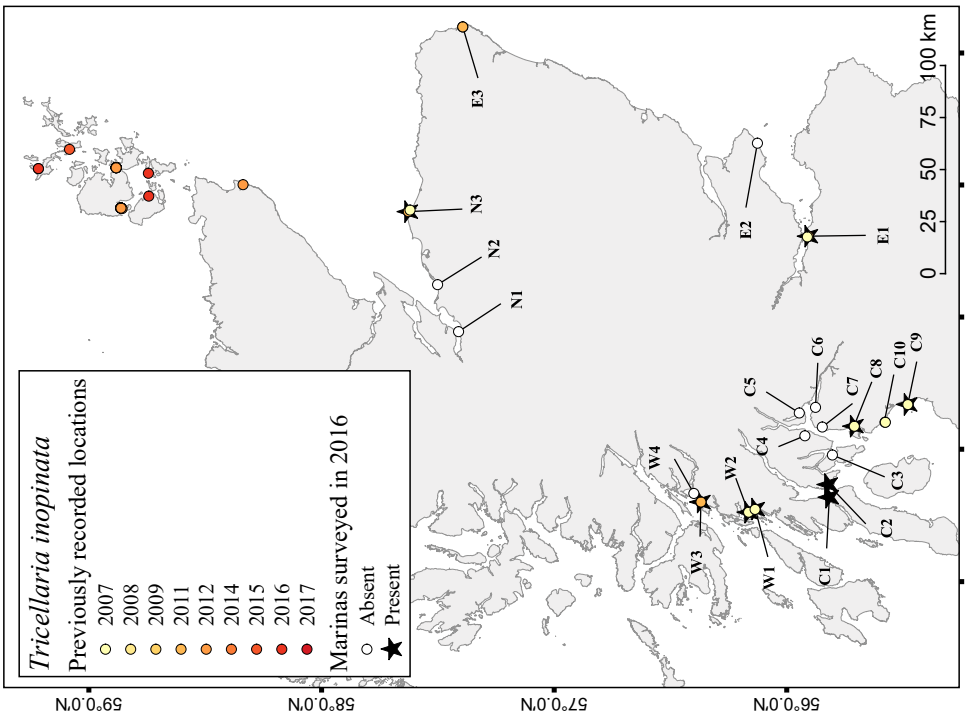


Figure 2.6: *Tricellaria inopinata distribution in Scotland. 2016 RAS results are shown with historic presence records compiled from the literature.*

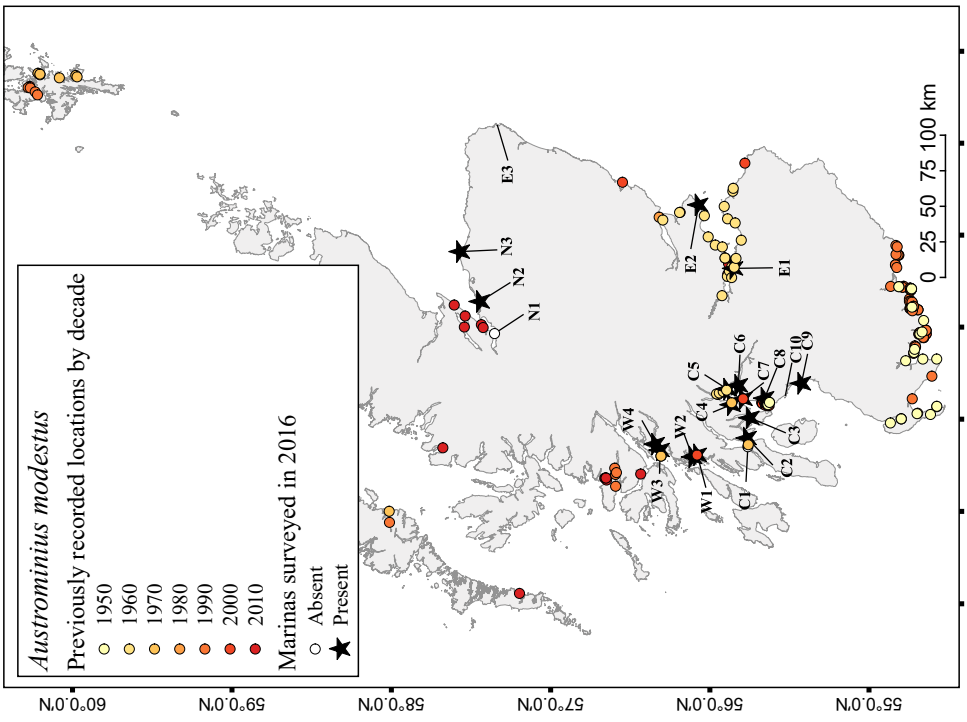


Figure 2.5: *Austrominius modestus distribution in Scotland. 2016 RAS results are shown with historic presence records compiled from the literature.*

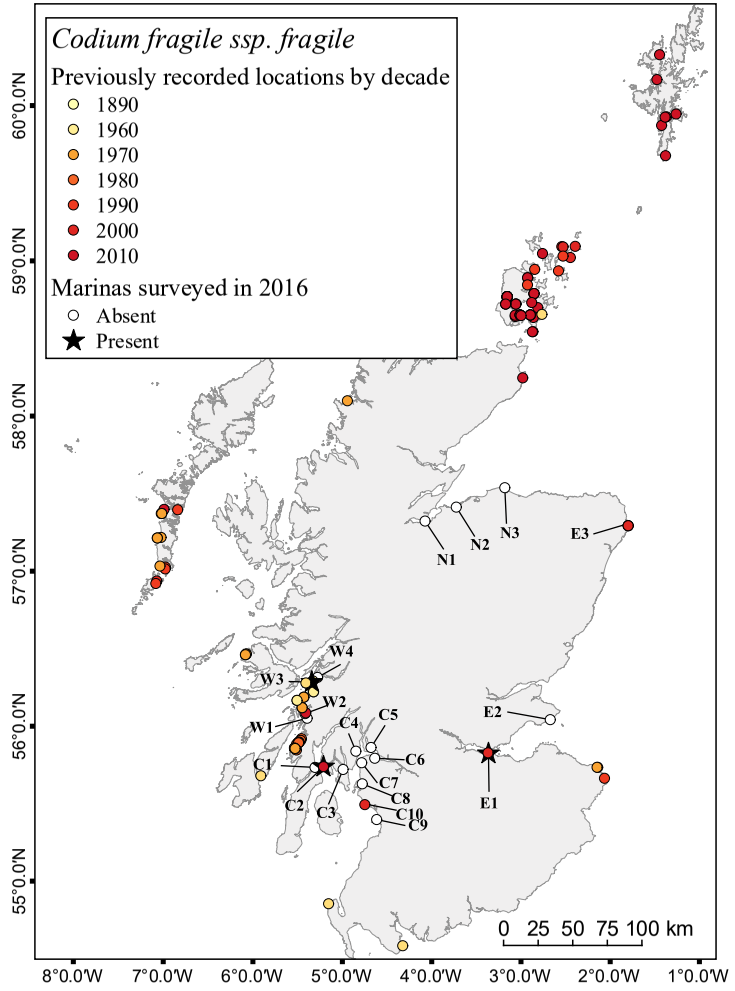


Figure 2.7: Codium fragile ssp. fragile *distribution in Scotland. 2016 RAS results are shown with historic presence records compiled from the literature.*

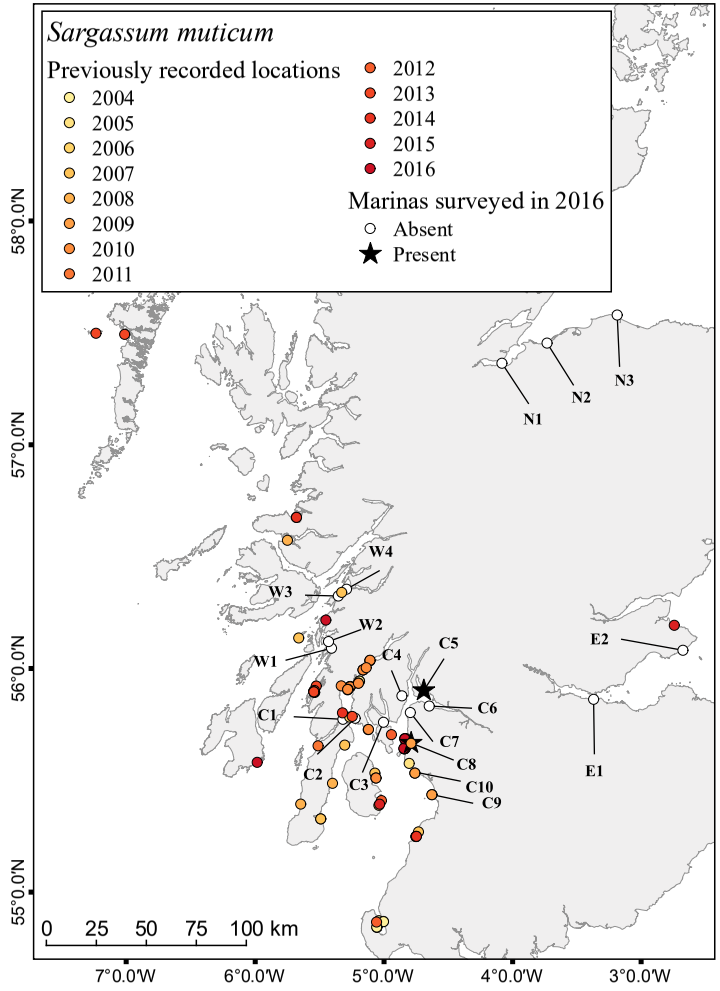


Figure 2.8: *Sargassum muticum distribution in Scotland. 2016 RAS results are shown with historic presence records compiled from the literature.*

simplex were confirmed by H. de Blauwe. The identification of suspected *Bugulina stolonifera* specimens has yet to be confirmed by a specialist. Identification of *D. vexillum* was confirmed through molecular sequencing of samples from both C2 and C8. The presence of *D. vexillum* in C2 was confirmed by a targeted survey conducted with Marine Scotland in July 2017.

The highest number of NNS was found at site W3 on the west coast of Scotland (10 species), followed by C2 in the Clyde and E1 in the Firth of Forth (8 species). Only site W4 contained no fouling NNS at the time of the 2016 survey (Figure 2.9). However, *C. mutica* was found in W4 during the RAS in 2006-2008 and on multiple occasions during more recent RAS training events (pers. comm. E. Cottier-Cook, 2016). *Austrominius modestus*, *C. mutica* and *Corella eumyota* were also found on settlement panels in W4 in 2015. As such, the fewest NNS observed overall was from site N1, where only *Cordylophora caspia* was observed. The total number of cryptogenic and NNS found in the Clyde was 14, 12 in the west sailing region, and eight in both the east and north (Figure 2.9). However, the number of sites surveyed in each region was not constant.

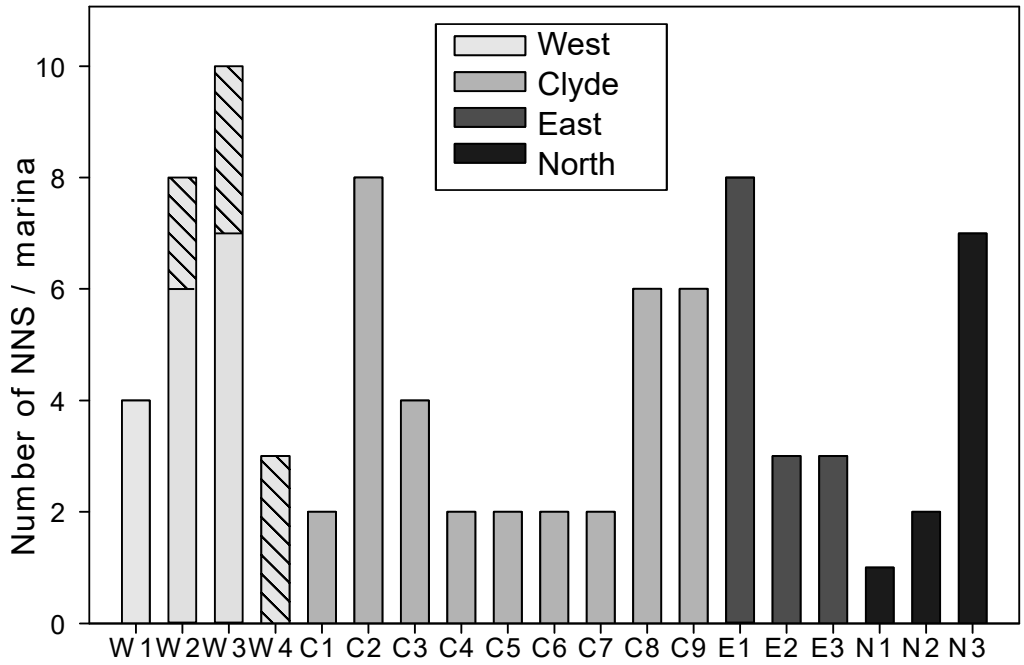


Figure 2.9: *Total number of targeted non-native species recorded in surveyed sites in 2016. Sites are divided by sailing region. Hashed bars indicate the addition of records from settlement panel surveys in 2015 in addition to RAS data. Data for site E3 was sourced from a separate RAS carried out in February 2016.*

2.3.3 Synthesis of Scottish Records

In total, 2054 records of 32 NNS and three cryptogenic species in Scotland have been compiled (Table A.2), an addition of 1020 records since C. Nall published the first version of this dataset in 2012. The new records are sourced primarily from published literature (585 records), and the National Biodiversity Network (NBN) (325 records), but also include the findings from surveys published in this thesis (80 records), as well as in other articles. The majority of published records are from monitoring work carried out by the Orkney Harbour Authority (Kakkonen et al., 2019). For species included in the original dataset, only records that have been made publicly available since 2012 have been added; however, this includes data that were collected before that date with the oldest record from July 1979. Looking at only records collected since 2012, 28 NNS and two cryptogenic species have been recorded (Table A.2).

The MNCR regions with the highest number of NNS and cryptogenic species are the west and the Clyde, with 20 NNS each, followed by Orkney with 18 (Figure 2.1). Areas with the highest number of NNS are those where surveys have been carried out in harbours and marinas (Figure 5.1). Due to the monitoring work carried out by the Orkney Harbour Authority, the largest number of new records were from Orkney (507 records total, of which 436 were from the monitoring programme). After Orkney, the Clyde (116/1019) and the west (85/1019) have had the greatest number of new records added since 2012. The most frequently recorded species is *Bonnemaisonia hamifera* (399 records), followed by *C. mutica* (285) and *A. modestus* (197) (Table A.2). In the last decade, *C. mutica* (Fig. A.8) and *Schizoporella japonica* (Fig. 2.11) have had the most records added due to their frequent recording by the Orkney monitoring surveys (Kakkonen et al., 2019).

Sixteen NNS are now identified as likely to be established in Scotland, and six NNS as being probably established (Table A.2). The establishment status of *B. violaceus* remains questionable due to uncertainties over identification. Of those species whose status was considered uncertain by Nall et al. (2015), *S. japonica* (Fig. 2.11), *C. eumyota* (Fig. 2.10), *M. gigas* (Fig. A.7), *D. vexillum* (Fig. A.2) and *T. inopinata* (Fig. 2.6) are now considered established.

An additional seven NNS in Scotland have been included in the updated dataset. These are *Boccardia proboscidea* Hartman 1940, *B. stolonifera* (Ryland, 1960), *C. caspia* (Pallas, 1771), *F. delicia* Winston, Hayward and Craig, 2000, *Potamopyrgus antipodarum* (Gray, 1843), and *U. pinnatifida* (Harvey) Suringar, 1873. The non-native snail *P. antipodarum* is primarily found in freshwater habitats, so records on NBN were filtered such that only marine records of

this species were added. Records of two cryptogenic species, *B. fulva*, and *Jassa marmorata*, identified by C. Nall in his survey of northern Scotland, have also been included. The cryptogenic comsmopolitan species *Diplosoma listerianum* was not included in the list due to historic confusion of records with *Diplosoma spongiforme*. However, there are 482 presence records in the NBN database, with the first record in the 1970s. Of these new species it is highly likely that *C. caspia* is established, but has been overlooked in surveys, or not included on target lists due to its association with brackish sites (Folino-Rorem et al., 2009). There are currently only isolated records for *B. stolonifera, F. delicia*, and *U. pinnatifida*. *Fenestrulina delicia* can potentially be confused with the native species *Fenestrulina malusii*, leading to uncertainty about its distribution (Wasson and De Blauwe, 2014). The records for the other species include relatively recent sightings, so it may be considered premature to determine their establishment status.

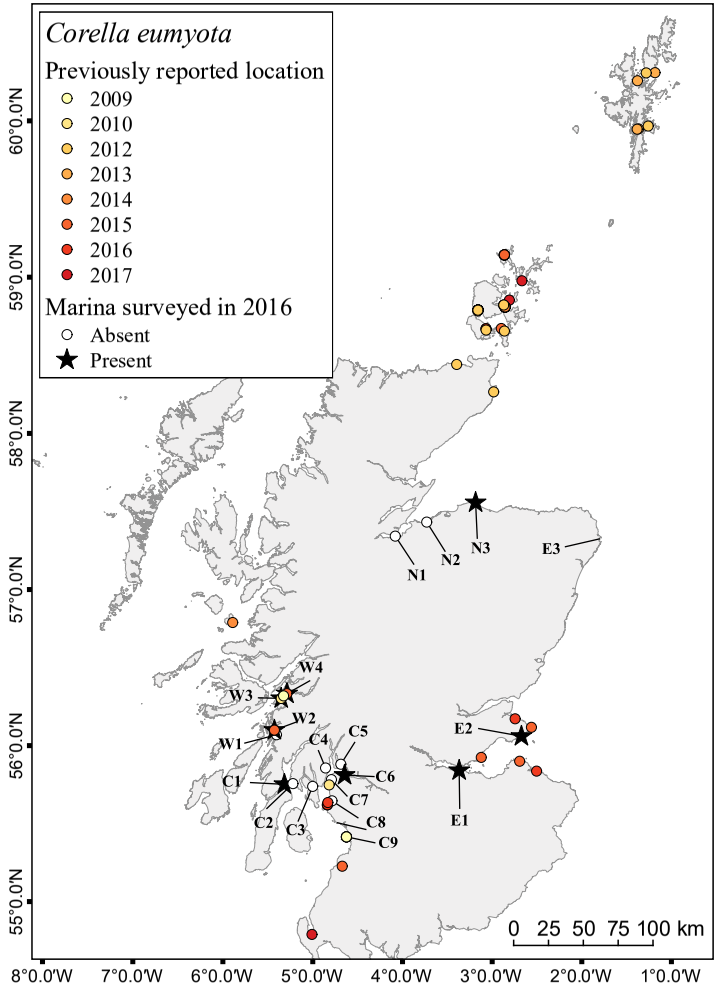


Figure 2.10: *Map showing Corella eumyota distribution in Scotland. 2016 RAS results are shown with historic presence records compiled from the literature.*

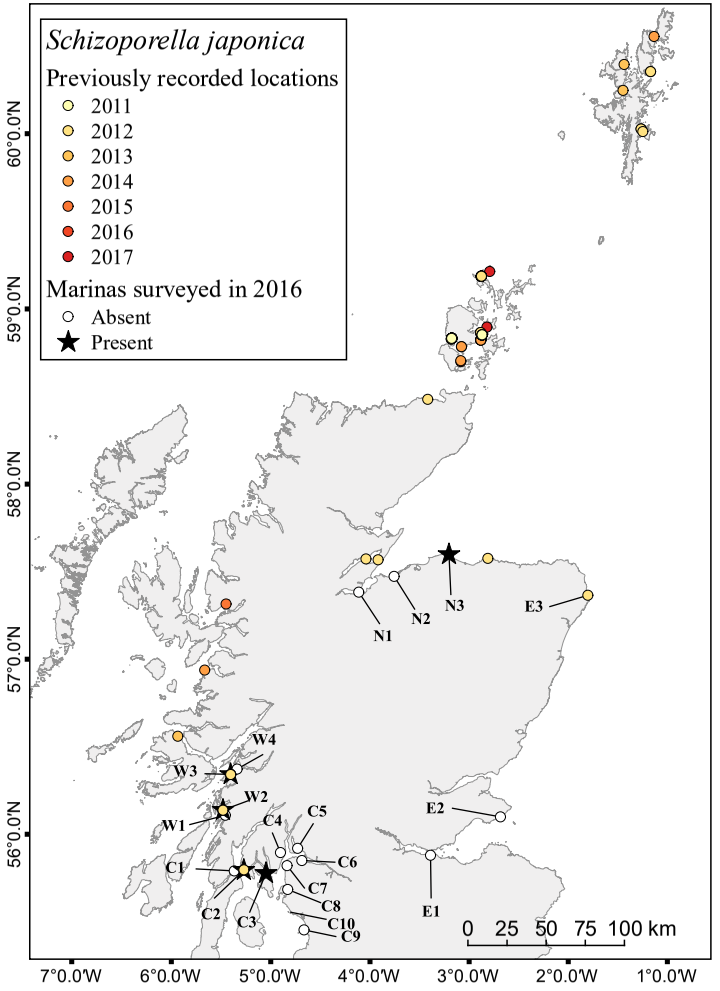


Figure 2.11: *Map showing Schizoporella japonica distribution in Scotland. 2016 RAS results are shown with historic presence records compiled from the literature.*

2.4 Discussion

In the decade between 2006 to 2016, the number of NNS recorded in the largest Scottish harbours and marinas has tripled from six to 18 species and over 800 new recorded sightings of marine and brackish NNS have been made. The number of NNS with records in Scottish waters now totals 32 species, with over half of these considered established. While some of these new species records are undoubtedly the result of increased survey effort over a wider geographical area, there are also a number of new marine NNS which have been introduced to Scotland since 2006.

2.4.1 Decadal comparison of NNS at specific sites

The number of NNS detected in the largest mainland marinas in Scotland has increased threefold from 2006 to 2016. During the surveys carried out in 2006 to 2008 there was no change in the overall number of NNS detected in the three years of repeated survey effort, but new site locations for known species were documented. In these surveys, scrape samples were collected at the same time as a RAS was performed. *Sargassum muticum* was found only during RAS', generally as unattached fronds explaining its absence from scrape samples. For other records, species that were found in a site using RAS one year, might only be found using scrape samples the next. The lack of coherence between RAS and the scrape sample at each sampling point shows that even when sampling the same habitat, interannual variation and differences between sampling methods may affect results. Repetition of surveys and the use of more than one sampling technique may be required for a complete baseline record to be achieved (Lehtiniemi et al., 2015b).

Comparing species patterns between 2006 and 2016 shows that the most common species across all sites have remained the same, with *Caprella mutica, Tricellaria inopinata* and *Austrominius modestus* the most frequently detected species. *Corella eumyota* and *Schizoporella japonica* have had the most rapid spread, found in over five sites in 2016 since they were first detected in Scotland in 2009 and 2011, respectively (Ryland et al., 2014; Nall et al., 2015).

Surveys were repeated in eight marinas, and the average number of NNS detected in resurveyed sites has increased from 2.0 in 2006 to 5.22 in 2016. Every resurveyed site has seen an increase in the number of NNS recorded. Bishop et al. (2015) resurveyed 10 sites in the English channel in 2004 and 2009/2010 and found that the average number of sessile

invertebrate NNS per site had increased from 6 to 7.6. This is a similar rate of increase as found in Scottish marinas, 1.6 species per 5 years, or 3.2 per 10 years. The average across all sites was lower at 4.37, as this incorporated a number of low salinity sites likely to have fewer marine NNS (Bishop et al., 2015, Chapter 3). Numbers of fouling NNS remain lower in Scottish sites than in the rest of the UK, with the average for marinas surveyed across the whole of England being 6.7 invertebrate NNS (Bishop et al., 2015; Minchin et al., 2013). Nevertheless, the increase in numbers of NNS across all sites shows that NNS continue to be spread around Scotland and the UK.

The number of new species introductions since 2006 varies between sites. Sites W4, C7, and W1 have lower than average numbers of NNS, and may be affected by high freshwater input producing unfavourable conditions for some marine species (see Chapter 3). E3 also has fewer NNS, although as sampling occurred in February instead of during the summer growth season, species may have been dormant and gone undetected. More information on whether variation in rate of introduction is a result of differences in propagule pressure or a function of the environmental suitability of sites for invasion, would enable more accurate estimation of the risk of species establishment in a site.

2.4.2 2016 Surveys

The 2016 surveys found records for 18 NNS and one cryptogenic species. The survey identified new location records for most species, although all species have previously been identified in Scotland, except for *Undaria pinnatifida*, which was identified in site E1 in September 2016 (Table A.2). This is the most northern record of *U. pinnatifida* in the UK so far, although its temperature tolerances indicate that it has the potential to spread further north (Epstein and Smale, 2017a). In the UK, there are relatively few records of *U. pinnatifida* in natural habitats with its establishment primarily restricted to artificial structures (Epstein and Smale, 2017b). However, *U. pinnatifida* is considered highly invasive (Lowe et al., 2000; Mineur et al., 2012), and recent research suggests that its spread to natural ecosystems is more likely when abundances in nearby marinas are high (James and Shears, 2016; Epstein and Smale, 2017b). It has been suggested that controlling abundance in artificial habitats (e.g. by manual clearing) may limit, to some extent, the impact of *U. pinnatifida* in natural habitats (Epstein and Smale, 2017b).

Across the sites surveyed in 2016, site W3 in the west of Scotland had the highest number of species (10). This may be confounded by the additional survey effort in sites W2, W3, W4,

and C7 where settlement panel data supplemented RAS data, leading to the addition of three species to the site total for W3. The data from the settlement panels recorded NNS not found in the 2016 RAS at three of the sites, and identified one species not included on the target list for the RAS (*Fenestrulina delicia*). The RAS' at sites W3 and W4 took place in late October 2016 due to delays, and as such, species recorded as present in 2015 may have been overgrown or present in lower abundances. Settlement panel data were not available for other marinas and this should be remembered when comparing NNS numbers between sites.

The highest number of NNS totalled over each Scottish sailing region was in the Clyde, followed by the west of Scotland. This is possibly confounded by survey effort, as more sites were surveyed in the Clyde than in any other region. However, there is also more sailing activity and infrastructure in these regions, the Clyde and West respectively account for 41% and 32% of resident vessel berths and are the primary destinations for visiting recreational vessels (EKOS Ltd., 2016). The presence of high NNS numbers in marinas across Scotland indicates that maritime activities which use marinas are playing an important role in the secondary spread of NNS. Given that many of the NNS which arrive in Scottish sites are already established in the UK and Ireland (Minchin et al., 2013), evaluating ways to tackle these secondary pathways should be an important part of Scottish biosecurity strategies.

A number of species may be under-represented in the survey results due to the RAS methodology and individual species habitat preferences. Species can go unnoticed if they have different habitat preferences or are present in very low abundances (Lehtiniemi et al., 2015b), especially those where identification in the field is difficult, such as with the bugulid bryozoans. *Didemnum vexillum* is often found in deeper waters and may not have been noticed if marinas had no static structures or removable trailing marina equipment (Cottier-Cook, *pers. comm.*), as the rapid assessment methodology is limited to the top 0.5 m of the water column and what can be pulled up from deeper depths (Ashton et al., 2006a). The record of *D. vexillum* in site C2 was confirmed by repeat visits in 2017 and 2018 which found colonies growing on submerged chains (Giesler, R. *pers. obs*), suggesting that the range of this species in Scotland is expanding (Beveridge et al., 2011, Cottier-Cook et al., 2019).

Of those species which were not recorded in the 2016 survey, *Styela clava* was identified in the 2006 - 2008 surveys; however, the marina with the most consistent *S. clava* recordings (C10) was not resurveyed in 2016 due to logistical reasons. There are additional records from a number of locations around the Clyde and west coast of Scotland, but only one of the surveyed sites had previously been recorded as containing *S. clava*. As it can frequently become obscured

by epibiotic species growth it may have been missed by the 2016 survey (Nall et al., 2015). Other species may represent genuine absences or limitations in the survey methodology. No records of *Watersipora subatra*, *Grateloupia turuturu*, or *Ficopomatus enigmaticus* were found either in this survey or the literature search so these species may not yet have spread to Scotland, despite their presence in England and Ireland (Minchin and Nunn, 2013; Bishop et al., 2015). The Chinese mitten crab has not been documented again since the remains of one specimen were found in the Clyde in 2014 (Yeomans and Clark, 2016), and there have only been very sporadic records of *Crepidula fornicata*. However, the RAS survey method may not have documented these species even if they were present as it concentrates on fouling organisms and does not incorporate a benthic survey (Ashton et al., 2006a).

A number of species detected in the 2016 survey are particularly likely to have a wider distribution than has been documented. *Cordylophora caspia* was found in four of the sampled marinas, all of which had some freshwater influence. Of Ponto-Caspian origin and found in freshwater and brackish areas all over the world, *Cordylophora* was first recorded in Scotland in 1984 (Folino-Rorem et al., 2009). It has likely either been overlooked in subsequent marina focused NNS surveys or not included on target lists due to its association with brackish sites (Folino-Rorem et al., 2009). Macroalgal species such as *Sargassum muticum*, *Codium fragile*, and *Colpomenia peregrina* were either infrequently recorded or absent from the marina surveys. These species are all considered established in Scotland and have relatively widespread distributions, but are more frequently recorded from natural habitats than on artificial structures (Mineur et al., 2012).

The bryozoan *Bugulina simplex* was identified in three locations in the Clyde, the first records for mainland Scotland since 1994 Ryland et al. (2011). The warm-water species has also been found in Shetland and Orkney since 2012, as well as in Northern Ireland (Sarmento et al., 2013; Nall et al., 2015). *Bugulina stolonifera* was identified in two locations in the Clyde and on the west coast, but the only other records for *B. stolonifera* comes from the west coast in 1982, and all three records should be considered unverified. Ryland et al. (2011) state that it is likely that both *B. stolonifera* and *B. simplex* have been under-recorded in much of western Europe due to difficulties with identification, despite having been identified as invasive in other parts of the globe. The detection of *Fenestrulina delicia* on settlement panels from one site on the west coast, is the third location record for this species in Scotland, following one record from the Clyde in 2009 and from Orkney in 2012 (Wasson and De Blauwe, 2014; Collin et al., 2015b). Identification of this species is likely confused with the similar species *F. malusii* and its

distribution is, therefore, likely more widespread than these three isolated records (De Blauwe et al., 2014). Detection of these fouling bryozoans in the 2016 survey shows that these species are present in Scotland, and that future NNS surveys should include laboratory identification of suspected bryozoan specimens.

2.4.3 Synthesis of Scottish records

There are now 2054 presence records of 32 marine NNS in Scotland. This study has added 940 records by reviewing published data, and 80 records from marina surveys. In the five years post-2012 more than 800 additional records were documented, the majority of these from Orkney where annual monitoring has been in place since 2012 (Kakkonen et al., 2019). Four additional fouling NNS have been added to the synthesised list collated by Nall et al. (2015), as well as three non-fouling NNS, and one cryptogenic microorganism. A detailed description of the arrival, distribution and status of the species documented in this list is provided by Kakkonen et al. (2019) and Bishop et al. (2015) and so is not replicated here; instead general trends are discussed. The total number of species remains lower than in other areas of the UK and Ireland, possibly as a result of temperature tolerance limits (Minchin and Nunn, 2013; Nall et al., 2015). Some species may have not established, as there have been no records of *Bugula neritina*, *Aulacomya atra*, *Amphibalanus amphitrite* or *Antithamnionella spp.* since the last review by Nall et al. (2015).

The geographic distribution of records is not uniform, with concentrations of records and higher numbers of NNS most likely representing increased survey effort in areas such as the west coast, Orkney and Shetland. This may in part be due to researchers actively surveying areas suspected to have high numbers of NNS (e.g. by looking at vector activity), it remains likely that if survey effort was to be intensified in other areas additional NNS would be recorded (Nall et al., 2015). Additional survey work could fill in some of the data gaps in less urbanised areas. However, as some types of survey do not include absence records, it can be hard to determine if gaps in the geographic distribution of species represent true absences. Including absence data in the list of compiled records would greatly improve its usefulness, but would require a more extensive analysis of the literature. An alternative source of information could be records from EIAs conducted by individual consultancies or companies, data from unofficial site visits by researchers, and data from statutory monitoring not included on the NBN database (Stebbing et al., 2014). This study did not actively seek out this information and, therefore, may not represent a fully comprehensive set of NNS records

for Scotland.

Nall et al. (2015) identified that 15 of the 23 fouling NNS recorded in their literature search had established (10) or probably established (5) self-sustaining populations. Of those not included, *B. violaceus*, *D. vexillum* and *S. japonica* were considered too recent an arrival to determine establishment status. Both *D. vexillum* and *S. japonica* have increased their range and can now be considered established (Loxton et al., 2017, Cottier-Cook *in press*). Of the five species that had only single or relatively few incidences reported, three have had no additional sightings since 2012 of (*A. atra*, *A. amphitrite*, or *B. neritina*). *Crepidula fornicata* has had one additional record added from the north-west through NBN. However, there have been additional records of *Magallana gigas* from the Firth of Forth and Shetland (Shelmerdine et al., 2017; Smith et al., 2015), with repeat recordings of this species in the Solway Firth where it is likely established (Figure A.7) (Cook et al., 2014a). Thus there are now sixteen species which can be considered established in Scotland, and six species which are probably established (Roy et al., 2014; Nall et al., 2015). Of these, some are restricted geographically to one area, but have the potential to increase their distribution.

2.4.4 NNS monitoring in Scotland

This study has updated marine non-native fouling records in Scotland, building on the work of Minchin et al. (2013) and Nall et al. (2015) and providing the most comprehensive record of marine NNS distributions in Scotland to date. The significant progress made on monitoring NNS distributions in Scotland over the last five years has contributed a large number of NNS records, and a much deeper understanding of individual species patterns than was available a decade ago. However, it is important this information is easily accessible and updated in a timely matter, otherwise it is of little use to managers or as a resource for future research (Stebbing et al., 2014; Lehtiniemi et al., 2015b). The compilation of these records allows analysis of patterns of NNS recording and distribution that are not possible with studies limited to one geographic area or species (Stebbing et al., 2014). This data will be shared with Marine Scotland, the body with responsibility marine NNS in Scotland, where it will hopefully be used to inform future surveillance and monitoring systems.

RAS have been used to sample high risk harbours and marinas around much of the UK coast (Minchin and Nunn, 2013; Bishop et al., 2015; Collin et al., 2015b; Nall et al., 2015) as these are not covered by statutory monitoring or by citizen science programmes (Stebbing et al., 2014). However, the sole reliance on RAS to fulfil legislative requirements is not

recommended, as some species will not be detected, no information on abundance of NNS is recorded, and the likelihood of early detection of new species is low (Rohde et al., 2017). As such, the use of other protocols has been advised, for example the HELCOM/OSPAR protocol (H/O) for port surveys which includes more extensive sampling with dive teams, in order to sample a variety of habitats including soft-bottom substrates and mobile species (HELCOM, 2003; Rohde et al., 2017). Statutory monitoring in New Zealand and Australia uses a variety of survey techniques to carry out monitoring and surveillance for target species in high risk areas identified through pathway analysis (Hewitt and Martin, 2001; Woods et al., 2017). The procedures used in these countries have influenced the development of monitoring programmes carried out in the Sheltand and Orkney islands. In Orkney, annual monitoring since 2012 has included RAS, scrape surveys, phytoplankton and zooplankton tows, and benthic grab samples (Kakkonen et al., 2019). This monitoring has amassed over 430 records of marine NNS since it started (Kakkonen, 2016; Kakkonen et al., 2019). Time and expense prevented the use of these methods for the 2016 surveys. A similar strategy combining comprehensive surveys at high risk sites, with additional less frequent, lower cost monitoring at low risk sites may be more feasible to establish at a national level in Scotland (Woods et al., 2017). This would help fill in gaps currently not covered by the ad-hoc monitoring programmes in place, and better address legislative requirements (Boyes et al., 2016).

Sites that persistently have lower numbers of NNS than nearby heavily invaded systems may have different environmental or hard infrastructure features that reduce the risk of a NNS successfully establishing and thus may require lower levels of monitoring (Payne et al., 2014; Lehtiniemi et al., 2015b). Information on areas where new arrivals are more frequently recorded could be combined with data on marine activities to determine intensity of pathway activity, as has been done in Shetland to help produce a NNS monitoring plan (Collin et al., 2015a; Shucksmith and Shelmerdine, 2015; Tidbury et al., 2016). Combining this with Scottish marine planning initiatives could highlight areas where biosecurity plans should be included as part of environmental impact assessments or where biosecurity measures should be included as part of marine protected area management plans.

CHAPTER 3

Variation in marina fouling communities

ABSTRACT

Managing the receiving environment of high risk sites could contribute to reducing the likelihood of non-native species (NNS) establishment post-arrival. Harbours and marinas can act as entry points for species introduction, facilitating secondary dispersal of introduced species within a region. Thus understanding the environmental and anthropogenic factors which affect susceptibility to invasion within marinas could minimise the spread and impact of NNS. This study investigated patterns of non-native and native species diversity in fouling communities on pontoon floats within Scottish marinas. Fouling community composition was investigated among marinas found on a spectrum of high to low freshwater influence. Forty-four taxa were identified from photographs of fouling communities, the majority of which were native species (35 species). Rapid assessment surveys identified 18 targeted NNS across all sites. Native species remain dominant in most biofouling communities in Scottish marinas, although NNS occupied more than 50% of biofouling cover in one site, and over 10% in another three. NNS and native species diversity were driven by the degree of freshwater influence in a site, with low salinity sites in close proximity to rivers having significantly fewer NNS. The expected salinity of a site derived from marina features and model data could be used as an indicator of the likelihood of species invasion, contributing to assignment of monitoring effort and development of management plans.

3.1 Introduction

The previous chapter has shown that the number of marine NNS in Scottish marinas has risen in the last decade, despite increasing effort to prevent species transfer. The RAS data contributes to a number of studies investigating the distribution of target NNS, but there has been no examination of patterns of native and non-native fouling species abundance and diversity between different sites in Scotland. Analysis of fouling community composition can be used to establish the degree of NNS dominance within a community, which can help infer the potential impact of NNS (Choi et al., 2016). Comparison of NNS diversity between sites can also help elucidate which factors may be influencing likelihood of NNS establishment in marina fouling communities.

Elucidating which factors may be responsible for current NNS distributions is complicated by the range of variables which affect the whole NNS introduction process (see Figure 1.3). The likelihood of introduction may vary depending on vector concentrations, while establishment may be influenced by the resident community, the environmental conditions, and availability of resources. This can make teasing the relative importance of individual variables difficult (Clarke Murray et al., 2014; Lord et al., 2015; Choi et al., 2016). However, insight into the factors which affect a site's vulnerability to invasion could help guide management and monitoring efforts (Clarke Murray et al., 2014). Studies from Canada, California and the Mediterranean have identified a variety of environmental and anthropogenic factors which influence the distribution of NNS within ports and marinas, including the latitude, sea surface temperature and salinity, harbour type, presence of floating structures, vessel activity and population size of the nearest settlement (Lord et al., 2015; López-Legentil et al., 2015; Choi et al., 2016). In the UK, Foster et al. (2016) analysed the association between physical features of marinas (length of vertical seawalls, pontoon length, marina entrance width, distance to the nearest river, length of sloping breakwaters, and presence of swing moorings) and NNS richness in surveyed UK marinas. Semi-enclosed marinas, and those with an intermediate length of seawall (200 - 550 m), were identified as more likely to have higher numbers of NNS. This study also identified that the probability of NNS presence in a marina increased as distance from a river increased, with marinas over a 1000 m away from a river having significantly higher numbers of NNS than those within 20 m (Foster et al., 2016). High freshwater input from nearby rivers was expected to create low salinity environments within marinas (Foster et al., 2016). However, Foster et al. (2016)

relied on NNS presence data collected by other researchers with different research objectives. Marinas expected to have lower salinities have been excluded from targeted RAS in the UK (including from data sets used by Foster et al.) and in the USA, as the likelihood of marine fouling NNS establishment is assumed to be lower in low salinity sites (Bishop et al., 2015; Lord et al., 2015).

Fouling NNS are thought to be less likely to survive introduction into low salinity ports and marinas as hull-fouling species would have to withstand the change from marine to very low salinity conditions during vessel transit (David et al., 2013; Moreira et al., 2014). Although susceptibility to salinity change varies between species, and even within populations, the likelihood of marine species being capable of surviving the transition from fully marine conditions to freshwater is deemed to be very low (David et al., 2013). However, marinas subject to large variation in salinity as a result of freshwater inflow could create highly disturbed conditions for native species as well, reducing native species diversity and biotic resistance by increasing open space for settlement of novel species (Dafforn et al., 2012). NNS have also been shown to be more tolerant of pollutants and environmental stress than native species, and disturbed assemblages may be more likely to be invaded than non-disturbed ones (Kenworthy et al., 2018; López-Legentil et al., 2015).

This study aimed to test whether low salinity marinas have fewer NNS by investigating NNS and native species presence and abundance in sites spanning a range of expected salinity conditions. Marinas and harbours in Scotland are frequently located within river mouths, estuaries, or near urban centres with storm drain runoff (Nall et al., 2015; Foster et al., 2016). The salinity in these environments can fluctuate greatly, varying over time dependent on daily, seasonal and annual variation in rainfall and tidal conditions, as well as spatially, influenced by site design and local geography (Floerl and Inglis, 2003; Smyth and Elliott, 2016). Variation in salinity over time may result from differences in rainfall, river flow, and tide, affecting both coastal waters more generally and local conditions within marinas. The variation in coastal salinity will therefore depend on the oceanic and climatic conditions of the region studied. For example, in San Francisco bay there is no rainfall between May and October, and the degree of winter rainfall has been shown to have significant impacts on summer fouling community composition (Chang, 2009; Chang et al., 2018). In Scotland, rainfall occurs throughout the year and is higher than in the rest of the UK. 73% of the total rainfall is estimated to run-off into the marine environment (Marine Scotland, 2011).

Spatial variation in salinity may be influenced by the position of a marina within an

estuary, source of freshwater input (e.g. river position within or near to a marina, presence of storm drains, marina outlets), and current dynamics within a site. The design of a marina will influence environmental conditions within a site, which may be substantially different from nearby stretches of natural coastline or bays (Mineur et al., 2012; Rivero et al., 2013; Johnston et al., 2017). Marinas enclosed or partially-enclosed by seawalls have been shown to have reduced flushing and water circulation (Floerl and Inglis, 2003; Kenworthy et al., 2018). This can result in changes to sediment deposition, pollutant levels and propagule pressure as well as salinity (Floerl and Inglis, 2003; Dafforn et al., 2009a; Crooks et al., 2010; Rivero et al., 2013). Highly enclosed marinas may develop a stratified layer of freshwater if there is sufficient input of freshwater, and sites with no river inflow have even been observed to develop a thin stratified layer following heavy rainfall (*pers. comm.* Cottier-Cook, 2015). The salinity conditions experienced by organisms within sites may thus be very different from salinity conditions outside the marina (Rivero et al., 2013). This leads to difficulties in estimating the range of salinity conditions experienced by fouling communities within sites.

A number of studies have identified salinity as one of the driving environmental factors affecting patterns of NNS distribution among harbours and marinas (Clarke Murray et al., 2014; Choi et al., 2016; Foster et al., 2016). However, the methods by which salinity data are collected range widely in the degree to which they capture spatial and temporal variation. For example, (Choi et al., 2016) took single point measurements using a refractometer. Clarke Murray et al. (2014) obtained sea-surface salinity and temperature data for their investigation of fouling NNS in British Columbia, Canada, from 18 monitoring stations which collect daily records, abstracting monthly averages and the minimum and maximum records over a 6 year period. In Scotland, routine monitoring data have been incorporated into a hydrodynamic model of Scottish shelf waters, the Scottish Shelf Model (SSM). This offers a potential route to estimating salinity at relatively fine spatial scales (current resolution is 1 km in coastal areas), but is unlikely to account for impacts of site design. However, capturing information on local sources river discharges (e.g. proximity to rivers, the number of rivers nearby, and river width) may help determine if sites will be subject to the effects of potentially rapid influxes of freshwater.

3.1.1 Research aims

This study aimed to investigate patterns of native and non-native fouling species abundance and diversity among fouling communities in Scottish marinas. Fouling assemblages on marina

pontoons were chosen for investigation as these communities are more likely to be colonised by NNS than static structures, and also offer easy access at all tidal states. Data was gather on biotic and abiotic features of eighteen of the largest mainland marinas to answer the following questions:

- Does native and non-native diversity vary among Scottish marinas and how dominant are NNS in fouling communities?
- Which environmental and anthropogenic marina features best correlate with native and non-native diversity?
- Do lower salinity marinas have fewer NNS?
- Can secondary data on salinity and river input be used to predict patterns of NNS distribution within Scottish marinas?
- Can native species diversity be used to predict patterns of NNS distribution within Scottish marinas?

Eighteen marina surveys were conducted to assess fouling community composition and salinity and temperature within each site. Secondary data expected to influence salinity within sites, including distance to and width of the nearest river, and the predicted salinity generated by the Scottish Shelf Model was also collected. Abiotic data was gathered on marina features such as the number of berths, degree of enclosure and connection to large ports. In answering these questions, this study aims to identify indicator species or abiotic variables which could be used to predict risk of NNS invasion and thus guide development of monitoring plans and biosecurity management options.

3.2 Methods

3.2.1 Marina surveys

Primary and secondary data on the biotic and abiotic characteristics of eighteen marinas in mainland Scotland was collected over a two month period from September to October 2016. The methods used to select the eighteen marinas are presented in Chapter 2 (Section 2.2.2), with the same codes used to identify marinas (Figure 2.1).

Biotic data on the fouling communities in each marina was collected using two different methods, a Rapid Assessment Survey (RAS) and a photo quadrat survey. These surveys were carried out on marina pontoons as they allow access to fouling communities at any tidal state. In each of the marinas surveyed, pontoon floats were made of hard plastic which varied in colour between sites. The RAS collected presence/absence data on a target list of NNS, while the photo quadrat survey allowed identification of both native and non-native taxa, and provided information on abundance.

3.2.1.1 Rapid Assessment Survey (RAS)

At each site, a RAS for target non-native species was conducted. The methodology followed for these surveys is described in Section 2.2.2. Data on NNS presence from settlement panels used in experiments in Chapter 4 was also included for 4 marinas (Section 4.3). While this amounts to an uneven survey effort between sites, it was considered that accounting for all known NNS was an important element of this study. Consideration of the limitations of the different survey techniques is presented in Section 2.4.

3.2.1.2 Biofouling photo quadrat survey

The fouling community present at each site was characterised by photographing 30 quadrats on the vertical side of floating pontoons. Photo quadrat survey methodology was adapted from previous studies of biofouling communities and hydroids on pontoons in marinas (Boswarva, 2015; Lord et al., 2015). Photographs were taken using a Hero4 Silver GoPro attached to a custom built apparatus, which allowed for the camera and quadrat to be lowered into the water and positioned against the vertical side of marina pontoon floats (Figure 3.1). The camera was thus fixed at a set distance (20 cm) away from the rectangular quadrat (measuring 18 x 23.5 cm), which was maintained within and between sites. A random number

generator and map of the marina pontoons was used to choose 30 random sections on the marina pontoons; the nearest pontoon float at each location was photographed, with the quadrat positioned before looking over the side to remove selection bias (Lord et al., 2015). If a section was inaccessible, the next nearest accessible section was photographed instead (Lord et al., 2015). The camera and quadrat were held in place while three photos of each quadrat were taken by using the time-lapse setting on the GoPro, set to a 5 second interval, to maximise the likelihood of obtaining a focused image.

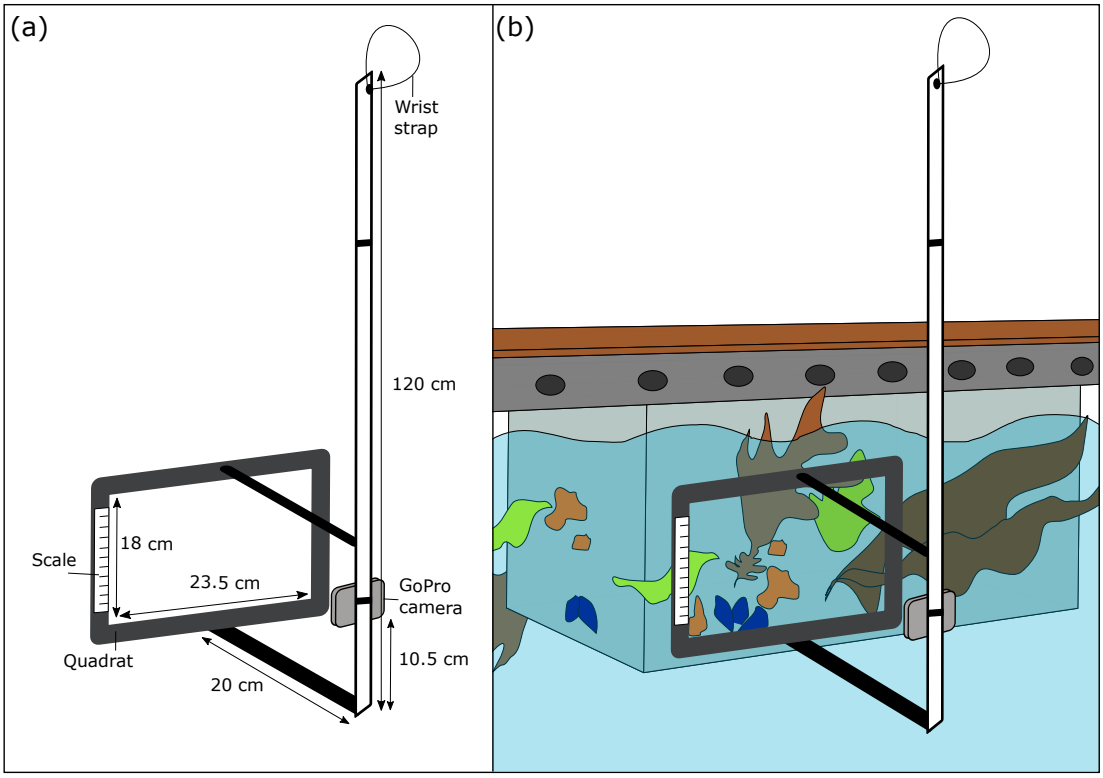


Figure 3.1: *Figure showing quadrat and camera apparatus (a) with a representation of how the quadrat would be positioned against a pontoon float (b).*

Photos were processed in Irfan-J using the Perspective Transformation Plug-in (Vicanek, Michael 2009 Perspective Transformations v8.2, MV's Plugins) to correct for the orientation of the photos (Boswarva, 2015). Photographs were analysed using Coral Point Count with Excel extensions (CPCE v4.1) (Kohler and Gill, 2006). Fifty points were overlaid on the photo, placed within a 21.5 x 16 cm quadrat, leaving a 1 cm gap between the border and quadrat edge, to reduce mis-identification based on distortion by the placement of the quadrat frame (Drummond and Connell, 2005; Atalah et al., 2016). A 5 x 10 point grid was automatically overlaid on each photo, with 1 point randomly placed in each grid square allowing for a

stratified random arrangement of the 50 points (Atalah et al., 2016). Organisms under each point were identified to the lowest taxonomic level possible by one researcher, and percentage cover of each taxon calculated for each photo. Notes made from species observations during the rapid assessment surveys were also used to inform taxon identification. Due to difficulties in identifying species solely from the photographs, identification down to species level was often not possible, and instead some organisms were grouped into functional assemblages (e.g. filamentous brown algae, kelp, arborescent bryozoa). If it was not possible to identify an organism it was classed as an 'unidentified species' (Jimenez et al., 2017). If areas of the photograph showed uncolonised pontoon float this was counted as 'blank space'.

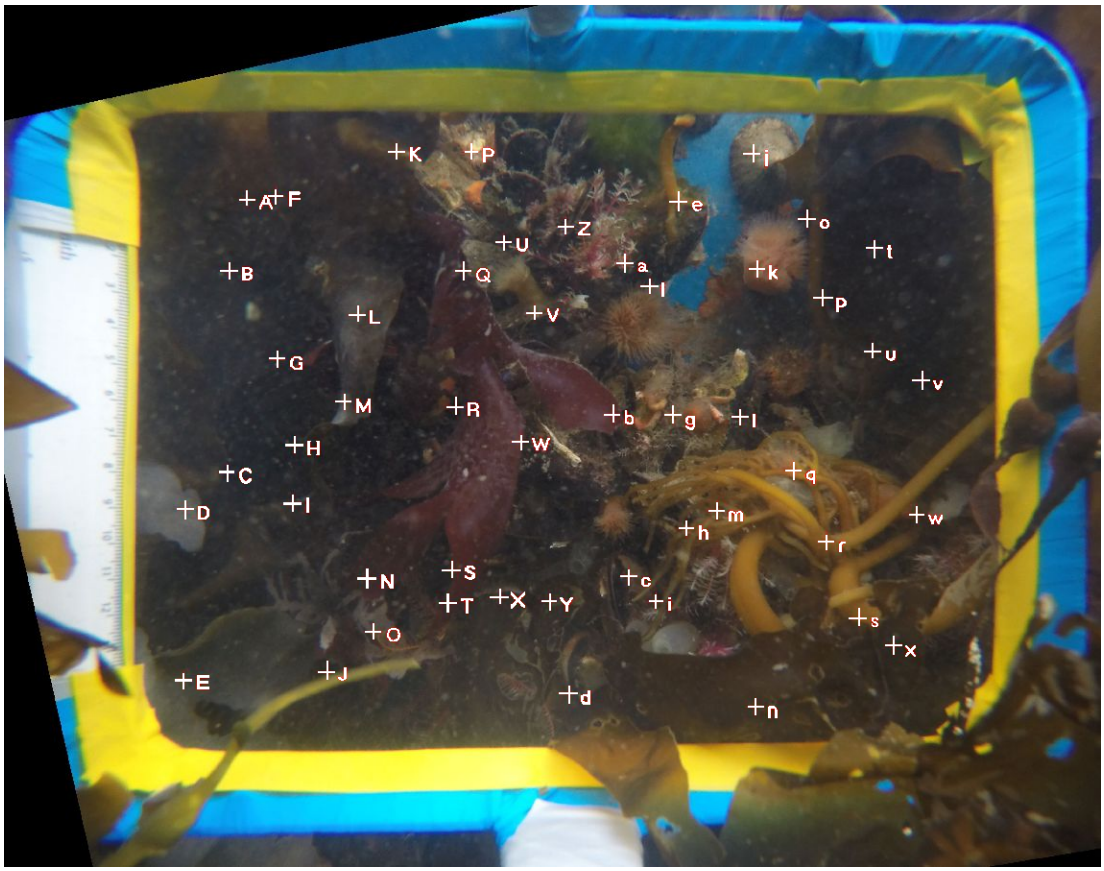


Figure 3.2: *Example of biofouling community photograph (from site W3) in the image analysis programme CPCE with 50 stratified random points overlaid. The species underneath points have been identified to lowest taxonomic level possible. The photo's orientation has been corrected to allow for analysis with CPCE.*

The photo quadrat surveys were not conducted at site E1, although a RAS was, due to poor water visibility as a result of high sediment suspension. Although a return trip was attempted, visibility remained very poor, despite the weather being much calmer on this

occasion. Reference to problems with siltation at this site, and necessary dredging, suggests that low water visibility due to sediment load may be characteristic of this marina, and of other east coast sites (Port Edgar Berth Holders' Association, 2018). As such, site E1 was excluded from statistical analyses comparing RAS results with data from the photo-quadrat and CTD surveys.

3.2.1.3 CTD sampling

Salinity and temperature data depth profiles were sampled at each marina using a handheld castaway-CTD from the pontoons (SonTek CastAway CTD, 2010). Ideally, continuous or very frequent monitoring would be best suited to capturing the effects of potentially rapid influxes of freshwater from rivers or storm drains on salinity conditions. However, the costs of continuous monitoring salinity loggers (>£800 per unit) was prohibitive in this study. CTD sampling was done at one time point for each marina, for the majority of the marinas this was at the same time as the rapid assessment survey was performed (Table 3.5). However, for four marinas (C2,C3,C7,C8) the measurements were taken approximately three weeks later due to malfunctioning equipment. At five locations within each site, the castaway CTD was used to take measurements from the surface to the bottom of the water column. Monitoring locations were chosen to cover as wide an area around the marina as possible, and usually involved two measurements taken near the pontoon access bridge and three in the outer region of the marina.

3.2.2 Secondary abiotic data

Supplementary data on marina features that have shown associations with NNS presence in studies in other areas of the world as well as within the UK was sourced from satellite images, marina plans, website information and sailing directories (Table 3.1) (Foster et al., 2016; Nall et al., 2015). In addition to latitude and longitude, information on the size of the marina (represented by number of berths) and minimum depth was collected from marina websites or the Practical Boat Owners Guide. Google Earth Pro was used to calculate distance measures using the 'Path' measuring tool and satellite pictures of marinas (see methods in Foster et al. (2016), Appendix D) (Google, 2018). Marinas were either enclosed by seawalls or open, so the entrance width was measured as an estimate of this. The entrance width of the marina was measured as the minimum distance between seawalls, or between breakwaters and the shore. The shortest distance between marinas and the nearest of the top 11 largest

Table 3.1: *Data collected within the 17 surveyed marinas, characterising biological communities, environmental data, and secondary data on marina features*

Variable	Unit	Details	Method
		Biotic data	
NNS presence / absence		For target list see Table 2.2	Rapid Assessment Survey (RAS) for target NNS
Biofouling community composition		30 photo-quadrat replicates of vertical pontoon biofouling communities per site	Photo quadrat survey
		Environmental data	
Salinity	PSU	Salinity measured through water column at 5 sample points within marina	CastAway CTD
Temperature	°C	Salinity measured through water column at 5 sample points within marina	CastAway CTD
		Secondary data	
LOCATION			
Latitude	Decimal degrees	Marina mid-point	Google Earth
Longitude	Decimal degrees	Marina mid-point	Google Earth
Port distance	m	Distance between marina mid-point to the nearest port (sourced from list of 11 largest ports in Scotland on NMPi)	Google Earth Path tool
SITE DESIGN			
Berths		Number of vessel berths	Marina website
Entrance width	m	Minimum distance between seawalls, or breakwaters and shore	Google Earth Path tool
Depth	m	Minimum depth at MLWS	Practical Boat Owner guide
FRESHWATER INFLUENCE			
River distance	m	Distance between marina mid-point to the nearest river mouth	Google Earth Path tool
River width	m	Distance across the river mouth of the nearest river	Google Earth Path tool
Number of rivers		Number of rivers within 2000m	Google Earth
Predicted salinity	PSU	Salinity at a point 200m seaward from the marina site generated by a run of the Scottish Shelf Model	Scottish Shelf Model

ports in Scotland was also measured, as ports represent sites where large volumes of ballast water may be exchanged which is an important vector for NNS introduction. Port information was sourced from the national marine planning interactive tool (NMPi) (Scottish Government, 2015).

The limitations of the collected salinity data (only one time point, collected on different dates between sites) meant that supplementary data on site characteristics which could inform salinity was sourced from also sourced from satellite images (see Table 3.1). Satellite images from Google Earth were used to count the number of rivers within 2000 m of the marina. The width of the nearest river was measured as the linear distance from bank to bank at the mouth. The distance to the nearest river was calculated from the centre point of the marina to the centre of the river mouth. River flow data was not available for any of the rivers that ran into or near to the surveyed marinas, and the financial limitations of the project meant that it was not possible to collect flow data in person, just as repeat salinity measurements were not feasible. Published literature on salinity around the Scottish coast was also examined to determine the extent of influence of each estuary on coastal salinities. This was supplemented by salinity data obtained for each site through the Scottish Shelf Model (Wolf et al., 2016).

The Scottish Shelf Model (SSM) is a three-dimensional hydrodynamic model of Scottish waters, developed for Marine Scotland Science (Wolf et al., 2016). The SSM uses a Finite Volume Community Ocean Model (FVCOM), an unstructured grid composed of triangular model elements, which allows the grid resolution to change to fit the complex Scottish coastline (O'Hara Murray, 2017). The SSM has been used to produce a full year climatological run, forced with climatologically averaged data for a 25 year period (O'Hara Murray, 2017). As summarised by O'Hara Murray (2017), the climate forcing data includes temperature, salinity, water level and 3D current velocities, atmospheric forcing data such as wind velocity, air pressure, precipitation and evaporation, as well as fresh water input from the Grid-to-grid hydrological model developed by the Centre for Ecology and Hydrology (CEH) which incorporates river discharge and diffuse run-off.

The SSM has limited interpretability very close to shore, so it was not possible to source annual predicted data for the site directly. Instead, for each site, a circle with a 1 km radius and a centre point located 200 m seaward of the centre of each marina was selected to represent the area nearest to each marina. Data was averaged for a full year run of the Wider SSM domain model and averaged across the 1 km area for each of 20 depth layers, although only the surface layer was used in this study. The width of the surface layer may vary between

sites, as the model divides the water column at each location into 20 depth layers, thus the width of the surface layer will vary depending on the bathymetry of each location.

3.2.3 Data analysis

Univariate analyses were conducted in R-Studio using the car package, with graphics produced using gg-plot2 (RStudio Team, 2018; Wickham, 2009). Multivariate analyses were performed using PRIMER v7 software (Anderson et al., 2008).

3.2.3.1 Differences in species diversity between sites

Percentage cover data generated from CPCE analysis of photographs was used to calculate the total cover of the photo occupied by biofouling organisms, and species diversity indices for each photo quadrat. The species diversity indices calculated were the number of taxa recorded (species richness) and the Shannon-Wiener diversity. For each photo quadrat, cover and diversity indices were calculated for the whole community and for subsets of species, based on their status as native or non-native and cryptogenic.

To establish whether species richness differed between sites, data on the number of species recorded in each quadrat was square-root transformed to account for non-linearity and an one-way ANOVA used to compare mean species richness of the whole community and of just native species between sites. A non-parametric Kruskal-Wallis test was used to assess differences in Shannon-Wiener diversity between sites. Differences in mean biofouling cover between sites were analysed with an one-way ANOVA.

Similar analyses were performed on the multivariate dataset of species percentage cover generated by the analysis of 510 photo replicates in CPCE. The amount of uncolonised space and unidentified species were documented but were not included in multivariate comparisons between samples as in one site the pontoons had been cleared approximately around two months before sampling took place, which could have led to uncolonised space influencing similarity measures. The percentage cover of each species within a quadrat was dispersion-weighted and then square-root transformed and a Bray-Curtis similarity matrix calculated. Data was transformed in order to account for over-dispersion in species counts, and to downweight the influence of the most abundant species (Clarke et al., 2014). A two-way nested ANOSIM test with performed with site nested within coast to determine if community composition differed between sites, and between the Atlantic (n=4) and North Sea (n=13) coasts.

3.2.3.2 Patterns of species diversity among sites

The total number of species counted was pooled across the 30 photo replicates taken at each site. Other species diversity indices were averaged across the 30 photo replicates at each site. The linear correlation between the number of NNS recorded in the RAS and the species diversity of the native community was calculated using Pearson's correlation coefficient. Pearson's correlation was also calculated for the number of NNS detected in the RAS and the pooled number of NNS detected in photo quadrats, to compare detection between the RAS and photo quadrats.

For the multivariate analysis, species percentage cover data was averaged across the 30 photo replicates for each site. This data was fourth-root transformed based on inspection of shade plots to downweight the influence of the most abundant data, and a Bray-Curtis similarity matrix calculated on all variables excluding unidentified species and uncolonised space (Clarke et al., 2014). The same routine was performed for only native species and only non-native and cryptogenic species. To analyse the presence/absence non-native species data recorded in the RAS, a Sorenson similarity coefficient was used to calculate a resemblance matrix (Clarke et al., 2014).

In order to compare patterns among sites and species subsets, hierarchical agglomerative cluster analysis using group-averages and SIMPROF testing were generated for the overall, native and non-native assemblages (Clarke et al., 2014; Choi et al., 2016). Clusters based on SIMPROF results were overlaid on an non-metric Multidimensional Scaling (nMDS) plot. The addition of the number of NNS detected in the RAS overlaid onto the native assemblage nMDS plot allows comparison of patterns between native and NNS. The SIMPER routine was run in order to distinguish taxa which contributed most to similarities within and between cluster defined groups.

3.2.3.3 Environmental data

Salinity measurements are presented in PSU throughout this chapter. Calibration of the castaway CTD resulted in a correction factor of -0.1 °C and +0.6 PSU being applied to the temperature and salinity data respectively. Salinity heatmaps showing salinity across each marinas and over the water column were produced in R by interpolating between CTD measurements taken at 5 sample points within each marina, and plotting the resulting data using ggplot2 (Wickham, 2009). Salinity and temperature data was also averaged at each

Table 3.2: *Abiotic factors included in BEST analysis, including information on the data source. A log transformation was applied to distance measures.*

Abiotic factors	Unit	Data transformation
Latitude	Decimal degrees	
Longitude	Decimal degrees	
Port distance	m	Log
Berths		
Entrance width	m	Log
Depth	m	Log
River distance	m	Log
River width	m	Log
Number of rivers		
Surface salinity	PSU	CTD surface salinity averaged across 5 sample points
Surface temperature	°C	CTD temperature averaged across 5 sample points
Minimum predicted salinity	PSU	Minimum salinity generated by annual run of SSM

sample point across the whole water column, and from 0 to 1.5 m depth. These values, and the surface salinity and temperature were then used to calculate the mean, variance, minimum and maximum salinity for each site.

Salinity data was scaled to account for biologically significant changes following methods from Collins and Williams (1982). The rationale for this is that as changes in salinity are expected to affect species in a non-linear way, scaling salinity allows for clearer identification of the differences between sites. Average minimum and surface salinity was classified as (1) <27, (2) 27 - <30, (3) 30 - <32, (4) 32 - <33, (5) 33 - <33.5, (6) 33.5 - <34, (7) >34 (Collins and Williams, 1982).

3.2.3.4 Influence of abiotic factors on species assemblages

The correlation between different abiotic variables and the biotic assemblages was tested using the BEST (bio-Env) procedure in PRIMER. Data on site location, design and environmental measurements was entered into PRIMER (see Table 3.2. Distance data (distance to nearest river, entrance width, river width and distance to nearest port) was log transformed, and then data was normalised to a common scale (Clarke et al., 2014; Choi et al., 2016). A global BEST procedure was run in PRIMER using 999 permutations, to test the statistical significance of the relationship between the biotic and abiotic variables. The BEST procedure was performed for the whole community, native and non-native species assemblages. Linktree cluster analysis was used to identify whether there were thresholds in the environmental variables which corresponded to differences in community similarity between sites (Clarke et al., 2014).

3.3 Results

3.3.1 Fouling assemblages in Scottish marinas

A total of 44 unique taxa were identified across all sites. Twenty-four were identified to species level, five to genus, and the rest to class or functional group. Of these taxa, thirty-five were native, seven non-native, and one cryptogenic. It was not possible to confirm the status of bugulid bryozoans from the photographs as both native and non-native bugulid species are found within fouling assemblages at the surveyed sites, thus this group was excluded when native and non-native subsets were analysed. The most abundant taxa across all sites were brown filamentous algae, *Mytilus edulis*, green turf algae, green filamentous algae, and kelp.

In the rapid assessment survey (RAS), 18 non-native species and one cryptogenic species were identified. The results of the RAS are examined in Chapter 2, section 2.3.

The quadrat photographs detected fewer NNS than the RAS, with presence records for only 8 NNS and cryptogenic species across all sites (Figure 3.3). The NNS which it was possible to detect in quadrat photographs were the macroalgae *Sargassum muticum* and *Codium fragile ssp. fragile*, the amphipod *Caprella mutica*, the bryozoans *Tricellaria inopinata* and *Schizoporella japonica* and the hydroid *Cordylophora caspia*. Identification of the cryptogenic colonial ascidian *Diplosoma listerianum* was also possible from the photographs, although this species was not included in the RAS target list at the time due to ambiguity over its introduction status. However, in the case of some of these species, it was only possible to confirm identity in the photographs due to microscopic identification of samples collected in the RAS. Nevertheless, despite the differences between survey methods there was a positive correlation between the total number of NNS from the RAS and the pooled number of NNS found in photo quadrats for each site ($r=0.839$, $p<0.001$).

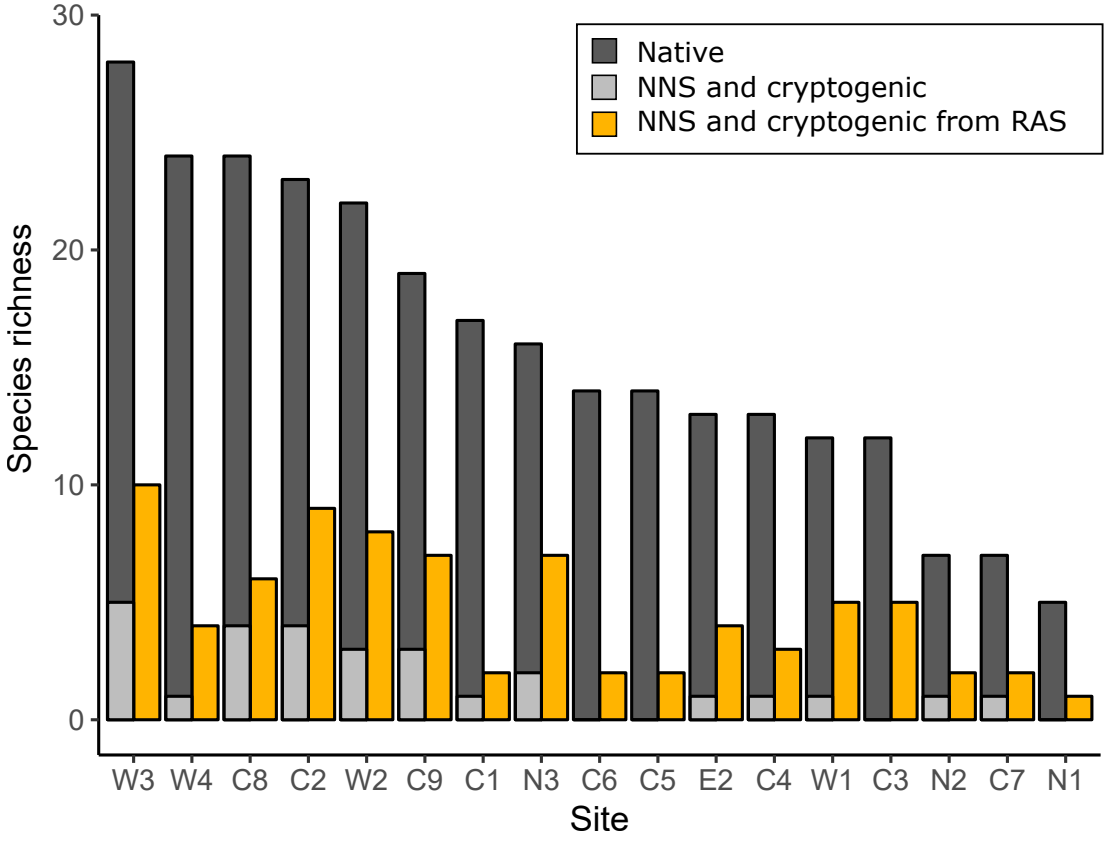


Figure 3.3: *Bar chart of the pooled number of taxa identified from photo quadrats in each site, with the proportion of native and non-native and cryptogenic species indicated by dark and light grey bars respectively. The number of non-native and cryptogenic species found in the RAS is also shown.*

3.3.1.1 Differences in species diversity between sites

Community assemblages appeared visually different between sites (Figure 3.4), and this was confirmed using univariate and multivariate analyses. The pooled number of taxa within each site ranged from five in site N1 (all native) to 28 in site W3 (23 native, five non-native) (Figure 3.3). The number of NNS documented in the RAS ranged from 1 to 10 per site (including the cryptogenic species *Bugulina fulva*) (Figure 3.3). Sites differed significantly in the average taxon richness of quadrats (ANOVA $F(16,492)=32.48$, $p<0.0001$), which ranged from 2.07 ± 0.78 species in site N1 to 7.83 ± 1.7 species in site C2 (Figure B.2).

Average species richness and pooled species richness were positively correlated ($r=0.833$, $p<0.001$). Shannon-Wiener diversity also differed significantly between sites ($\tilde{\chi}^2(16)=227.3$, $p<0.0001$) (Table 3.3). Multivariate analysis of the 510 replicate photographs showed that overall community composition differed significantly between sites (ANOSIM $R=0.59$, $p(perm) = 0.0001$), but was not significantly different between coasts (Figure B.1).

Sites also differed when only native species were considered. Native species richness (ANOVA F(16,493)=30.45, p<0.0001) and Shannon-Wiener diversity ($\tilde{\chi}^2$(16)=216.92, p<0.001) differed significantly between sites, as did similarity of the native fouling community (ANOSIM R=0.537, p(perm)=0.0001) (Table 3.3). Average non-native species richness documented in photo quadrats was low (0 - 5 species) compared to native species richness, but also differed significantly between sites ($\tilde{\chi}^2$(16)=233.12, p<0.001).

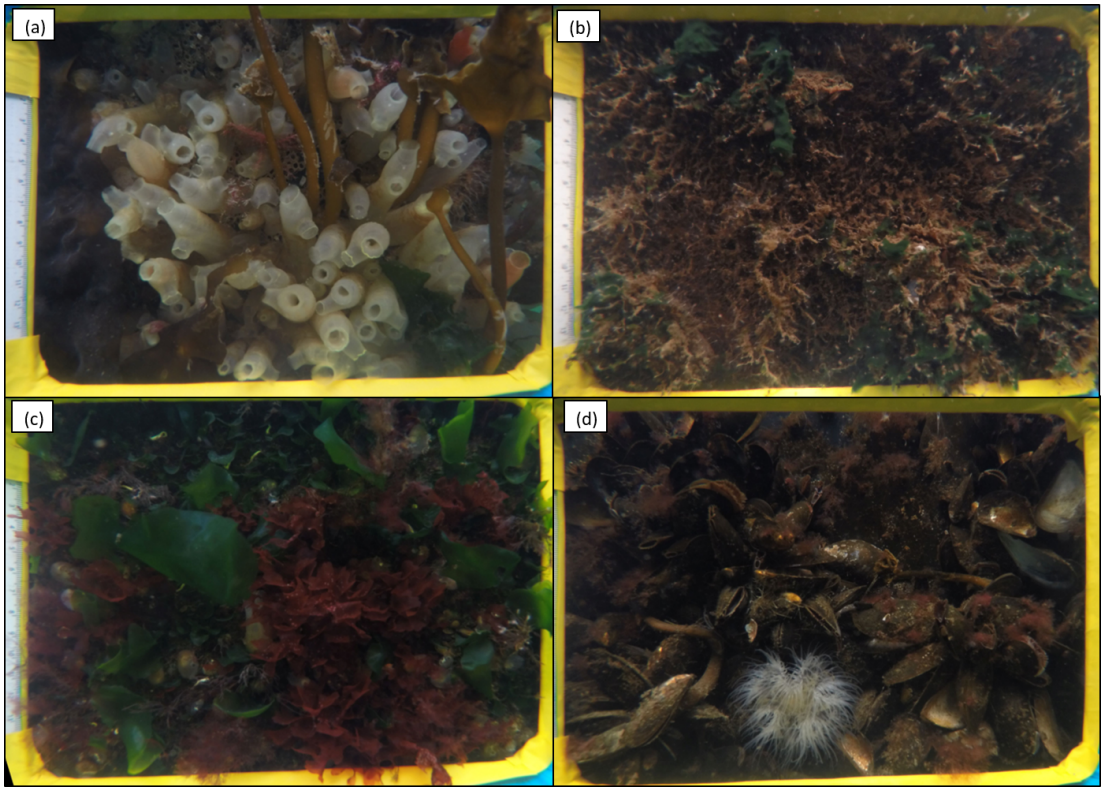


Figure 3.4: *Figure showing four photos of the typical biofouling community in site W3 (a), C7 (b), W4 (c), and C6 (d). Photos were taken using a GoPro Hero4, and altered using IrfanJ to correct for minor distortion.*

In all sites excluding one, over 90% of the photographed quadrat area was occupied by fouling species. The mean percentage of occupied space varied between sites ($\tilde{\chi}^2$(16)=178.03, p<0.001), with sites C2 and E2 standing out as having significantly lower cover than all other sites (Figure 3.5). The contribution of NNS to overall biofouling cover varied between marinas but was not correlated with increasing NNS richness. Site C7 is notable as the only site where the contribution of NNS to the total biofouling cover (54.57%) was higher than that of native species as a result of cover of a single non-native species, the brackish hydroid *Cordylophora caspia* (Figure 3.5). The most abundant NNS across all sites were the hydroid *C. caspia*, the bryozoans *Tricellaria inopinata*, *Schizoporella japonica* and the cryptogenic colonial

ascidian *Diplosoma listerianum*. The site with the highest mean abundance for both *T. inopinata* and *S. japonica* was C2, where they accounted for over 12.6% and 4.1% of the overall mean percentage cover respectively.

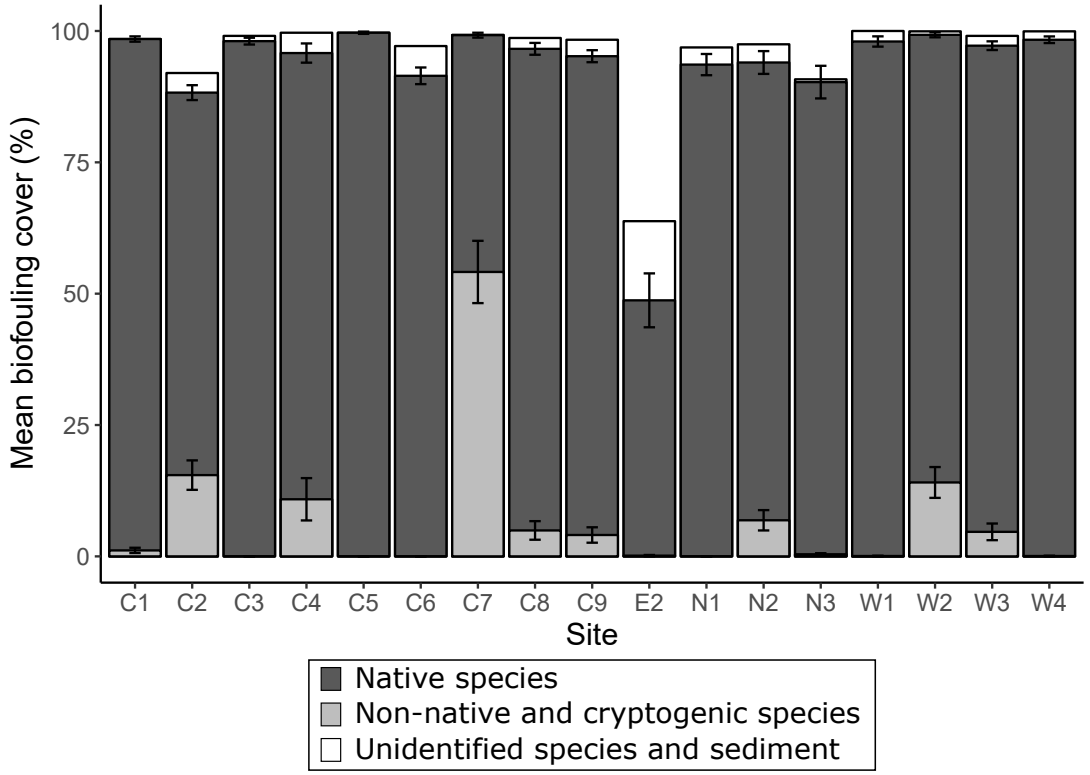


Figure 3.5: *Bar chart of mean biofouling cover from photo quadrats in each site. The mean cover (± SE) of native and pooled non-native and cryptogenic species is shown. Also included is the mean cover of species which it was not possible to identify due to their small size, or which were obscured by a film of sediment. The water in site E2 had high sediment load which made analysis of photographs from this site difficult.*

3.3.1.2 Patterns of community composition among sites

Patterns of fouling community similarity among sites were investigated using multivariate analysis based on the overall fouling community, and native and non-native species subsets. Cluster analysis showed that grouping of sites varied depending on the clustering method used and on if the whole biofouling assemblage or subsets of native or non-native species were considered. Sites C7, N1, N2, and C4 were most consistently separated from other sites, which can also be seen in nMDS plots of the sites (Figure 3.7). However, the separation between the rest of the sites is less clear cut, with a more gradual spectrum of dissimilarity in fouling community assemblages (Figure 3.7). This results in different groups of sites depending on

which biotic data is considered.

Looking at the similarity of sites based on the whole community assemblage, four groups of sites were identified (Figure 3.7a). SIMPER analysis showed that sites in Group A had low diversity with just four taxa responsible for 85% of the similarity between sites, green turf algae (34.5%), brown filamentous algae (30%), and *Cordylphora caspia* and other hydroids (Table B.1). Sites in Group B had high abundances of green, brown, and red filamentous algae, while those in site C were distinguished by the high abundances of *Mytilus edulis*, brown filamentous algae and kelps. Group D had the most diverse assemblages, with fourteen taxa responsible for 85% of the similarity between sites (Table B.1).

Sites with higher numbers of native species were also shown to have higher number of non-native species recorded in RAS survey (r=0.661, p=0.003) (Figure 3.6). The number of NNS was also positively correlated with native Shannon-Wiener diversity (r=0.663, p=0.003). The separation of sites based on RAS presence/absence data supports these results, as sites C7, N1, N2, C4 are shown to be significantly dissimilar to other sites, although the clusters produced by RAS and native species data differ in the grouping of C3 and C5.

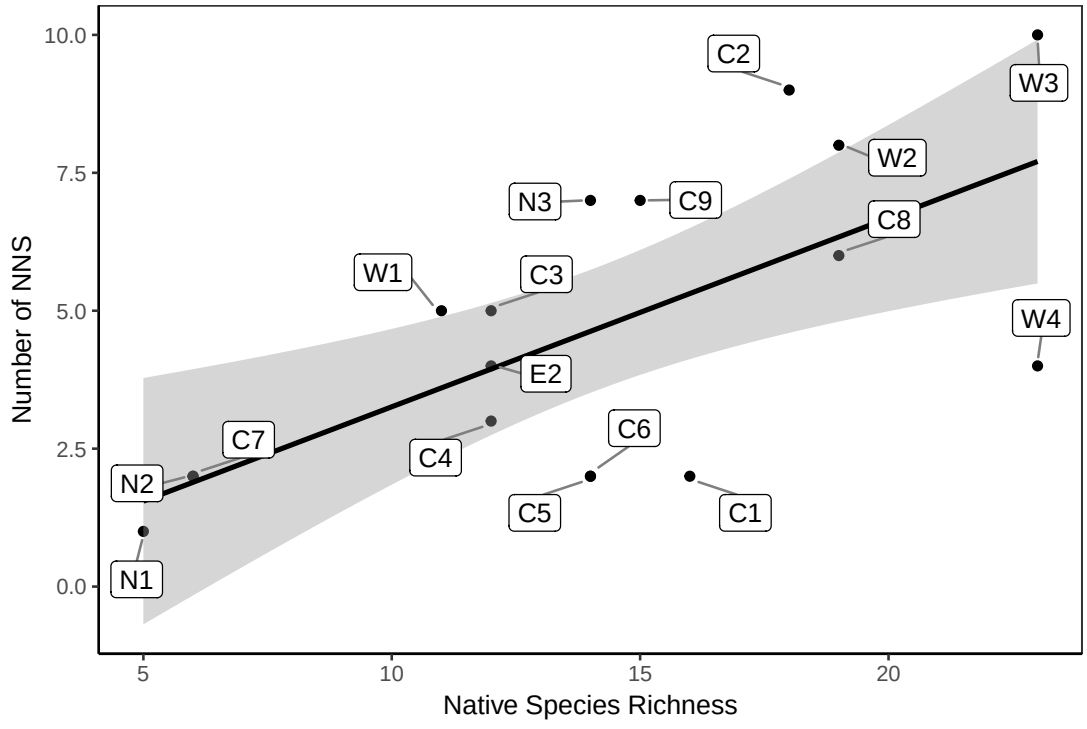


Figure 3.6: *Scatter plot of the number of NNS against native species richness in each site.*

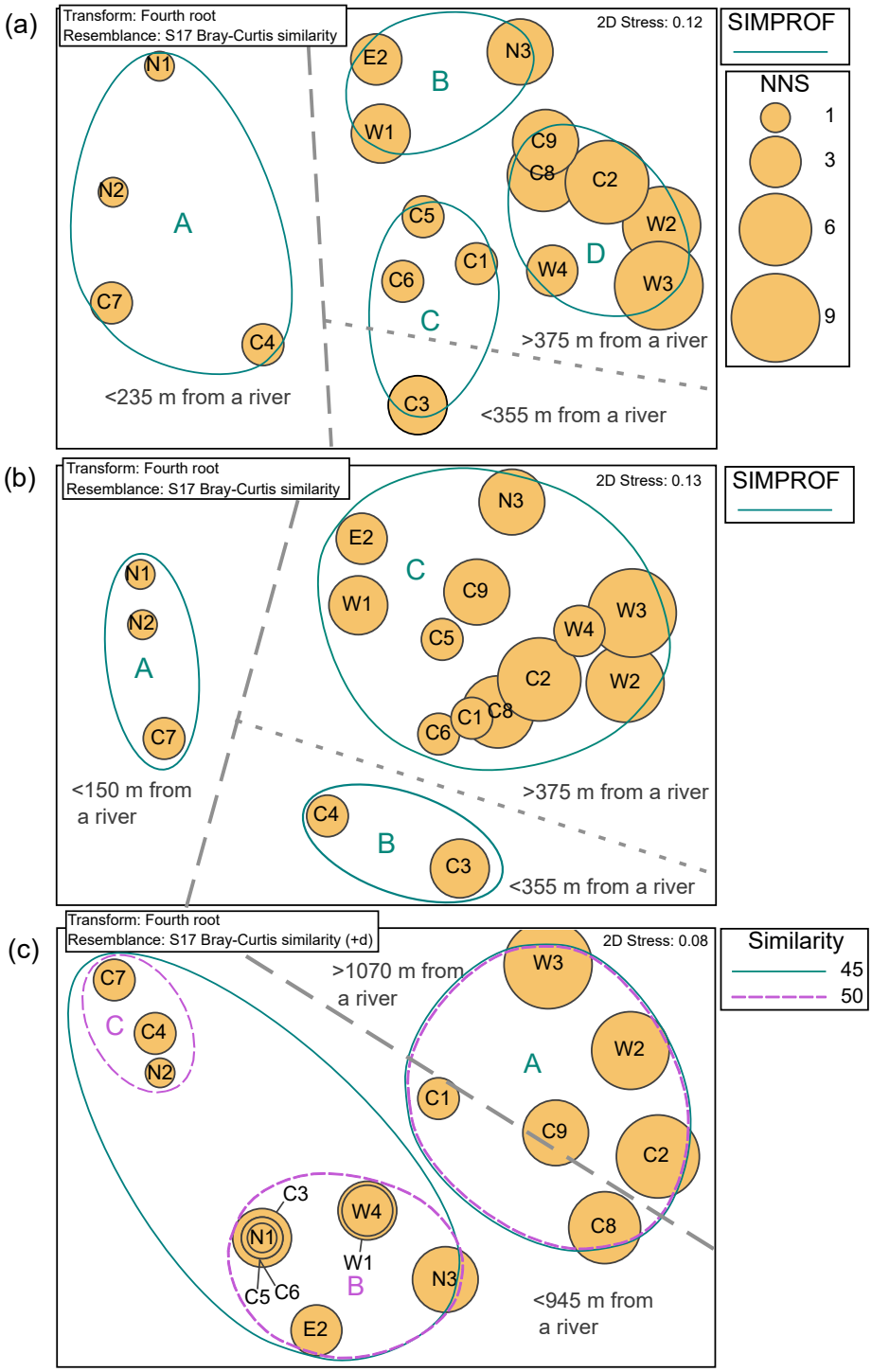


Figure 3.7: *nMDS plots of sites based on similarity of the whole community (a), only native species (b), and only non-native and cryptogenic species (c). nMDS ordination of sites is based on Bray-Curtis similarity matrices calculated from fourth-root transformed mean cover (%) data from quadrat photos. Bubbles indicate the number of NNS recorded in the RAS survey in each site. Overlaid on the nMDS plots are sites groups based on cluster analysis (see Figure B.4 for details), solid green lines indicate results supported by SIMPROF tests. Grey dotted lines indicate site groupings based on LINKTREE analysis identification of thresholds in the proximity to rivers.*

Table 3.3: *Metrics of biological diversity of surveyed marinas. Species richness was pooled for each site across photo replicates, and the overall, native and non-native totals are given. RAS totals are for the combined number of NNS and cryptogenic species. The overall biofouling cover for each site (percentage cover averaged across replicates) is presented with the percentage contribution of native, non-native species (NNS), and cryptogenic species (Cryp.). The species richness and Shannon Diversity Index (H') averaged across photo replicates is presented for the whole community and for native species only.*

Site	Species richness				Cover of fouling species (%)	Contribution to total % cover			Mean number of species		Shannon-diversity	
	Overall	Native	NNS	RAS Cryp.		Native	NNS	Cryp.	Overall	Native	Overall	Native
C1	17	16	1	2	98.47 ± 2.76	98.84	1.15	0	4.23 ± 1.89	4.03 ± 1.73	0.94 ± 0.41	0.91 ± 0.39
C2	23	18	4	9	92 ± 6.21	78.91	16.88	0.681	7.83 ± 1.7	6.43 ± 1.28	1.67 ± 0.3	1.49 ± 0.32
C3	12	12	0	5	99.07 ± 2.21	98.99	0.00	0	3.2 ± 0.96	3.2 ± 0.96	0.62 ± 0.32	0.62 ± 0.32
C4	13	12	1	3	99.67 ± 1.3	85.22	11.34	0	3.07 ± 1.11	2.6 ± 1.1	0.63 ± 0.34	0.51 ± 0.33
C5	14	14	0	2	99.67 ± 1.18	100.00	0.00	0	3.87 ± 1.57	3.87 ± 1.57	0.9 ± 0.35	0.9 ± 0.35
C6	14	14	0	2	97.13 ± 4.38	94.17	0.00	0	3.83 ± 1.44	3.83 ± 1.44	0.86 ± 0.4	0.86 ± 0.4
C7	7	6	1	2	99.27 ± 2.55	45.39	54.57	0	2.5 ± 0.57	1.6 ± 0.62	0.58 ± 0.21	0.28 ± 0.32
C8	24	19	4	6	98.67 ± 4.85	91.08	4.78	0.422	5.23 ± 2.3	4.57 ± 1.83	1.01 ± 0.51	0.91 ± 0.47
C9	19	15	3	7	98.33 ± 3.6	92.14	3.80	0.493	5.4 ± 2.01	4.83 ± 1.62	1.24 ± 0.41	1.16 ± 0.38
E2	13	12	1	4	63.8 ± 35.25	76.18	0.27	0	2.2 ± 1.3	2.17 ± 1.21	0.42 ± 0.39	0.41 ± 0.38
N1	5	5	0	1	96.87 ± 5.24	96.63	0.00	0	2.07 ± 0.78	2.07 ± 0.78	0.46 ± 0.32	0.46 ± 0.32
N2	7	6	1	2	97.47 ± 5.87	89.40	7.31	0	2.5 ± 1.01	2.1 ± 0.71	0.69 ± 0.37	0.56 ± 0.33
N3	16	14	2	7	90.8 ± 17.04	98.97	0.07	0.369	3.13 ± 1.55	3 ± 1.49	0.66 ± 0.52	0.64 ± 0.51
W1	12	11	1	5	100 ± 0	97.93	0.00	0.068	2.67 ± 1.3	2.63 ± 1.25	0.51 ± 0.4	0.51 ± 0.4
W2	22	19	3	8	99.93 ± 0.37	85.27	6.45	7.723	5.87 ± 1.33	4.53 ± 1.31	1.27 ± 0.32	1.01 ± 0.4
W3	28	23	5	10	99.07 ± 1.64	93.41	1.44	3.361	6.8 ± 2.85	6.03 ± 2.3	1.39 ± 0.46	1.31 ± 0.4
W4	24	23	1	4	99.93 ± 0.37	98.33	0.00	0.068	4.93 ± 1.93	4.9 ± 1.92	1.05 ± 0.47	1.05 ± 0.46

3.3.2 Abiotic characteristics of marinas

3.3.2.1 Measured salinity and temperature

The CTD measurements showed differences in temperature and salinity among sites, although it should be remembered that sites were surveyed over the course of two months and so comparison based on this data is limited. The mean temperature ($\pm$ SD) over the top 1.5 m of the water column ranged from 10.34 $\pm$ 0.43 °C (N2) to 15.27 $\pm$ 0.06 °C (W1). The mean salinity ($\pm$ SD) over the top 1.5 m of the water column ranged from 12.20 $\pm$ 7.32 (N1) to 33.93 $\pm$ 0.003 (W3), while the mean surface salinity ranged from 4.53 $\pm$ 1.02 (N1) to 33.93 $\pm$ 0.01 (W3). The lowest recorded salinity was 3.34 in site N1.

Salinity varied over the water column in some sites (Figures 3.8, 3.9, 3.10), with sites N1 and N2 showing the greatest change in salinity across depth as well as the lowest recorded salinities (<20). The river width is larger in both these sites than in C7, the only other enclosed site with a river running directly into it (Table 3.4). In site N1 differences in salinity were also visible across the marina, dependent on sample location (Figure 3.8). The surface salinity variance was negatively correlated with the distance of a site from the nearest river (r=-0.846, p<0.001). Sites C7, C1, W4, C5, and C6 also showed variation in salinity over the water column, with high stratification of the water column in sites C7, C1, and W4. Sites C5 and C6 stand out as having relatively low salinities (<30) but well-mixed throughout the water column, likely a reflection of their position within the Clyde estuary.

3.3.2.2 Influence of abiotic factors on community similarity

Of all the abiotic factors, the distance from the nearest river was the factor which best correlated with biotic species distributions. Overall community composition (BEST rs = 0.681, p=0.001) and native species community composition (BEST rs = 0.65, p=0.002) were positively correlated with the distance of a site from the nearest river (Figure B.3. For non-native community composition (generated by RAS presence/absence data), a combination of the distance from the nearest river and the mean surface salinity predicted by the Scottish Shelf Model were correlated with community composition of NNS (BEST rs=0.609, p=0.001). The photo-quadrat generated community composition of NNS identified a combination of the distance from the nearest river, number of berths and depth as the abiotic factors which best correlated with similarities between sites (BEST rs = 0.47, p=0.001).

Linktree analysis of the overall community assemblage and native and NNS subsets was

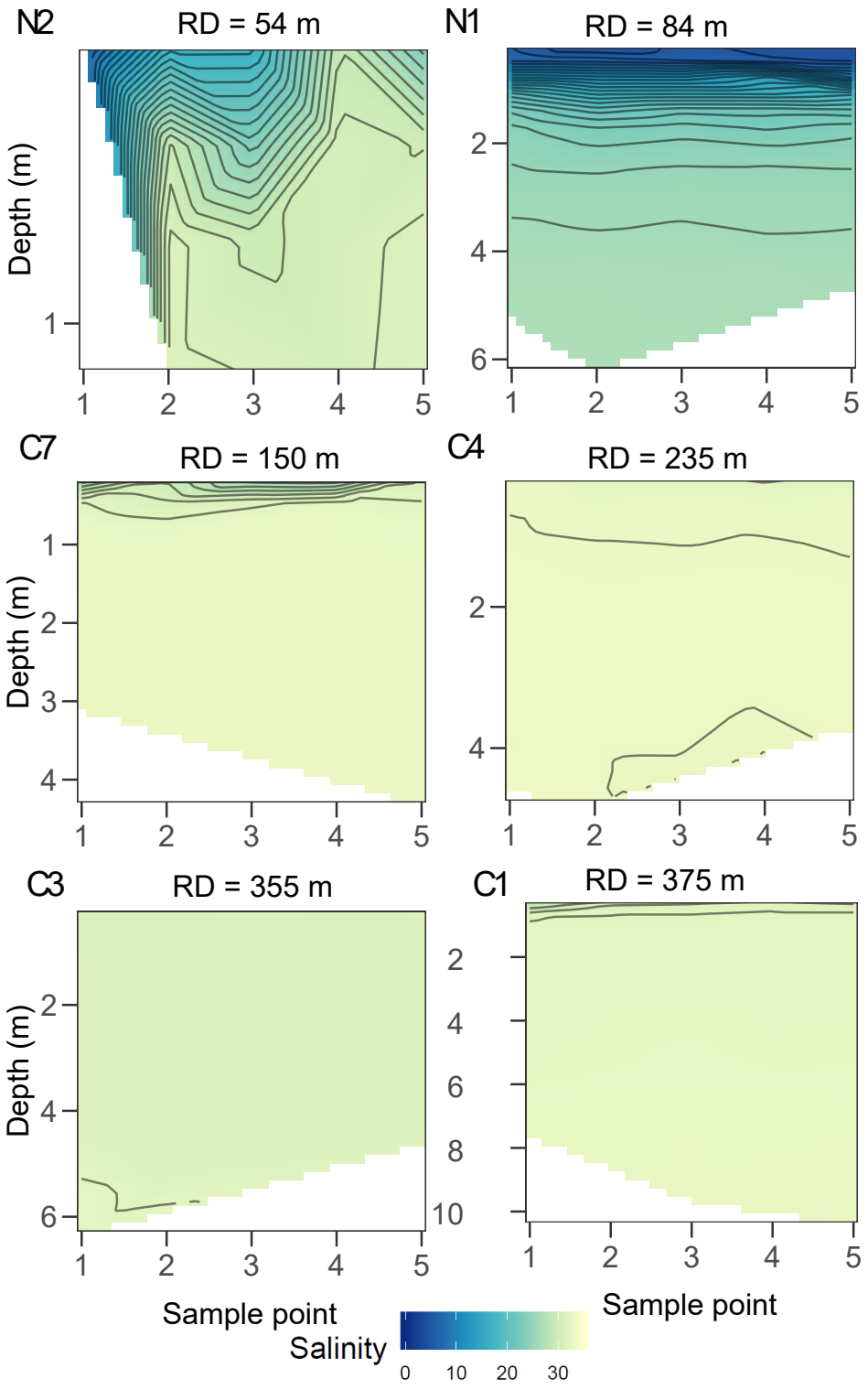


Figure 3.8: *Salinity (PSU) depth profile of six marinas. Salinity data is interpolated between 5 sample points where CTD measurements were taken in each marina. Contour lines represent a 1 PSU change in salinity. Marinas are ordered in increasing distance from the nearest river (RD), measured in metres*

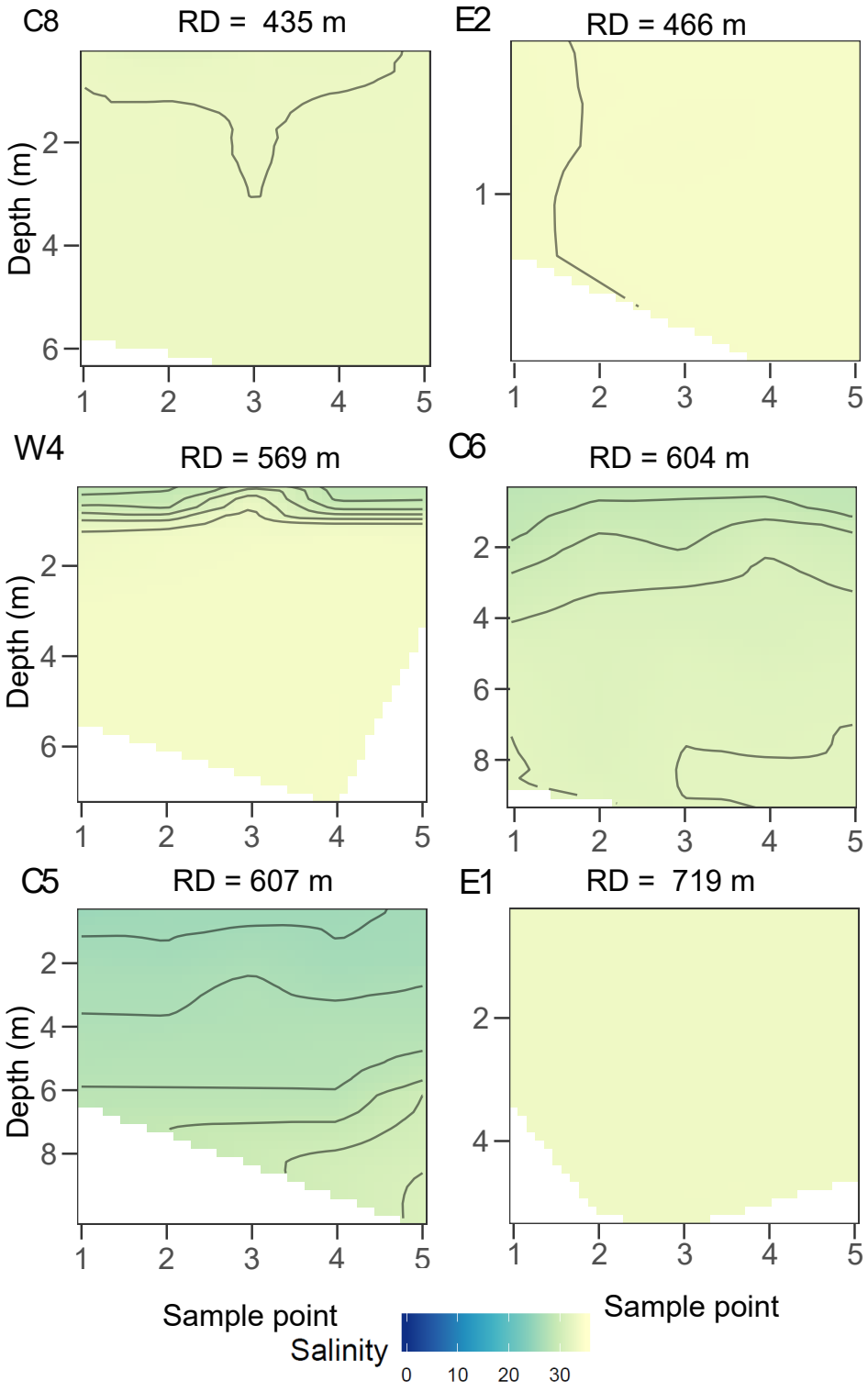


Figure 3.9: *Salinity (PSU) depth profile of six marinas. Salinity data is interpolated between 5 sample points where CTD measurements were taken in each marina. Contour lines represent a 1 PSU change in salinity. Marinas are ordered in increasing distance from the nearest river (RD), measured in metres.*

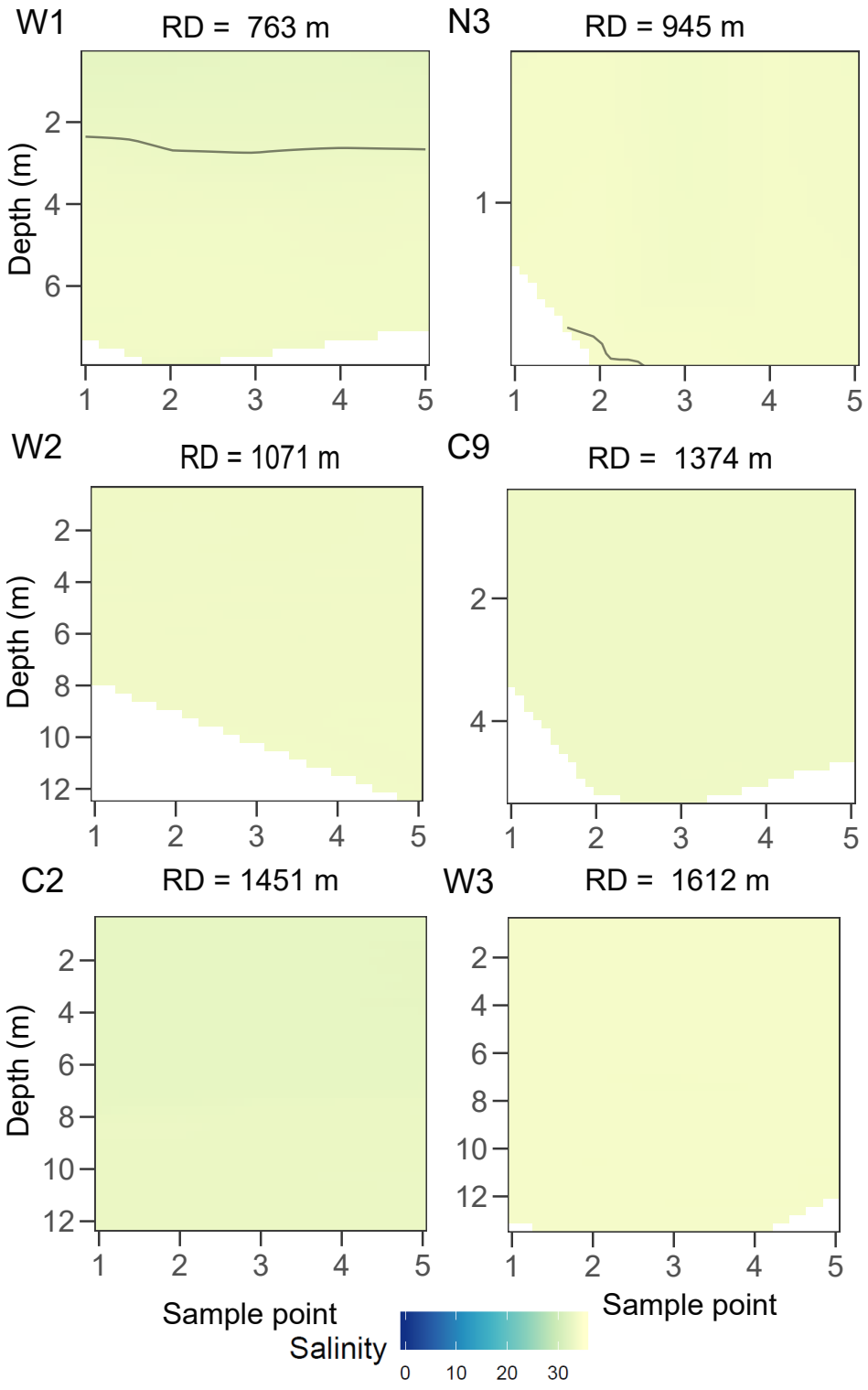


Figure 3.10: *Salinity (PSU) depth profile of six marinas. Salinity data is interpolated between 5 sample points where CTD measurements were taken in each marina. Contour lines represent a 1 PSU change in salinity. Marinas are ordered in increasing distance from the nearest river (RD), measured in metres*

Table 3.4: *Data collected on site salinity from primary (CTD data) and secondary sources (see Table 3.1 for data sources). Data is colour coded based on its expected effect on marina salinity. Values expected to create very low salinity conditions are coloured blue, low salinity conditions teal, and moderate conditions green, and high salinity conditions yellow. This has been used to rank sites based on the expected site salinity class (column 6). The CTD mean surface salinity data is also colour codes to provide a comparison with expected salinity, and to make identification of outliers easier (e.g. site C4 which has a high measured salinity but is expected to be subject to low salinity periods based on geographic and model data).*

	Secondary data					Primary data	
Site	**No. of rivers**	**River width (m)**	**Distance to nearest river (m)**	**SSM mean predicted salinity (PSU)**	**Expected site salinity**	**Mean surface salinity ± SD (PSU)**	**Mean surface temp ± SD (°C)**
N1	2	153.07	84	27.29	Very low	4.53 ± 1.02	11.02 ± 0.28
N2	1	35.75	54	31.84	Very low	18.11 ± 8.76	10.30 ± 0.43
C7	2	22.1	150	31.14	Very low	27.54 ± 2.59	11.91 ± 0.35
C4	4	44.25	235	29.67	Very low	32.18 ± 0.3	12.55 ± 0.12
C6	1	2.6	604	26.30	Low	28.58 ± 0.26	12.13 ± 0.06
C5	3	3.0	607	26.55	Low	25.79 ± 0.24	12.70 ± 0.07
E1	2	1.0	719	16.18	Low	31.09 ± 0.16	11.71 ± 0.03
W4	3	4.2	569	30.99	Low	28.89 ± 0.55	12.47 ± 0.37
C3	2	3.4	355	31.64	Moderate	31.26 ± 0.03	13.48 ± 0.02
C1	2	2.6	375	32.71	Moderate	29.42 ± 0.84	13.26 ± 0.02
W1	4	12.75	763	33.56	High	32.38 ± 0.10	15.37 ± 0.06
C8	1	2.7	435	32.15	High	32.75 ± 0.48	12.35 ± 0.06
E2	1	14.18	466	33.51	High	34.05 ± 0.09	12.07 ± 0.01
N3	1	18.42	945	33.41	High	33.73 ± 0.13	11.55 ± 0.01
W3	1	3.4	1612	32.21	High	33.93 ± 0.01	13.03 ± 0.01
C9	1	16.84	1374	32.63	High	33.31 ± 0.02	11.90 ± 0.01
C2	1	3.0	1451	32.76	High	32.50 ± 0.01	11.73 ± 0.04
W2	1	7.5	1071	33.66	High	33.41 ± 0.03	14.70 ± 0.01

used to identify thresholds in the the abiotic variables which matched differences in biological assemblages among sites (Figure B.3). Linktree analysis separated sites N1, N2, C4 and C7 based on these sites being less than 235 m from a river for the assessment of the whole community data and NNS RAS data. SIMPER analysis on these groups found that the non-native species which characterised these groups were the presence of *Cordylpohora caspia* in sites close to rivers and the presence of *Caprella mutica, Tricellaria inopinata* and *Corella eumyota* in sites further from a freshwater source.

Univariate analysis showed that as the distance of a site from a river increases, the number of native species richness per site increased ($F(1,15)=11.97$, $R^2=0.444$, $p<0.01$) as did the number of NNS recorded in the RAS ($F(1,15)=51.16$, $R^2=0.758$, $p=0.001$) (Figure 3.11). The number of NNS also increased with the scaled minimum salinity calculated for each site ($F(1,15)=26.32$, $R^2=0.637$, $p<0.001$). A simple linear regression of the number of NNS based on the scaled minimum salinity, shows that an additional 1.2 species can be expected for each

Table 3.5: *Abiotic site data collected on marina features for surveyed marinas. The rapid assessment survey date is provided, all surveys were done in 2016. For details of data sources, see Table 3.1.*

Site	Date	Coast	Latitude	Longitude	Port distance (m)	Berths	Entrance width (m)	Depth (m)
C1	25-Sep	Atlantic	55.86608	-5.41095	77360	200	127	8
C2	22-Sep	Atlantic	55.87340	-5.31370	71865	230	14	12
C3	26-Sep	Atlantic	55.86140	-5.08610	29809	150	464	2.4
C4	22-Sep	Atlantic	55.98350	-4.94780	15174	250	180	6
C5	30-Sep	Atlantic	56.01177	-4.77409	8304	210	276	12
C6	21-Oct	Atlantic	55.94280	-4.72600	1314	130	22	5.1
C7	21-Oct	Atlantic	55.91020	-4.87487	15733	600	27	3.4
C8	23-Sep	Atlantic	55.77368	-4.85824	30587	730	32	3
C9	21-Sep	Atlantic	55.54543	-4.67639	67836	400	20	3
E1	15-Oct	North Sea	55.99466	-3.41239	2746	300	218	2
E2	15-Oct	North Sea	56.22171	-2.69875	70057	102	15	0
N1	11-Oct	North Sea	57.49390	-4.23360	39962	150	35	3
N2	11-Oct	North Sea	57.59050	-3.86180	23638	84	18	0.5
N3	13-Oct	North Sea	57.72389	-3.27957	53465	102	17	1.5
W1	02-Sep	Atlantic	56.18248	-5.53081	62440	90	256	5
W2	26-Aug	Atlantic	56.21161	-5.55772	50422	250	37	15
W3	28-Oct	Atlantic	56.41820	-5.49750	17417	115	537	12
W4	24-Oct	Atlantic	56.45068	-5.43363	14457	250	833	5

increase in salinity class ($F(1,15)=26.32$, $R^2=0.637$, $p<0.001$). Scaled minimum salinity was not found to be significant in predicting the number of native species.

Linktree analysis and hierarchical agglomerative cluster analysis of similarities between native species assemblages both identified three groups, those less than 150 m from a river (Group A), those less than 355 m from a river (Group B), and those further than 375 m from a river (Group C) (Figure B.3b). SIMPER analysis was used to identify native species which might be able to characterise sites susceptible to higher rates of invasion. The taxa which distinguished the high salinity sites in group C from the very low salinity sites in group A, were the presence of red filamentous algae, green flat algae, and kelp in high abundances in sites in group C. High abundances of *Mytilus edulis* distinguished sites in group B from those in A and C (Table B.4).

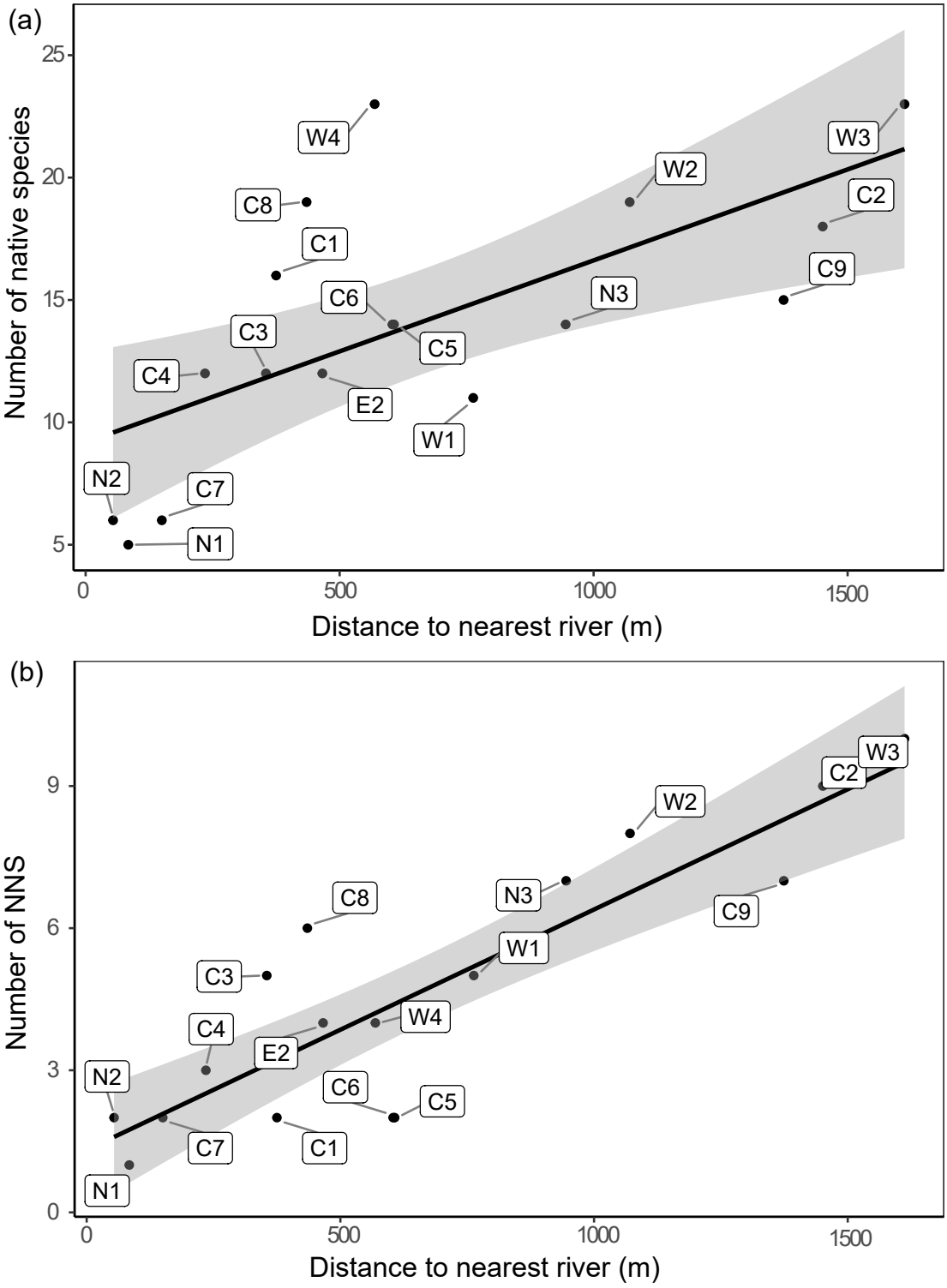


Figure 3.11: *Scatter plot of the number of non-native species recorded in the 17 sites and the pooled species richness of the biofouling community plotted against the distance a site to the nearest river (m), calculated from analysis of satellite photos. A regression line has been plotted for each graph.*

3.4 Discussion

3.4.1 Fouling assemblages in Scottish marinas

Native species dominate marina fouling communities in Scotland, both in the number of species and abundance. The majority of taxa identified from photographs of the biofouling community were native species (35/44), with seven NNS and one cryptogenic species. Fouling assemblages differed significantly among marinas, with the pooled species richness varying from five species in the least diverse site to 28 in the most diverse. The rapid assessment survey (RAS) found that the number of non-native and cryptogenic species in a site ranged from one to ten species, whereas it was only possible to identify eight non-native species in the quadrat photographs. These were either larger species (e.g. the large macroalgae *Codium fragile ssp. fragile* and *Sargassum muticum*), or species found in high abundances where it was possible to confirm identification through comparison with specimens collected in the RAS. Species identification was limited due to the poor resolution of some photographs, which also limited identification of native species in photos, thus many species were only identifiable to higher taxa. The degree of taxonomic resolution possible in the analysis of the quadrat photographs varied between taxa, with distinction between filamentous macroalgae especially difficult (Drummond and Connell, 2005). This study thus enables comparisons between sites, but should not be taken as a comprehensive analysis of fouling community variation.

Variation between species identified in the RAS and in quadrat photographs may also be due to the structures sampled by each method. The photo quadrat survey focused exclusively on communities growing on pontoon floats, whereas the RAS also investigated trailing marina equipment such as ropes and buoys, as well as fixed concrete columns and anchor chains. Species such as *Didemnum vexillum* are known to be predominantly at greater depths (Beveridge et al., 2011), and in site C8 and C2 were observed either on concrete columns or submerged pontoon anchor chains (Giesler, R. *pers. obs.*).

Pontoon floats had high volumes of biofouling in most sites, with the fouling species on average covering more than 90% of the photographed space in all sites except one. The site which had the highest amount of uncolonised space was site E2, with biofouling species only covering $63 \pm 35\%$ of the photographed quadrat space. It was not possible to obtain any information regarding maintenance at this site, so it is uncertain whether the pontoons were new or had been recently been scraped clean. Other factors which may affect fouling species

growth in this site include the low depth in this marina resulting in sections of the pontoons exposed at low tide, and high sediment levels in the water (Giesler, R, *pers. obs.*) (Hopkins et al., 2016; Forrest and Blakemore, 2006). High sediment load is common on the east coast and prevented camera surveys in another site in the Firth of Forth (site E1) (Port Edgar Berth Holders' Association, 2018). Site N3, the only other site on the east coast with high salinity, also had slightly lower levels of fouling cover (90.8 $\pm$ 17 % cover), with patches of pontoon float clearly visible. An alternative suggestion for why the pontoon floats in these two sites are clearer than other sites is provided by close examination of photos in site N3, where it is possible to see limpet grazing patterns in algal growth on bare pontoon surfaces (Figure B.6). Site E2 has much higher abundances of the limpet *Patella vulgata* L. (0.7% averaged across the whole site) than all other sites (<0.01%), so grazing by this species in sites E1 and N3 may contribute to reducing colonisation by fouling species dominant in other sites (Coleman et al., 2006; Jenkins et al., 2005).

Native species accounted for the majority of biofouling cover in most sites. Previous studies have used the relative abundance of native and NNS as a measure of the impact of invasive species within a site. Choi et al. (2016) for example, calculate an invasive species index, using a threshold value where NNS abundance is over 60% of the total community abundance as an indicator of sites where impact of NNS is likely to start becoming visible. In this study, NNS abundance did not exceed the threshold abundance of NNS impact identified by Choi et al. (2016); however, in three sites non-native species accounted for over 10% of the total cover and in site C7, NNS represented nearly 55% of the total biofouling cover.

In site C7 in the Clyde, non-native species cover was greater than that of native species. The non-native hydroid *C. caspia* accounted for, on average, 55% of the biofouling cover in quadrats in site C7. *Cordylophora caspia* is a euryhaline colonial hydroid which is capable of tolerating and adapting to a broad range of salinities (Figure 3.12) (Folino-Rorem and Renken, 2018). In this study, it was recorded in four sites (C4, C7, N1, and N2) with the records in the northern sites representing the first records of this species on the east coast of Scotland. In the other sites, abundance was much lower than is site C7 but still high, over 10% of biofouling cover in site C4, and 6% in site N2. The actual species delineations for the genus *Cordylophora* are uncertain, and molecular taxonomic work recently identified four clades within the genera, characterised by differences in environmental salinity tolerance (Folino-Rorem et al., 2009). The UK population used for molecular taxonomic identification by Folino-Rorem et al. (2009) fell into clade 1A, which were solely found in freshwater environments. The presence of

C. caspia in brackish marinas suggests that there have been multiple introductions to UK waters, supported by multiple introduction events in other invaded areas, and the presence of populations belonging to the brackish clade in Ireland. The high abundance of this species suggests that there may be a vacant niche in artificial brackish environments, where lower levels of competition from native species allow dominance by this NNS (Folino-Rorem and Renken, 2018). In the USA and other areas of Europe, high levels of fouling by this species have caused problems such as clogging water intake pipes (Folino-Rorem and Indelicato, 2005). As this species is likely more generally distributed in the UK than records show, including this species in future targeted monitoring is recommended, especially if low or freshwater habitats are to be surveyed (Folino-Rorem and Renken, 2018).

The most abundant non-native species were the hydroid *Cordylophora caspia* and the bryozoans *Tricellaria inopinata* and *Schizoporella japonica*. Given that both bryozoans species were introduced to Scotland relatively recently (2007 and 2011 respectively), the high abundance of these species in fouling communities may warrant further investigation (Loxton et al., 2017; Cook et al., 2013b, Chapter 2). The rapid spread of these species in other areas of their introduced range have prompted concerns over their impact on native species (Cook et al., 2013b). In this study, while native bryozoans were more frequently recorded across all sites, abundances of *T. inopinata* were much higher than for other bryozoans, echoing surveys from other areas of the UK and Europe where *T. inopinata* was shown to be the dominant bryozoan species (Arenas et al., 2006; Cook et al., 2013b).



Figure 3.12: *An image of Cordylophora caspia hydroids under the microscope*

3.4.2 Salinity conditions in marinas

The CTD data showed that differences in salinity were observable between marinas. The lowest surface salinities were recorded in sites N1 and N2 (<20 PSU), which are both enclosed marinas located within river mouths. A river also runs into site C7, although it is narrower than the rivers at sites N1 and N2, which may account for the higher salinity recorded in this site and the higher stratification observed. Sites C1 and W4 also showed stratified layers, which when compared with secondary data on river proximity and width confirms that these sites could be expected to be influenced by river discharge. Sites C5 and C6 stand out as having lower salinities throughout the water column, yet are located further away from rivers than sites with higher average salinities (Figure 3.9). These two sites are located higher within the Firth of Clyde, closer to the Clyde river than other sites within the Firth of Clyde, and can thus be expected to have salinities which reflect the high freshwater input from the river Clyde (Crowther and Chen, 2001). This is confirmed through examination of the mean predicted salinity generated by a run of the Scottish Shelf Model over the course of a year (Table 3.4). As the SSM data has been generated for a 1 km area encompassing the marina, it is expected that the predicted salinity will represent variation in estuary conditions and the influence of large river discharges, but may not encompass highly localised freshwater influences.

Interpretation of the CTD surface salinity measurements is limited as the data was collected at a single time point on separate days in different sites and thus only represents a snapshot of environmental conditions in each site (López-Legentil et al., 2015, Chapter 4). Long-term monitoring of salinity within a site would better identify the temporal and spatial salinity variation within a site, but this scale of sampling takes a long time to collect and could quickly become costly if the number of sites is high due to the costs of equipment. Thus parameters such as proximity to rivers, river width and position within estuaries could give useful indicators of the salinity conditions, and could be estimated from satellite data or models without the need for site visits (Foster et al., 2016). In this study, river distance was positively correlated with measured surface salinity. However, the low salinity in site C5 and C6 is not well represented by river proximity, with the surface salinity generated by the SSM instead identifying these sites as likely to be subject to estuarine conditions.

As a tool for predicting coastal salinity, the SSM accounts for a variety of hydrodynamic and climatic variables which influence salinity, such as fluvial and surface water discharge, and currents and tidal dynamics. However, the SSM surface salinity does not correspond

well with the measured salinity in sites subject to high river discharge (e.g. N1,N2,C7) as the salinity within these sites is highly localised as they are enclosed sites either located within river mouths or with rivers entering into them. As improvements to the resolution of this model are made (already complete for a section of the west coast) such that the resolution can be as high as 200 m as opposed to 1 km it may be that the salinity predictions generated by model runs more accurately identify salinity within urbanised coastal sites. Validating model generated data with measured salinities at different periods of the year in marinas and harbours would help to determine whether the SSM could be relied upon in future to predict salinities within these sites.

3.4.3 Patterns of native and non-native species diversity

Native and non-native species diversity were positively correlated in this study, suggesting that in Scottish marinas environmental conditions drive species diversity patterns as opposed to competitive processes (Levine and Antonio, 1999; Choi et al., 2016). This contrast with results from experimental studies which have shown that the success of NNS invasions is lower in communities with high native species diversity (Stachowicz et al., 2002). More diverse communities have been hypothesised to have higher biotic resistance as availability of limiting resources is lower, prevention establishment of new species (Levine and Antonio, 1999). Landscape-scale studies of NNS distribution have found the opposite trend, and the explanation of this paradox may be a result of variation in environmental conditions across large geographic scales as opposed to controlled experimental conditions (Choi et al., 2016; Levine, 2000; Stachowicz et al., 2002; Bulleri et al., 2008).

Native and NNS richness were both correlated with salinity parameters. NNS richness was correlated with scaled surface salinity measurements from CTD data, while both native and NNS distance increased as the distance to the nearest river increased. Salinity conditions within marinas are thus likely to impact both native and NNS diversity, supporting previous results from Clarke Murray et al. (2014); Foster et al. (2016) and Choi et al. (2016). In sites with high volumes of freshwater inflow, few native or NNS are likely to be able to tolerate the large variation in salinity conditions (Moreira et al., 2014; David et al., 2013; Smyth and Elliott, 2016). The similarity of native species fouling communities was primarily influenced by just one factor, the proximity to a river, with three clusters of sites identified. Sites N1, N2, and C7 are the sites located at river mouths (<150 m), and are characterised by very low species diversity, with high abundances of filamentous brown and green algae and hydroids, whereas

sites C3 and C4 at an intermediate distance (between 150 m and 355 m) were characterised by high abundances of *Mytilus edulis*. Sites furthest away from rivers had the highest diversity of species with high abundances of kelp and red and green algae. Sites with different levels of riverine freshwater input may thus be characterised by assessment of the native fouling community, with very low diversity communities representative of sites with very low salinity conditions, and mussel dominated communities found in sites with brackish conditions. As a group, these sites had the highest number and abundance of NNS (excluding the high abundance of *C. caspia*, and the differences in dominant native species could be used to identify sites most vulnerable to new species introductions. This information could be incorporated into national or regional biosecurity plans, or used to develop a short list of species likely to arrive in a site.

The NNS community composition generated by RAS presence/absence data was correlated with salinity metrics (distance from the nearest river and the mean surface salinity predicted by the Scottish Shelf Model (SSM). The transition hull-fouling species experience when vessels move from coastal marine conditions to less saline conditions in marinas with high freshwater inflow may prevent establishment of most marine species introduced via this vector (David et al., 2013; Moreira et al., 2014). However, NNS with a broader salinity tolerance, like *C. Caspia* and the barnacle *Austrominius modestus* (the only two NNS found in very low salinity sites), are more likely to be able to survive transfer (Folino-Rorem and Renken, 2018; Harms, 1986). A table containing known salinity tolerances for NNS identified in this study can be found in the Appendix (Table B.5). Changes in salinity conditions may even induce spawning in some brackish species (Minchin and Gollasch, 2003). Thus while these sites have been shown to be less susceptible to establishment of marine NNS, they are not immune. Nevertheless, these sites may require less frequent monitoring and should not be used as sentry sites to detect introduction of new NNS to the region (Inglis, 2001; Gittenberger et al., 2011).

Sites C5 and C6 are notable in having low numbers of NNS but mid-range levels of native species (Figure 3.6). These sites had lower recorded salinities from CTD measurements and lower predicted surface salinities in the SSM, reflecting the influence of estuarine conditions in the Firth of Clyde as opposed to proximity to a river. This could potentially explain observed differences in NNS and native species diversity in these sites, with few NNS able to tolerate changes in salinity during transport to the marina, but with estuarine conditions stable enough for higher native species diversity compared with sites such as C7, N1, and N2 subject to high

river discharge. This represents a tentative explanation for these observed differences in native and NNS diversity in these sites, but further investigation of temporal changes in salinity and fluvial discharge over an annual cycle with a larger sample of sites would be necessary to test this.

3.4.3.1 Other abiotic factors affecting NNS distribution

The focus of this study was on identifying the effect of variation in salinity conditions, and the sites were chosen to reflect a spectrum from low to high freshwater influence. In this study, similarity between sites based on the whole community and native assemblage data found no influence of location (latitude, longitude), distance to the nearest port, or elements of marina design. The significant effect of salinity on resulting community composition may have obscured other factors which contributed to differences in invasion patterns (Nall et al., 2015).

The similarity of the NNS fouling assemblage between sites based on data from the quadrat photos was influenced by proximity of the nearest river, marina depth at low tide, and the number of berths. The importance of the number of berths may be overemphasised due to the high abundance of NNS in site C7, which is also one of the largest marinas. However, the number of berths can be used as a proxy for vessel activity, which could influence propagule pressure and physical disturbance of fouling communities, and has previously been shown to influence NNS richness in Scotland (Nall et al., 2015). It may be that these factors influence success of a NNS within a community (NNS assemblage abundance data), but have less influence on NNS establishment within a site (RAS data) (Clark and Johnston, 2009).

The prevalence of a species may also be affected by management practices in a site. Information on maintenance activities was not collected in a routine way in this study, but in site C2, researchers were informed by the marina manager that the pontoon floats had been scraped clear of biofouling material in the month preceding the survey. This offers a potential explanation for why cover of the biofouling community was relative low (92%), and the discrepancy between site C2 having the highest average species richness in quadrat photographs, but not the highest pooled species richness at the site level. Scraping of pontoon floats may act as a disturbance event, reducing abundance of dominant species and increasing diversity on the floats, if not at the level of the whole site (Hopkins and Forrest, 2008; Airoldi and Bulleri, 2011). Scraping pontoon floats may enhance recolonisation as a result of a number of mechanisms, including increasing the abundance of space for settlement, release of chemical

and or physical cues that prompt settlement of certain taxa, and reducing competition from dominant taxa (Hopkins and Forrest, 2008). Increased rates of recolonisation have been found following in-water cleaning of vessel hulls without reapplication of anti-fouling paint (Hopkins and Forrest, 2008; Floerl et al., 2005). Furthermore, scraping of floats may increase abundance of NNS. The cover of NNS was high in site C2 (17% of the total biofouling cover), predominantly due to high abundances of the non-native bryozoans *Schizoporella japonica* and *Tricellaria inopinata*. *Schizoporella japonica* has been shown to be able to rapidly grow following disturbance (Loxton et al., 2017; Cook et al., 2013b). This anecdotal evidence supports previous research which suggests that timing of maintenance activities on artificial structures may facilitate establishment and dominance of marine NNS in these systems (Airoldi and Bulleri, 2011). Consideration of the impact of maintenance in facilitating the establishment of these species may thus be important to consider in biosecurity plans for individual sites (Cook et al., 2014b; Payne et al., 2014; Viola et al., 2017).

The distribution of NNS among sites will also be determined by a species' introduction history as well as differences in susceptibility to invasion between sites (Jimenez et al., 2017). A species may have been introduced multiple times or established in one location and undergone further secondary spread (Clarke Murray et al., 2014). For example, the non-native colonial ascidian *Didemnum vexillum* was first detected in site C8 in 2009, and was found to be established on nearby subtidal structures soon after (Beveridge et al., 2011). The discovery of *D. vexillum* in site C2 within the Clyde is more likely compared with marinas in other regions, especially those on the east coast as the nearest records of *D. vexillum* in the east are in Kent (NBN, 2019). However, given that likelihood of species transfer by vectors is not necessarily linked with the physical distance between sites, knowledge of site connectivity may be a more important factor to include in future studies than distance between sites (Lord et al., 2015; López-Legentil et al., 2015).

3.4.4 Conclusions

This study has shown that the diversity of native and non-native species in fouling communities in Scottish marinas is primarily driven by environmental conditions. Fouling communities in marinas in close proximity to large rivers are more likely to experience lower and more variable salinity conditions. Salinity has been identified as an important factor correlated with NNS distribution patterns in ports, marinas and natural habitats in other parts of the world (Epelbaum et al., 2009; Genovesi et al., 2014; Clarke Murray et al., 2014; Ruiz et al.,

2000). Information on proximity to rivers could be combined with salinity data generated by the Scottish Shelf Model to estimate the salinity conditions within Scottish marinas, and thus to estimate risk of fouling NNS establishment. The presence of highly diverse fouling communities could be used to identify sites which require frequent monitoring and should be kept under surveillance to enable rapid detection of new species. However, while low salinity sites may need require less frequent monitoring, they should not be excluded from RAS entirely, as they may host high abundances of species capable of tolerating these conditions.

CHAPTER 4

Investigating hypo-saline treatment of fouling communities

ABSTRACT

Fouling on recreational vessels is an important vector of spread for marine non-native species (NNS). Hull cleaning for recreational vessels can be a considerable expense for owners, so finding cost-effective methods to reduce biofouling could limit the spread of introduced species. Freshwater ports have historically been used to purge biofouling from larger shipping vessels. Salinity shock with freshwater has also been shown to be an effective treatment option for a number of invasive marine NNS. Two field-experiments using fouling communities grown on settlement panels in marinas on the west coast of Scotland were conducted to investigate whether short-term immersion of fouling communities in hypo-saline water could be an effective tool for removing biofouling and controlling NNS. In the first experiment, the effectiveness of different salinities and durations of treatment on reducing biofouling cover was investigated. Results showed immersion in salinities lower than 10 PSU is effective in removing 20 - 50% of biofouling cover (combined cover of native and NNS), although rapid recovery occurred over the course of the eight-week monitoring period. However, treatment with hypo-saline water caused a permanent shift in community composition compared with ambient controls. The effect of changing immersion time from one to four hours was negligible. The second experiment investigated whether response to treatment with freshwater varied between fouling communities with different levels of environmental tolerance as a result of variation in freshwater inflow. The largest changes in biofouling community assemblages were seen in the marina with the most stable natural fully saline conditions, while little effect of treatment was observed in the marina with the highest freshwater inflow. Over both experiments, native species were more detrimentally affected

by treatment than non-native species. Response to hypo-saline treatment varied between different NNS, with some species controlled by freshwater treatment and others increasing in abundance over the monitoring period, possibly as a result of reduced competition from previously dominant native species. These findings highlight the importance of considering post-treatment processes and local community dynamics when planning control of fouling marine species using freshwater.

4.1 Introduction

Hull fouling on small vessels is an unregulated pathway of NNS spread, facilitating the transfer and establishment of marine NNS in ports and harbours (Roberts and Tsamenyi, 2008; Clarke Murray et al., 2011; Roche et al., 2015; Foster et al., 2016). Preventative measures such as the regular application of anti-fouling paint are less well adhered to within the recreational boat sector than for commercial vessels, and fouling may be exacerbated by slower speeds and longer periods spent stationary (Clarke Murray et al., 2011; Foster et al., 2016). Cost effective ways to treat contaminated vessels and infrastructure without releasing viable propagules or fragments are needed to both prevent fouling species spread and control species post-arrival (Forrest et al., 2009; Roche et al., 2015). As most marine species are unable to survive rapid changes in salinity, treatment with low salinities could offer mechanism for preventative removing of biofouling and as a tool to control or eradicate organisms once a NNS has been detected (Moreira et al., 2014).

To date, the use of hypo-saline treatments have been more widely examined for use in the aquaculture industry compared with the boating sector (Forrest and Blakemore, 2006; NSPMMPI, 2013), a result of the need for survival of the aquaculture stock species limiting the use of chemical treatments (Denny, 2008). Guidance to the Australian aquaculture industry recommends two hours of freshwater immersion combined with 12 hours air exposure as a cost-effective and environmentally friendly way to treat pests on aquaculture stocks and gear (NSPMMPI, 2013). In the recreational boating industry, freshwater could be used to reduce biofouling on whole vessels or other movable elements of marina infrastructure, such as ropes and equipment or sections of pontoon. Vessels deemed to be at risk of carrying marine NNS (e.g. those arriving from international ports or known NNS hot-spots) could be directed to marinas known to have very low salinity. The risk of marine NNS establishing in these environments is lower, and the low salinity could help slough off high risk species before the vessel was allowed to travel elsewhere.

Freshwater ports and rivers are known to purge marine biofouling from vessels (Minchin and Gollasch, 2003). In 1998, Brock et al. (1999) moored a decommissioned battleship in a river in Oregon, USA for nine days in order to reduce the likelihood of NNS being transferred when the ship was moved to Hawaii. Mooring the vessel in salinities between 2-10 ppt resulted in 90% of the hull cleared of fouling organisms, with the remaining species deemed to be of low threat or not capable of surviving the voyage. However, uncertainty about the effectiveness

of this treatment was raised by Apte et al. (2000) who observed that the mussel, *Mytilus galloprovincialis* survived the period in low salinity waters and went on to spawn upon arrival in the new port (Apte et al., 2000; Minchin and Gollasch, 2003). This shows the importance of monitoring changes in community composition during the recovery period post-treatment.

Freshwater could also be used to target specific areas of vessels where it is difficult to apply anti-foul paint, and which are hard to manually clean. For example, de Castro et al. (2018) have proposed flushing with freshwater as a method of removing biofouling from sea chests, and have demonstrated that it is possible to lower the salinity of sea chests to below 7 PSU by pumping in freshwater. Encapsulation methods could allow for the application of freshwater treatments to the whole vessel, or affected parts of static infrastructure. Roche et al. (2015) have developed an in-water encapsulation berth to allow treatment of contaminated vessels, but this type of system is not yet commercially available. Wrapping or encapsulating vessels has previously been used to treat NNS invasions by creating an anoxic environment or by applying chemicals (Roche et al., 2015; Atalah et al., 2016). Treating vessels with freshwater is likely to have quicker results than waiting for the creation of anoxic conditions (which can take a number of days to develop), and does not risk release of chemicals into the wider environment (Roche et al., 2015). In addition there are normally no associated permits needed to add freshwater into the marine environment. Freshwater is widely available in marinas and ports as it is used by boat owners for refilling or washing vessels.

A number of ex-situ experiments have shown that osmotic shock with freshwater can be used to kill specific NNS. Experimental freshwater immersion has been trialled on *Caprella mutica* (Ashton et al., 2007), *Caulerpa taxifolia* (Theil et al., 2007), *Didemnum vexillum* (Denny, 2008; McCann et al., 2013), *Sabella spallanzanii* (Jute and Dunphy, 2017), *Styela clava* (Minchin and Duggan, 1988), *Tubstreaea* spp. (Moreira et al., 2014), and *Undaria pinnatifida* (Forrest and Blakemore, 2006). These studies have generally focused on a target organism, been carried out in aquaria or laboratory settings, and have not considered the effect of treatment on the wider fouling community of which the target species may be a part. The results have demonstrated the feasibility of controlling individual invasive NNS using freshwater immersion, and shown that the duration of treatment required for 100% mortality varies between species. Tolerance to osmotic stress varies between species depending on their osmoregulatory capacity (O'Loughlin et al., 2006; Moreira et al., 2014; Hauton, 2016; Pauli and Briski, 2018). Successful treatments have been shown following as little as 20 minutes exposure for some species (e.g. *S. spallanzanii*), two to four hours for others, while some require much

longer periods (>24 hours) (McCann et al., 2013; Moreira et al., 2014; Jute and Dunphy, 2017). Taxa with shells or exoskeletons within which they can retreat are more likely to be able to survive episodic environmental stress (Smyth and Elliott, 2016).

Variation in species' survival may result in shifts in species assemblages, and episodic environmental stress has previously been shown to cause long term changes in community composition (Sorte et al., 2010a,b). In San Francisco bay, salinity drops in years with very high rainfall have been shown to change the dominant species in fouling communities the following summer (Chang et al., 2018; Chang, 2009). Shifts to greater dominance of NNS following episodic osmotic stress have also been recorded (Sorte et al., 2010a; Chang et al., 2018), potentially as a result of NNS demonstrating greater tolerance to environmental disturbance than native species (Crooks et al., 2010; Rivero et al., 2013). In environments frequently exposed to salinity stress, the community is more likely to be made up of species capable of tolerating periods of low salinity. These organisms are likely to be less susceptible to hypo-saline treatments, and the efficacy of treating NNS which originated from brackish environments with freshwater is likely to be lower.

Targeting a single organism within a fouling community is often challenging, thus frequently the whole fouling community will be treated in order to eradicate or control a specific invasive NNS (Holt and Cordingley, 2011; Atalah et al., 2016). As ports and harbours are often host to a number of different NNS, variation in the recovery and recolonisation of native and non-native fouling species post-treatment could result in unanticipated shifts in fouling community assemblages (Sorte et al., 2010a). This study thus aimed to assess the impact of low salinity treatments on entire fouling communities, and to investigate changes in native and NNS cover over the recovery period post-treatment. At the time this study was conducted (June - October 2015), no previous experimental analysis of hypo-saline treatment on marina fouling communities had been conducted. A similarly designed study was carried out by de Castro et al. (2018) in 2016 in Plymouth, coincidentally using similar methods, which presents a useful comparison for the results of these experiments.

Two field experiments were designed by adapting methodology used to test the effects of chemical baths and heat exposure on fouling communities (Crooks et al., 2010; Sorte et al., 2010a). Field experiments were carried out on fouling organisms grown on settlement panels to assess the practicality of low salinity treatments in similar conditions to which might be experienced in a management scenario. The first objective of the study was to test the efficacy of different salinities and different immersion periods on reducing biofouling cover of native,

non-native, and cryptogenic species. The immersion periods were chosen based on results of previous single-species studies which showed mortality following treatment for between 20 minutes to several hours (McCann et al., 2013; Moreira et al., 2014; Jute and Dunphy, 2017), and to reflect time periods that would be practicable for boat owners to implement without restrictive delays to sailing schedules (Roche et al., 2015). The second part of the study aims to assess the response to freshwater treatment of fouling communities with different levels of tolerance to low salinity conditions. Together these experiments explore whether short-term treatment of fouling communities with low salinities could be an effective management option for reducing biofouling and controlling NNS.

4.2 Methods

4.2.1 Site description

Salinity experiments were carried out in four recreational marinas in the west of Scotland, UK (Table 4.1). Site E1 (56.4181° N, 5.4999° W), located in Ardantrive Bay on the Isle of Kerrera, was selected to carry out the investigations into the effect of exposure to different salinities for variable amounts of time (Experiment 1). It is an open water marina with an average depth of 12 m and receives little freshwater input, apart from minimal surface water run-off from the Isle of Kerrera (Figure 4.1).

Table 4.1: *Site details for the four marinas within which salinity experiments were conducted.*

Experiment	Site	Latitude	Longitude	Salinity	Marina design
E1	E1	56.4181 °N	5.4999 °W	Marine	Open
E2	A	56.2102 °N	5.5562 °W	Marine	Enclosed
E2	B	56.4490 °N	5.4312 °W	Moderate freshwater	Open
E2	C	55.9093 °N	4.8714 °W	High freshwater	Enclosed

Experiment	Site	SST (°C)		Surface Salinity (PSU)	
		Mean	Range	Mean	Range
E1	E1	12.65	11.53 - 13.55	33.57	32.44 - 33.64
E2	A	13.27	12.21 - 14.53	33.56	32.62 - 33.99
E2	B	12.99	12.12 -13.76	28.77	24.49 - 32.10
E2	C	12.98	11.70 - 14.01	23.365	8.89 - 31.90

To compare the response of communities exposed to different environmental conditions (Experiment 2), freshwater immersion experiments were conducted in three different sites (A, B and C) (Figure 4.1). Marinas were chosen due to differences in environmental salinity conditions between sites. However, as there was no specific data available on salinity within each site, characterisation of salinity patterns was thus based on site location, design and previous observations of freshwater inflow at each site (Cottier-Cook, *pers. comm.*).

Site A (56.2102° N, 5.5562° W) is located on the north shore of the Craignish peninsula in an enclosed and very sheltered bay. This marina receives little run-off and has no freshwater source nearby and was thus expected to have a similar salinity to coastal marine waters. Site B (56.4490° N, 5.4312° W) is an open-water marina, located near the Lynne of Lorne at the mouth of Loch Etive. The site receives freshwater input from a small river near the pontoons, and from freshwater run-off from Loch Etive. The outflow of water from Loch Etive also

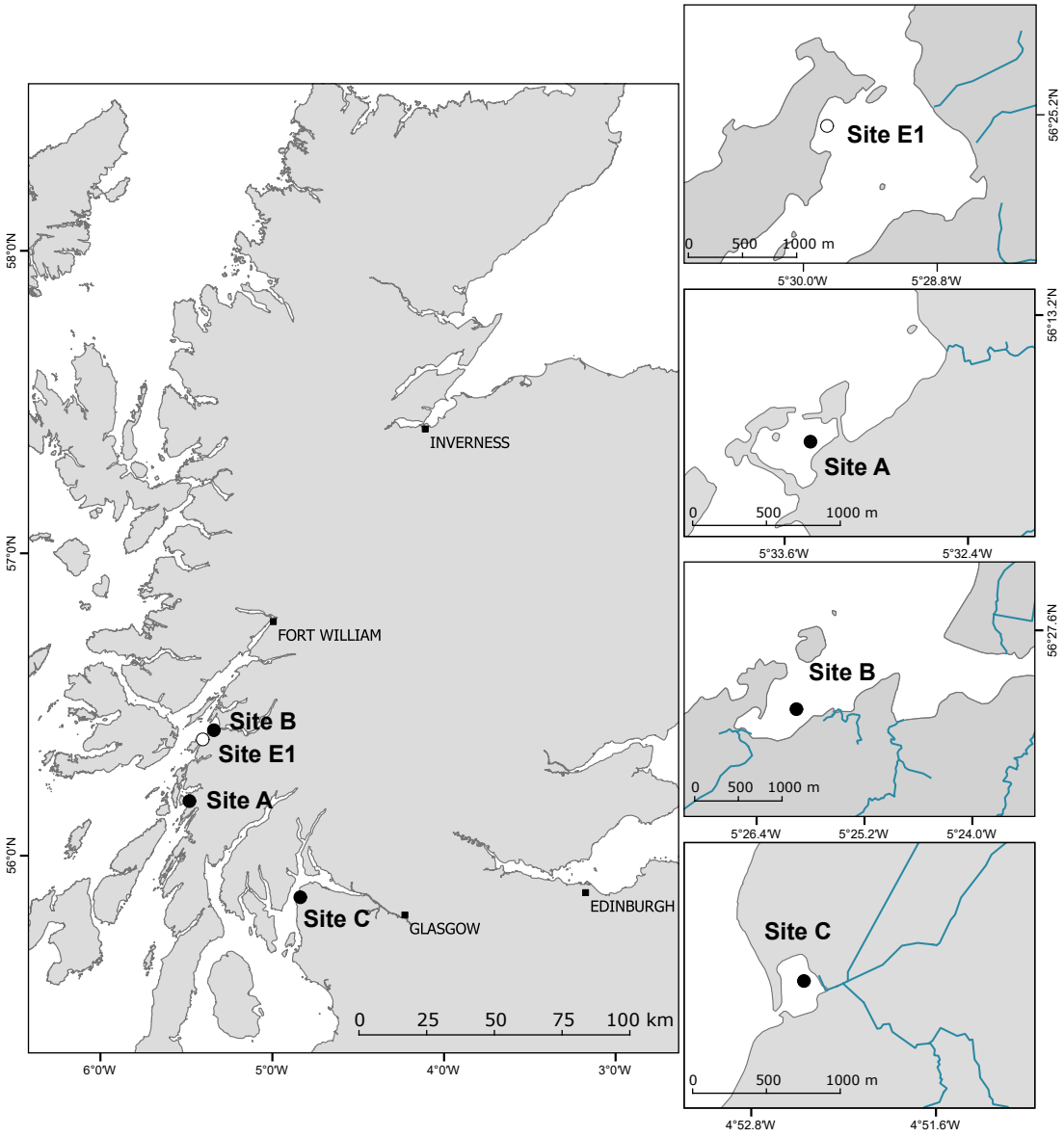


Figure 4.1: *Map of Scotland showing location of site E1 (○) where the first experiment was located, and sites A, B, and C where the second experiment took place (●). Panels on the right show the local geographic context of marinas where the experiments were carried out, including the enclosed nature of site A and C, and the location of nearby rivers (blue lines). Note that scales vary between sites.*

creates an eddy within the bay, running clockwise on the ebbing tide, and anti-clockwise on the flood tide (Ashton et al., 2010). Located in the Firth of Clyde, site C (55.9093° N, 4.8714° W) is a very enclosed marina with entry to the marina restricted to a 20m wide channel. A river runs directly into the marina and during periods of heavy rainfall, a freshwater top layer can form with a visible halocline. Water visibility can also be very low in site C due to high sediment loads (*pers. obs.*).

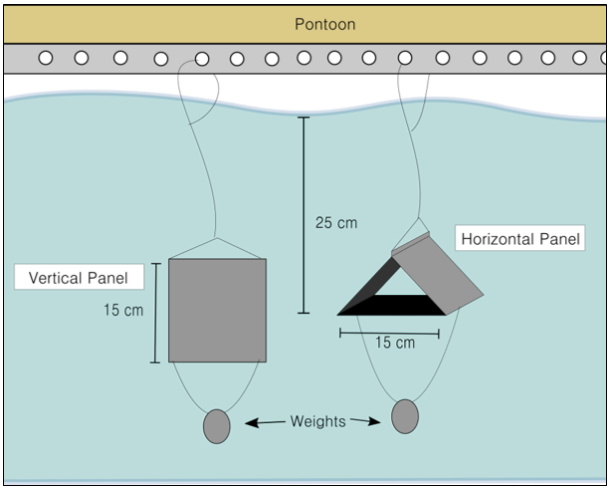


Figure 4.2: *Figure showing set-up of settlement panels on marina pontoons*

4.2.2 Environmental Monitoring

Environmental monitoring was carried out at 5 locations within site E1, and four locations within sites A, B and C. Monitoring points were located either at the start or end of pontoons on which the experimental settlement panels were deployed. At each monitoring point, salinity, temperature and depth data were recorded using a YSI Castaway-CTD every 2 weeks for the duration of the study period (n=8) (SonTek, 2010, San Diego).

4.2.3 Field communities

CoreX Settlement panels (15x15 cm) were deployed in each marina in June (Site E1) and July (Sites A, B, and C) 2015 and left for 8 weeks to develop representative fouling communities. Panels were hung both vertically and horizontally (downward facing) in the water column to mimic pontoon biofouling communities, as clear differences in biofouling community assemblage have previously been observed between orientations (Figure 4.2) (Glasby, 2000; Glasby and Connell, 2001). Panels were hung from the side of floating pontoons using rope

and cable ties, and weighted by lead shipping weights or beach cobbles in plastic netting (Figure 4.2). The panels were hung from pontoons so that they were positioned at 0.25 m depth to represent communities growing in the upper layer of the water column (i.e. those that are most frequently exposed to variations in salinity). 130 panels were set out in site E1, and 50 in each in sites A, B, and C (Figure 4.3).The community on the lower side of the horizontal panels were later analysed, and the side of the vertical panels to be analysed was identified before deployment and indicated by the placement of the panel label.

4.2.4 Experimental design

Experiments 1 and 2 followed broadly similar procedures, with key differences in the treatments settlement panels were exposed to. A summary of the design of both experiments is provided in Figure 4.3.

4.2.4.1 Experiment 1: Salinity exposure experiment

This experiment was designed to test the response of fouling community exposure to different salinities (freshwater <0.5, 10, 17 and ambient seawater) for varied periods of time (1, 2 and 4 hours). Salinity treatments reflected thresholds of salinity change to which marine invasive NNS are known to be susceptible (McCann et al., 2013; van der Gaag et al., 2016). The duration of treatments (exposure) were designed to reflect biosecurity treatments which might be carried out on visiting vessels (Roche et al., 2015).

The experiment took place over the course of a week in site E1. Experiments were carried out on marina pontoons in order to reflect ambient marina salinities and temperatures, and to reduce unnecessary disturbance of experimental panel communities from transportation (Hopkins et al., 2016). Experimental treatment design inspired by experiments on the influence of heavy metals and heat treatment on fouling communities carried out by Sorte et al. (2010a) and Crooks et al. (2010).

Sixty horizontal and sixty vertical pre-fouled panels were randomly assigned to one of 12 treatments (n=5) in a crossed factorial experimental design with two variables; salinity (4 levels) and exposure (3 levels). Treatments were randomly assigned across panel location in the marina, but blocked by experiment day (5 blocks) (Figure 4.3). Each panel was placed in a separate 20 L opaque plastic container, containing 10 L of water. Lids were placed on top of the containers to prevent rain from influencing the salinity inside the container. Specific salinities were achieved by mixing specific quantities of ambient seawater from the marina

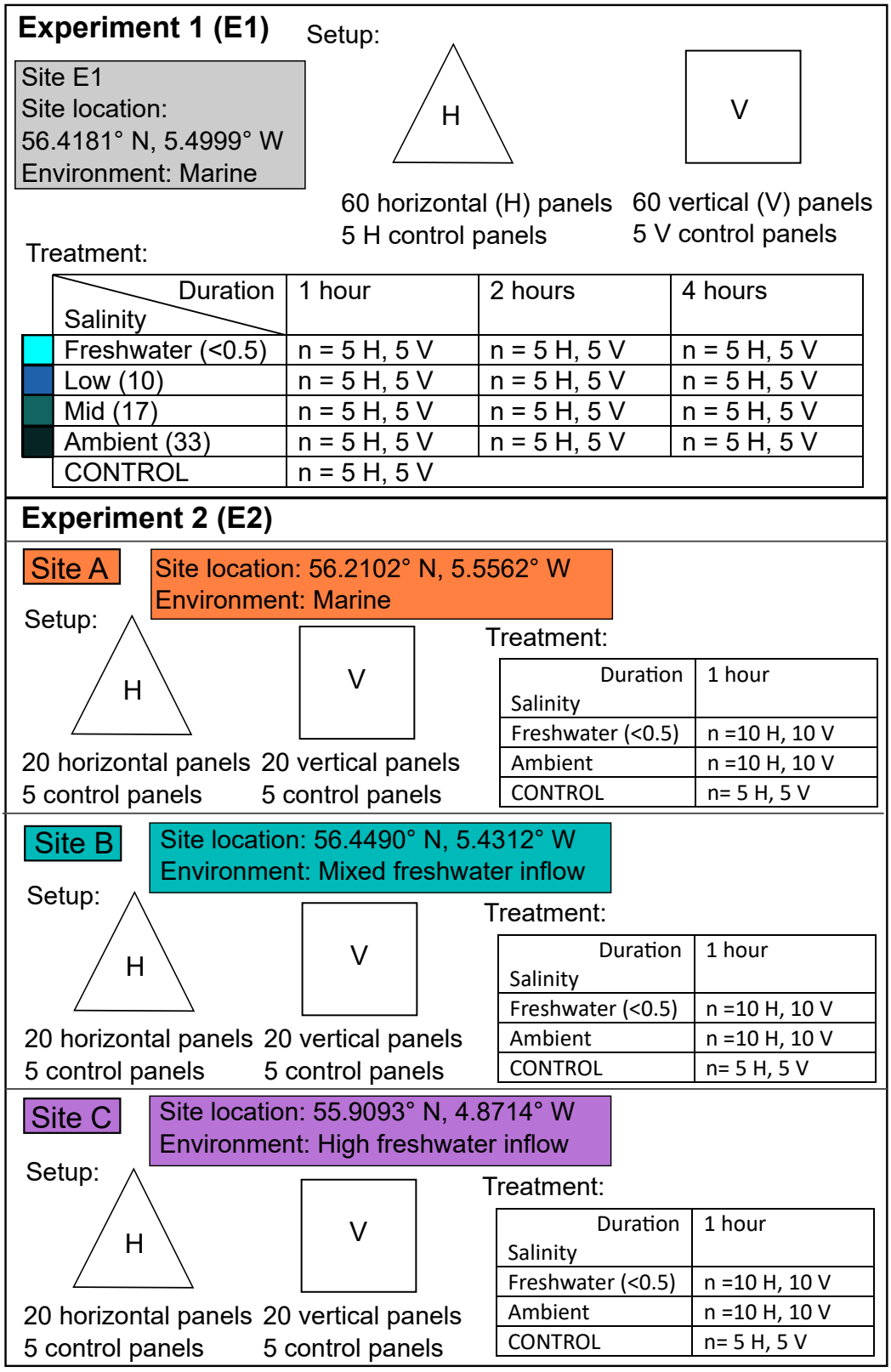


Figure 4.3: *Graphic representation of experimental design and set-up of each experiment*

with freshwater available on the pontoons. Very slight variation in ambient salinity resulted in average salinities of 0.22 (±0.15), 10.27 (±0.47), 17.24 (±0.36), and 33.22 (±0.13).

The water was replaced after 2 hours for panels in the 4 hour treatment, in order to compensate for lowered oxygenation of the water. Salinity and temperature was checked at the start of the experiment using a YSI castaway CTD to perform a point measurement. Temperature was also monitored continuously during the experiment using a iButton temperature logger in each experimental container. Treatment of panels was staggered by 5 minutes to allow for the removal from the pontoon at the start of the experiment, and for photography and reattachment post-treatment.

Panels were suspended in the same orientation as in the field, using a fixed anchor point at the bottom of the container and a system of hooks and dowels at the top. Panels were left submerged for the length of their respective treatment before being removed, photographed, and reattached to the pontoons in their original positions. Photographs were taken using a Nikon Coolpix AW100 set a standardised distance from the panels.

4.2.4.2 Experiment 2: Freshwater immersion experiment

This experiment was designed to test the response of fouling communities grown on horizontal and vertically orientated panels in three marinas with very different salinity regimes, to a brief period (one hour) of immersion in freshwater conditions. In each marina (n=3), 20 horizontal and 20 vertical panels were randomly assigned to either the freshwater (n=10) or ambient control (n=10) treatment (Figure 4.3). Experiments took place in each marina over the course of two days, and timing was blocked by pontoon (half day per block).

Experiments across the marinas took place on successive days over the course of a week (31/08/2015 - 06/09/2015), in the same order in which panels were deployed such that experiments took place after exactly 8 weeks. Treatment of panels were staggered by 5 minutes to allow for the removal from the pontoon at the start of the experiment, and for photography and reattachment post-treatment.

Experimental set up followed the same design as that in Experiment 1, except treatments were only applied for a one hour period. Each panel was placed in a separate 20 L plastic container, containing 10 L of either freshwater or ambient seawater from the marina. Freshwater was sourced from the marina supply, and ambient seawater was drawn from the marina at the approximate depth the panels were hanging at (0.25 m). The mean ambient salinity across the treatments varied between the marinas, and was highest in site A (33.41 ±

0.27), followed by site B (26.99 ± 1.08), and lowest in site C (25.18 ± 1.13).

4.2.5 Community recovery and analysis

Treatment effect on communities was monitored in the same way for both experiments. Following exposure to the respective treatment, panels were replaced back in the marina in their original positions. Panels were then removed from the water, photographed and replaced, after 7, 28, and 55 days (1, 4 and 8 weeks) (Figure 4.4).

In order to compare establishment of fouling species following the experiment with normal recruitment rates, control panels (n=10) were deployed alongside experimental panels in each marina for 8 weeks and then scraped clean at the time of the experiment (Figure 4.3). These blank control panels were set out at the same time as experimental panels were returned to the pontoons, in order to determine which species were recruiting to bare substrate during the recovery period. Blank panels were photographed at the same time as experimental panels after 1, 4 and 8 weeks.

Eight weeks after treatment, panels were removed from the pontoons and placed in 70% Industrial Methylated Spirit (IMS). Panels were analysed under a microscope in the lab, and identified to species level where possible. Percentage cover was estimated by placing an 100 point grid (14x14 cm), over the panels. Where more than one species was present, both species were recorded, and total percentage cover could thus reach over 100%. Species not present under a point were recorded as occupying 0.05% to generate a full species list. Algae were grouped in analysis of the panels, as the majority of records were from small filamentous algae, which were difficult to identify due to bleaching effects of the preservation process.

The photographs were analysed to assess recovery over time using the count software Coral Point Count Extension (CPCE) (Kohler and Gill, 2006). An 100 point grid was placed over photographs of the panels and standardised by the size of the panels (Figure 4.5). Where two species overlaid one another, only the top fouling organism visible was counted. Identification of organisms was aided by the records from the microscope analysis of panels, but identification to species level was still not always possible from the photographs. Macro-algae were grouped into red, green or brown seaweeds.

Mobile species were not included in either analysis, as recolonisation following treatment could have been immediate, and identification in photographs was not possible.

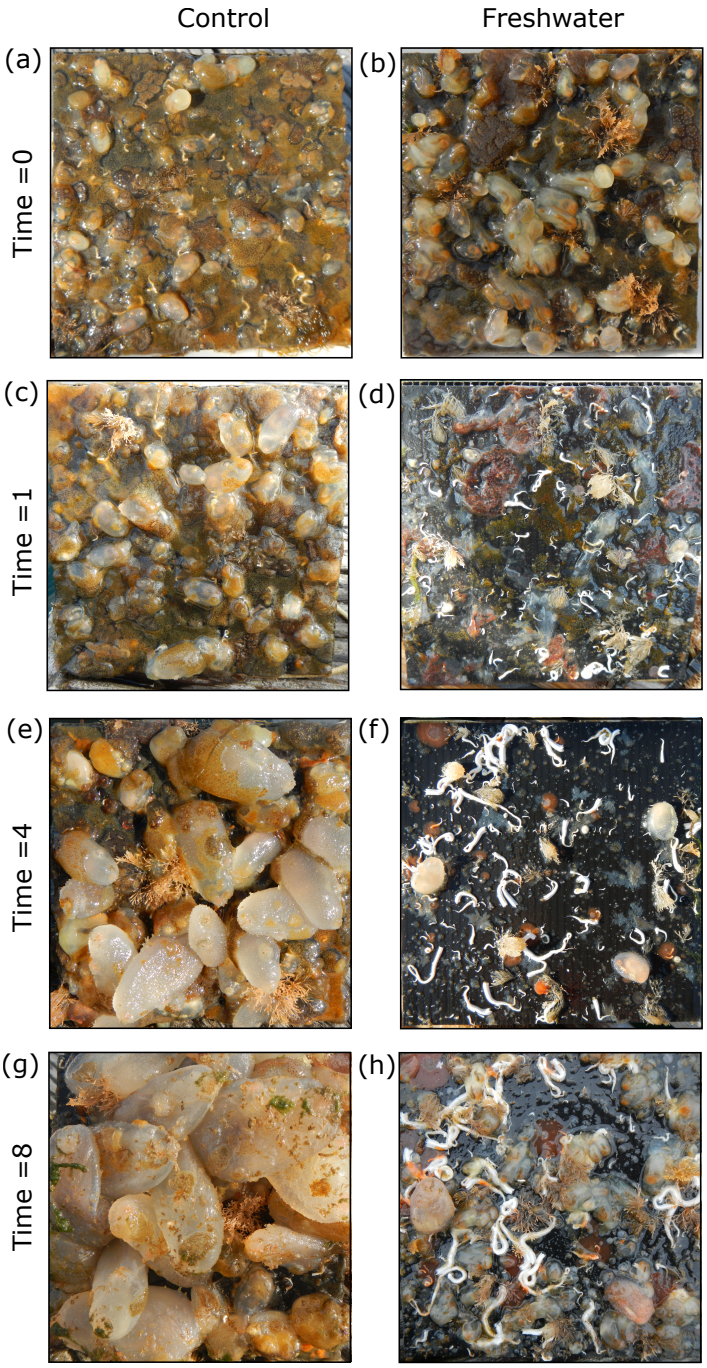


Figure 4.4: *Figure showing photographs of two example horizontal panels from site A taken at the start of the experiment (a,b), and 1 (c,d), 4 (e,f), and 8 (g,h) weeks after replacement in the marina. The photographs on the left show a panel subjected to the ambient seawater control, and on the right a panel subjected to the freshwater treatment. These photographs are typical of horizontal panels from site A, and show the loss of biofouling cover following freshwater treatment. Examples of panels from sites B and C are located in Appendix C (Figures C.2, C.3).*

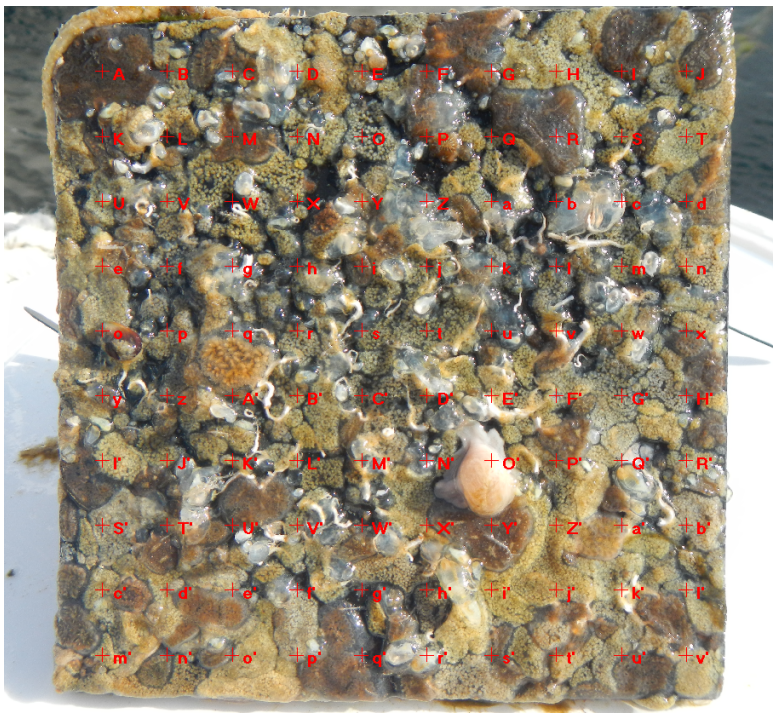


Figure 4.5: *An 10 x 10 point grid overlaid on a panel photograph from site A by Coral Point Count Extension (CPCE) software.*

4.2.6 Data analysis

Multivariate and univariate analyses were conducted using PRIMER v7 statistical software, with the PERMANOVA add-on (Anderson et al., 2008). Univariate figures were produced using ggplot2 in the statistical software 'R Studio' (RStudio Team, 2018; Wickham, 2009). Univariate metrics (total biofouling cover and species richness) for each panel were calculated from percentage cover data for individual species generated either through analysis of photographs in CPCE, or from analysis of physical panels under the microscope.

4.2.6.1 Preliminary data exploration

To assess whether pre-existing differences between panel communities may affect detection of treatment effect, multivariate analyses were performed on Bray-Curtis similarity matrices calculated on fourth-root transformed percentage cover data. Data from photographs taken immediately before and after treatment in experiment 1 were compared to see if effect of salinity treatment was immediately detectable.

Pre-treatment differences in communities on panels suspended horizontally and vertically were assessed using a non-metric multidimensional scaling (nMDS) plot of

community similarity and a two-way ANOSIM test on the crossed factors 'Orientation' and 'Marina'. A similarity percentage anlalysis (SIMPER) was used to identify which species contributed most to dissimilarities between communities on horizontal and vertical communities at each site. For experiment 1, these analyses were run on data from photos taken before treatment, and for experiment 2 analyses were run on photos taken immediately after treatment, on the assumption that affect of treatment was not immediately detectable.

4.2.6.2 Analysis of photograph data

Analyses of the CPCE data were run separately for panels from each site and for horizontal and vertical panels due to pre-treatment differences in community composition. Univariate analyses were run on the total percentage cover and species richness for each panel photograph, with data summarised from the multivariate dataset. Multivariate analyses were run on bray-curtis similarity matrices calculated from fourth-root transformed percentage cover data (Mayer-Pinto et al., 2016), with the amount of uncolonised space included as a variable. Univariate analyses were run on euclidean distance matrices (Mayer-Pinto et al., 2016).

Both univariate and multivariate analyses were performed using the PERMANOVA routine in PRIMER. The PERMANOVA routine was run with 9999 permutations of residuals under a reduced model, and Type III sums of squares to account for the unbalanced design which occurred as a result of panels being lost during the recovery period. PERMANOVA designs varied depending on the experiment (see Table 4.2). The highest-order interaction term was excluded to account for the repeated-measures design (Anderson et al., 2008). If interaction terms relevant to the research questions were found to be significant, post-hoc PERMANOVA t-tests were run on the significant interaction term. The PERMDISP routine was also run on significant terms to test heterogeneity of dispersion between groups (9999 permutations, distances between centroids (Anderson et al., 2008; Nall et al., 2015). If significant heterogeneity was detected, results were interpreted with caution.

The PERMANOVA routine for univariate data differs from traditional repeated-measures ANOVA analyses in that it uses permutations to calculate the equivalent R statistic and p-value and therefore does not have all the assumptions of traditional univariate analyses. The complexities of the data, non-linear response, non-normal distribution of the residuals, and difference in the variation of data between factor levels, meant that traditional univariate approaches to data analyses were not appropriate (Anderson and Millar, 2004). While the

Table 4.2: *PERMANOVA design for analyses in Experiment 1 and 2*

Experiment 1:			
Salinity (Sa)	4	Fixed	
Exposure (Ex)	3	Fixed	
Time (Ti)	4	Fixed	
Panel (Pa)	120	Random	Nested within Salinity and Exposure
Excluded term: TimexPanel(SalinityxExposure)			
Experiment 2:			
Treatment (Tr)	2	Fixed	
Pontoon (Po)	3	Fixed	
Time (Ti)	4	Fixed	
Panel (Pa)	40	Random	Nested within Salinity and Pontoon
Excluded term: TimexPanel(SalinityxPontoon)			

PERMANOVA routine is not insensitive to heterogeneity of variance within the data, this weakness was considered as still generating results of scientific interest, and was therefore interpreted as part of the response (Anderson et al., 2008).

Tests were also run on taxon groups and individual non-native and cryptogenic species using the same routines outlined above. The PERMANOVA design differed from tests for the whole community in that tests were not run separately for panels of different orientations, instead orientation was included as an additional factor. The rationale for this was that the response of species to treatment might be expected to be the same on both orientations, but recovery might be affected by response of other species which could differ between orientations. Thus the impact of orientation could not be removed from the test as it was considered a biologically significant factor.

The same procedure was used to run tests for percentage cover data generated from analysis of panels under the microscope. Univariate and multivariate analyses were run using the same analysis procedures as outlined above.

4.2.6.3 Environmental data

CTD temperature and salinity data was averaged across sample locations within each site to produce average measurements for each depth sampled. Temperature and salinity heatmaps were produced in R using by interpolating between CTD measurements taken two weeks apart, and plotting the resulting timeseries using ggplot2 (Wickham, 2009).

Differences in mean temperature between salinity treatments were assessed using Wilcoxon t-tests as data was non-normally distributed.

4.3 Results

4.3.1 Experiment 1: Varied salinity experiment

4.3.1.1 Site Characteristics

Site E1 showed minimal variation in salinity with an average surface salinity of 33.57 (range 32.44-33.64) over the 16 week monitoring period (Table 4.1). The lowest salinities were observed in early September (Figure C.1a). The average sea surface temperature over the monitoring period was 12.75 °C, with temperature increasing over the summer period from a minimum of 11.53 °C in June to 13.55 °C in mid-October (Figure C.1b).

Forty-nine fouling taxa were recorded at site E1 (excluding mobile species) based on microscope analysis of fouling panels eight weeks after the treatment was applied. Algal species were grouped together for this data set. Most of the species were either bryozoans (19 species) or ascidians (12 species), with other taxa including serpulid worms, hydroids and anemones, sponges, mussels and saddle oysters, and filamentous algae. The total pooled species richness recorded from the CPCE analysis of photos at the end of the monitoring period was 36 taxa. Species richness was greater on horizontally orientated panels than vertically orientated panels, both at the start of the experiment (CPCE data, Pseudo-F=74.47, $p<0.001$) and at the end of the monitoring period (Microscope data, Pseudo-F=21.43, $p<0.001$).

Following the eight week settlement period, horizontal and vertical panels had significantly distinct community assemblages prior to the start of the experiment (ANOSIM R=0.3, $p<0.001$) (Figure C.4). Horizontal panels were most consistently characterised by higher average abundances of the encrusting bryozoan *Electra pilosa* and the colonial ascidian *Diplosoma listerianum* compared to vertical panel communities had higher abundances of the native hydroid belonging to the genus *Obelia* as well as showing greater variability in community similarity (PERMDISP F=36.5, $p(perm)<0.001$) (Table C.2). There were no pre-treatment differences in community similarity between salinity (ANOSIM R=-0.018, p=0.69) and exposure (ANOSIM R=-0.029, p=0.79) treatment levels.

There were ten suspected non-native species and cryptogenic species recorded in site E1; *Austrominius modestus*, the solitary ascidians *Asterocarpa humilis* and *Corella eumyota*, and the bryozoa *Fenestrulina delicia*, *Bugulina stolonifera*, *Schizoporella japonica*, and *Tricelleria inopinata*. The mobile non-native amphipod *Caprella mutica* was also recorded but was not included in the

analysis. The cryptogenic species were *Amathia gracilis*, *Bugulina fulva*, *Displosoma listerianum*.

4.3.1.2 Biofouling community response

Biofouling cover at the start of the experiment was significantly greater on horizontally orientated panels (Mean cover ± SE = 73.8 ± 18.2 %) than vertically orientated ones (57.9 ± 32.4 %), although there was greater variation on vertically orientated panels. On vertically orientated panels, there were no significant differences in bioufouling cover found between salinity treatments over the course of the recovery period (Pseudo-F=1.88, p=0.055), but significant differences in cover between salinity treatments were observed for horizontal panel communities (Pseudo-F=7.547, p<0.0001) (Table C.3).

Immersion in the low (10) and freshwater treatment caused a significant (p<0.01) reduction in biofouling cover on horizontal panels (Figure 4.6). One week post-treatment, cover on the horizontal panels had been reduced by 30% as a result of treatment with freshwater, and by 22% by the low salinity treatment. By the fourth week, recovery had begun, but cover on the freshwater treated panels was still significantly (p<0.01) lower than all other treatments, while cover on the low salinity treatment was significantly (p<0.0001) lower than on the ambient treated panels (Figure 4.6). Eight weeks after the experiment, there was no significant difference in mean cover between treatments. Microscope analysis of the panels confirmed that there were no significant differences in biofouling cover between treatments on either horizontal or vertical panels at the end of the recovery period (Pseudo-F=0.475, p=0.7).

Significant interaction effects of salinity level and duration of exposure to treatment were found for cover on horizontal panel communities (Pseudo-F=2.786, p=0.024). Pair-wise tests revealed that one week after treatment, cover was significantly lower on panels subjected to the medium (17) salinity treatment for four hours than those only exposed for one hour (t=2.313, p=0.0475). Panels subjected to the low (10) salinity treatment for two hours also had significantly lower cover both immediately after (t=3.052, p=0.0142) and one week after (t=4.493, p=0.024) treatment than those subjected to the treatment for four hours, likely due to pre-existing variation between panels (Figure C.5).

Multivariate analysis of community response indicated that while there was complete recovery of biofouling cover, community composition remained altered by low salinity treatment at the end of the monitoring period. Community composition was significantly affected by salinity treatment over the course of the recovery period on both horizontally (Pseudo-F=3.859, p<0.001) and vertically (Pseudo=F=2.495, p<0.001) orientated panels.

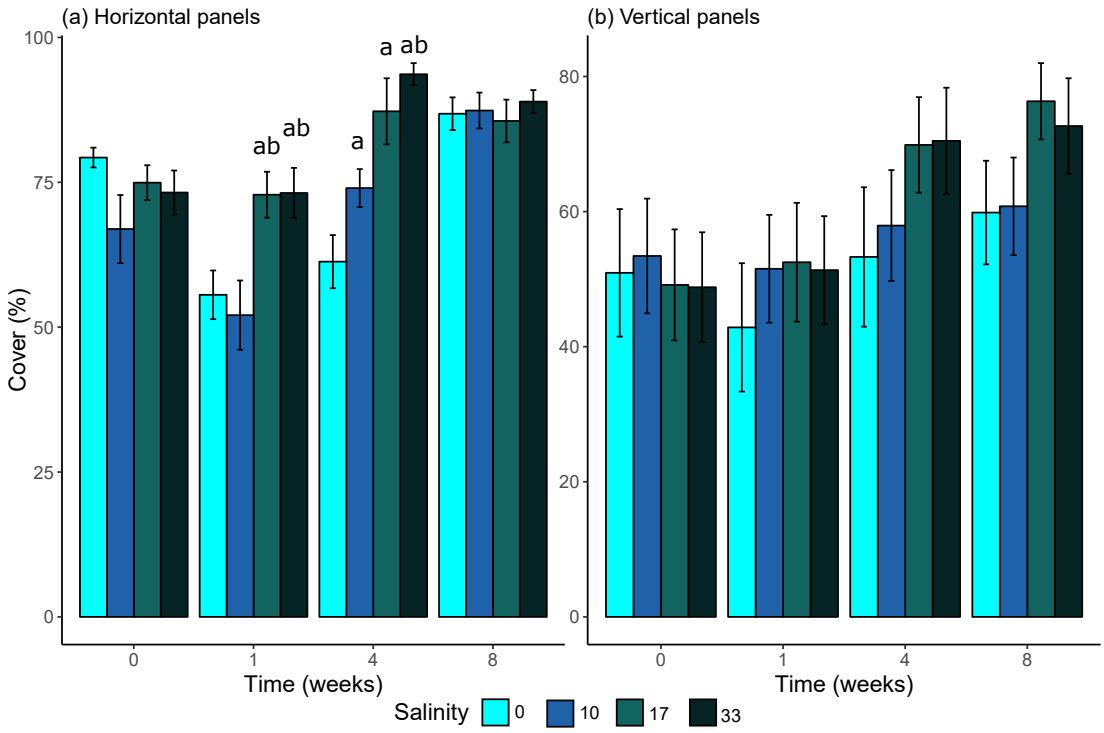


Figure 4.6: *Bar chart showing biofouling cover on horizontally (a) and vertically (b) orientated panels. The mean (±SE) percentage cover on panels exposed to different salinity treatments is shown at each recovery monitoring time point at 0,1,4 and 8 weeks post-treatment. Results are grouped across different exposure times. Significant pair-wise test results are indicated on the graphs (a - significant difference from freshwater treatment ($p<0.01$), b - significant difference from low (10) salinity treatment ($p<0.01$)).*

Significant differences between the lower (0, 10), and higher (17, 33) salinity treatments were seen by week one on horizontal panels, and by week four on vertical panels. These differences were still evident eight weeks after the experiment on panels of both orientations (Table C.5).

4.3.1.3 Response of non-native and cryptogenic species

Prior to the start of the experiment, the majority of biofouling cover was composed of native species (44.2 $\pm$ 26.6%), followed by cryptogenic species (22.6 $\pm$ 16.4%), with only an average 0.15 $\pm$ 0.44% cover of NNS. Salinity treatment had no significant effect on NNS cover. Native species cover was significantly affected by treatment (Pseudo-F=7.088, p<0.001), horizontal panels subject to freshwater treatment had significantly lower cover of native species than other salinities, four (p<0.05) and eight (p<0.001) weeks after treatment (Figure 4.7a). Pooled cryptogenic and non-native species cover was significantly affected by salinity treatment on both horizontal (Pseudo-F=8.109, p<0.001) and vertical panels (Pseudo-F=4.97,p<0.001). On horizontal panels low salinity and freshwater treatment caused cover to decline in the first week. However, by the end of the monitoring period cover had recovered on the low salinity treated panels, and was significantly higher on the freshwater treated panels compared to other salinity treatments. On vertical panels, cover was significantly higher on the mid- and ambient salinity treated panels in weeks 4 and 8 (Figure 4.7c). These results were confirmed by microscope analysis of panels at the end of the eight-weeks monitoring period. Cover of non-native and cryptogenic species was dominated by the colonial tunicate *Diplosoma listerianum*, which accounted for over 88% of the total non-native and cryptogenic species cover at the end of the monitoring period.

The cryptogenic *Diplosoma listerianum* showed a decline in cover on the freshwater and lower-salinity treated panels in the first week compared to the higher salinity treatments. Recovery on the freshwater-treated horizontal panel communities following the initial decline led to these panels having significantly higher cover of *D. listerianum* compared to the other treatments by week eight (Figure 4.8). There was no effect of treatment with different salinities on the mean percentage cover of the non-native bryozoans *Schizoporella japonica* (Pseudo-F=1.44, p=0.82), or *Tricellaria inopinata* (Pseudo-F=1.349, p=0.22).

4.3.1.4 Response of individual taxa

Species richness was significantly affected by salinity treatment on horizontal (Pseudo-F=2.767, p<0.01) but not vertical panels, with low and freshwater treatment causing a decrease

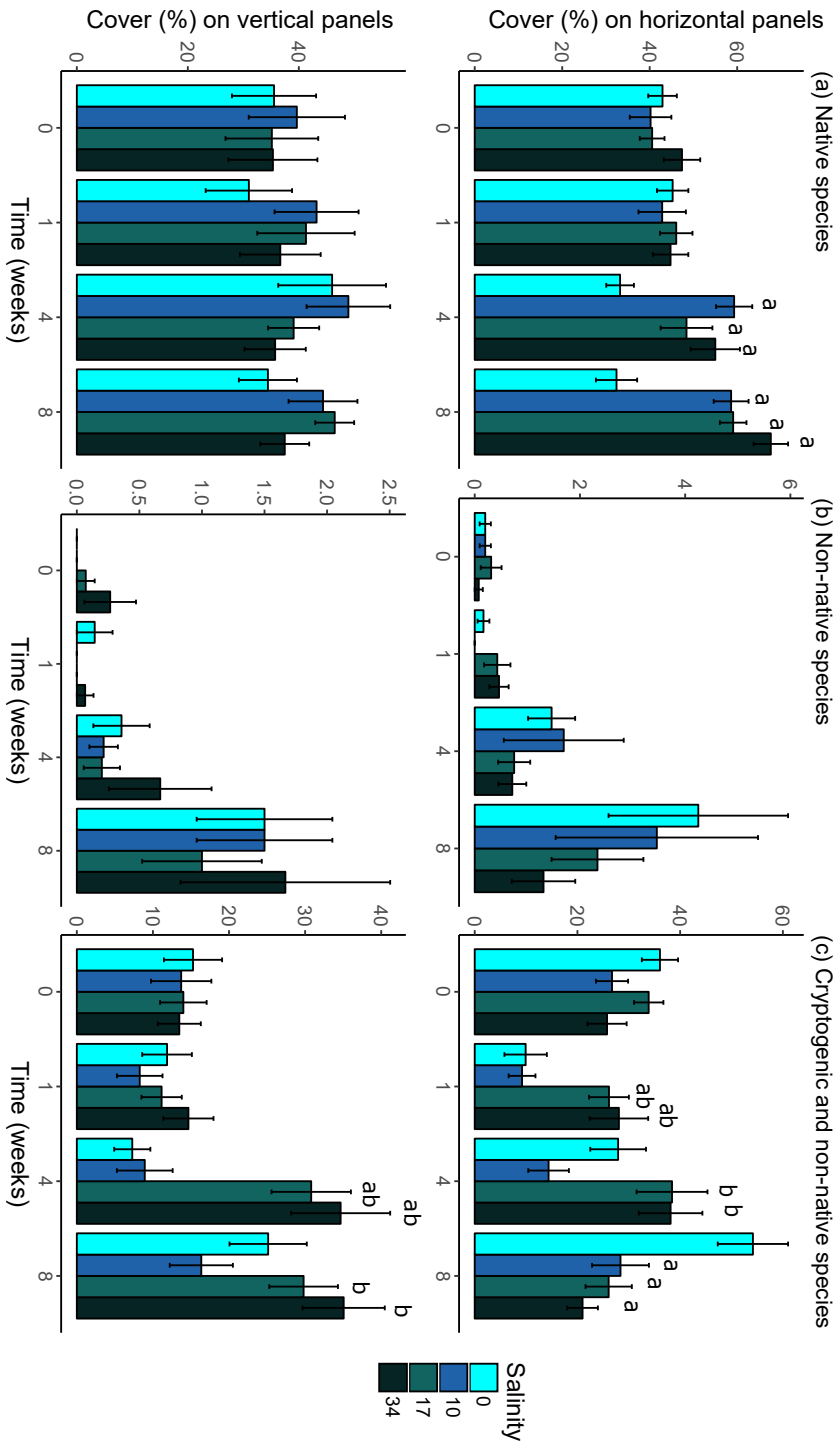


Figure 4.7: *Bar chart showing mean (±SE) percentage cover of (a) native, (b) non-native, and (c) cryptogenic and non-native species over the course of the monitoring period on horizontally and vertically orientated panels, exposed to different salinity treatments. Results are grouped across different exposure times as no interaction effect of exposure time and salinity was found in the RM-ANOVA. Significant pair-wise test results are indicated on the graphs (a - significant difference from freshwater treatment (p<0.05), b - significant difference from low (10) salinity treatment (p<0.05)).*

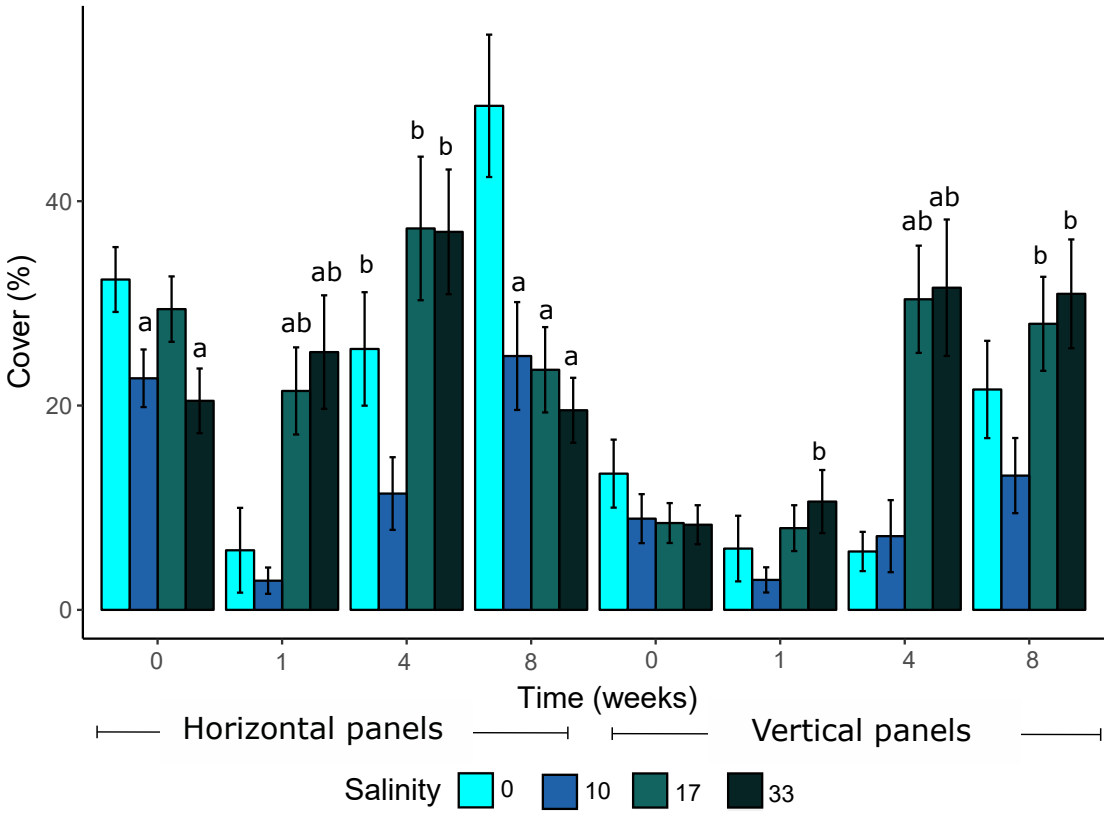


Figure 4.8: *Bar chart showing cover of Diplosoma listerianum on horizontally and vertically orientated panels. The mean (±SE) percentage cover on panels exposed to different salinity treatments is shown at each recovery monitoring time point at 0,1,4 and 8 weeks post-treatment. Results are grouped across different exposure times as no interaction effect of exposure time and salinity was found in the RM-ANOVA. Significant pair-wise test results are indicated on the graphs (a - significant difference from freshwater treatment (p<0.05), b - significant difference from low (10) salinity treatment (p<0.05)).*

in the number of species after one week (Figure C.6). Different taxa responded to treatment in different ways, shifting community composition on low salinity treated panels. Ascidian species were the most detrimentally affected by immersion in low salinities, with treatment causing significant differences in cover of both solitary (Pseudo-F=7.311, $p<0.001$) and colonial (Pseudo-F=4.967, $p<0.001$) ascidians on horizontal and vertical panels over the recovery period. Ascidian specimens showed an immediately observable response to lower salinity treatments, taking on a whitish appearance visible in the photographs taken immediately after the experiment. Low salinity and freshwater treatment of panels caused a significant decrease in ascidian cover in the week following treatment as ascidian species died and fell off the panels (Figure 4.9). Solitary ascidian cover increased over the monitoring period on the medium and ambient treated panels, such that in comparison cover on low and freshwater treated panels was significantly lower at the end of the monitoring period (Figure 4.9a). Colonial ascidians showed greater recovery from dieback following freshwater and low salinity treatment on horizontal panels compared to vertical panels (Figure 4.9b). At the end of the monitoring period cover of colonial ascidians was significantly higher on the freshwater-treated horizontal panels than all other treatments.

Significant differences in cover of other taxa were observed during the recovery period at the four and eight week monitoring points as a result of the interaction between panel orientation and salinity treatment, specifically for serpulids (Pseudo-F=5.056, $p<0.001$), encrusting bryozoa (Pseudo-F=6.075, $p<0.001$) and macroalgae (Pseudo-F=1.997, $p<0.05$) (Figure C.7). At these monitoring points, significant differences in serpulid cover were observed between the extreme treatments (0, 33) and the mid-range treatments (10, 17) on horizontal panels, and between the freshwater and all other treatments on the vertical panels. Cover of encrusting bryozoa was significantly higher on low (10) salinity treated horizontal panels. There was significantly higher algal cover on the freshwater treated panels compared to the ambient treated panels. Some taxa showed no response to salinity treatments. Cover of hydroids and arborescent bryozoans declined over time but was not significantly different affected by salinity treatment or panel orientation.

4.3.1.5 Recruitment during the recovery period

Blank control panels were set out at the same time as experimental panels were returned to the pontoons post-treatment. Biofouling cover and species richness on blank control panels increased over the recovery period as species settled and grew on both horizontal and vertical

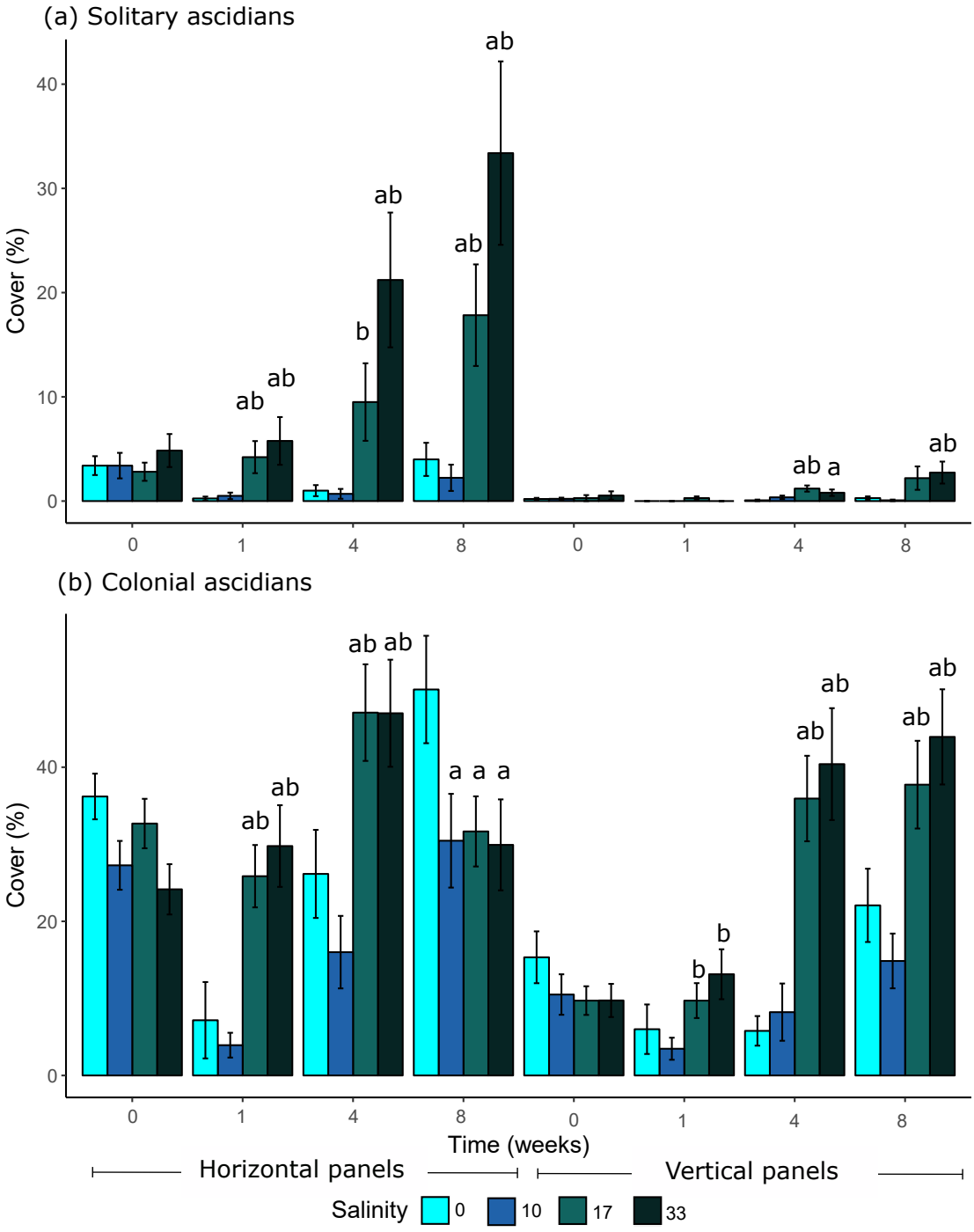


Figure 4.9: *Bar chart showing (a) solitary and (b) colonial ascidian cover on horizontally and vertically orientated panels. The mean (±SE) percentage cover on panels exposed to different salinity treatments is shown at each recovery monitoring time point at 0,1,4 and 8 weeks post-treatment. Results are grouped across different exposure times as no interaction effect of exposure time and salinity was found in the RM-ANOVA. Significant pair-wise test results are indicated on the graphs (a - significant difference from freshwater treatment ($p<0.05$), b - significant difference from low (10) salinity treatment ($p<0.05$)).*

panels (Figure C.8). Twenty-seven species had recruited on horizontal panels, and fifteen on vertical panels at the end of the recovery period, but the only species with >1% mean cover were *Diplosoma listerianum* (45 $\pm$ 24.13%) and *Electra pilosa* (30.6 $\pm$ 26.52%) on horizontal panels and *D. listerianum* (34 $\pm$ 34.1%) and grouped macroalgae (7.61 $\pm$ 7.7%) on vertical panels (Figure C.9). Rates of species detection were lower in the analysis of photographs compared with the microscope analysis, recording only 10 and 11 species (pooled total = 18) on horizontal and vertical panels respectively.

4.3.2 Experiment 2: Freshwater immersion experiment

4.3.2.1 Environmental monitoring

Salinity varied between sites and over the course of the monitoring period (Table 4.1). Salinity in site A remained stable over time and throughout the water column with an average surface salinity of 33.81 (range 32.62-33.93) (Figure 4.10a). In site B, lower salinities were observed down to 7m deep and the average surface salinity of 28.77 (range 24.49 - 32.10) is below the expected salinity values for coastal waters (30-34 PSU) (Figure 4.10b). In site C, when freshwater input is high the water column is highly stratified and a visible freshwater layer forms at the water surface, which has been observed to reach 0.5 m deep. The average surface salinity in site C was 23.365 (range 8.89-31.90) with the lowest salinities recorded in the surface water (Figure 4.10c). Salinity also varied across site C, with the lowest salinities recorded near the river mouth. Higher salinities are observed in both sites B and C in September when the lowest rainfall was also observed in the west of Scotland (data from Dunstaffnage rainfall gauge).

The average sea surface temperature over the 16 week period in site A was 13.27 °C (12.21-14.54), 12.99°C (12.12-13.76) in site B, and 12.98 °C (11.7-14.01) in site C. Temperature in sites A and B increased from mid-August through to mid-October, reaching a maximum of 14.5 °C in site A, and around 14 °C in sites B and C (Figure C.10).

4.3.2.2 Biofouling communities

Biofouling communities differed between the three sites, with significant differences in the composition of communities which had settled on panels prior to the start of the experiment following eight weeks of recruitment (ANOSIM R=0.915, p<0.001). Communities also varied between panel orientations in each site (ANOSIM R=0.488, p<0.001) (Figure C.11). Following

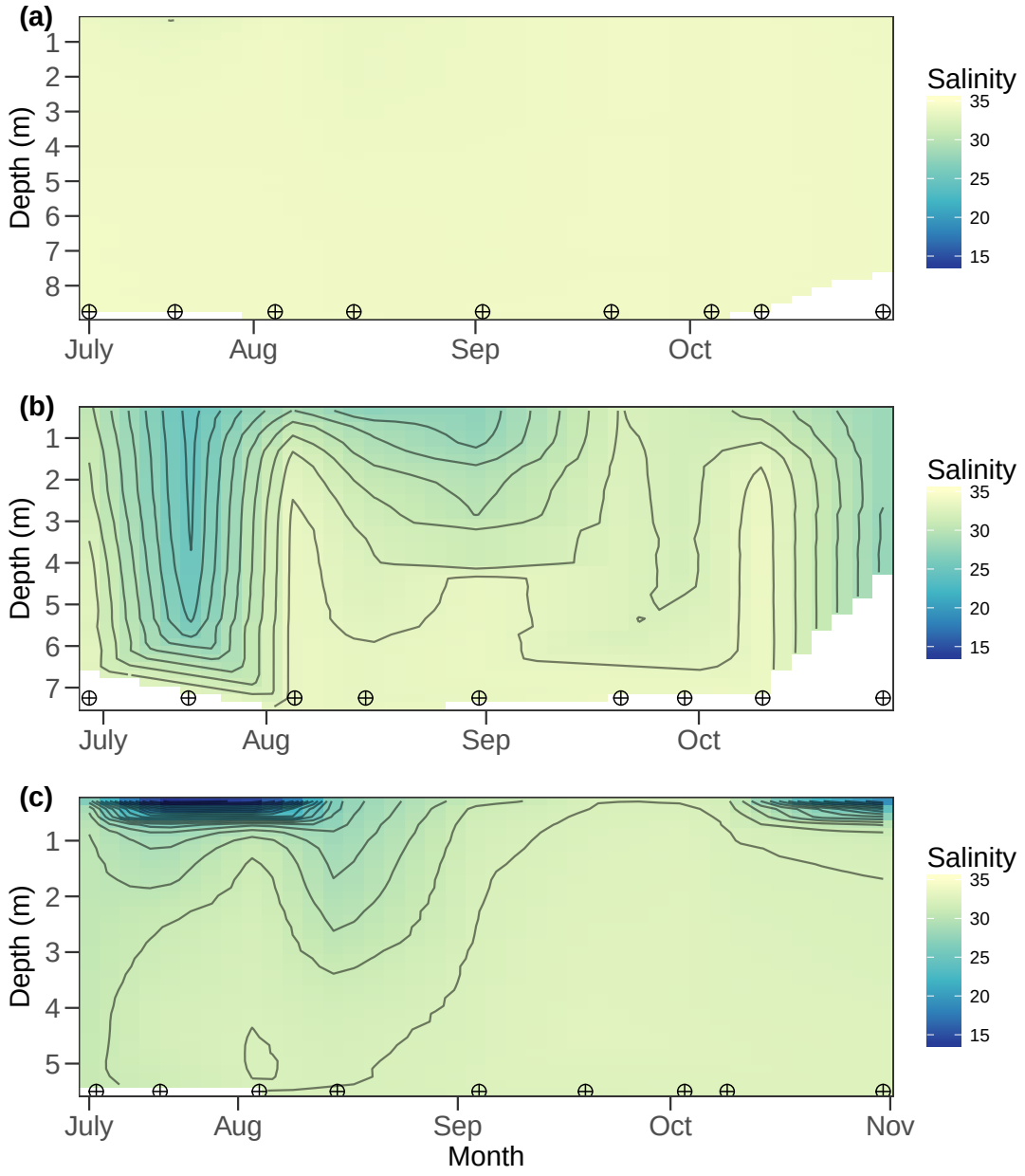


Figure 4.10: *Figure showing the salinity depth profile of sites A (a), B (b), and C (c). Salinity data is interpolated from 8 CTD measurements taken every 2 weeks over 4 months (exact sample time is indicated by* $\oplus$ *). Contour lines represent a 1 unit change in salinity.*

eight weeks settlement, cover on panels in site A was dominated by solitary ascidians (>50% cover) on horizontal panels and by colonial asicidans (>30% cover) on vertical panels, with both arborescent and encrusting byrozoans also present in high numbers. Vertical panels also had a greater amount of uncolonised space. In site B, there was a higher dissimilarity between panels of each orientation (56.23%), with a high abundance of brown filamentous algae on vertical panels, and of the encrusting bryozoan *Electra pilosa* on horizontal panels. In site C, panels were dominated by the non-native hydroid *Cordylophora caspia*, with vertical panels also having high abundances of brown filamentous algae.

Microscope analysis of panels at the end of the experiment identified 36 non-mobile fouling taxa in sites A and B, and 14 taxa in site C (see Tables C.6, C.7, and C.8 for full species lists). In site A most species were either ascidians (10 species) or bryozoans (13 species) and panels were dominated by three species, *Ascidiella aspersa*, *Botrylloides schlosseri*, and the cryptogenic *Diplosoma listerianum*. Site B also had high numbers of ascidians (11 species), bryozoans (7 species), and hydrozoans (7 species), while panels were dominated by the encrusting bryozoan *Electra pilosa*, brown filamentous algae, and the saddleoyster *Anomia epphipium*. There were far fewer species overall in site C, with only three ascidian species and four bryozoans, with panels dominated by filamentous brown algae, the arborescent bryozoan *Amathia gracilis* and the non-native hydroid *Cordylophora caspia*. Barnacles, and serpulid keel worms were present at all sites.

There were ten non-native and cryptogenic species identified across all sites in the microscope analysis of panels, nine at site A, five at site B, and four at site C. These totals included the invasive amphipod *Caprella mutica* which was found in both site A and B, but was not included in experimental analysis as it is a mobile species. The non-native barnacle *Austrominius modestus*, and the cryptogenic *A. gracilis* was found at all three sites. The non-native solitary ascidian *Corella eumyota* and cryptogenic colonial ascidian *Diplosoma listerianum* were found at sites A and B. At site A, there were also three non-native (*Bugulina simplex*, *Schizoporella japonica*, and *Tricellaria inopinata*) and two cryptogenic bryozoans (*A. gracilis*, *Bugulina fulva*). The non-native hydrozoan *Cordylophora caspia* was only found at site C.

4.3.2.3 Effect of treatment on biofouling cover

Treatment with freshwater caused significant loss of biofouling cover on both horizontal (Pseudo-F=40.225, $p<0.001$) and vertical (Pseudo-F=24.338, $p<0.001$) panels in site A. In the four weeks following treatment, 47% of biofouling cover was lost from horizontal panels,

and 59% from vertical panels (Figure 4.11a). Ambient-treated panels showed no decline in cover, and still showed significant differences with freshwater-treated panels 8 weeks after the experiment. Multivariate analysis of community similarity showed that treatment caused significant differences in community composition on both horizontal and vertical panel communities from one week after the experiment to the end of the monitoring period (H: Pseudo-F=14.082, $p<0.001$; V: Pseudo-F=7.016, $p<0.001$). On vertical panels there were also differences in community assemblage between treatments at the first monitoring time point. The analysis of the microscope data confirmed that differences in community assemblages between treatments and orientations were still present at the end of the monitoring period (Psuedo-F=3.573, $p<0.01$).

In site B, a significant effect of treatment was only observed on horizontal panels (Pseudo-F=38.495, $p<0.001$). Biofouling cover did not show the same losses in response to freshwater treatment as in site A, instead mean cover increased over time (from 47% to 86%) on ambient panels, but not on freshwater-treated panels (Figure 4.11b). In site B, treatment with freshwater affected community composition on horizontal panels but not vertical panels (Pseudo-F=3.732=3, $p<0.001$), with significant differences observed from the first week to the end of the monitoring period. Microscopy results showed significant differences in community similarity between treatments and orientations but no interaction between the two (Tr: Pseudo-F=5.505, $p<0.001$; Or: Pseudo-F=11.778, $p<0.001$).

In site C, significant differences in biofouling cover between treatments were present on horizontal panels (Pseudo-F=3.68, $p<0.05$) but not vertical panels immediately after treatment and 1 week following treatment (Figure 4.11c). Similarly, significant differences in community similarity between treatments in site C were only observed on horizontal panels immediately after the experiment and at the one week count (Pseudo-F=3.485, $p<0.01$), and not on vertical panels. However, microscopy results do identify significantly different communities between treatments and orientation, although with no interaction between the two (Tr: Pseudo-F=3.586, $p<0.01$; Or: Pseudo-F=21.239, $p<0.001$).

4.3.2.4 Response of non-native and cryptogenic species

In site A, native and cryptogenic species dominated cover on panels at the start of the experiment while cover of NNS was lower, contributing to less than 10% of the total cover. Freshwater treatment resulted in significantly lower native species cover from the one week monitoring point onward (Figure 4.12(a)). Treatment with freshwater caused significant loss of

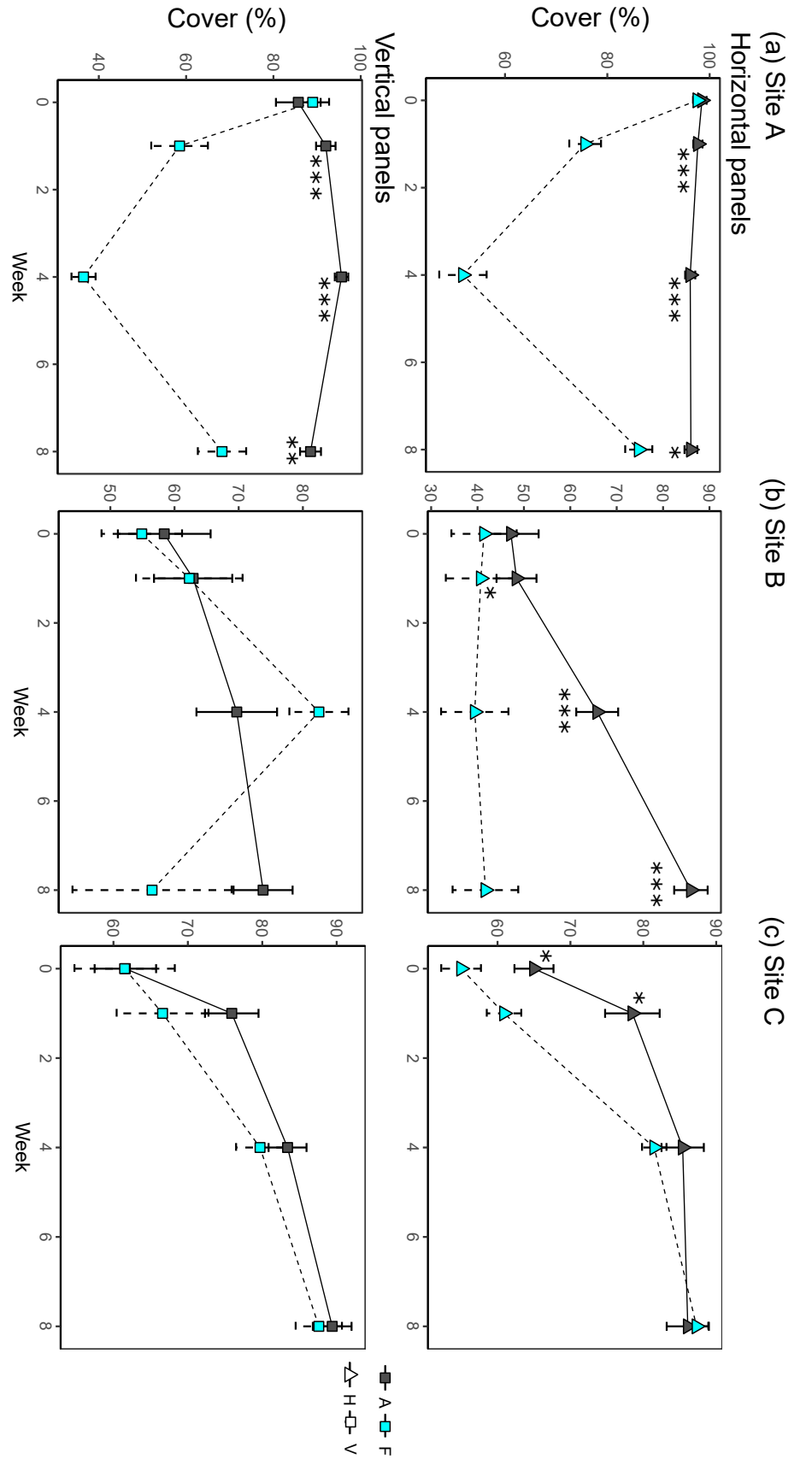


Figure 4.11: *Line graph showing biofouling cover in site A (a), site B (b), and site C (c) on horizontal and vertical panels. The mean (±SE) percentage cover on panels exposed to the freshwater (F) and ambient control (A) treatment is shown at 0,1,4 and 8 weeks post-treatment. Significant pair-wise test results are indicated on the graphs (*p<0.05, **p<0.01, ***p<0.001).*

combined cover of cryptogenic and NNS up to four weeks post-treatment (H: Pseudo-F=6.486, p<0.001; V: Pseudo-F=6.675, p<0.01), although by the end of the monitoring period there were no longer significant differences between treatments (Figure 4.12(a)). Cryptogenic cover in site A was dominated by *Diplosoma listerianum*, which was significantly affected by treatment (TrxTi, Pseudo-F=6.47, p<0.001), with freshwater treatment causing a significantly greater decline in *D. listerianum* cover over the four weeks post-treatment (Figure 4.13). There were no significant differences in NNS cover between treatments, but cover of NNS increased for both treatments over the course of the monitoring period and significant differences were observed for individual species. Treatment and panel orientation resulted in significant differences in the cover of two non-native bryozoans in site A, *Schizoporella japonica* (TrxTixOr, Pseudo-F=4.6, p<0.01) and *Tricellaria inopinata* (TrxTixOr, Pseudo-F=5.28, p<0.01) over the monitoring period. Response differed between the two species, while freshwater treatment resulted in an increase in cover of the encrusting *S. japonica* over time, it prevented further growth of the arborescent *T. inopinata* (Figure 4.13).

Cover of non-native and cryptogenic species was much lower than native cover in site B. There was no effect of treatment on non-native and cryptogenic species, only on native species on horizontally orientated panels (Pseudo-F=24.159, p<0.001), where freshwater treatment resulted in significantly lower cover at the four and eight week monitoring counts (Figure 4.12(b)). There was no effect of treatment on the cryptogenic species *Amathia gracilis*, and abundances of the non-native *Austrominius modestus* and cryptogenic *D. listerianum* were too low (<1% cover) to be analysed.

In site C, non-native and cryptogenic species had much higher cover than native species, due to the dominance of the non-native hydrozoan *Cordylophora caspia*, which on average accounted for 82.4% cover on horizontal panels, and 29.4% on vertical panels at the start of the experiment. There were significant differences in NNS and cryptogenic species cover on horizontal panels immediately after treatment and at week 1 (Pseudo-F=6.25, p<0.01) (Figure 4.12(c)). Looking at individual NNS, cover of *C. caspia* and the cryptogenic *A. gracilis* varied between horizontal and vertical panels (*C. caspia*: pseudo-F=58.18, p<0.001; *A. gracilis*: pseudo-F=32.18, p<0.001), but was not affected by freshwater treatment. The non-native barnacle *A. modestus* increased in cover on freshwater treated panels, but not on ambient treated panels (pseudo-F=7.445, p<0.001) irrespective of panel orientation (Figure C.12(c)).

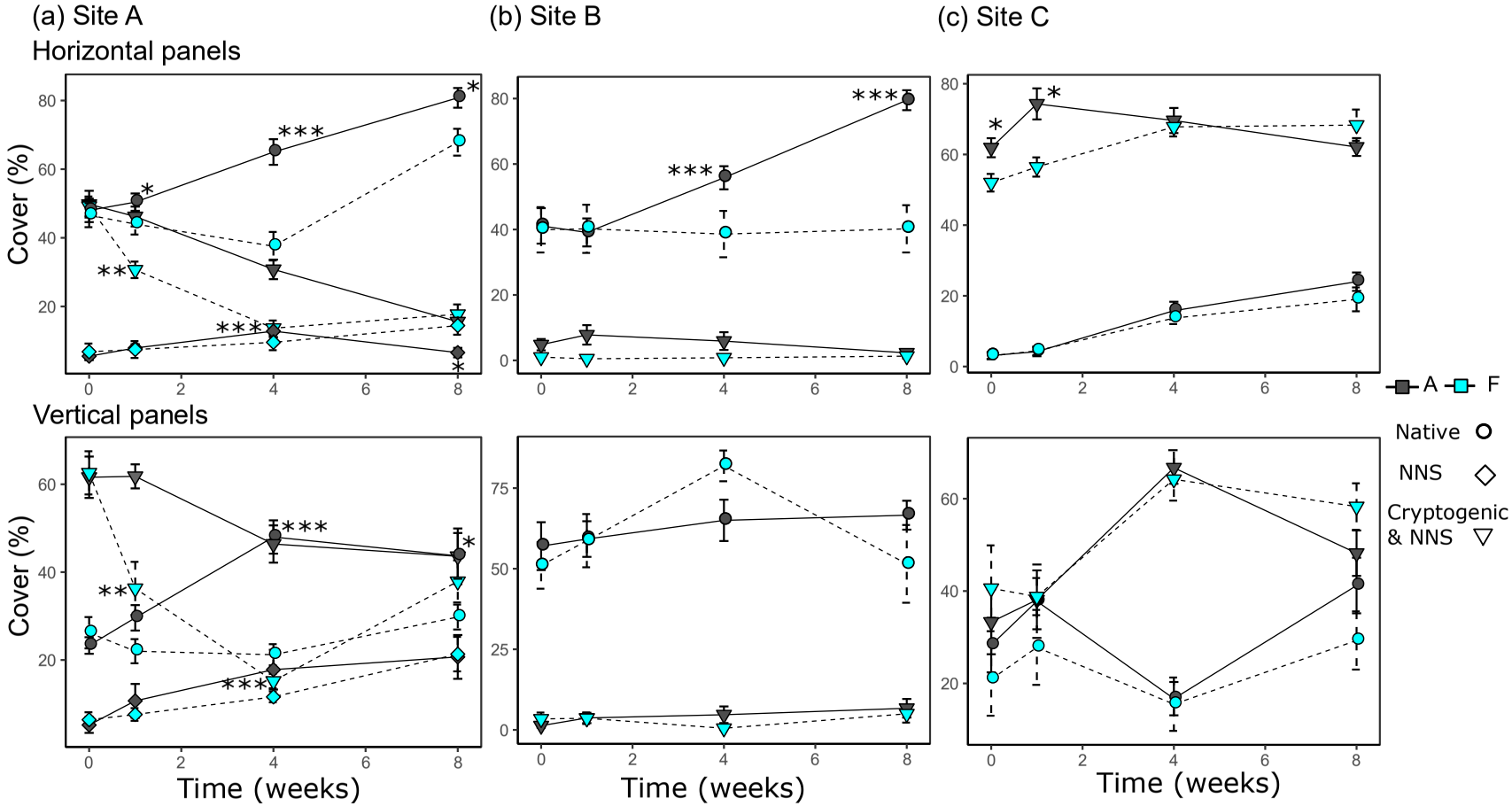


Figure 4.12: *Line graph showing cover of native, non-native and cryptogenic species in site A (a), site B (b), and site C (c) on horizontal and vertical panels. The mean (±SE) percentage cover on panels exposed to the freshwater (F) and ambient control (A) treatment is shown at 0,1,4 and 8 weeks post-treatment. Significant pair-wise test results are indicated on the graphs (*p<0.05, **p<0.01, ***p<0.001).*

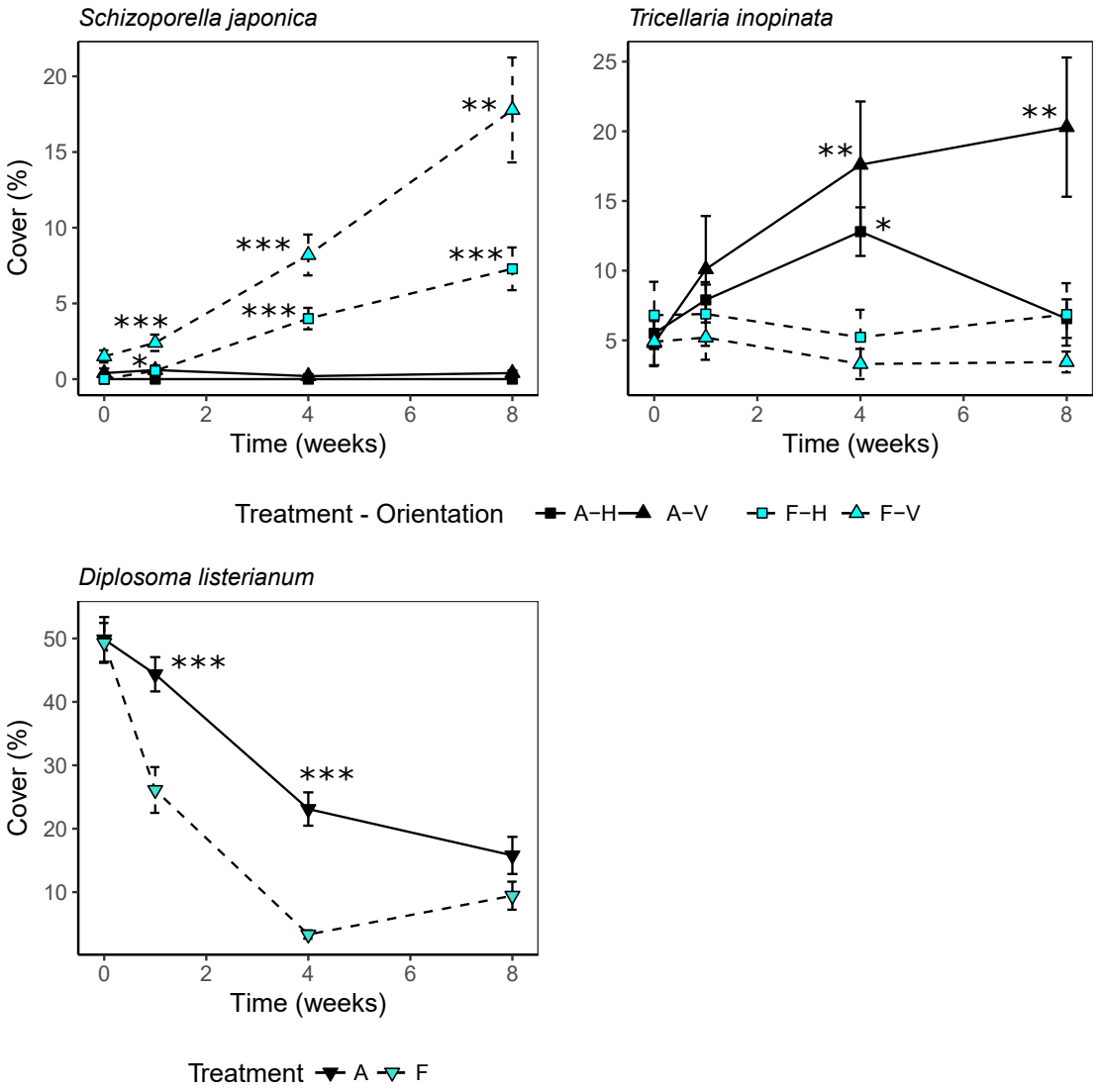


Figure 4.13: *Figure showing treatment effect on mean percentage cover of Schizoporella japonica (a), Tricellaria inopinata (b) and Diplosoma sp. (c) in site A. Treatment is classed as ambient seawater (A) or as freshwater (F), and orientation of panels in the field is shown as horizontal (H = △) or vertical (V = □). Cover is merged between panel orientations for Diplosoma sp. as there was no significant difference in cover between horizontal and vertical panels. Significant results of pairwise t-tests are shown for treatment (c): *p<0.05, **p<0.01, ***p<0.001.*

4.3.2.5 Response of individual taxonomic groups

Species richness differed significantly between freshwater and ambient treatments on horizontal panels in site A (Pseudo-F=4.329, p<0.05) and on vertical panels in site B (Pseudo-F=5.334, p<0.01), while there was no impact of treatment on species richness in site C (Figure C.13). Freshwater treatment caused an increase in species richness over time relative to the ambient treatment in site A, whereas in site B species richness increased on ambient but not freshwater treated panels (Figure C.13). The microscope analysis of panels from site A confirmed that freshwater treated panels had a significantly higher species richness irrespective of panel orientation at the end of the monitoring period (Tr: Pseudo-F=25.391, p<0.001), with an average of 19.8 $\pm$ 2.3 species on freshwater treated panels compared to 15.4 $\pm$ 2.8 species on ambient treatment panels. In site B, the microscope data showed significant differences in species richness between horizontally (14.1 $\pm$ 3.9) and vertically (10.5 $\pm$ 4.4) orientated communities but no difference between salinity treatments (Or: Pseudo-F=7.197, p<0.05).

Ascidian species were the taxon most affected by freshwater treatment in sites A and B, although ascidian abundances in site C were too low for analysis (<1% mean cover). Freshwater immersion caused large losses in cover of solitary ascidians in sites A and B (Figure 4.14), resulting in significant differences between treatments over time (A: Pseudo-F=12.356, p<0.001; B: Pseudo-F=4.88, p<0.01). For colonial ascidians, freshwater treated panels in Site A had significantly lower cover compared to ambient treated panels one and four weeks after treatment (Pseudo-F=4.324, p<0.001, Figure 4.15). Cover of colonial ascidians in site B was too low for assessment (<1% mean cover).

There were significant differences in cover of encrusting bryozoa between treatments over time in site A (Pseudo-f=6.061, p<0.01)and site B (Pseudo-F=16.301, p<0.001). Encrusting bryozoans showed a similar response in site A as in site E1 (see Section 4.3.14), with a large increase in cover over time on freshwater-treated panels but not on ambient-treated (Figure C.14). Freshwater treatment only significantly affected cover of arborescent bryozoa in site A (Pseudo-F=5.228, p<0.01), where cover increased over time on ambient treated panels but not on freshwater-treated panels.

Cover of serpulid worms was significantly affected by treatment in sites A (TxOrxTi Pseudo-F=5.214, p<0.01), B (TxOrxTi Pseudo-F=3.25, p<0.05), and C (TrxTi Pseudo-F=6.154, p=0.001), but showed different responses to freshwater treatment in each site (Figure C.15).

Some taxa were unaffected by freshwater immersion in all sites, with no significant

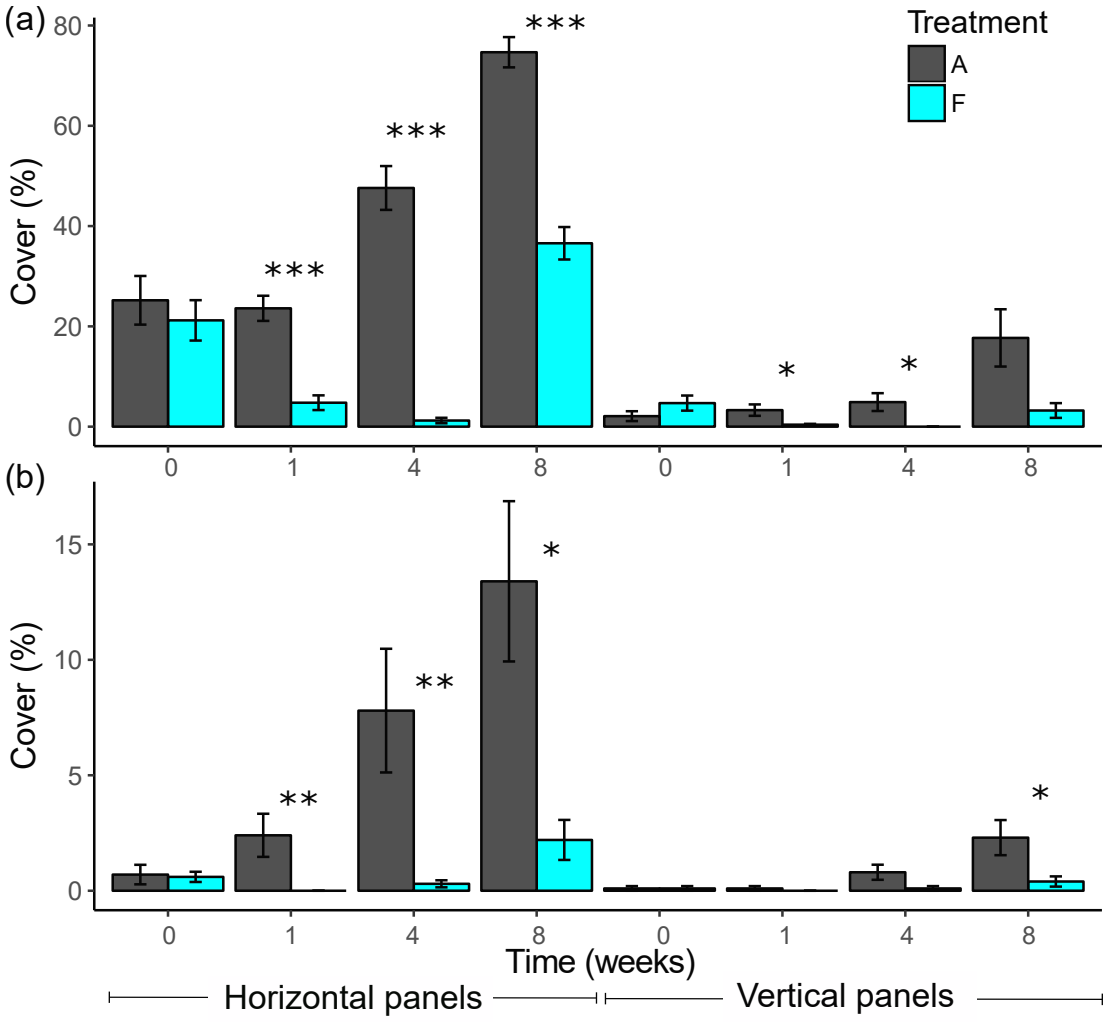


Figure 4.14: *Bar chart showing mean (±SE) percentage cover of solitary ascidians in sites A (a) and B (b) on horizontally and vertically orientated panels respectively. Cover is shown at each recovery monitoring time point at 0,1,4 and 8 weeks post-treatment. Significant results of pairwise t-tests between treatments at each time point are shown: *p<0.05, **p<0.01, ***p<0.001.*

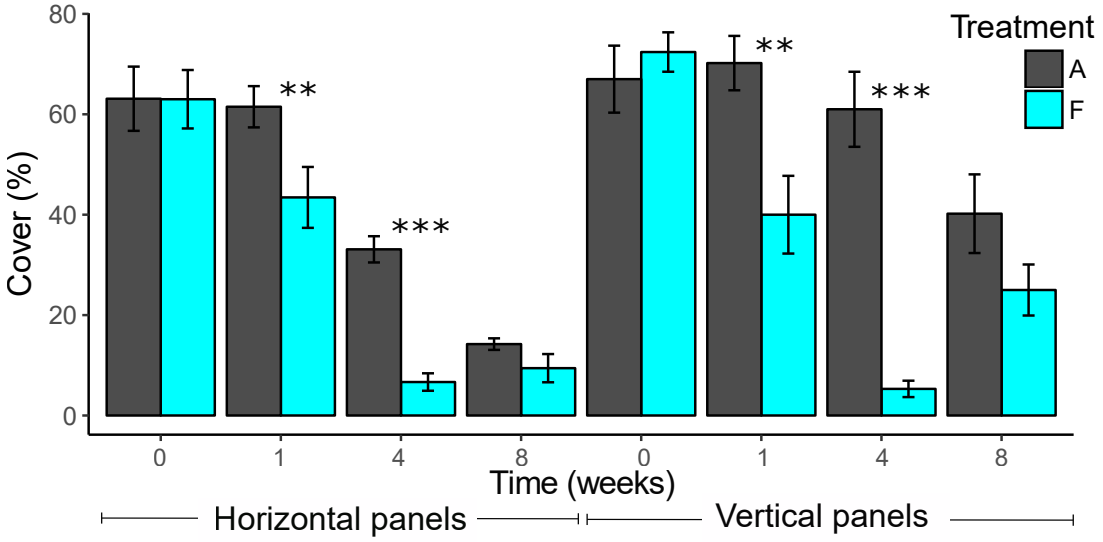


Figure 4.15: *Bar chart showing mean (±SE) percentage cover of colonial ascidians in site A on horizontally and vertically orientated panels. Cover is shown at each recovery monitoring time point at 0, 1, 4 and 8 weeks post-treatment. Significant results of pairwise t-tests between treatments at each time point are shown: *p<0.05, **p<0.01, ***p<0.001.*

differences in cover between treatments (Cnidaria, Macroalgae). Across all sites, macroalgae were present predominantly on vertical compared with horizontal panels, and only in site B were significant differences in cover observed between treatments on vertical panels (Pseudo-F= 2.876, p<0.05) four weeks after the experiment. Cover of other taxa was generally too low for trends to be detected.

4.3.2.6 Recruitment during the recover period

Settlement on blank control panels was assessed to determine which species were recruiting to bare substrate during the recovery period. Cover and species richness increased over time in all sites (Figure C.16). In site A, analysis of panels under the microscope showed that 22 species had recruited to horizontal panels by the end of the monitoring period and 21 to vertical panels (pooled total = 26 species). Twelve species contributed at least 1% cover at the end of the monitoring period. Space was dominated by *Diplosoma listerianum* which covered on average over 69% of available space on horizontal panels, and 86% on vertical panels. On horizontal panels, the next most abundant species were *Electra pilosa* and *Botryllus schlosseri*, and on vertical panels *Tricellaria inopinata* and *Obelia spp.* (Figure C.16). In site B, 23 and 9 species had recruited to horizontal and vertical panels respectively (pooled total = 25) by the end of the recovery period, with 8 species contributing at least 1% average cover. Space

was dominated by *Anomia epphipium* (18.2 $\pm$ 8.59%) on horizontal panels and by filamentous algae on vertical panels (31.72 $\pm$ 38.82%) (Figure C.16). In site C, 12 species had recruited to panels by the end of the recovery period, of which 7 had on average more than 1% cover. On horizontally orientated panels, the non-native barnacle *Austrominius modestus* had the highest average cover (16 $\pm$ 8.33%), while filamentous algae only recruited to vertical panels where they accounted for 28.8 $\pm$ 29.2% of space (Figure C.16).

4.4 Discussion

This study investigates the effectiveness of short-term immersion in low salinities as a tool for removing biofouling and reducing spread of certain fouling non-native species.

4.4.1 Low salinity immersion as a tool to remove biofouling

Immersion in low-salinity water (≤ 10) caused fouling species to die and slough off horizontal panels, resulting in reduced biofouling cover and species richness in the four weeks following treatment. Freshwater treatment resulted in loss of 29% of total cover, while low salinity treatment resulted in a loss of 22% in the week following the experiment. There were no differences in cover detected between the mid-salinity and ambient treatments, or between the low (10) and freshwater treatments in the first week following treatment. The lack of differences between the low (10) salinity and freshwater treatments suggest that for the durations tested, using completely freshwater may not be necessary to achieve treatment aims. This increases the possible uses of low salinity treatments, as de Castro et al. (2018) were able to show that salinities as low as 7 could be achieved by pumping freshwater into sea chests. They also showed that treatment with water at 7 PSU was sufficient to kill most fouling species when they were immersed for 2 hours, while treatment with 20 PSU was largely ineffective (de Castro et al., 2018).

Increasing exposure time from one hour to two or four hours had no effect on the impact of the low and freshwater salinity treatments. While differences between panels subject to immersion in the low salinity water for two and four hours were observed immediately and one-week after treatment, these differences were likely due to pre-existing variation in cover between panels as effects of treatment on cover were not immediately detectable. Duration of treatment did influence the impact of immersion in the mid-range (17) salinity treatment, with significant lower cover found on four-hour treated panels compared to one-hour treated panels at the one week monitoring point. This suggests that if higher salinity is used, the length of treatment may need to be increased. For instance, Minto et al. (2015) found that immersion in water with a salinity of 24 ppt caused a decrease in biofouling cover following three days of treatment, although greater reductions were observed following immersion in 12 ppt.

The differences in response between the different salinity treatments used in this study

suggest lowering salinity to below 10 rather than extending the duration of treatment would be a more efficient way to increase removal of biofouling. However, results of individual species tests do show large variation in the duration of exposure to freshwater required for 100% mortality to be achieved. Denny (2008) found that dipping *Didemnum vexillum* in freshwater for just two minutes resulted in 74% mortality, increasing to 87% mortality if the treatment duration was 10 minutes. However, other species can tolerate up to 48 hours immersion in low salinities (Forrest and Blakemore, 2006). The treatment times used in this study were chosen based on results of single species studies (McCann et al., 2013; NSPMMPI, 2013; Moreira et al., 2014), and also represent timings which would not cause undue costs to boat owners and reflect natural low salinity periods which might occur within one tidal cycle (Roche et al., 2015). Nevertheless, combining very low salinity (<10) immersion with exposure times >24 hours would likely decrease the likelihood of species surviving treatment.

Biofouling community composition differed between panel orientations, as has been observed in other studies of fouling communities (Glasby, 2000; Glasby and Connell, 2001). The lower face of horizontal panels was dominated by *Diplosoma listerianum* and *Electra pilosa*, while on vertical panels hydroids belonging to the genus *Obelia spp.* were dominant. Response to low salinity treatment varied between panel orientation, with no significant effect of low (10) and freshwater treatment on overall biofouling cover or species richness detected on vertical panels. However, differences in community composition between lower and higher salinity treatments were observed on vertically orientated panels four and eight weeks post-treatment. This was likely caused by loss of solitary and colonial ascidians, as significantly lower cover of these taxa was also detected on vertical panels (Figure 4.9). Thus, low salinity treatments did cause loss of biofouling organisms on vertical panels, but this was not detectable in analysis of overall cover. Across both orientations, there was a lag time between an organisms' death and the time required for an organism to slough off the panels. Evidence of discoloured or decaying solitary and colonial ascidians and empty bryozoan skeletons was visible on panels one week after treatment, and these organisms still occupied a large amount of space on panels which may have prevented settlement of new larvae on the panel substrate (de Castro et al., 2018). Vertical panels initially had much lower overall cover than horizontal panels, and thus had a higher availability of bare substrate on which taxa could settle immediately after panels had been returned to the pontoons post-treatment. New recruitment on vertical panels may thus have counteracted losses of affected taxa. Biofouling cover on vertically orientated panels was also more variable than on horizontal panels, which might have prevented detection of

trends (Clarke et al., 2014).

4.4.2 The effect of environmental salinity variation on freshwater treatment

Replication of freshwater treatment in different sites showed that community response to treatment was dependent on the environmental characteristics of each site. Brief (1 hour) exposure to freshwater can reduce biofouling in sites where natural salinity variation is low. Where environmental variability is higher, communities show greater tolerance to episodic salinity stress. Sites E1 and A had similar salinity profiles with little variation in salinity over the 16 week monitoring period, and little variation with increasing water depth. These sites were expected to receive minimal freshwater input, and recorded salinities matched the expected values for well-mixed coastal waters in this area (Conor et al., 2006). In Site B, the average salinity was on the lower end of the expected salinity values for coastal waters, which ranges from 30 to 34 on the west coast of Scotland (Conor et al., 2006). During periods of high freshwater influence, salinities as low as 24 were observed down to the bottom of the water column. Water is relatively well mixed at this site, potentially due to strong current flow in the bay and the influx of mixed water from the nearby sea loch (Ashton et al., 2010). The highly stratified salinity profile observed in site C is likely a result of the highly enclosed nature of the marina design, reducing water circulation and exchange (Floerl and Inglis, 2003; Rivero et al., 2013). Salinity in the surface waters reaches below 10, and the species found at this site are those which are capable of surviving short reductions in salinity.

Community response to freshwater immersion in Site A was similar to that observed for the lower salinity treatments in Site E1. Freshwater immersion caused significant losses of fouling species on both horizontal and vertical panels in site A, reducing biofouling cover and species richness over the four weeks post-treatment. Freshwater immersion also resulted in loss of biofouling cover in site B, although a response was only detected on horiontal panels. Cover of fouling species was much lower in site B in general, and much more variable on vertical than horiontal panels. Response may also have been underestimated in site B due to the dominance of the encrusting bryozoan *Electra pilosa*. The calcareous exoskeleton of encrusting bryozoans remains even when the zooids have died, and as response was only evaluated through examination of photographs, counts of *E. pilosa* cover may have included individuals which did not survive freshwater treatment. Comparison of sequential photos of the same panel post-analysis reveal that by the eight-week monitoring point, *E. pilosa* colonies on freshwater-treated panels had sloughed off panels while on ambient-treated panels colony

size had increased (Figure C.2).

In Site C, communities showed little response to freshwater. Differences in cover between the freshwater and ambient treated panels were observed immediately after and one-week following treatment, but it is likely that these were due to pre-existing variation between panels as no immediate effect of treatment was detected in site E1, where communities would be expected to show a larger response to low-salinity treatment than the site C community. Species growing at shallow depths in this marina are exposed to large changes in salinity due to the presence of high volumes of freshwater. However, some brackish species may still struggle with sudden exposure to freshwater (salinity < 0.5) (David et al., 2013; Moreira et al., 2014; Smyth and Elliott, 2016).

4.4.3 Community recovery post-treatment

Low salinity treatment resulted in differences in community assemblage between low and freshwater salinity treated panels and the 17 and ambient treatments in site E1, and between freshwater and ambient treatments in sites A and B. These differences in community assemblage persisted until the end of the monitoring period. This was driven by variation between species tolerance to osmotic shock and different recruitment patterns for species during the recovery period. Episodic environmental stress has previously been shown to cause long term changes in community composition (Sorte et al., 2010a,b). In San Francisco bay, salinity drops in years with very high rainfall have been shown to change the dominant species in fouling communities the following summer (Chang, 2009; Chang et al., 2018).

Tolerance to environmental stress varies between taxa and individual species (Dijkstra and Simkanin, 2016; Hauton, 2016). Taxa with shells or exoskeletons within which they can retreat are more likely to be able to survive episodic environmental stress (Smyth and Elliott, 2016). Mussels and bryozoans were observed to be able to survive hypoxic conditions created by encapsulation for up to four days longer than other fouling taxa, in experiments conducted by Atalah et al. (2016). Soft-fouling species are likely to be easier to remove, and our results demonstrated this. Ascidian species were most affected by low salinity treatment across all sites and showed an observable response to lower salinity treatments, taking on a whitish appearance visible in the photographs taken immediately after the experiment. Both colonial and solitary ascidians showed high mortality following immersion in the low salinity and freshwater treatment. Solitary ascidians did not recover to the same levels as on ambient salinity panels. This had significant effects on community composition, as growth on ambient

control panels in sites E1 and A was dominated by solitary ascidians, primarily *Ascidiella aspersa* and *Ciona intestinalis*.

Solitary ascidians have been shown to dominate fouling communities and suppress overall diversity (Chang, 2009). Experiments by Chang (2009) showed that when disturbance results in the loss of solitary ascidians, space was opened up for the recruitment of other species. This was replicated in our study in site A and E1, where treatment with freshwater caused solitary ascidians to die and fall off panels, creating open space for recruitment and growth of encrusting byrozoa, serpulid worms and colonial ascidians. In site E1, the colonial ascidian *Diplosoma listerianum* and the encrusting bryozoan *Electra pilosa* both showed high recruitment rates on the blank control panels as well as showing high recovery on the low-salinity treated panels. Thus even though cover of colonial ascidians was greatly reduced by low salinity treatment in site E1, recruitment post-treatment resulted in increased cover of this taxa relative to the higher salinity treatments. Solitary asicidians showed higher recovery rates in site A than site E1, accounting for nearly 40% cover on horizontal freshwater treated panels at the end of the recovery period. In site B, recovery rates were lower overall, potentially due to the longer delay in time for encrusting bryozoa to slough off panels, leaving less time for new species to settle. As species settlement can vary on a weekly basis and shows high levels of seasonality (Berntsson and Jonsson, 2003; Nall, 2015), the timing of treatment will likely affect which species colonise bare space created by low-salinity immersion.

4.4.4 Response of non-native and cryptogenic species

The sites with low freshwater influence (A and E1) had higher numbers of non-native and cryptogenic species, 10 in site E1 and 9 in site A. However, cover of NNS was higher in Site C due to dominance of the biofouling community by *Cordylophora caspia* and *Amathia gracilis*, which are tolerant of brackish conditions (Meek et al., 2012; Folino-Rorem and Renken, 2018). It is possible that there are fewer native species capable of living in these high disturbance environments, leaving vacant niches which non-native species can colonise (Paavola et al., 2005). Research suggests that *C. caspia* is not a single species but multiple cryptic species, with different genotypes adapted for freshwater and brackish conditions, and each genotype has had multiple introductions around the world (Folino-Rorem et al., 2009).

Low salinity treatment had no effect on overall cover of NNS in any sites, although the low volume and high variability in NNS cover on panels in sites E1 and B might have obscured treatment effects. The pathways by which introduced species are spread may exert

selection pressure for organisms which have broader physiological tolerances or are better able to recover following disturbance events (Piola and Johnston, 2008; Gröner et al., 2011; Cook et al., 2015; Pauli and Briski, 2018). In terms of tolerance to salinity changes, some NNS have been shown to have broader salinity ranges and be better able to recover from salinity shock than related native species (Gröner et al., 2011; Cook et al., 2015). Testing the response to episodic salinity stress, Lenz et al. (2011) found that NNS displayed higher survival rates and less deviation from normal respiratory levels under stressful conditions.

In site A, some NNS were affected by freshwater treatment, but response differed between *Schizoporella japonica* and *Tricellaria inopinata* such that these patterns were obscured when species were pooled by their introduction status. Cover of *T. inopinata* increased on ambient panels but not on freshwater treated panels, while cover of *S. japonica* significantly increased on freshwater treated panels but not on ambient control panels. In site E1, no significant effect of treatment was observed for either *S. japonica* and *T. inopinata*, but cover of both species was much lower at the time treatment was applied. As a group, studies into the effect of heat-shock have shown non-native bryozoans to be particularly resistant to environmental stress (Wasson et al., 2001). *Schizoporella japonica* is tolerant of salinities from 15 to 36, and thus may be able to survive short periods of freshwater immersion (Loxton et al., 2017). The loss of dominant ascidian species likely reduced competition for food and space, enabling growth of the encrusting bryozoan.

The colonial ascidian *Diplosoma listerianum* accounted for the majority of cryptogenic species cover in sites E1 and A, and response of this species drove the trends observed when pooled cryptogenic and NNS response was analysed. The other cryptogenic species, the arborescent bryozoan *Amathia gracilis* showed no differences in cover between treatments in sites E1, B, or C and cover was too low in site A for analysis. Low salinity treatment resulted in most colonies of *D. listerianum* dying and falling off panels, with cover reduced by freshwater treatment one to four weeks post-treatment. After this, recruitment of new zooids meant that cover of *D. listerianum* increased on low salinity treated panels, such that by the end of the monitoring period the freshwater-treated panels had significantly higher cover of this species than all other treatments. That this species recovered as a result of new settlement, not growth of surviving colonies is suggested by the high levels of recruitment of *D. listerianum* to the blank panels set out at the same time as the experimental panels were returned to pontoons. The capacity of *D. listerianum* for rapid growth may enable it to more rapidly expand on bare substrate following disturbance events (Altman and Whitlatch, 2007; Vance et al., 2009;

de Castro et al., 2018).

4.4.5 Low salinity as a management tool

Hypo-saline treatment of fouled vessels is envisioned as an effective way to reduce the risk of moving NNS between areas, as part of a wider biosecurity plan. The results of this study suggest that low salinity treatments could be useful in removing biofouling from vessels or static structures, especially of soft fouling species in environments with low salinity variation. Treatment times would have to be increased from those used in this study in order to ensure that hard-fouling taxa were also removed. However, patterns of species recovery suggest that treatment could render vessels more susceptible to colonisation by other NNS following treatment. The timing of treatment may thus be crucial to achievement of biosecurity management goals (de Castro et al., 2018).

Low salinity treatments could nevertheless play a useful role in removing biofouling from vessels pre-departure in order to prevent further spread of species. This would result in vessels leaving before most taxa have sloughed off, reducing opportunities for recolonisation. Low salinity treatments could also be used as part of biosecurity plans for marina operators, to treat equipment and hard infrastructure that has arrived from other sites. As species have been shown to be capable of surviving transport over land for a number of days (Kauano et al., 2016), marina operators should follow similar guidance as aquaculture operators to increase biosecurity at a site. Guidelines to the aquaculture industry in Australia recommend a combined treatment of a two hour period of freshwater immersion followed by 12 hours of dessication to remove unwanted fouling species (NSPMMPI, 2013).

Low salinity immersion has also been investigated as a tool for eradicating or controlling specific marine invasive NNS (McCann et al., 2013; Moreira et al., 2014; Jute and Dunphy, 2017). Response to osmotic shock is species specific and thus using freshwater immersion to eradicate an species requires knowledge of individual species tolerances to determine the salinity and duration of exposure required to achieve 100% mortality (Moreira et al., 2014). Salinity tolerance tests conducted to investigate potential range limits or to profile risk of species invasion may be useful in guiding decisions on whether freshwater immersion may be an appropriate management tool for specific species (Steen, 2004; Epelbaum et al., 2009; Gröner et al., 2011; Keller et al., 2011; Bollen et al., 2016; Rocha et al., 2017). However, the increase in cover of *Diplosoma listerianum* and *Schizoporella japonica* in sites E1 and A following freshwater treatment shows that the disturbance could instead facilitate growth of non-target

non-native species.

The pathways by which introduced species are spread may exert selection pressure for organisms which have broader physiological tolerances or are better able to recover following disturbance events (Piola and Johnston, 2008; Gröner et al., 2011; Cook et al., 2015; Pauli and Briski, 2018). In terms of tolerance to salinity changes, some NNS have been shown to have broader salinity ranges and be better able to recover from salinity shock than similar native species (Gröner et al., 2011; Cook et al., 2015). Testing the response to episodic salinity stress, Lenz et al. (2011) found that NNS displayed higher survival rates and less deviation from normal respiratory levels under stressful conditions. This study found that while *D. listerianum* was susceptible to low salinity treatment, settlement of new propagules meant this species was able to increase its abundance on the bare space that was created as a result of the disturbance. In the case of *S. japonica*, colonies survived treatment and the removal of dominant competitive species allowed for growth during the recovery period. This highlights the need for continued monitoring following treatment and the potential necessity of repeat treatments for more tolerant NNS (Holt and Cordingley, 2011).

4.4.6 Conclusions

The use of lowered salinity as a tool to reduce spread of fouling NNS was trialled using manipulative field experiments. Treatment with salinities lower than 10 significantly reduced biofouling cover on horizontally oriented panels. The duration of time panels were exposed to treatment only affected panels exposed to the 17 salinity treatment. Nevertheless, longer or repeated treatments with low salinities (<10) would be necessary to target more tolerant hard-fouling taxa. Effectiveness of freshwater treatment has been shown to vary between sites with different levels of environmental freshwater input, with brackish species in site C showing little response to treatment. Response and recovery varied between species and taxa, with treatment resulting in shifts in community assemblage on affected panels that persisted until the end of the recovery period. This loss of dominant solitary ascidians in sites E1 and A led to cover of the non-native encrusting bryozoan *Schizoporella japonica* and cryptogenic *Diplosoma listerianum* increasing on freshwater-treated panels, indicating that treatment could have unintended consequences as NNS may be better able to recover following disturbance. Timing of treatment may thus be a very important consideration in order to achieve biosecurity management goals, and hypo-saline treatment may be more appropriate to rid vessels of biofouling pre-departure or when applied within an enclosed environment to treat high-risk

vessels (Roche et al., 2015; de Castro et al., 2018). The potential for facilitation of NNS should also be considered when hypo-saline treatments are used to target specific invasive NNS species, as eradication treatments are often carried out in environments where more than one NNS may be present.

CHAPTER 5

General Discussion

This study has investigated fouling non-native species (NNS) in marinas in Scotland. Studies from around the world have shown that artificial structures in urbanised environments like harbours and marinas can support fouling communities characterised by a high prevalence of NNS (Bulleri and Chapman, 2010). High vector activity in harbours and marinas combined with availability of hard substrate for settlement and high levels of physical, chemical and environmental disturbance make these sites highly susceptible to NNS establishment (Ruiz et al., 2009). These sites can thus be a useful focal point for investigations into marine NNS, allowing detection of newly arrived species and investigation into the biological and abiotic factors which might affect establishment and spread.

This study has investigated the distribution and abundance of fouling NNS within and between sites, to update baseline information on NNS spread in Scotland and to identify patterns in NNS establishment over time and across the country. This study focused on a fundamental environmental parameter, salinity, which affects the biological processes of all aquatic organisms, native and non-native species alike. The applications of natural salinity variation and experimentally induced salinity change for managing marine NNS in Scotland were investigated using field surveys and experiments.

Prior to this thesis, low salinity ports and marinas in Scotland had been suggested as low risk sites for the establishment of marine NNS (Payne et al., 2014). However, no studies had investigated the distribution or abundance of NNS in these sites, with previous studies of NNS in marinas in the UK excluding sites suspected to have low salinities (Foster et al., 2016). There is great variability in salinity conditions along Scotland's coasts due to high rainfall, large input from rivers and restricted water flow between Scottish lochs and open water (Marine Scotland, 2011). However, the salinity conditions experienced in individual marinas may be affected by a number of factors include river discharge, current and tidal

dynamics, the degree of openness or enclosure of a site by seawalls (Mineur et al., 2012; Rivero et al., 2013; Johnston et al., 2017). Investigating the salinity conditions within marinas and their affect on marine fouling community composition and vulnerability to invasion has not been well studied anywhere in the world, including in the UK. Where the influence of salinity has been incorporated into investigations of habitat invasibility in harbours and marinas, methods used have been insufficient to characterise spatial and temporal variability. This study has aimed to address this through incorporation of geographic data and hydrodynamic model data, as well as examination of native fouling community composition.

The work presented has addressed the central objectives (Section 1.4) of this study as follows:

- *Chapter 2:* Assessed patterns of individual non-native species establishment and spread in Scotland over the last decade.
- *Chapter 3:* Analysed marina fouling community composition to assess native and non-native species diversity and the influence of salinity variation and other abiotic factors on NNS establishment.
- *Chapter 4:* Examined the efficacy and limitations of short-term treatment with hyposaline water as a management tool for reducing biofouling and controlling NNS.

The following sections summarise and discuss the main findings of each chapter, putting the outputs into a more global context. Recommendations on ways in which monitoring and surveillance efforts could be improved to better meet legislative requirements are provided, and the opportunities offered by post-arrival control and management are highlighted. A description of the limitations of this study and opportunities for future research needed to further inform these decisions concludes this chapter.

5.1 Summary of findings

5.1.1 Chapter 2: Characterising non-native species in Scottish marinas

Rapid assessment surveys (RAS) were conducted in eighteen mainland Scottish marinas, resurveying eight sites first visited in 2006 and providing baseline data for seven new sites. Records were compiled from unpublished data, the online National Biodiversity Network (NBN) database and published literature to update a synthesised list of marine non-native species records.

- The surveys conducted in this study provided 80 new presence records for eighteen non-native and one cryptogenic species in Scottish marinas. This is triple the number of fouling NNS recorded in 2006-2008. Of the 30 targeted species, six bryozoans, four ascidians, five macroalgae and four other invertebrate species were identified. The number of targeted species recorded in a single marina ranged from two to ten species.
- Since 2006, nine new marine non-native species have been recorded in Scotland. The most recent arrival is *Undaria pinnatifida* for which there is currently only one record in the Firth of Forth. A NNS not targeted in the 2016 RAS, the bryozoan *Fenestrulina delicia*, was recorded on settlement panels set out in 2015. This represents only the third record of this species in Scotland and the first in a marina. Other recent arrivals, the bryozoans *Schizoporella japonica* and *Tricellaria inopinata*, have undergone rapid spread and were among the top five most frequently found species.
- The eight marinas first surveyed in three sequential years from 2006-2008 were resurveyed in 2016. In these sites, the average number of NNS increased from 2.0 to 5.2. This represents the introduction of 1.6 species every five years, a rate comparable to that seen in other marinas in England and Wales. The average number of species in each site is now higher than the maximum number of species recorded in one site in 2006-2008.
- A review of published literature, survey data, and citizen science records collected in the last five years resulted an additional 1019 records being added to a synthesised list of Scottish marine and brackish NNS records. This resulted in location records for 32 marine NNS, and three cryptogenic species. Twenty-two species are considered established or probably established, an increase of seven species since the last synthesis

of Scottish marine NNS in 2012. The greatest number of new records have come from Orkney, where annual monitoring has contributed over 400 new presence records.

This chapter updates baselines information on the distribution of NNS in Scotland. Furthermore, compilation of marine non-native records in one place allows comparison of species distribution maps and can be used to direct future monitoring and surveillance effort and to inform meaningful decisions about biosecurity and pathway management.

5.1.2 Chapter 3: The influence of salinity on variation in marina fouling communities

This study aimed to investigate patterns in NNS diversity among sites with different levels of freshwater inflow. Field surveys were conducted to collect data on the composition of fouling communities on floating pontoons, NNS presence on artificial structures, and the environmental conditions within marinas. Information on marina design, location, and river input was sourced from satellite images and online sources, to determine which anthropogenic and environmental features influenced non-native and native species diversity among sites.

- Forty-four taxa were identified from photographs of fouling communities across seventeen sites, the majority of which were native species. Pontoon floats had high biofouling cover in most sites, with fouling assemblages in all marinas, with the exception of one site, dominated by native species cover. The most abundant taxa across all sites were macroalgae and the edible mussel *Mytilus edulis*, and the most abundant NNS were *Cordylophora caspia*, *Schizoporella japonica* and *Tricellaria inopinata*. The abundance of NNS in most sites is relatively low, but investigation of the impact of *Cordylophora caspia* dominance in one site in the Clyde (C7) may be warranted.
- Fouling assemblages differed significantly among marinas. The pooled species richness ranged from 5 species in the least diverse site to 28 species in the most diverse. Non-native and cryptogenic species richness within sites ranged from 1 to 10 species. Sites with high native diversity also had high non-native species diversity.
- Species composition patterns for the whole community assemblage, and when only native species were considered, were best correlated with one environmental variable, the distance of a site from the nearest river. River width was not significantly correlated with fouling community composition. Differences among fouling communities were not influenced by the location, distance to nearest port or temperature.

- Clustering of sites by similarity of their native species assemblage divided sites into groups which could be characterised by their distance from the nearest river. Sites with high freshwater influence (i.e. less than 150 m from a river), were characterised by low species diversity, high abundances of filamentous brown and green algae, hyrdoids, and the presence of the non-native species *Cordylophora caspia*. Sites further than 375 m from a river had high species diversity of both native and non-native species, and could be distinguished by the presence of native taxa such as large abundance of kelp, red filamentous algae and green flat algae. Mid-range sites C3 and C4 were dominated by *Mytilus edulis* and could be further distinguished from higher salinity sites by the absence of red filamentous algae, and lower abundances of encrusting bryozoa.

- The NNS presence/absence data from the RAS was only correlated with salinity metrics (i.e. distance from the nearest river and the minimum salinity identified by the Scottish Shelf Model). NNS richness also rose with increasing salinity, calculated from transformed CTD measurements taken in each site. The only NNS found in very low salinity sites were the brackish hydroid *Cordylophora caspia* and the barnacle *Austrominius modestus*.

- When only the NNS community composition was assessed using data obtained through the photo-quadrat surveys, similarity between sites was correlated with river distance, marina depth at low tide, and the number of berths. The abundance of NNS within a site may thus be influenced by additional factors. Number of berths is a metric representing the size and connectivity of a marina. These differences in patterns of NNS richness and abundance warrant further investigation, as dominance of a species once it has established can occur in both low- and high salinity sites.

The diversity of native and NNS in fouling communities in Scottish marinas is primarily driven by environmental conditions. The distance to the nearest river, salinity data generated by the Scottish Shelf Model, and the composition of the native species community could be used to identify sites vulnerable to species invasion, using minimal information obtainable without the need for site visits. This information could be incorporated into national or regional biosecurity plans, or used to develop a 'watch' list of species likely to arrive at a particular site.

5.1.3 Chapter 4: Experiments investigating hypo-saline treatment of fouling communities

In this study, two experiments were conducted to investigate and compare responses of native and non-native fouling species to treatment with hypo-saline water. The first experiment investigated the effectiveness of different salinities and duration of treatment on reducing biofouling cover. The second experiment investigated whether response to treatment with freshwater varied between fouling communities with different levels of environmental tolerance, as a result of variation in freshwater inflow.

- Treatment with salinities lower than 10 PSU significantly reduced biofouling load, resulting in loss of 20 - 50 % of biofouling cover.
- No significant effect of increasing treatment duration from one to four hours was seen for the lower salinity treatments, with the only difference observed between the one and four hour mid-salinity (17) treatments. Nevertheless, repeated exposure or increasing treatment duration by a significant amount would likely be necessary to target more tolerant taxa.
- Recruitment and growth of tolerant taxa led to cover increasing to pre-treatment levels relatively rapidly (<8 weeks), but the composition of community assemblages remained altered at the end of the monitoring period compared with ambient salinity treated assemblages. This is a result of response to salinity treatments varying between taxa, with dominant ascidian species most affected by treatment with hypo-saline water.
- As a group, non-native species were not affected by treatment, although an effect of hypo-saline treatment on percentage cover was observed for individual non-native species. Response varied between species, with survival of some species decreased by immersion in low salinities, while other species showed greater growth on low salinity treated panels.
- Response to freshwater treatment varied between sites, suggesting that consideration of the community tolerance to environmental change is important when planning treatment strategies. Fouling community response to freshwater treatment was significantly greater in sites with less freshwater influence.

The ability of some NNS to quickly recolonise and grow following disturbance could, therefore, lead to hypo-saline treatment having unintended consequences, facilitating growth

of tolerant NNS by reducing the cover of dominant native species or by increasing availability of resources such as space. Nevertheless, hypo-saline treatment represents a useful method of removing biofouling load, as long as due consideration is given to the timing of treatments and environmental tolerance of the treated community.

5.2 Discussion of findings

5.2.1 Spread of marine NNS in Scotland

The number of invasive NNS in British and European waters is increasing (Minchin et al., 2013). Scotland now has over twenty-two established marine and brackish NNS, with presence records for another ten (Chapter 2). Over five of these species have been introduced in the last decade. However, in comparison with the rest of the UK, the total number of Scottish NNS remains low, as does the average number of NNS found in each marina (Minchin and Nunn, 2013; Bishop et al., 2015). It has been suggested that cooler sea surface temperatures (SST) in Scottish waters may restrict the distribution of some NNS, and the distance from mainland Europe and lower vector activity may also factor into reducing secondary spread from NNS introduced to major European ports (Nall et al., 2015). However, resurveyed sites demonstrate that the average number of NNS in marinas is increasing, at a similar level of increase as sites in the English channel (Bishop et al., 2015). Rising SST may be causing some species to spread northwards, increasing the environmental range within which a species can survive and reproduce. The warming of Scottish coastal waters, shown to have increased by 0.4-0.5 °C per decade over the last 25 years, is thought to be the reason why the Pacific oyster *Magallana gigas* may now be able to spawn succesfully in Scotland (Shelmerdine et al., 2017; Hopkins et al., 2010) .

The average contribution of NNS to overall fouling community composition is far lower than has been documented in other temperate regions around the world. Choi et al. (2016) suggested a threshold value of 60% when assessing if the contribution of NNS to the overall community assemblage was likely to cause significant impacts, based on observations of noteable changes in productivity, richness and resource availability when NNS abundance exceeded this level. In a survey of Canadian marinas, over half the sites surveyed from the Atlantic coast exceeded this threshold, although only 1 of those on the Pacific coast did (Choi et al., 2016). In this study, the highest percentage contribution of NNS was of *C. caspia*, which reached 54% of the overall fouling cover in site C7, a very low salinity site, and accounted for 11% of total cover in site C4, a low salinity site. The two sites with the next highest contribution of NNS were both marine sites, C2 and W2, which also had far higher NNS richness. Thus high contributions of NNS to the overall community were found at both high and low salinity sites. NNS richness was significantly lower in low salinity sites, but represented a similar

percentage of the overall species diversity, due to native and NNS diversity following similar patterns among sites.

There are relatively few studies to provide comparative data, but in San Francisco bay where there is wide salinity variation, NNS were found to represent up to 78% of total species richness in hard substrate communities in low salinity environments, and 79% of species richness in high salinity environments (Jimenez et al., 2017). In soft sediment communities, the percentage of total species diversity accounted for by NNS was 40 and 60% in low and high salinity sites respectively (Jimenez et al., 2017). In hard substrate communities, NNS also occurred in a higher percentage of samples than native species did, accounting for 28% in high salinity and 37% of low salinity occurrences in samples (Jimenez et al., 2017). Thus while fewer NNS may establish in low salinity environments, once a species has established there is still potential for impact on native species. While low salinity sites may not represent good spots for detection of novel species invasion, research into NNS establishment in these environments should not be discounted. Further work to establish if these species are recruiting into natural systems, and how increased abundance of NNS might be changing interactions with resident species is required to determine fouling NNS impact in Scotland.

5.2.2 Impact of invasive species in Scotland

While there have been few quantifiable negative impacts on the environment in Scotland so far (Davison, 2008), among the species established are a number known to have caused significant impacts in other areas of their introduced range. The rapid spread of *Schizoporella japonica* and *Tricellaria inopinata* around Scotland pose risks to native biodiversity, as they are capable of overgrowing other organisms and displacing native bryozoans (Cook et al., 2013b; Loxton et al., 2017). *Codium fragile ssp. fragile* and *Sargassum muticum* are found in natural habitats along the west coast of Scotland (Trowbridge and Todd, 1999), and are known to compete with and overshadow native macroalgae and seagrass in other areas of their introduced range (Britton-Simmons, 2004; Watanabe et al., 2009). No evidence of quantitative impact in Scotland has been identified so far (Davison, 2008).

The identification of *D. vexillum* in an additional site in the Clyde coincided with its discovery in a protected loch on the west coast of Scotland (RAPID, 2018). *Didemnum vexillum* is considered a species of high concern in Scotland as it is capable of rapid growth, smothering sessile and benthic species as well as anthropogenic structures. The arrival of this species in Loch Creran prompted the Scottish Government to put in place the first species

control agreement under the WANE Act, allowing swift management action to be taken due to concern over its potential impact on local protected biodiversity features (RAPID, 2018). Species control agreements and orders can be used by government agencies to set out voluntary or mandatory measures which land owners or responsible parties must implement to control or eradicate an invasive species (Scottish Government, 2012). The spread of *D. vexillum* from its initial site of detection in the Firth of Clyde in 2010 despite the management actions taken to control it is concerning (Beveridge et al., 2011), and demonstrates the vulnerability of the rest of the west coast of Scotland to further spread of this species.

The detection of *Undaria pinnatifida* in the Firth of the Forth marks the arrival of this species in Scottish waters (Chapter 2). *Undaria pinnatifida* is included on the list of the world's '100 worst invasive species', and may reduce native species diversity by competing with other macroalgae (Wotton et al., 2004; MacLeod et al., 2016; Epstein and Smale, 2017a). In southern England, *U. pinnatifida* has become wide spread along rocky shores following establishment on nearby artificial structures, including in a protected SAC (Heiser et al., 2014; Epstein and Smale, 2017b, 2018). Furthermore, spread of this species on to other artificial structures such as aquaculture facilities could result in increased husbandry costs (James and Shears, 2016). The potential for this species to cause impacts on protected species or habitats in Scotland requires further assessment. Establishing whether this species has spread into natural systems within the Firth of Forth through targeted surveys may represent the best starting point for this inquiry.

5.2.3 Preventing species arrival

The potential risks of these established species, and from species not yet introduced to Scotland, illustrates the need for adequate biosecurity in the marine environment. As covered in Chapter 1, the best strategy for reducing invasive species impact is to prevent arrival of new species in the first place (Genovesi, 2005; Molnar et al., 2008; Hewitt and Campbell, 2007). This requires identification of the main routes of species arrival, as well as development of measures to prevent transfer (Molnar et al., 2008). This study has confirmed previous analysis of the recreational boating sector as an important contributor to the spread of marine NNS in Scotland (Ashton et al., 2006b), as even relatively remote marinas with few other vectors had high numbers of NNS (e.g. site C2) (Ulman, 2018). However, there is still little regulation covering the recreational boating sector (Clarke Murray et al., 2011), although following the introduction of the WANE Act, vessel owners and marina operators may have be liable or

have to pay control costs if they are found to have not followed best practice in biosecurity planning and to have contributed to the spread of NNS, even if their role was unintentional (Payne et al., 2014).

The research presented in Chapter 4 identified that quarantine of a vessel in low salinity environments could potentially remove up to 50% of fouling in just one hour, and could offer an effective method for removing soft-bodied organisms such as non-native ascidians. More tolerant species would likely require immersion for much longer periods, potentially over 48 hours, which could reduce uptake of freshwater treatment as an easy to apply treatment for vessel owners (Roche et al., 2015). However, this research has also demonstrated the danger that treatment with freshwater could inadvertently increase NNS abundances by increasing the availability of space for settlement and reducing competition from resident dominant species. Other types of disturbance that result in removal of established epifaunal assemblages on artificial structures such as maintenance activities have also been shown to facilitate non-native species establishment (Altman and Whitlatch, 2007; Britton-Simmons and Abbott, 2008; Bugnot et al., 2016; Viola et al., 2017). Thus tolerant NNS, or those within the wider propagule pool, could recover or re-establish in greater abundances than at pre-treatment levels. Timing of management interventions may thus be critical, either to treat vessels or infrastructure prior to transport or reapplication of anti-foul paint, or for management activities to occur in winter when fewer species may have reproductive propagules in the water column (Airoldi and Bulleri, 2011; Viola et al., 2017).

This research is part of a growing field investigating how best to manage biofouling to reduce spread of NNS. However, novel pathways for species spread are emerging which are far harder to regulate, including the rafting of organisms attached to marine litter or debris (Gregory, 2009; Minchin et al., 2013; Miralles et al., 2018). This represents a global threat, including to more remote locations with less human activity and fewer traditional pathways of species introduction (Barnes, 2002; Barnes and Milner, 2005; Campbell et al., 2017). Drifting of species attached to damaged infrastructure represent a further threat. The 2011 earthquake and resulting tsunami in Japan enabled large amounts of debris to travel across the Pacific Ocean. This debris later arrived on the west coast of the USA and Hawaii with a number of living eastern Pacific species still attached (Calder et al., 2014; Carlton et al., 2017).

Storm damage can also disrupt species control efforts. A storm in the UK resulted in fragments of damaged pontoon from a marina containing *Didemnum vexillum* drifting into open waters, potentially disrupting efforts to contain *D. vexillum* within the marina (Scottish

Government, 2018). As climate change is expected to increase the intensity of storms, the frequency of this sort of event is likely to increase (Patricola and Wehner, 2018). Most of the plastic pollution in the ocean comes from land-based sources, but debris from aquaculture and coastal infrastructure may be particularly concerning for the spread of NNS, as well as the colonisation of plastic debris by NNS once it reaches coastal environments (Campbell et al., 2017).

5.2.4 Identifying factors influencing NNS distribution

Salinity has been identified as a driving factor influencing the composition of fouling communities and the distribution and abundance of NNS in Scotland (Chapter 3), supporting results from other areas such as in the USA and Canada (Lord et al., 2015; López-Legentil et al., 2015; Choi et al., 2016). Variation in salinity and the proximity of a site to rivers is likely to affect community composition due to different salinity tolerance ranges between species. Consideration of the influence of salinity variation may be especially important as climate change is predicted to change rainfall patterns in the UK, increasing winter rainfall in Scotland and decreasing precipitation in the south of England during the summer (Winder et al., 2011). The effects of climate change could result in more frequent hyposaline events, with increased storms and changes in rainfall resulting in more freshwater run-off (Pardo et al., 2011; Cook et al., 2013a; Vuorinen et al., 2015; Dijkstra and Simkanin, 2016).

The results of two experiments to test episodic osmostic stress as a management tool (Chapter 4), also demonstrate the effect environmental disturbance may play in changing natural biofouling assemblages (Sorte et al., 2010a,b; Bradley et al., 2012; Cockrell and Sorte, 2013). This study shows that even short-term osmotic shock can alter community assemblages up to eight-weeks post-disturbance, and in some sites increased the proportion of cryptogenic and NNS cover. Results of studies investigating the impact of low salinity events in urbanised areas on native communities depend on the species under consideration (Irlandi et al., 1997; Cox and Foster, 2013). In San Francisco bay, inter-annual winter extremes in rainfall are thought to trigger large changes in the fouling community composition, shifting dominance from solitary ascidians and branching bryozoa to colonial ascidians and encrusting bryozoans (Chang, 2009; Chang et al., 2018). This study shows that episodic run-off could cause significant changes to marine fouling community assemblages in sites where this type of disturbance is infrequent. Sites exposed to frequent low salinity events may be more resistant to osmotic shock, but are likely to have less diverse communities and could still have high

abundances of NNS.

Anthropogenic alterations to coastlines result in changes to how freshwater enters marine systems, high rainfall events could contribute to storm sewer overflow and higher surface water run-off from urbanised coastal towns and cities, lowering localised salinity but also increasing release of chemical pollutants and contaminants into the marine environment (Winder et al., 2011; Cox and Foster, 2013; Sheahan et al., 2013; Smyth and Elliott, 2016). Increased pollutant load, sediment suspension, nutrient availability and oxygen levels are all factors which could be associated with river discharge into coastal environments (Sheahan et al., 2013). None of these factors were accounted for in this study, and future investigations would be advised to include analysis of these parameters, as observations during marina surveys in 2016 confirmed that sites located within river mouths had much lower and more variable visibility depending on rainfall. NNS are expected to be more tolerant of physical and chemical disturbance, so differences in these variables between sites, or as a result of climate change, could also contribute to the establishment success of NNS (Altman and Whitlatch, 2007; Lenz et al., 2011; Clarke Murray et al., 2012). Climate change has also been predicted to interact with the introduction of NNS through impacts of temperature increases and heat waves (Sorte et al., 2010a,b), and by facilitating and restricting the ranges of both native and non-native species (Cottier-Cook et al., 2017). The interaction between climate anomalies such as extended periods of drought or intense periods of rainfall and habitat degradation may facilitate NNS establishment (Winder et al., 2011).

Returning to the influence of factors affecting the current distribution of NNS, the relative importance of individual environmental and anthropogenic variables has been demonstrated to vary between regions. While salinity plays an important role in the composition of fouling communities in Scotland, the coastal environment around Scotland is heavily influenced by high volumes of rainfall discharging back into the marine environment through river discharge and surface water run-off (Marine Scotland, 2011). This thesis demonstrated that the salinity range within marinas can vary significantly between sites, influenced by proximity to rivers and the position of a site within estuaries (Chapter 3). In the Mediterranean, proximity to the Suez canal, shipping ports and temperature were found to influence NNS richness in marinas, while no influence of freshwater source was found (Ulman, 2018). Ulman (2018) suggested the proximity to the Suex canal, a major corridor for species introduction to the Mediterranean sea may have obscured salinity effects, although differences in results may also be a result of scale, as marinas were identified as having riverine input if a site was within

5 km of a river. The data from this thesis has demonstrated the influence of riverine input may be much more restricted than a 5 km radius, as did the results from Foster et al. (2016) which showed an increase in NNS in sites more than 2 km from a river. Nevertheless, this does not counteract the argument that different regions may have different factors which structure NNS distribution. Thus, development of biosecurity plans and monitoring schemes must take account of regional variation to develop a tailored approach to controlling NNS spread (Choi et al., 2016; Ulman, 2018).

Identification of the factors which influence NNS establishment within a site could also inform planning decision made about the environmental impacts of marine developments (Dafforn et al., 2015a; Bishop et al., 2017; Johnston et al., 2017). In areas where the risk of NNS establishment is determined to be high, adjustments to marina design could be made which minimise the likelihood of NNS establishment (Bulleri and Chapman, 2010; Mayer-Pinto et al., 2017). The volume of floating infrastructure could be reduced, maintenance activities which disturb native communities restricted, and facilities to contain fouling when vessels are dry-docked and cleaned included (Nall et al., 2015; Foster et al., 2016). This study found that in Scotland, marinas located within river mouths have fewer marine NNS, although they may also be more susceptible to establishment of tolerant species compared to fully saline sites (Paavola et al., 2005, Chapter 3). This research supports a suggestion by Minchin and Gollasch (2003), that altering marina design or siting to include a freshwater source could potentially offer a way to reduce site susceptibility to NNS establishment. Alternative suggestions have included incorporating include lock gates or encapsulation berths into marinas, thus allowing for quarantine and treatment of either the whole marina or individual vessels (Bax et al., 2002; Holt and Cordingley, 2011), although this approach is more reactive than precautionary and could have additional environmental impacts (Floerl and Inglis, 2003; Rivero et al., 2013). An approach similar to trials of eco-engineering studies could be used to identify changes which encourage growth of native communities and increase biotic resistance (Bugnot et al., 2018; Dafforn et al., 2016; Johnston et al., 2017).

5.2.5 Improving marine biosecurity in Scottish waters

Eradication and control of novel species may depend on rapid action following introduction. This requires quick detection of new arrivals, and relies upon effective monitoring and surveillance regimes. This thesis contributed to the collation of NNS records for all known marine species in Scottish waters, building on work by Nall et al. in 2012. This synthesised

list now includes over 2000 records of marine NNS in Scotland, and provides a resource not currently available anywhere else in the UK. The information provided in this database will be shared with Marine Scotland and could be used to guide development of a national scale monitoring plan which targets areas with higher rates of NNS introduction. In Shetland, a baseline assessment of NNS distributions was conducted to help inform development of a biosecurity plan (Collin et al., 2015a,b). However, to conduct such as assessment at a national level would require significant monetary investment and time and may not be necessary as high risk sites have already been identified through surveys conducted in this thesis and by other researchers (Beveridge et al., 2011; Macleod, 2013; Nall, 2015; Collin et al., 2015b; Cook et al., 2015; Kakkonen et al., 2019)

The collated distribution data can be used to identify hotspots of NNS establishment (Figure 5.1). Analysing the distribution of records around Scotland, shows that in general marinas and harbours have higher numbers of NNS than areas of natural coastline, although there is also a strong sampling bias present in that surveys have primarily been carried out in these locations. Other areas of known high NNS density include areas with historically high volumes of aquaculture (e.g. Loch Sween and areas on the west coast) and others near major ports (e.g. Cromarty Firth, Scapa Flow, and Loch Ryan) (Nall et al., 2015; Kakkonen et al., 2019, Chapter 2). There are also significantly more NNS (and more records) on the west coast than the east coast (Figure 5.1). While there are undoubtedly a number of gaps in survey effort, especially on natural shores, so far novel species have been principally identified in association with artificial structures (Ryland et al., 2014; Cottier-Cook et al., 2019).

Combining the distribution data gathered in this thesis with data on the ecological, economic, social and cultural value of the marine environment around Scotland can be used to establish priority areas of monitoring and surveillance of NNS and inform prevention strategies (Inglis, 2001; Hulme, 2009). For example, identification of hotspots of NNS establishment may also be useful in highlighting where onward risk of further spread by other vectors is high, e.g. sites associated with the oil and gas or renewable energy industry (Nall et al., 2015). In New Zealand, a global leader in marine biosecurity, development of a national surveillance and monitoring scheme involved identification of sites where:

1. The risk of NNS introduction is high (e.g. high concentrations of vector activity near ports, large marinas, or aquaculture sites);
2. The risk of impact on vulnerable ecosystems is high (i.e in the UK this could include sites designated as a SAC, SPA, SSSI, MPA for species or habitats which might be impacted

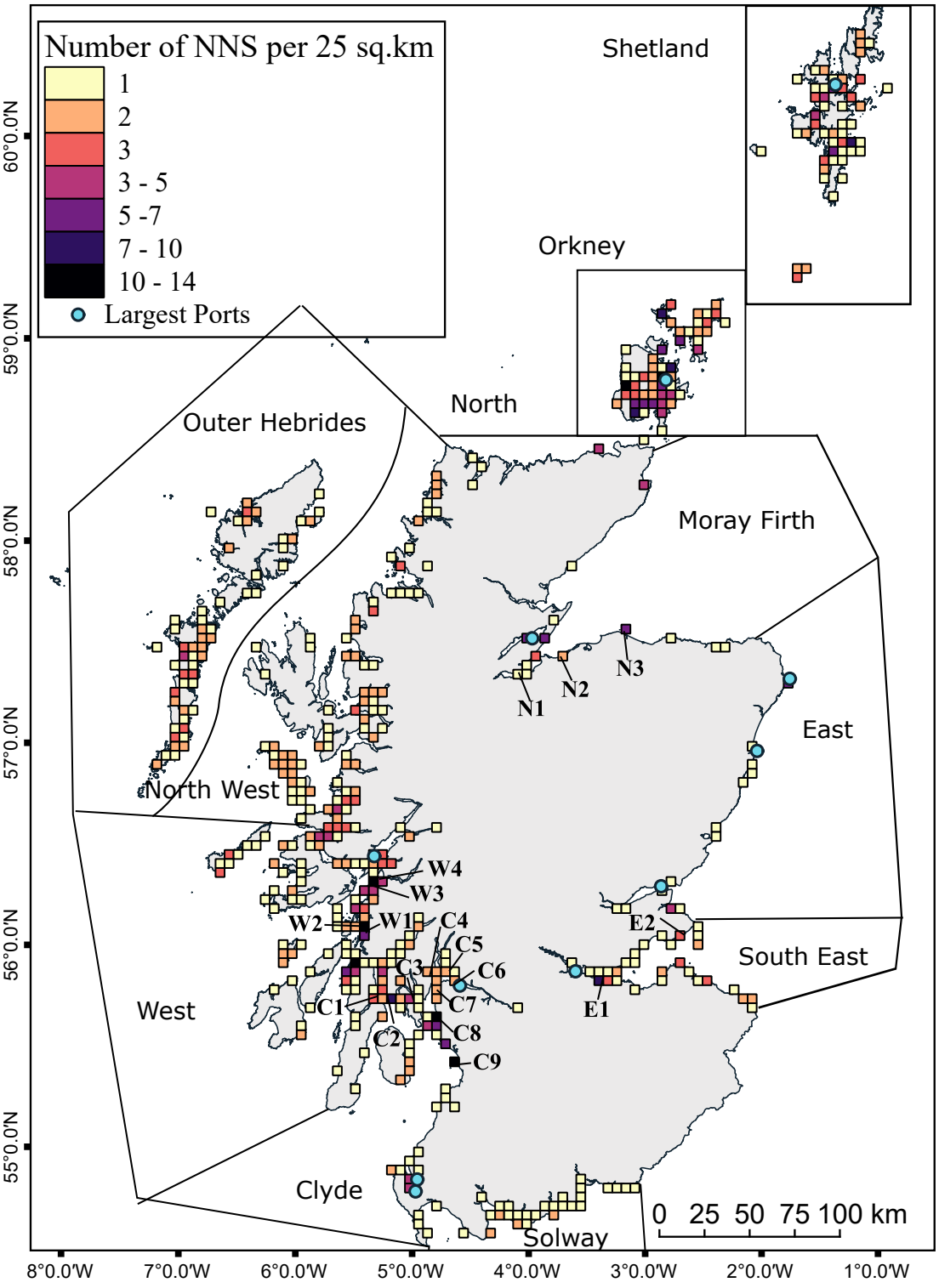


Figure 5.1: *Map showing the total number of marine non-native species within 5 x 5 km grid squares (Note: map shows the number of species, not the number of records). Sections of coastline with no grid squares represent unsurveyed areas. The location of the top 11 largest ports in Scotland is shown (Marine Scotland, 2016). Scottish marine regions identified in the Marine Nature Conservation Review (MNCR) and designated for the development of regional marine plans (Marine Scotland, 2018).*

by NNS introduction);

3. The risk of economic or social impacts are high (e.g. popular recreational water areas, aquaculture stock)
4. Environmental conditions increase likelihood of species establishment (Hewitt and Martin, 2001; Woods et al., 2017).

The results of Chapter 3 identified that some sites may have a lower risk of species introduction due to lower salinity conditions. This information can be directly incorporated into biosecurity plans, for example it would not be recommended to use very low salinity sites (N1, N2 or C7) or those known to experience low salinity conditions as a result of their position in the Firth of Clyde (C5 or C6) as surveillance sites for new arrivals. Chapter 3 also indicated that data on river distance and predicted salinity generated by the average climatological run of the Scottish Shelf Model could be combined to give a rough indication of expected salinity regimes within harbours and marinas. This could be used to assess risk in smaller sites where baseline NNS surveys have not yet been carried out.

Further information on vector activity and sensitive habitats has already been gathered as part of the development of Scotland's National Marine Plan, which was supported by an assessment of the condition of Scotland's seas (Baxter et al., 2011). This resulted in the collection of a large volume of information on Scotland's maritime industries, environment, and natural biodiversity, which has been brought together in one place and made accessible through the NMP interactive portal (NMPi). The biosecurity plan for the Shetland isles has already demonstrated that integration of anthropogenic, environmental and biological data to identify the areas most vulnerable to invasion is possible (Collin et al., 2015a; Shucksmith and Shelmerdine, 2015). This should be replicated at a national scale to develop a national NNS monitoring strategy, or incorporated into regional marine plans to build up a national level cover.

5.2.6 Methods to assess NNS distribution and impact

The methods used to survey NNS in this thesis made use of some established survey methods, as well as using novel equipment and analysis techniques to identify the distribution and abundance of species. Rapid assessment surveys (RAS) are one of the most common methods of surveying for marine NNS, especially within harbour and marina environments, where easy access to fouling communities is provided by pontoons and slipways (Minchin and Nunn,

2013; Bishop et al., 2015; Collin et al., 2015b; Nall et al., 2015). Rapid surveys carried out by experts or those trained in the identification of target species offer a very quick system to search high risk sites for specific target species (Bishop et al., 2015). This can be much more efficient in confirming arrivals of novel species or checking for horizon species than using camera surveying equipment or standardised scrape sampling. In this thesis, when surveying fouling NNS the RAS was found to be better than photo-surveys for identifying individual NNS, as the photo-quadrat technique did not allow for identification of organisms requiring analysis under the microscope to distinguish between native and NNS (e.g. some bryozoan species) (Chapter 3). Combining photographic surveys with dive or RAS and collection of suspect organisms is an established method of surveying NNS, and has been used to survey benthic environments, vessel hulls and hard structures (Clarke Murray et al., 2011).

The disadvantage of these traditional techniques is that all still require taxonomic expertise and time to survey large areas or quantities of samples or photos. There are also difficulties associated with correctly identifying cryptic species, which may not be resolvable through traditional microscopic analysis (Thomsen and Willerslev, 2015; Zhan et al., 2015). For instance, some of the ascidian species considered native in this study are considered NNS in other parts of the globe (*Ciona intestinalis* and *Botryllus schlosseri* have recently been identified as species complexes (Zhan et al., 2015). These are morphologically cryptic species which can only be distinguished through genetic analysis. Surveys which do not collect physical specimens run the risk that taxonomic changes in the future can not be cross-checked against stored specimens.

The development of environmental DNA (eDNA) sampling techniques could offer a novel way to detect NNS in both freshwater and marine environments, either through targeted species identification using qPCR assays or using metabarcoding to characterise whole communities from a single water or sediment sample (Holman et al., 2019). However, the field requires more work to identify the factors affecting species detectability, as choices made during sample design can have significant impacts on results. Nevertheless, studies have identified high congruence between eDNA and traditional techniques, and use of these methods for monitoring is likely to greatly increase in the coming decade (Holman et al., 2019). Molecular survey techniques also enable monitoring of species in locations where traditional surveys are more costly and harder to carry out, such as in polar or remote regions (Lacoursiere-Roussel et al., 2018). The use of different techniques will ultimate depend on the research question of interest; however, for national surveillance and monitoring schemes

combining eDNA analyses with targeted expert surveys of high risk sites may soon become the most cost-effective method for detection of new introductions.

To establish whether NNS are impacting their resident communities, an indication of the abundance of NNS within an area is required. RAS techniques can be adapted to include a rough estimate of abundance, but only provide a rough indication of community dynamics. For example, Ashton et al. (2006a) score species abundance based on estimate of cover for algae or colonial species, and counts of individuals. In Chapter 3, the Hero4 GoPro enabled rapid characterisation of the fouling communities on marina pontoons, with photographs of a high enough quality to identify some but not all organisms to species level. This method of surveying provides useful information on species cover and the respective contribution of native and NNS to communities, as well as allowing standardised comparison of communities between sites. The use of CPCE to analyse these photos improved analysis time over other methods such as Image-J through use of species codes. However, the manual processing of this data was still extremely slow as a result of the need for a trained expert to identify every species under a point-count. In America, researchers are using a citizen science project (Invader ID) to help process photos of settlement tiles (Smithsonian Environmental Research Center (SERC), 2019). While the taxonomic resolution may not be as high as through microscopic analysis of photos, the ability to process large quantities of data on community composition is enabling investigation of change in coastal areas of wider spatial and temporal scales (SERC, 2019). Citizen science projects have already shown some success in recording coastal biodiversity in the UK, so future studies could incorporate similar models to increase the data analysis potential of comparative community studies.

5.3 Limitation of the study and recommendations for future research

This thesis has aimed to address key knowledge gaps to find ways to improve the management of marine NNS in Scotland. The following section considers some of the key challenges and limitations in the research presented, as well as outlining opportunities for future research.

- *Synthesised list of marine NNS*

  The synthesised list of records could be improved in a number of ways. The data collected for this list was based on the framework established by Nall et al. (2015). Where new fields were added (e.g. date of recording) this information was collected for new records but was not filled in for records added by Nall et al. (2015) due to time constraints. It would be useful to backtrack and add this data, to allow analysis of factors such as seasonality of recording different species. Furthermore, while gathering presence records is the highest priority, the addition of absence records for targeted NNS would allow confirmation of genuine absences in species distribution and areas which have reduced survey effort. Collecting this data may be difficult for some types of survey (e.g. seasearch dives on NBN), but is possible for targeted RAS.

- *Distribution of marine NNS in natural environments*

  This thesis has significantly updated the information on the distribution of NNS, particularly focusing on marinas, as they represent sites with a high-risk of NNS establishment. However, the synthesised list of NNS has shown that a number of species are well established in natural environments, mostly macroalgae and the barnacle *Austrominius modestus*. As some of these species are known to have a negative impact on native biodiversity, an analysis of spread within vulnerable sites is recommended, especially given the recent arrival of *Undaria pinnatifida* in the Firth of Forth, and of *Didemnum vexillum* in Loch Creran (Cottier-Cook et al., *in review*).

- *Salinity monitoring at localised scales*

  The salinity within marinas has been shown to influence the composition of marina biofouling communities (Chapter 3) and the sensitivity of these communities to experimentally applied osmotic shock (Chapter 4). The design of a marina may influence salinity and circulation patterns, with features such as proximity to a river,

presence of storm drain, seawalls or breakwaters, creating environmental conditions within a site very different to those outside (Floerl and Inglis, 2003; Foster et al., 2016; Rivero et al., 2013). The salinity within a marina may change on an hourly or daily basis, influenced by changes in tidal regime, rainfall, and circulation patterns (Floerl and Inglis, 2003). Models such as the Scottish Shelf Model do not take account of the very near-shore localised conditions within urbanised areas (Wolf et al., 2016). Thus, characterising the salinity within a site is challenging, requiring repeated monitoring for each site.

In this study, measurements of salinity were made using a handheld castaway CTD, with measurements taken over a number of locations within a marina to provide a picture of variation in salinity within a site and through the water column. The limited number of sites in Chapter 4 allowed these measurements to be repeated over 16 weeks to provide a time-series of salinity change over summer months; however, the large number of sites over a wider geographic area in Chapter 3 meant that this was not possible. Instruments that can continuously monitor salinity are available, and use would have greatly enhanced the results of this research, but the cost of salinity monitoring loggers was prohibitive to their use in this study. This study has confirmed previous research which indicated that the distance to the nearest river may be a useful proxy, but validation of these results with longer and more in depth salinity data for a larger number of sites would be useful.

- *Further investigations into hypo-saline treatment of NNS*

  This thesis has shown how hypo-saline immersion may offer a useful tool for reducing biofouling load on vessels or equipment in certain circumstances (Chapter 4), and merits further research. In this study, field experiments were used to test the effect of hypo-saline immersion with experiments conducted on marina pontoons to replicate conditions which might be experienced in a management scenario. However, conducting experiments in the field reduced the possibility of collecting high resolution data on individual species survival rates and increased the potential for confounding variables, such as temperature and water quality, to influence the results. Replication in similar studies in aquarium facilities supports the results found in this study (de Castro et al., 2018), but a more detailed assessment of the mortality of individual organisms would increase the number of inferences that can be drawn from future studies.

  Further research into other factors, which might affect response to hypo-saline

treatment, could investigate variables such as age and depth of the fouling community. Studies of biofouling community response to other environmental stresses have shown variation as a result of the age of the community (juvenile vs adult). The timing of the treatment over the course of the year could also be investigated, as recovery of the community will depend on which species are present in the recruitment pool. Susceptibility to salinity change has also been shown to vary with temperature, so tolerance of some species to lower salinity may be lower in winter months.

5.4 Concluding remarks

The number of marine NNS in Scotland is increasing, with over five new species introduced in the last decade alone. A number of these species are becoming increasingly widespread, as well as increasing in abundance within fouling communities in Scottish marinas. Establishment of NNS in these artificial environments could enable further spread into natural habitats, where some may have negative impacts on native biodiversity if they spread into vulnerable habitats (Cottier-Cook et al., 2019). Advances in invasive species policy in Scotland are being driven by a holistic approach to sustainable management of marine resources through the implementation of national and regional marine plans. The findings of this study contribute to a growing body of research on marine NNS in Scotland by increasing the spatial and temporal resolution of data on fouling NNS distributions. This thesis also presents the first data comparing native and NNS contributions to fouling community composition, showing that this may vary as a result of differences in salinity between sites. The natural variation in salinity found in Scottish ports and marinas should be capitalised on to aid in implementing cost-effective monitoring of NNS. Application of freshwater treatments as a management measure to reduce fouling on vectors or treat contaminated infrastructure has the potential to offer a low cost solution; however, the potential negative consequences of increasing abundance of tolerant NNS indicates that this treatment method requires further investigation before implementation.

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Appendices

A - Chapter 2

Table A.1: *List of horizon species for which a literature and NBN search was carried out. No records of these species in Scotland were found on the NBN database at the time the search was performed (1st March 2018).*

Horizon species
Botrylloides diegenesis Ritter & Forsyth, 1917
Ficopomatus enigmaticus (Fauvel, 1923)
Grateloupia turuturu Yamada, 1941
Gracilaria vermiculophylla (Ohmi) Papenfuss, 1967
Hemigrapsus sanguineus (De Haan, 1835)
Hemigrapsus takanoi Asakura & Watanabe, 2005
Perophora japonica Oka, 1927
Watersipora subatra (Ortmann, 1890)

Table A.2: *Full species list from synthesised list of marine non-native species records for Scotland, divided by MNCR regions. The total number of species and records for each region is shown. Species which are considered established are highlighted in dark grey, those considered probably established in light grey (Nall et al., 2015).*Species have had new presence records added since 2012.*

Species	Clyd.	E	MF	N	NW	OH	Ork.	SE	Shet.	Sol.	W	No. of records	No. of MNCR regions
Invertebrates													
Amphibalanus amphitrite									1			1	1
*Asterocarpa humilis**							20		1		4	25	3
Aulacomya atra			1									1	1
*Austrominius modestus**	45	5	7		5	3		25	11	84	12	197	9
*Boccardia proboscidea**						3	1					4	2
*Botrylloides violaceus**	3		3		1	1	1	1				10	6
Bugula neritina	2											2	1
*Bugulina simplex**	3				1		3		2			9	4
*Bugulina stolonifera**	1										2	3	2
*Caprella mutica**	20	4	7	1	14	11	158	6	28		36	285	10
*Cordylophora caspia**	4		2			1					2	9	4
*Corella eumyota**	10	4	1	1	1	1	39	4	6	2	6	75	11
*Crassostrea gigas**	1					1	1	2	1	19	2	27	7
*Crepidula fornicata**	1				1			1	1			4	4
*Diadumene lineata**							4		1		37	42	3
*Didemnum vexillum**	9										1	10	2
*Eriocheir sinensis**	1											1	1
*Fenestrulina delicia**	1								1		1	3	3
*Potamopyrgus antipodarum**	2	5				30	15			1		53	5
*Schizoporella japonica**	3	1	4	1	2		76		8		7	102	8
*Styela clava**	24				3						10	37	3
*Tricellaria inopinata**	12	2	3				31	3			9	60	6
Macroalgae													
Antithamnionella spirographidis	9				18				6		17	50	4
Antithamnionella ternifolia						2		1			2	5	3
*Asparagopsis armata**	8	1			15	15	28		8		27	102	7
*Bonnemaisonia hamifera**	3	7	4		47	42	83	14	117		82	399	9
*Codium fragile ssp fragile**	4	3			1	14	51	7	8	1	43	132	9
*Colpomenia peregrina**	9	1		3	15	16	28	2	9	1	36	120	10
*Dasysiphonia japonica**	14		3		31		28		5		14	95	6
*Melanothamnus harveyi**	6	3		1	1	3	12	1			5	32	8
*Sargassum muticum**	72	1			3	2				2	19	99	6
*Undaria pinnatifida**								2				2	1
Cryptogenic Species													
*Bugulina fulva**			2	1	2	1	6	1			4	17	7
*Jassa marmorata**	1		6		4		4				5	40	6
*Karenia mikimotoi**							1					1	1
Total number of records	268	37	43	8	165	146	590	70	214	110	383	2054	
Total number of species	26	12	12	6	18	16	20	14	17	7	24	35	

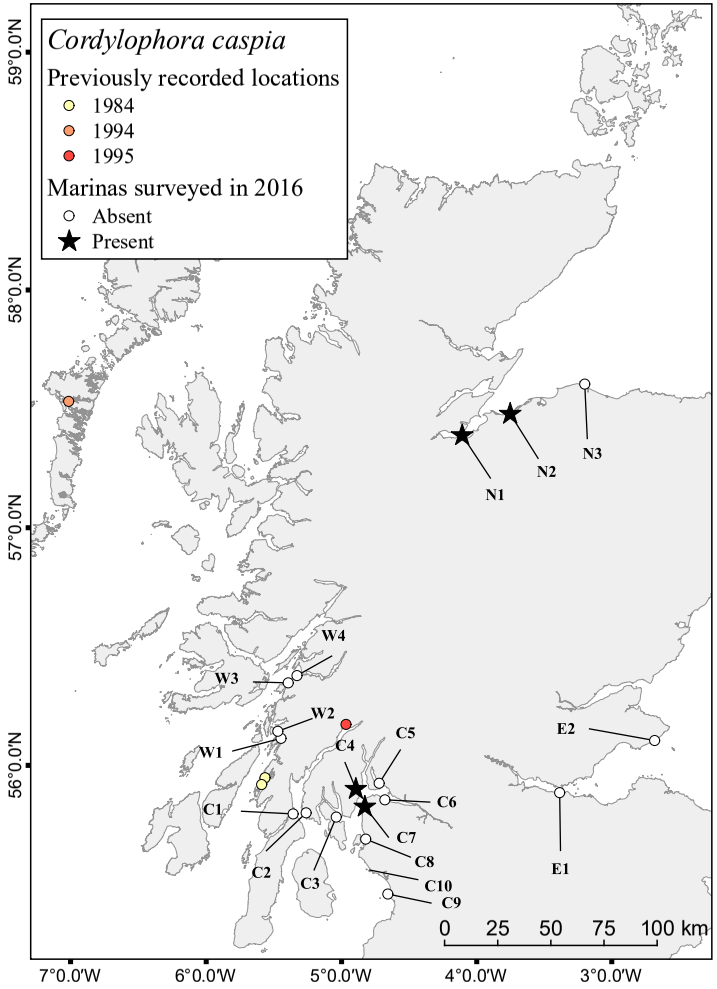

Figure A.1: *Map showing Cordylophora caspiadistribution in Scotland. 2016 RAS results are shown with historic presence records compiled from the literature.*

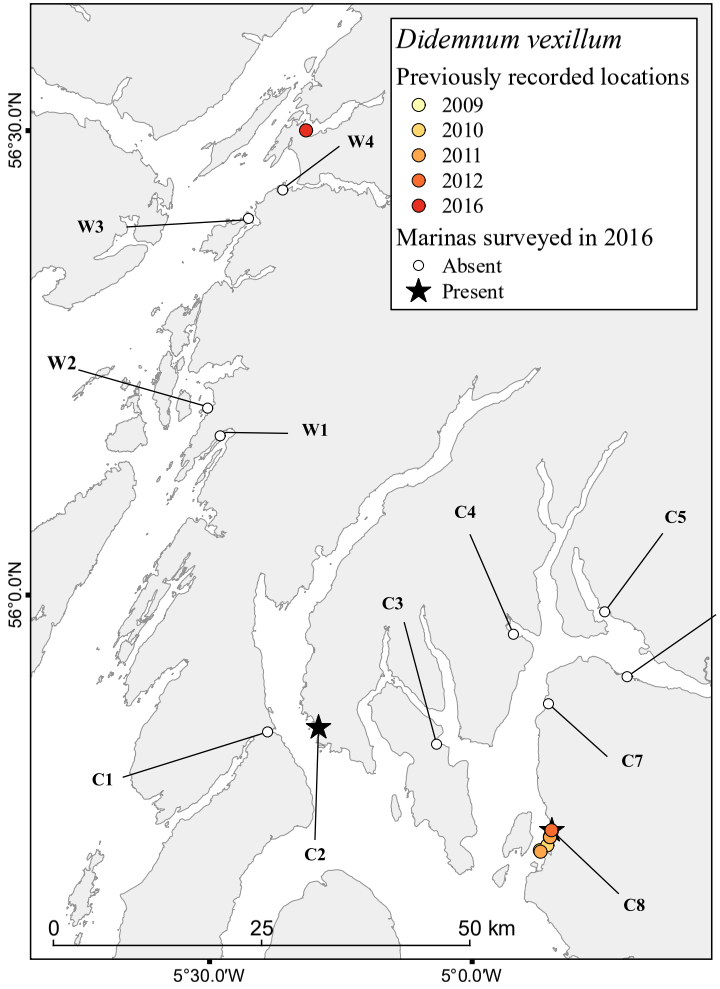

Figure A.2: *Map showing Didemnum vexillum distribution in Scotland. 2016 RAS results are shown with historic presence records compiled from the literature.*

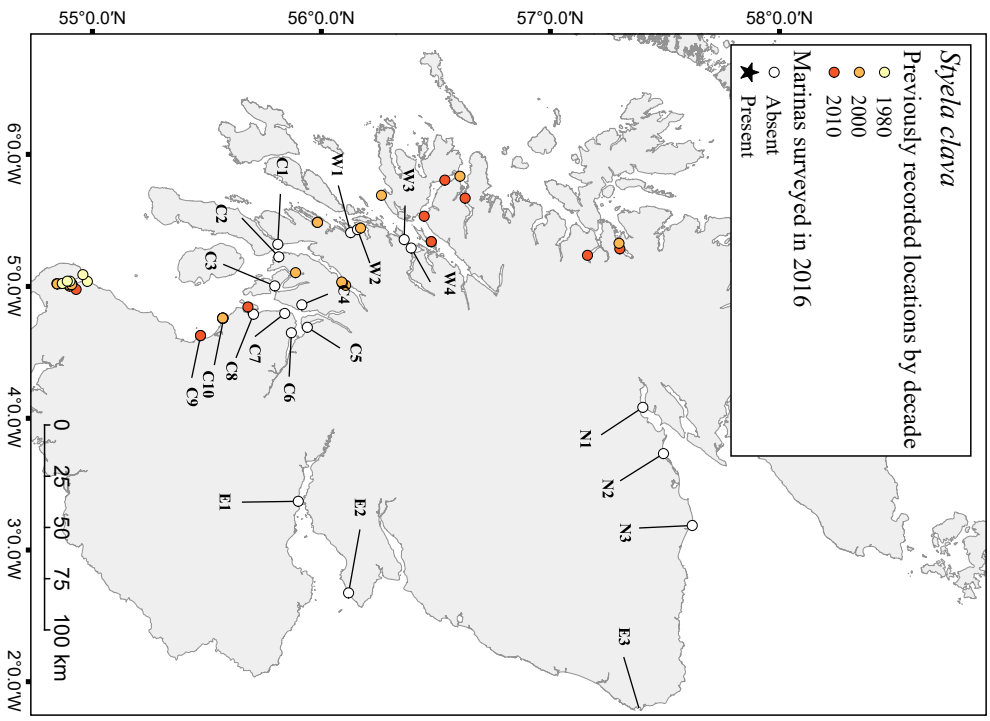

Figure A.3: *Map showing Styela clava distribution in Scotland. 2016 RAS results are shown with historic presence records compiled from the literature.*

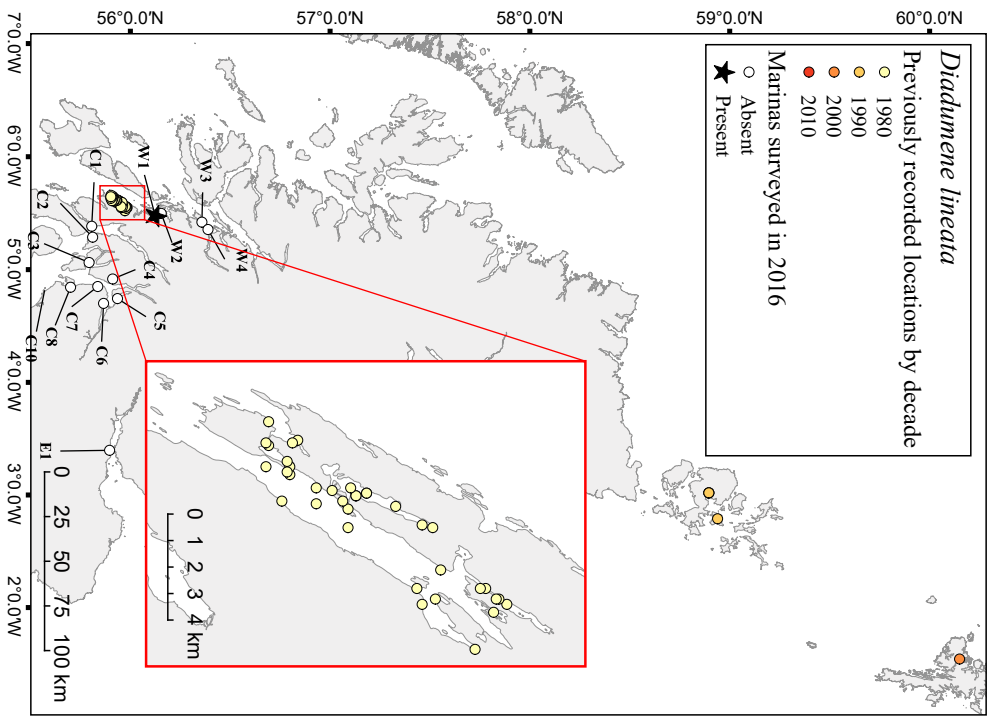

Figure A.4: *Map showing Diadumene lineata distribution in Scotland. 2016 RAS results are shown with historic presence records compiled from the literature.*

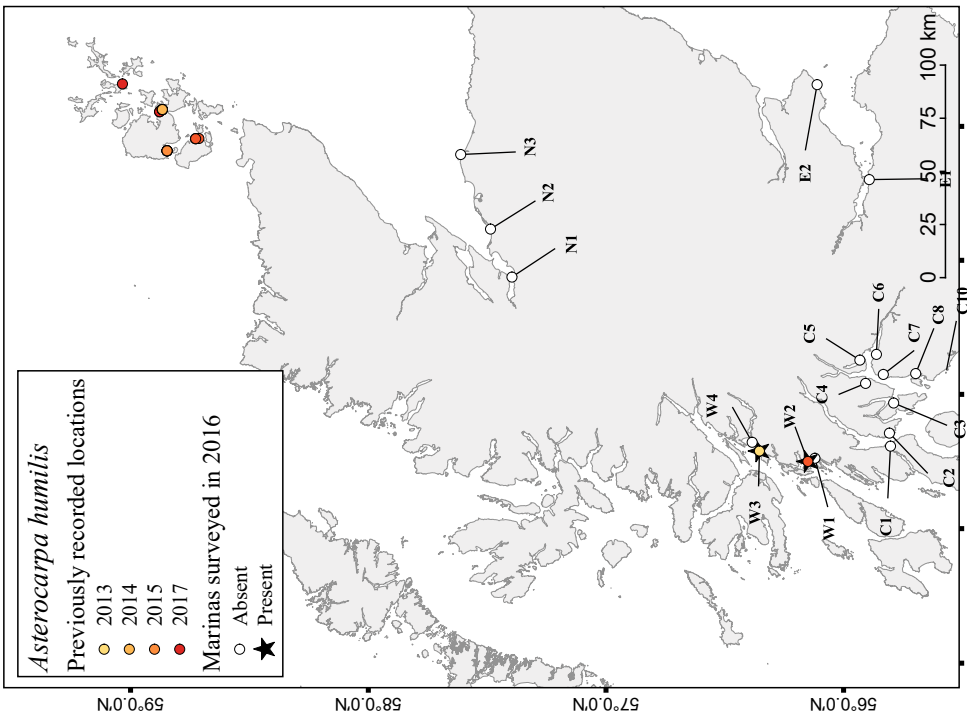

Figure A.6: *Map showing Asterocarpa humilis distribution in Scotland. 2016 RAS results are shown with historic presence records compiled from the literature.*

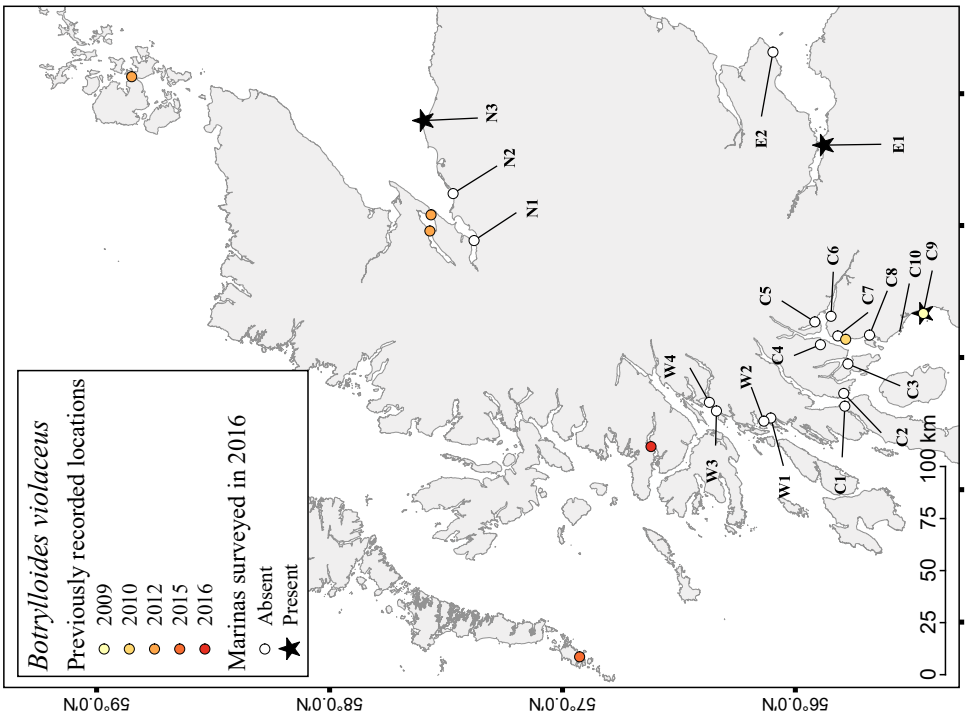

Figure A.5: *Map showing Botyrlloides violaceus distribution in Scotland. 2016 RAS results are shown with historic presence records compiled from the literature.*

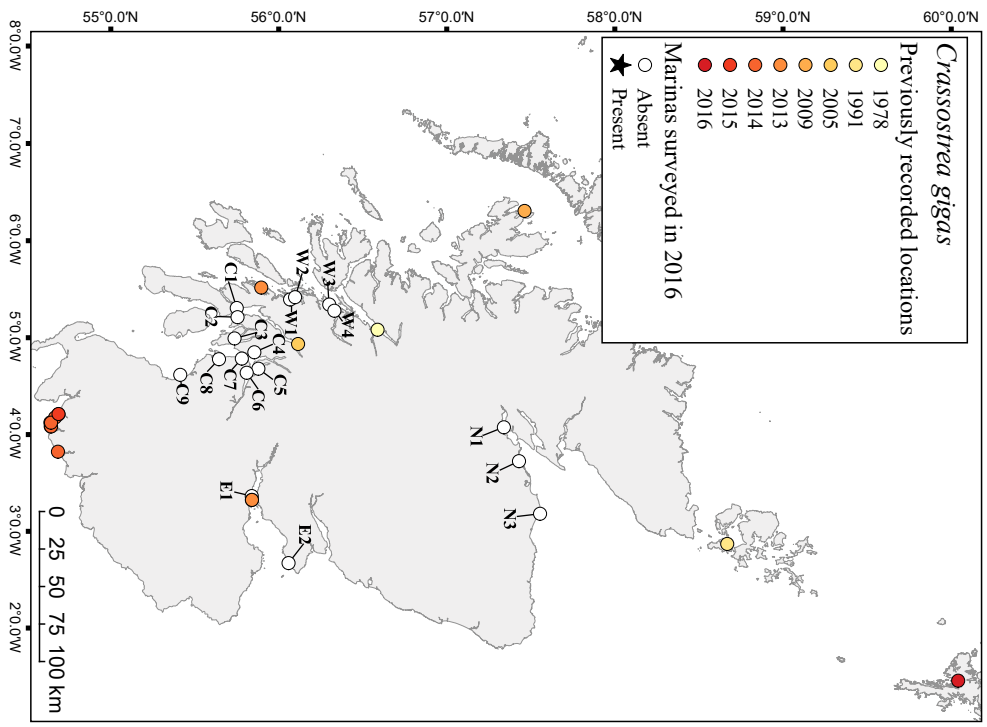

Figure A.7: *Map showing Crassostrea gigas distribution in Scotland. 2016 RAS results are shown with historic presence records compiled from the literature.*

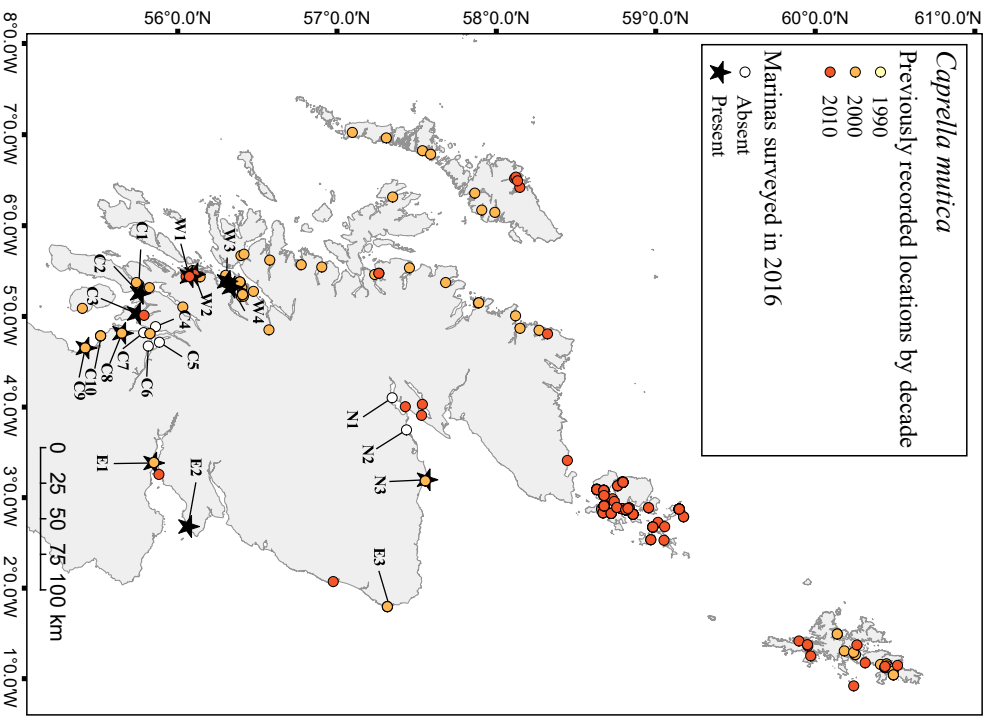

Figure A.8: *Map showing Caprella mutica distribution in Scotland. 2016 RAS results are shown with historic presence records compiled from the literature.*

B - Chapter 3

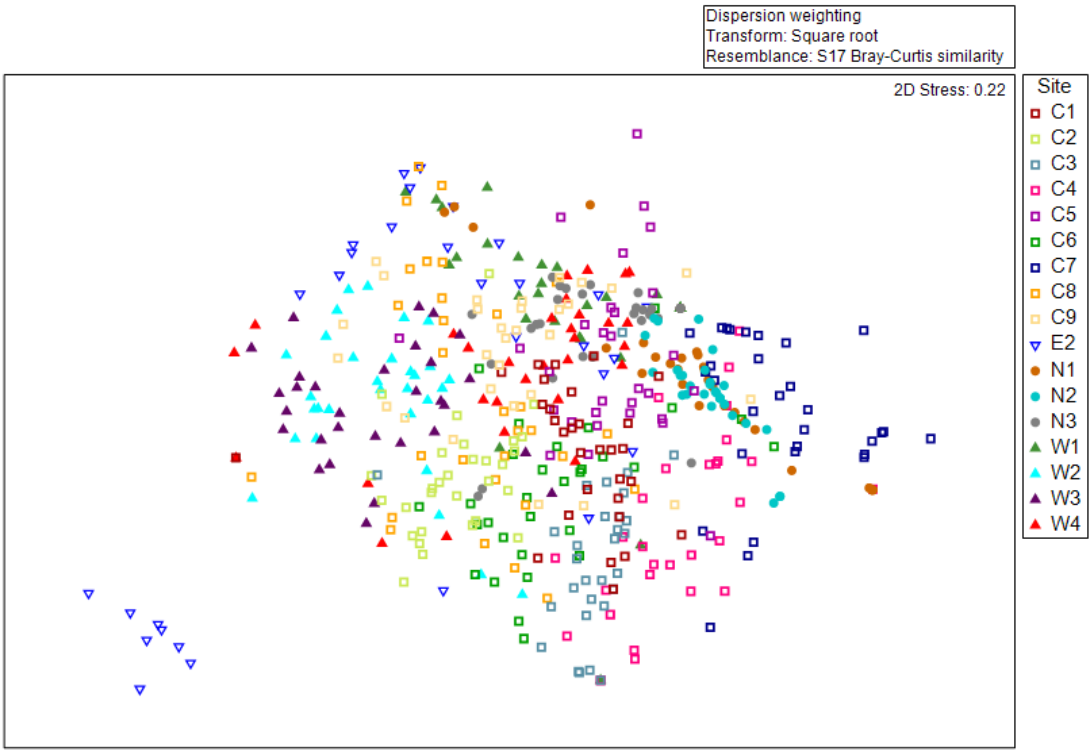

Figure B.1: *A 2-dimensional non-metric Multi-Dimensional Scaling (nMDS) plot based on Bray-Curtis similarities on dispersion-weighted square-root transformed percentage cover data for each of the 30 replicated photos of biofouing communities at the 17 sampled sites. Sites are shown by individual symbols and colours.*

Table B.1: *SIMPER analysis showing the contribution of each taxon to similarity of sites within groups. Groups were identified by the cluster analysis of similarity of the overall fouling community for each site. Taxa are ordered by decreasing contribution, with abundances representing fourth-root transformed average percentage cover for each group.*

Species	Avg. Abundance	Avg. Similarity	Sim/SD	% contribution	Cumulative % contribution
Group A - Average similarity: 62.47					
Spongy green algae	2.41	21.54	5.62	34.47	34.47
Brown filamentous algae	2.19	18.68	3.45	29.89	64.37
Cordylophora caspia	1.54	7.22	0.91	11.56	75.93
Hydroid	0.81	5.89	6.88	9.43	85.36
Group B - Average similarity: 60.01					
Brown filamentous algae	2.39	13.98	6.96	23.3	23.3
Green filamentous algae	2.14	12.78	5.29	21.3	44.6
Red filamentous algae	1.08	6.02	10.9	10.03	54.63
Green flat algae	0.88	5.15	5.14	8.59	63.22
Balanoid barnacle	1.2	5.1	1.83	8.49	71.71
Hydroid	1.01	5.04	20.5	8.41	80.12
Mytilus edulis	0.87	3.45	16.3	5.75	85.86
Group C - Average similarity: 66.66					
Brown filamentous algae	2.2	12.13	16.04	18.2	18.2
Mytilus edulis	2.23	10.7	3.07	16.05	34.25
Kelp	1.6	7.35	2.81	11.03	45.28
Hydroid	1.49	6.27	6.92	9.4	54.68
Metridium senile	1.05	4.84	4.52	7.27	61.95
Green flat algae	0.86	4.4	4.69	6.61	68.56
Spongy green algae	1.21	4.29	0.91	6.43	74.99
Green filamentous algae	0.87	4.18	2.93	6.28	81.26
Spirobrancus spp.	0.76	3.57	6.96	5.36	86.62
Group D - Average similarity: 63.78					
Kelp	1.97	6.91	5.51	10.83	10.83
Green filamentous algae	1.81	6.01	4.03	9.43	20.26
Brown filamentous algae	1.81	5.89	4.27	9.24	29.5
Red filamentous algae	1.68	5.66	3.25	8.87	38.37
Mytilus edulis	1.5	5.06	6.35	7.93	46.3
Hydroid	1.13	4	8.66	6.28	52.58
Green flat algae	1.37	3.96	2.71	6.21	58.79
Tricellaria inopinata	1.14	2.98	1.18	4.67	63.46
Diplosoma listerianum	1	2.93	3.5	4.59	68.05
Spongy green algae	0.93	2.84	4.13	4.45	72.5
Ciona intestinalis	1.11	2.77	2.14	4.35	76.85
Spirobrancus spp.	0.86	1.97	1.26	3.1	79.95
Electra pilosa	0.65	1.87	1.35	2.94	82.88
Balanoid barnacle	0.76	1.8	1.27	2.82	85.7

Table B.2: *SIMPER analysis showing the contribution of each NNS to similarity of sites within groups. Groups were identified by the cluster analysis of similarity of the NNS assemblage based on RAS presence/absence data for each site. Taxa are ordered by decreasing contribution.*

Species	Avg. Abundance		Avg. Similarity	Sim/SD	% Contribution	Cumulative % contribution
Group A - Average similarity: 48.33						
Cordylophora caspia	0.8		31.33	1.1	64.83	64.83
Austrominius modestus	0.6		13	0.62	26.9	91.72
Group B - Average similarity: 47.78						
Austrominius modestus	0.92		14.7	1.8	30.77	30.77
Caprella mutica	0.83		10.94	1.36	22.9	53.66
Corella eumyota	0.58		6.98	0.6	14.61	68.27
Tricellaria inopinata	0.67		6.68	0.8	13.99	82.26
Diplosoma listerianum	0.5		4.17	0.53	8.73	90.99
Groups A and B - Average dissimilarity = 80.19						
	Group A	Group B				
Cordylophora caspia	0.8	0	11.79	1.46	14.7	14.7
Caprella mutica	0	0.83	10.41	1.89	12.99	27.69
Corella eumyota	0	0.58	9.55	0.98	11.91	39.6
Tricellaria inopinata	0	0.67	8.54	1.13	10.65	50.25
Diplosoma listerianum	0.4	0.5	7.16	0.9	8.94	59.19
Austrominius modestus	0.6	0.92	6.59	0.75	8.21	67.4
Schizoporella japonica	0	0.42	4.42	0.81	5.52	72.92
Sargassum muticum	0.2	0.08	3.53	0.53	4.41	77.32
Bugulina simplex	0	0.25	2.74	0.56	3.42	80.74
Bugulina fulva	0	0.25	2.46	0.57	3.07	83.81
Dasysiphonia japonica	0	0.17	1.9	0.42	2.37	86.18

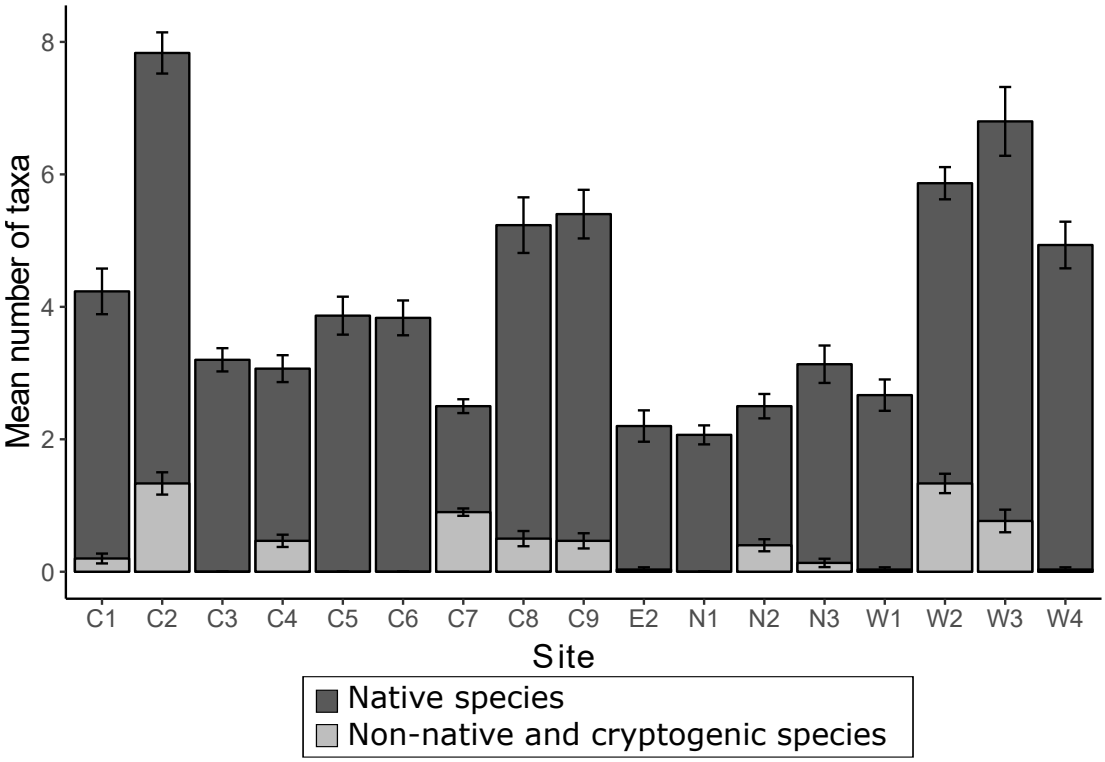

Figure B.2: *Bar chart of the mean species richness from photo quadrats in each site. The mean number of taxa (± SE) of native and pooled non-native and cryptogenic species in each site is shown.*

Table B.3: *SIMPER analysis showing the contribution of native species to similarity of sites within groups identified by a cluster analysis. Hierarchical agglomerative clustering was used to group sites based on similarities in their native assemblage. Taxa are ordered by decreasing contribution.*

Species	Avg. Abundance	Avg. Similarity	Sim/SD	% contribution	Cumulative % contribution
Group A - Average similarity: 74.06					
Spongy green algae	2.45	28.67	18.11	38.71	38.71
Brown filamentous algae	2.34	27.19	13.95	36.72	75.43
Group B - Average similarity: 58.31					
Mytilus edulis	2.73	19.06		32.68	32.68
Brown filamentous algae	1.82	12.7		21.79	54.47
Green flat algae	1.1	7.86		13.47	67.94
Hydroid	1.13	7.4		12.7	80.64
Group C - Average similarity: 61.91					
Brown filamentous algae	2.08	9.76	3.28	15.77	15.77
Green filamentous algae	1.64	6.83	2.06	11.03	26.8
Kelp	1.61	6.27	1.82	10.13	36.93
Red filamentous algae	1.42	6.06	4	9.78	46.71
Mytilus edulis	1.47	5.85	2.39	9.45	56.16
Hydroid	1.23	5.25	4.31	8.48	64.64
Green flat algae	1.1	4.42	4.02	7.13	71.77

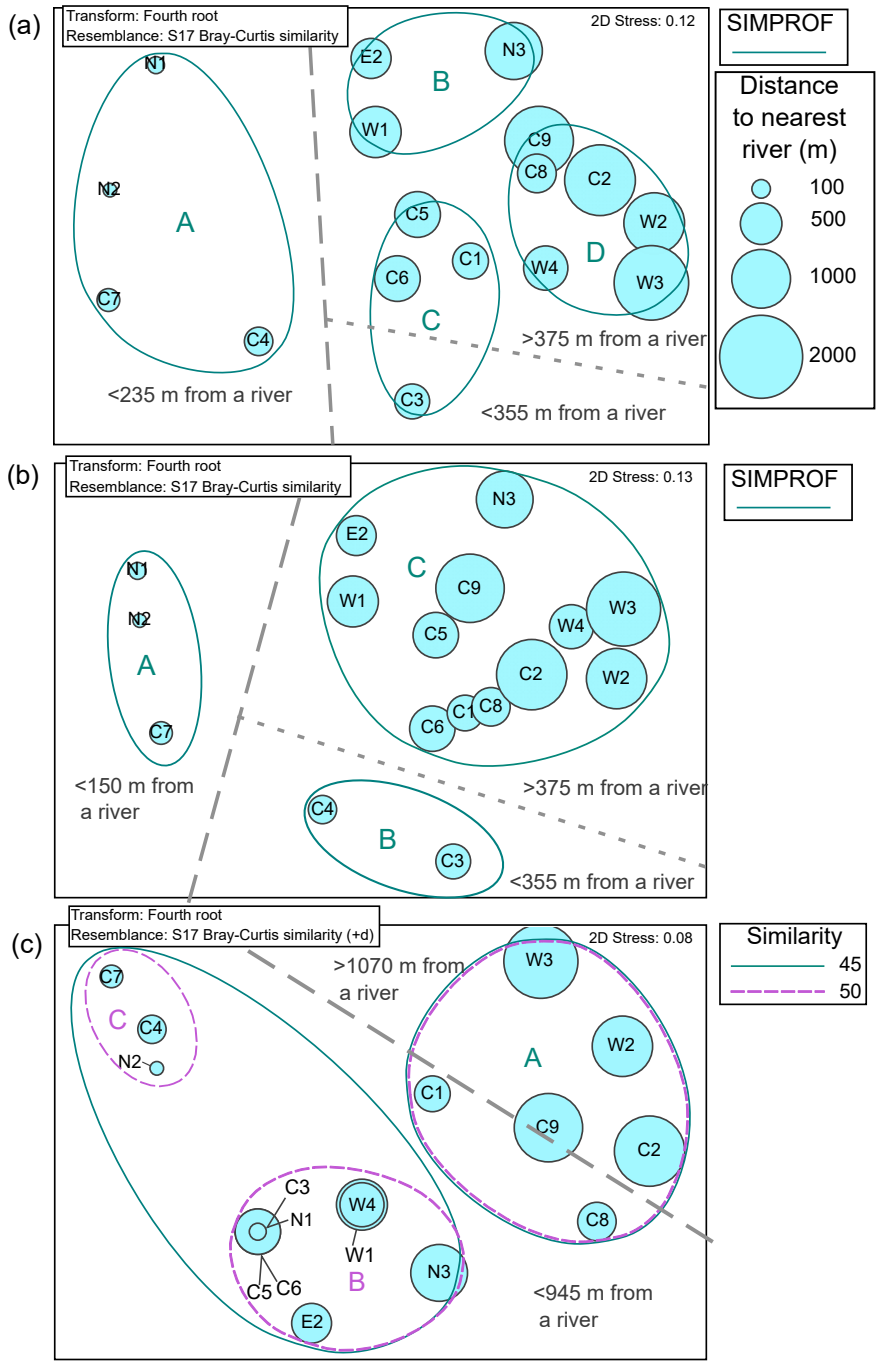

Figure B.3: *nMDS ordination plots of site similarities based on data from the whole community (a), only native species (b), and only non-native and cryptogenic species (c). nMDS ordination of sites is based on Bray-Curtis similarity matrices calculated from fourth-root transformed mean percentage cover data generated by CPCE analysis of photographs. Bubbles indicate the distance of sites to the nearest river (m). Overlaid on the nMDS plots are sites groups based on hierarchical cluster analysis (see Figure B.4 for details), solid green lines indicate results supported by significant SIMPROF tests. Grey dotted lines indicates splits of the fouling communities based on LINKTREE identification of existing thresholds in the abiotic data (river distance).*

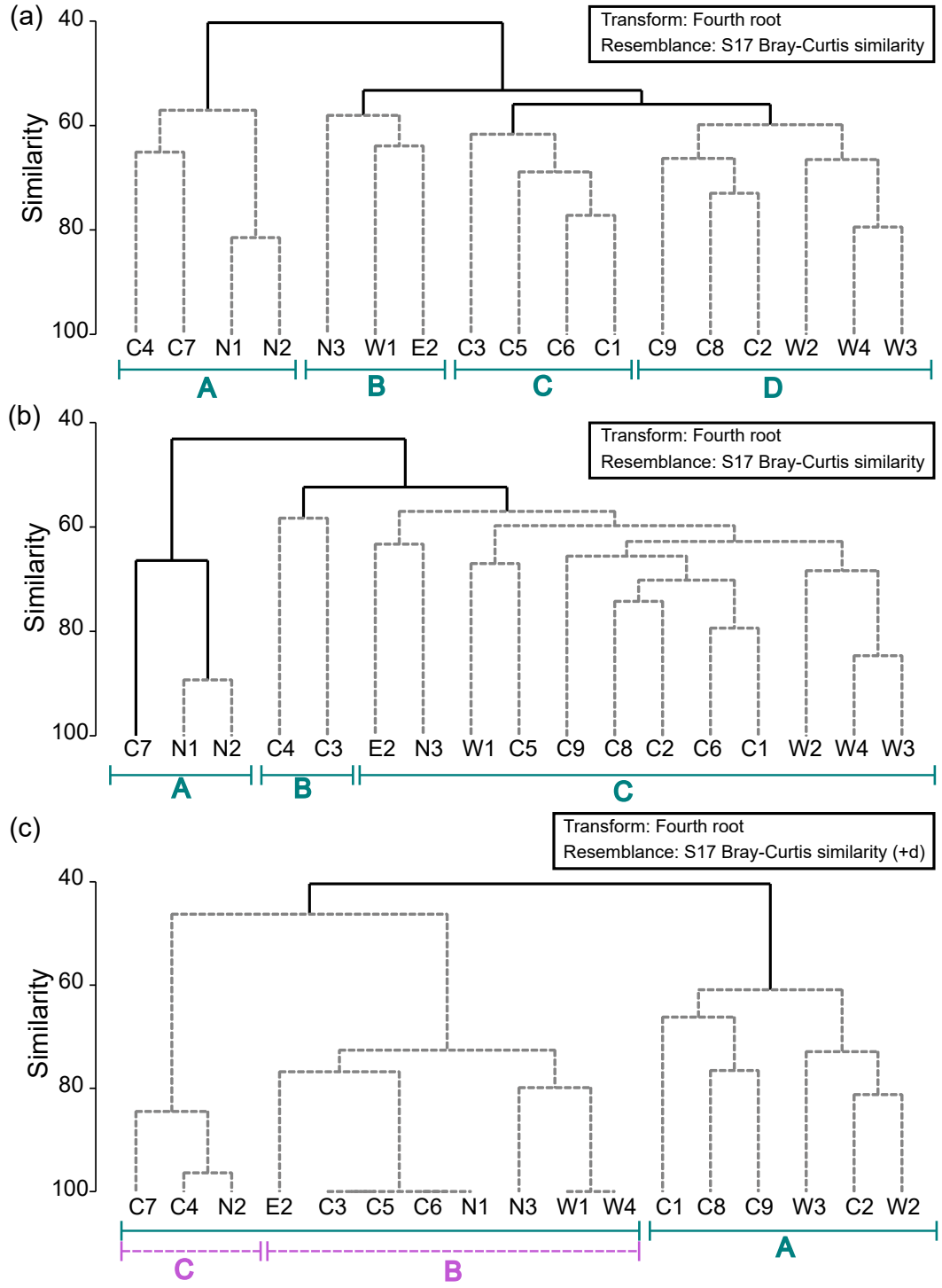

Figure B.4: *Dendrogram of the 17 sites using group-average hierarchical clustering. Clusters of sites were generated using average percentage cover data from the whole community (a), only native species (b), and only non-native and cryptogenic species (c). Clustering was performed on Bray-Curtis similarity matrices calculated from fourth-root transformed mean percentage cover data generated by CPCE analysis of photographs. Solid black lines indicate results supported by significant SIMPROF tests, which have been used to group sites.*

Table B.4: *SIMPER analysis showing the contribution of native species to dissimilarity between groups identified by a cluster analysis. Hierarchical agglomerative clustering was used to group sites based on similarities in their native assemblage. Taxa are ordered by decreasing contribution.*

Species	Avg. Abundance		Avg. Dissimilarity	Diss/SD	% contribution	Cumulative % contribution
Groups B & A - Average dissimilarity = 53.50	Group B	Group A				
Mytilus edulis	2.73	0.42	10.64	3.72	19.89	19.89
Green turf algae	1.14	2.45	6.04	1.09	11.28	31.18
Green flat algae	1.1	0	5.1	14.17	9.53	40.7
Green filamentous algae	0.54	1.08	4.18	1.27	7.81	48.51
Metridium senile	0.76	0	3.42	0.91	6.39	54.91
Encusting bryozoa	0.63	0	2.95	4.07	5.52	60.43
Fucus spp.	0.97	0.53	2.89	1.55	5.4	65.83
Brown filamentous algae	1.82	2.34	2.42	2.17	4.52	70.35
Groups C & A - Average dissimilarity = 57.42	Group C	Group A				
Kelp	1.61	0	5.8	2.56	10.1	10.1
Green turf algae	1.02	2.45	5.37	2.47	9.36	19.46
Red filamentous algae	1.42	0.17	4.54	2.89	7.91	27.37
Mytilus edulis	1.47	0.42	4.24	1.61	7.39	34.76
Green flat algae	1.1	0	4.01	3.15	6.99	41.75
Green filamentous algae	1.64	1.08	3.58	1.22	6.23	47.98
Barnacle	0.89	0	3.43	1.5	5.97	53.95
Spirobrancus spp.	0.72	0	2.61	1.39	4.55	58.5
Ciona intestinalis	0.79	0.44	2.51	1.25	4.37	62.87
Hydroid	1.23	0.66	2.21	1.07	3.85	66.72
Metridium senile	0.62	0	2.13	1.12	3.71	70.44
Groups C & B - Average dissimilarity = 47.64	Group C	Group B				
Red filamentous algae	1.42	0	4.33	3.89	9.08	9.08
Mytilus edulis	1.47	2.73	4.01	1.77	8.42	17.5
Kelp	1.61	0.4	3.92	2.09	8.23	25.73
Green filamentous algae	1.64	0.54	3.75	1.48	7.87	33.61
Green turf algae	1.02	1.14	3.58	2.1	7.52	41.13
Metridium senile	0.62	0.76	2.36	1.25	4.95	46.08
Fucus spp.	0.43	0.97	2.25	1.57	4.72	50.8
Ciona intestinalis	0.79	0.25	2.12	1.15	4.45	55.25
Barnacle	0.89	0.33	2.1	1.23	4.4	59.65
Brown filamentous algae	2.08	1.82	1.44	1.45	3.03	62.68
Spirobrancus spp.	0.72	0.51	1.44	1.51	3.03	65.71
Encusting bryozoa	0.31	0.63	1.44	1.58	3.02	68.73
Electra pilosa	0.47	0	1.4	1.04	2.94	71.67

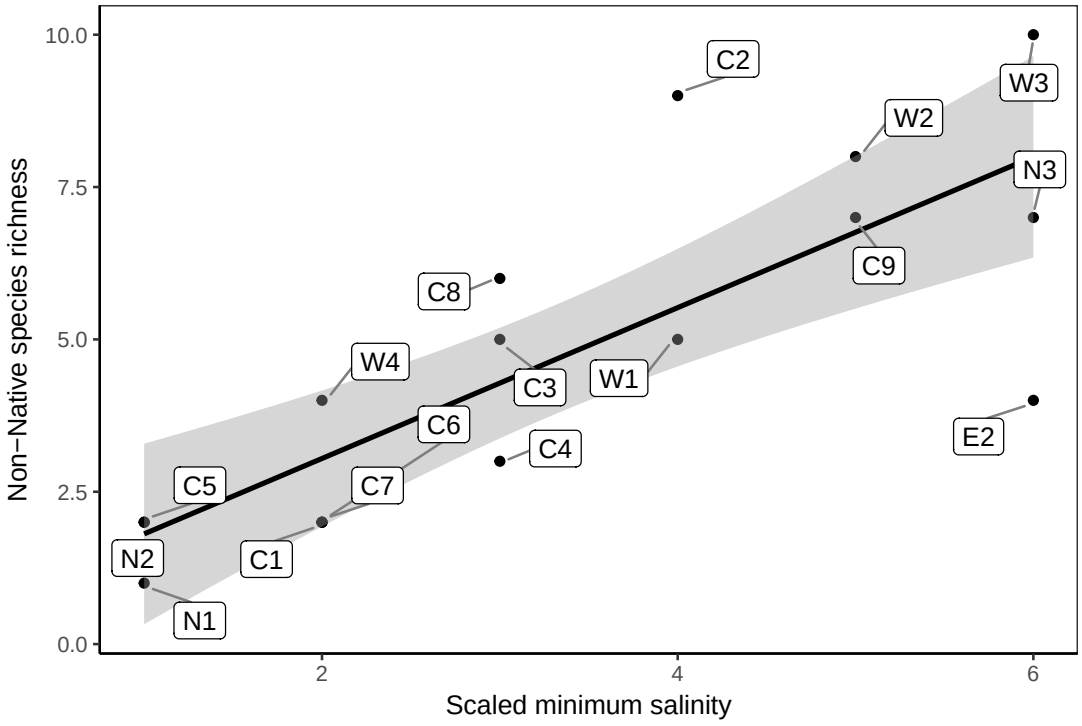

Figure B.5: *Scatter plot of the number of NNS against the scaled minimum salinity in each site. Salinity has been scaled from low (1) to high (6) based on the biological significance of the level of change.*



Figure B.6: *Photo of fouling species in site N3, showing presence of the limpet Patella vulgata and characteristic grazing patterns.*

Table B.5: *Salinity tolerance ranges found in the literature for target NNS identified in Scottish marinas. Tolerances should only be taken as guides, as data is patchy with methods used to determine ranges obtained through different methods, including through identification of known distribution limits. There is also a lack of consensus between some authors, thus database data has been preferentially relied upon where possible as these sources can be updated.*

Species	Salinity tolerance	Reference
ARTHROPODA		
Austrominius modestus	19-40 PSU	O'Riordan (2015)
Caprella mutica	<15 PSU	Ashton et al. (2007)
ASCIDIANS		
Asterocarpa humilis	No available data	
Botrylloides violaceus	20-38 ppt	Epelbaum et al. (2009)
Ciona intestinalis	11-42 PSU	O'Loughlin et al. (2006)
Corella eumyota	No tolerance of fresh or brackish conditions	Cardeccia et al. (2018)
	30-40 ppt	Narščius (2019b)
Didemnum vexillum	No tolerance of fresh or brackish conditions	Cardeccia et al. (2018)
Styela clava	10 - 36	O'Loughlin et al. (2006)
BRYOZOA		
Bugulina fulva	18-40 PSU	Fofonoff et al. (2018)
Bugulina simplex	18-30 PSU	Narščius (2019a)
Bugulina stolonifera	26-40 PSU	Fofonoff et al. (2018)
Fenestrulina delicia	No available data	
Schizoporella japonica	15-36 PSU	Loxton et al. (2017)
Tricellaria inopinata	20-35 PSU tolerated	Occhipinti-Ambrogi (2009)
CNIDARIA		
Cordylophora caspia	0.5-8.8 PSU	Jašinskas (2019)
Diadumene lineata	>7 PSU	Podbielski et al. (2016)
ALGAE		
Codium fragile ssp. fragile	17.5 - 40 PSU	O'Loughlin et al. (2006)
Melanothamnus harveyii	18-30 PSU	Narščius (2019c)
Dasysiohnia japonica	10 PSU lethal poor growth	Bjærke and Rueness (2004)
Sargassum muticum	< 15 ppt unable to reproduce, <25 ppt less competitive	Harries et al. (2007)
Undaria pinnatifida	Freshwater	Forrest and Blakemore (2006)
	0-27 PSU	O'Loughlin et al. (2006)

C - Chapter 4

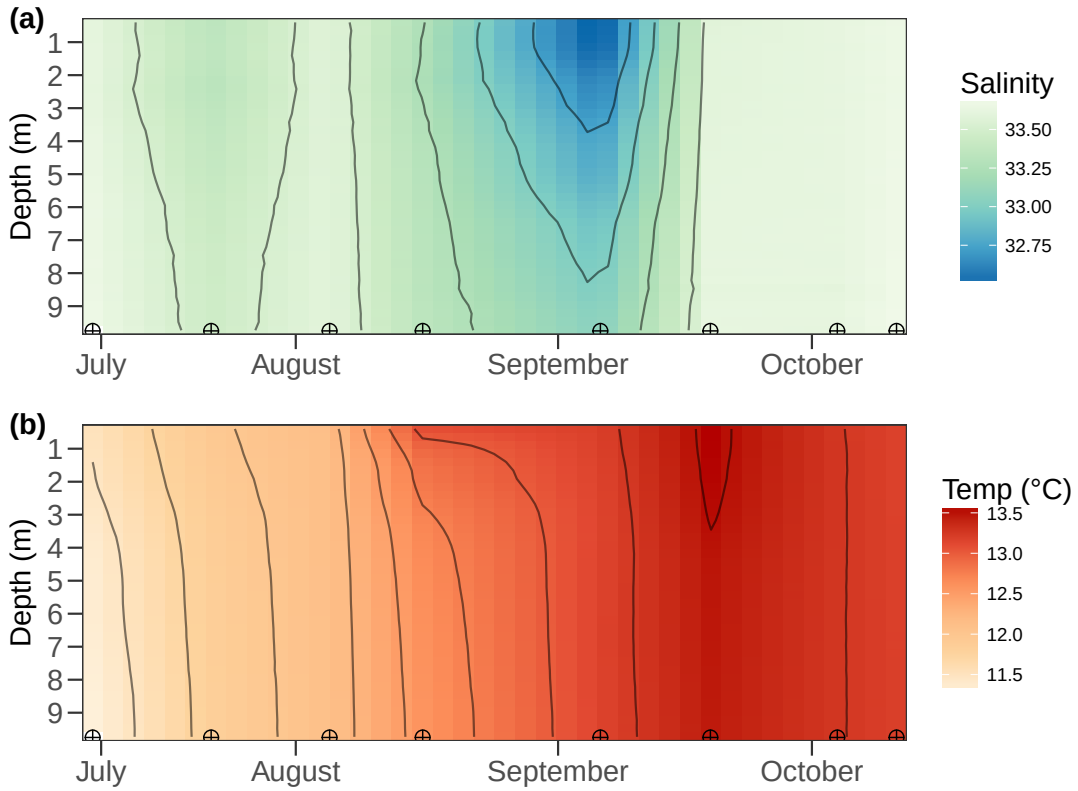

Figure C.1: *Figure showing the salinity (a) and temperature (b) depth profile of site E1. Salinity and temperature data is interpolated from 8 CTD measurements taken every 2 weeks over 4 months (exact sample time is indicated by ⊕). Data from CTD casts at 5 sites in site E1 has been averaged across the marina for each sample day at each depth. Contour lines represent a 1 unit change in salinity, and a 0.25 °C change in temperature.*

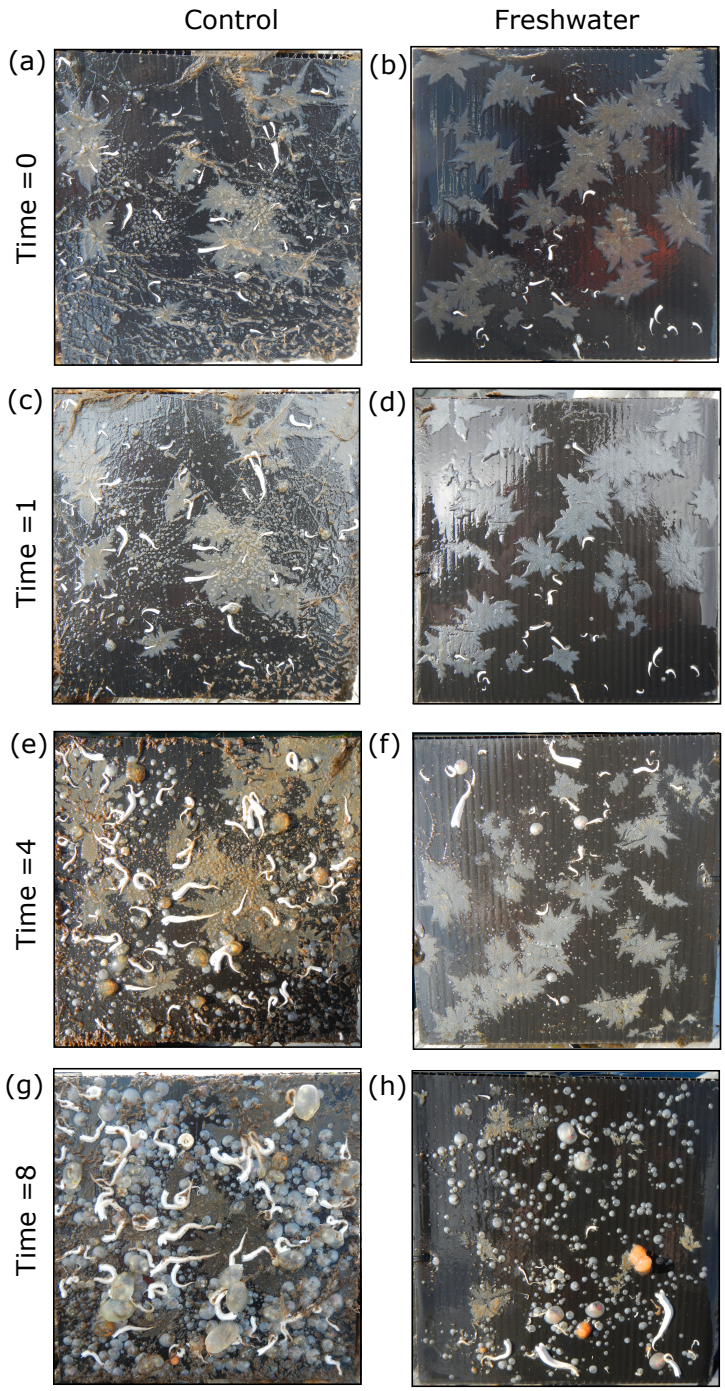

Figure C.2: *Figure showing photographs of two example horizontal panels from site B taken at the start of the experiment (a,b), and 1 (c,d), 4 (e,f), and 8 (g,h) weeks after replacement in the marina. The photographs on the left show a panel (H7) subject to the ambient seawater control, and the right show a panel (H39) subjected to the freshwater treatment. These photographs are examples of horizontal panels from site B.*

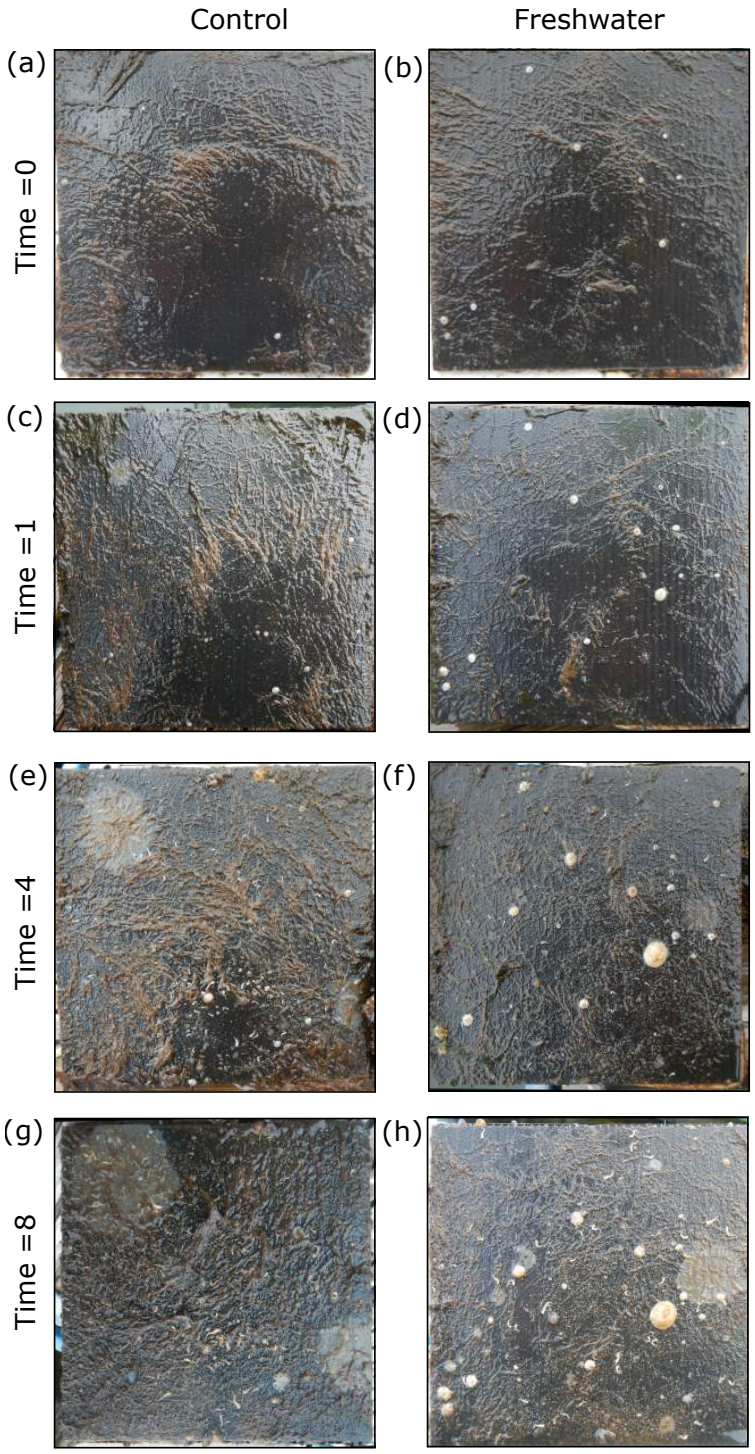

Figure C.3: *Figure showing photographs of two example horizontal panels from site C taken at the start of the experiment (a,b), and 1 (c,d), 4 (e,f), and 8 (g,h) weeks after replacement in the marina. The photographs on the left show a panel (H15) subject to the ambient seawater control, and the right show a panel (H21) subjected to the freshwater treatment. These photographs are typical of horizontal panels from site C.*

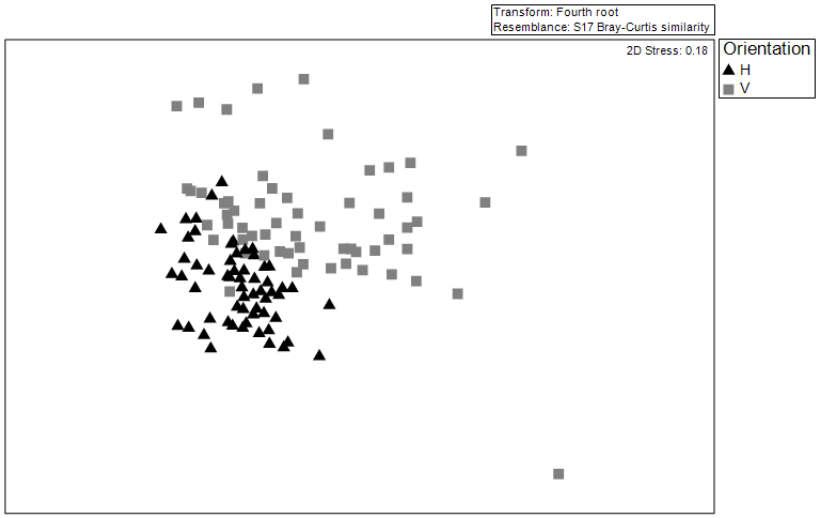

Figure C.4: *nMDS of pre-treatment biofouling community in site E1. Calculated on fourth-root transformed Bray-Curtis data, with orientation of panel communities shown as either Horizontal (H) or Vertical (V). The nMDS clearly shows a separation between pre-treatment 8 week-old biofouling communities grown on horizontal and vertically orientated panels, confirmed by results of an ANOSIM test.*

Table C.2: *Table showing the SIMPER results classifying the contribution of individual species to similarity between horizontal (a) and vertical (b) panels, and the dissimilarity between horizontal and vertical panels (c).*

Horizontal & Vertical panels - Average dissimilarity = 49.77

	Group H	Group V				
Species	Avg. Abund	Avg. Abund	Avg. Diss	Diss/SD	Contrib%	Cum.%
Electra pilosa	1.93	0.74	5.06	1.50	10.17	10.17
Obelia spp.	1.27	1.64	4.46	1.23	8.96	19.13
Amathia gracilis	1.05	0.83	3.78	1.28	7.59	26.72
Botryllus schlosseri	1.09	0.42	3.72	1.31	7.47	34.19
Diplosoma listerianum	2.11	1.47	3.27	1.09	6.56	40.76
Clytia hemisphaerica	0.83	0.09	3.23	1.14	6.50	47.25
Spirobranchus spp.	1.18	0.67	3.04	1.22	6.10	53.35
Uncolonised space	2.16	2.28	2.83	1.11	5.69	59.04
Ciona intestinalis	0.64	0.05	2.39	0.99	4.81	63.85
Brown filamentous algae	0.07	0.49	2.04	0.60	4.11	67.96
Green filamentous algae	0.02	0.49	1.93	0.61	3.88	71.85

Table C.1: *Species list for site E1 with percentage cover averaged shown for freshwater and ambient and treatment treated panels*

Taxon	Species	Horizontal		Vertical	
		Freshwater	Ambient	Freshwater	Ambient
Algae	Algae (grouped)	0.01 ± 0.01	0 ± 0	30.114 ± 22.86	8.57 ± 14.98
	Hydroides norvegicus	0 ± 0	0.165 ± 0.37	0.004 ± 0.01	0.073 ± 0.26
Annelida	*Pomatoceros sp.*	7.85 ± 5.77	9.308 ± 8.75	1.661 ± 2.33	6.273 ± 4.56
	Spirorbis (Spirorbis) spirorbis	0 ± 0	0 ± 0	0.004 ± 0.01	0.017 ± 0.02
Arthropoda	*Jassa spp. (tubes)*	1.23 ± 3.42	0 ± 0	0 ± 0	0 ± 0
	Amphiblestrum flemingii	0 ± 0	0.081 ± 0.28	0 ± 0	0 ± 0
	Amathia gracilis	0 ± 0	0.004 ± 0.01	0 ± 0	0 ± 0
	Callopora dumerilli	0.95 ± 1.74	1 ± 1.29	0.011 ± 0.02	0.003 ± 0.01
	Celeporella hyalina	2.004 ± 3.72	1.619 ± 3.43	0.375 ± 1.08	0.81 ± 1.26
Bryozoa -	*Clytia hemisphaerica*	1.008 ± 3.6	0.308 ± 1.11	0.357 ± 1.08	0 ± 0
	Conopeum reticulum	0.004 ± 0.01	0.004 ± 0.01	0 ± 0	0 ± 0
encrusting	*Cryptosula pallasiana*	0.412 ± 0.86	0.092 ± 0.27	0.014 ± 0.02	0.007 ± 0.02
	Electra pilosa	25.538 ± 13.48	24.154 ± 20.66	2.582 ± 3.27	11.203 ± 17.2
	Fenestrulina delicia	0.012 ± 0.02	0.077 ± 0.28	0.004 ± 0.01	0.067 ± 0.26
	Microporella ciliata	0.169 ± 0.37	0.012 ± 0.02	0.007 ± 0.02	0 ± 0
	Schizoporella japonica	5.077 ± 4.8	0.55 ± 1.45	1.161 ± 2.87	0.54 ± 0.74
	Bugula spp.	0.004 ± 0.01	0 ± 0	0 ± 0	0 ± 0
	Bugula flabellata	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Bryozoa	*Bugula fulva*	0.008 ± 0.02	0.546 ± 0.96	0.214 ± 0.8	0.4 ± 1.3
	Bugula stolonifera	0.238 ± 0.6	0.392 ± 0.87	0.071 ± 0.27	0 ± 0
(arborescent)	*Scrupocellaria scruposa*	0.077 ± 0.28	0 ± 0	0 ± 0	0 ± 0
	Scruparia ambigua	0.469 ± 0.87	0.077 ± 0.28	0.004 ± 0.01	0.407 ± 1.55
	Scruparia chelata	0.169 ± 0.55	0.004 ± 0.01	1.796 ± 2.91	0.21 ± 0.41
	Tricellaria inopinata	0.7 ± 1.31	0.542 ± 1.2	0.439 ± 0.85	1.277 ± 3.41
	Ascidian unident.	0.004 ± 0.01	0 ± 0	0 ± 0	0 ± 0
Chordata -	*Botryllus schlosseri*	3.085 ± 4.76	16.388 ± 16.65	0.375 ± 0.62	14.67 ± 14.9
Tunicata (colonial)	*Botrylloides leachii*	0 ± 0	0 ± 0	0 ± 0	0 ± 0
	Early stage Didemnid (unconf.)	0.158 ± 0.37	0 ± 0	0 ± 0	0.003 ± 0.01
	Diplosoma listerianum	56.077 ± 25.43	27.692 ± 11.88	29.293 ± 23.7	36.933 ± 26.33
	Ascidiella aspersa	0.788 ± 1.22	11.308 ± 12.42	0.004 ± 0.01	2.337 ± 4.37
	Ascidiella scabra	0.477 ± 0.87	9.696 ± 11.62	0.014 ± 0.02	1.077 ± 1.79
	Asterocarpa humilis	0.088 ± 0.27	0.085 ± 0.28	0.018 ± 0.02	0.01 ± 0.02
Chordata -	*Ciona intestinalis*	1.558 ± 3.01	15.396 ± 17.95	0.229 ± 0.42	0.267 ± 0.59
Tunicata (solitary)	*Corella eumyota*	0.473 ± 0.77	0.165 ± 0.55	0.004 ± 0.01	0 ± 0
	Dendrodoa grossularia	0 ± 0	0.388 ± 0.77	0 ± 0	0 ± 0
	Molgula sp.	0.012 ± 0.02	0.012 ± 0.02	0 ± 0	0.067 ± 0.26
	Austrominius modestus	0.088 ± 0.27	0.004 ± 0.01	0.004 ± 0.01	0.003 ± 0.01
Cirripedia	*Balanus crenatus*	0.085 ± 0.28	0 ± 0	0.004 ± 0.01	0.007 ± 0.02
	Verrruca stroemia	0 ± 0	0 ± 0	0.004 ± 0.01	0.003 ± 0.01
Cnidaria	*Metridium senile*	0 ± 0	0 ± 0	0 ± 0	0 ± 0
- Anthozoa	Anemone (unid.)	0.312 ± 1.11	3.392 ± 8.07	0 ± 0	0 ± 0
	Bougainvilla ramosa	0.154 ± 0.55	0 ± 0	0.286 ± 0.83	0 ± 0
Cnidaria	*Obelia spp.*	1.615 ± 3.8	0.927 ± 2.29	6.929 ± 9.52	3.8 ± 6.58
- Hydrozoa	*Turbularia indivisa*	0.7 ± 1.97	0.077 ± 0.28	0.004 ± 0.01	0.003 ± 0.01
Mollusca	*Anomia ephippium*	4.55 ± 5.23	0.496 ± 0.95	0.107 ± 0.26	0.23 ± 0.55
- Bivalvia	*Mytilus edulis*	0.104 ± 0.27	0.012 ± 0.02	0.371 ± 0.84	0.08 ± 0.26
	Leucosolenia botryoides	0.004 ± 0.01	0.096 ± 0.27	0.071 ± 0.27	0.15 ± 0.51
Porifera	*Leucosolenia complicata*	0 ± 0	0 ± 0	0 ± 0	0 ± 0
	Sycon ciliatum	0.023 ± 0.03	0.169 ± 0.37	0.154 ± 0.53	0.083 ± 0.25
na	BLANK SPACE	12.308± 9.4	7.615± 6.64	40.143± 31.38	27.333 ± 25.83

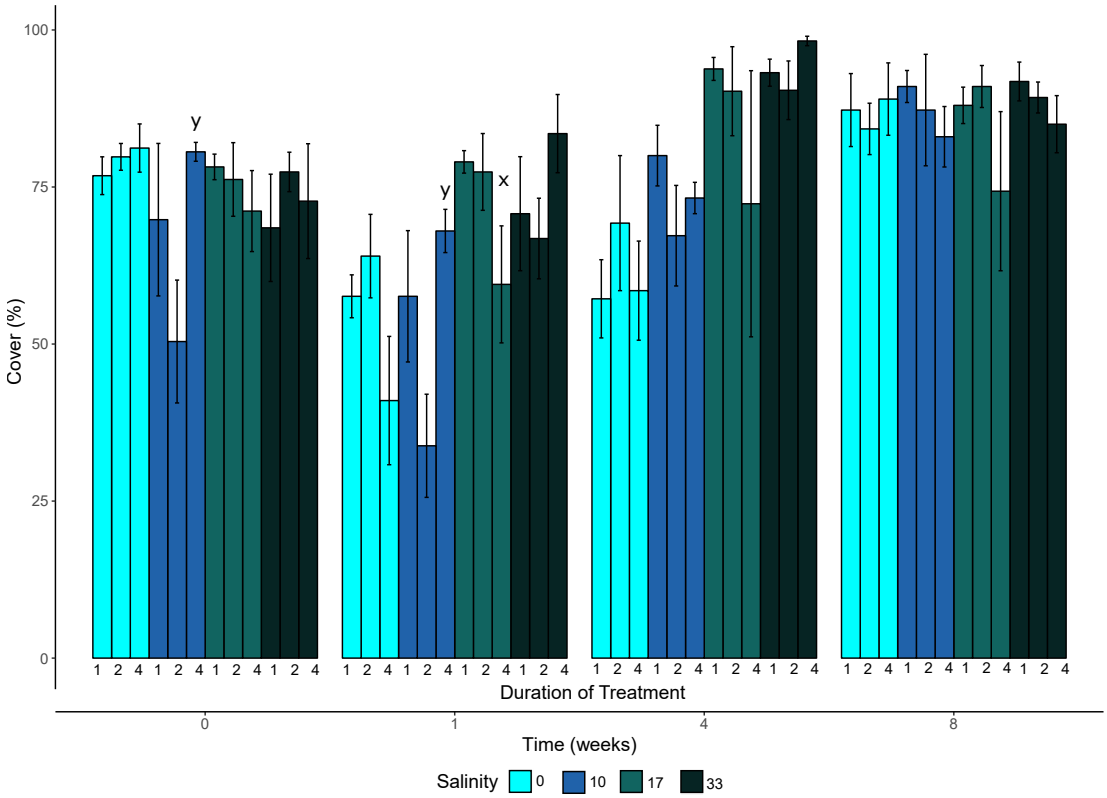

Figure C.5: *Bar chart showing biofouling cover on horizontally orientated panels at each recovery monitoring time point at 0,1,4 and 8 weeks post-treatment. The mean (±SE) percentage cover on panels exposed to different salinity treatments for different durations of treatment is shown. Significant pair-wise test between durations of exposure for each salinity treatment are indicated on the graphs (x - significant difference from 1 hour exposure period ($p<0.05$), y - significant difference from 2 hour exposure period ($p<0.05$)).*

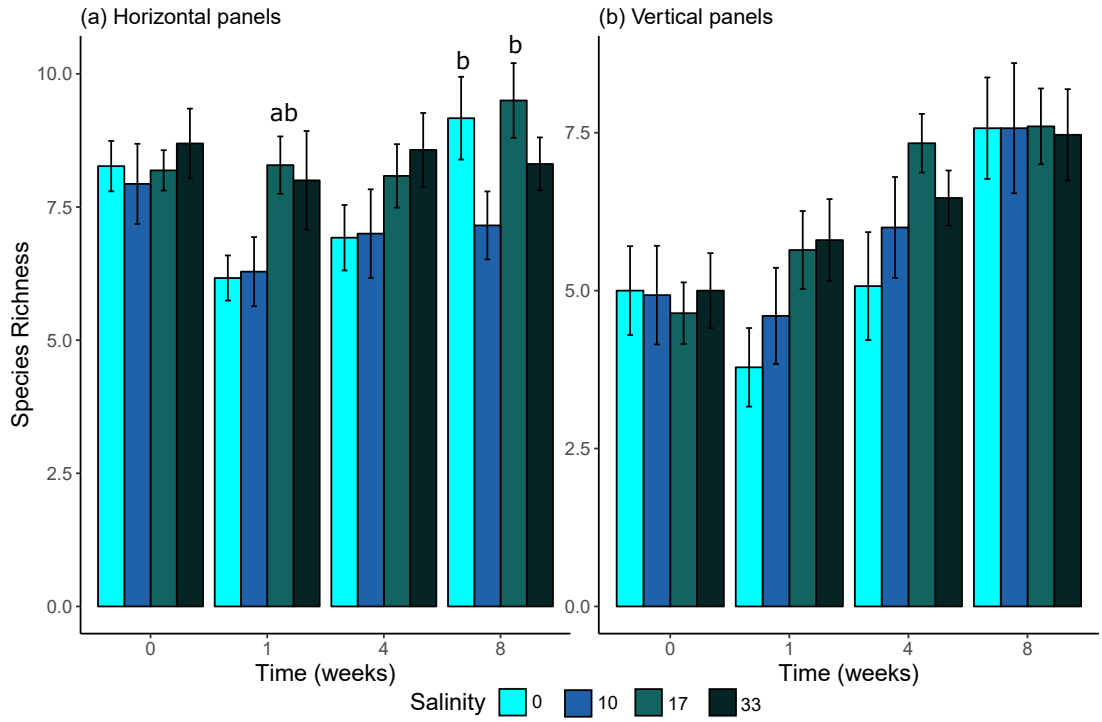

Figure C.6: *Bar chart showing species richness on horizontally (a) and vertically (b) orientated panels. The mean (±SE) species richness on panels exposed to different salinity treatments is shown at each recovery monitoring time point at 0,1,4 and 8 weeks post-treatment. Results are grouped across different exposure times as no interaction effect of exposure time and salinity was found in the RM-ANOVA. Significant pair-wise test results are indicated on the graphs (a - significant difference from freshwater treatment (p<0.01), b - significant difference from low (10) salinity treatment (p<0.01)).*

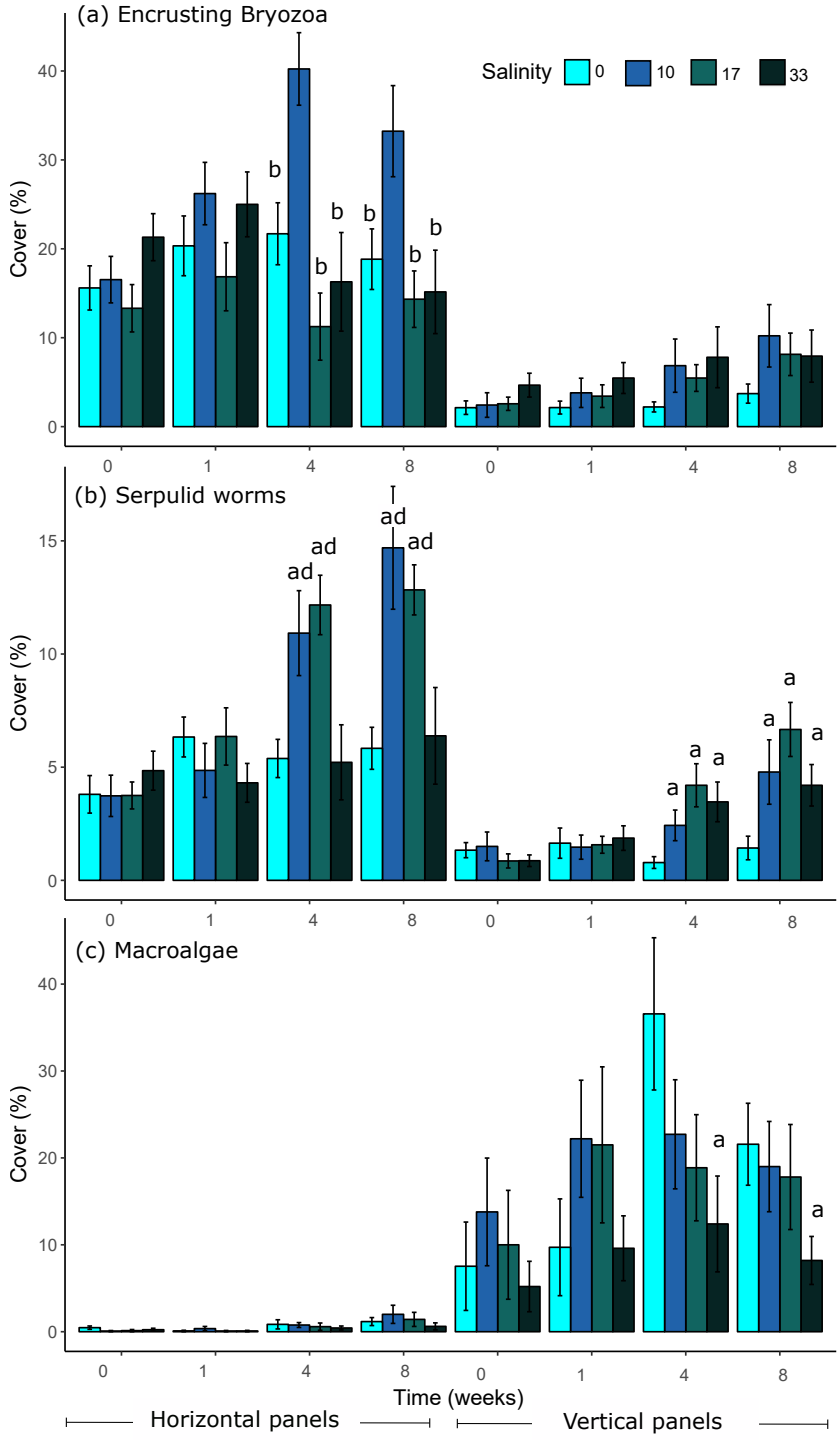

Figure C.7: *Bar chart showing cover of (a) encrusting bryozoans, (b) serpulid worms, and (c) macroalgae on horizontally and vertically orientated panels. The mean (±SE) percentage cover on panels exposed to different salinity treatments is shown at each recovery monitoring time point at 0,1,4 and 8 weeks post-treatment. Results are grouped across different exposure times. Significant pair-wise test results are indicated on the graphs (a - significant difference from freshwater treatment ($p<0.05$), b - significant difference from low (10) salinity treatment ($p<0.05$)), and d - signficant difference from ambient salinity treatment.*

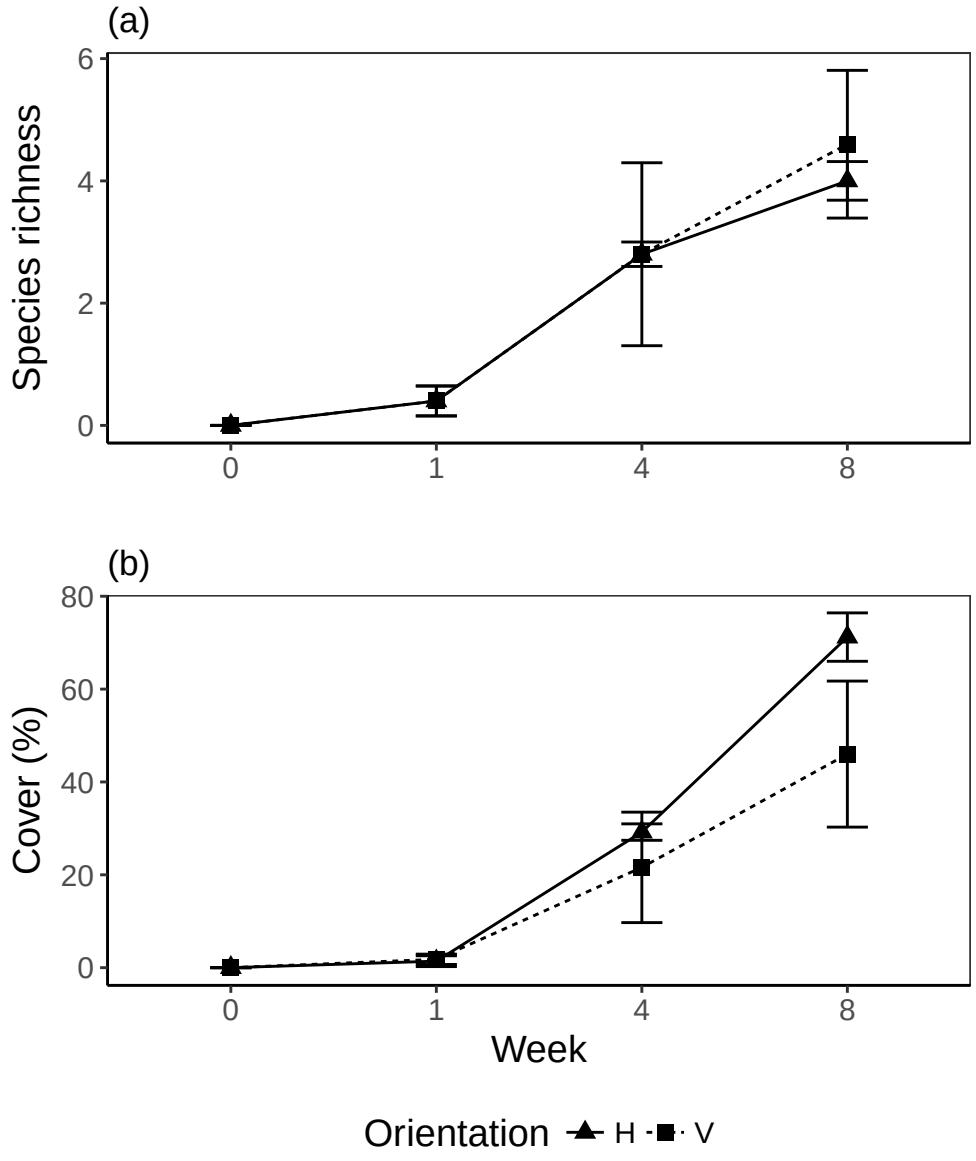

Figure C.8: *Stacked bar chart showing cover of different species on blank control panels set out at the beginning of the recovery period. Percentage cover of each species with more than 1% average cover is shown averaged for vertically and horizontally orientated panels.*

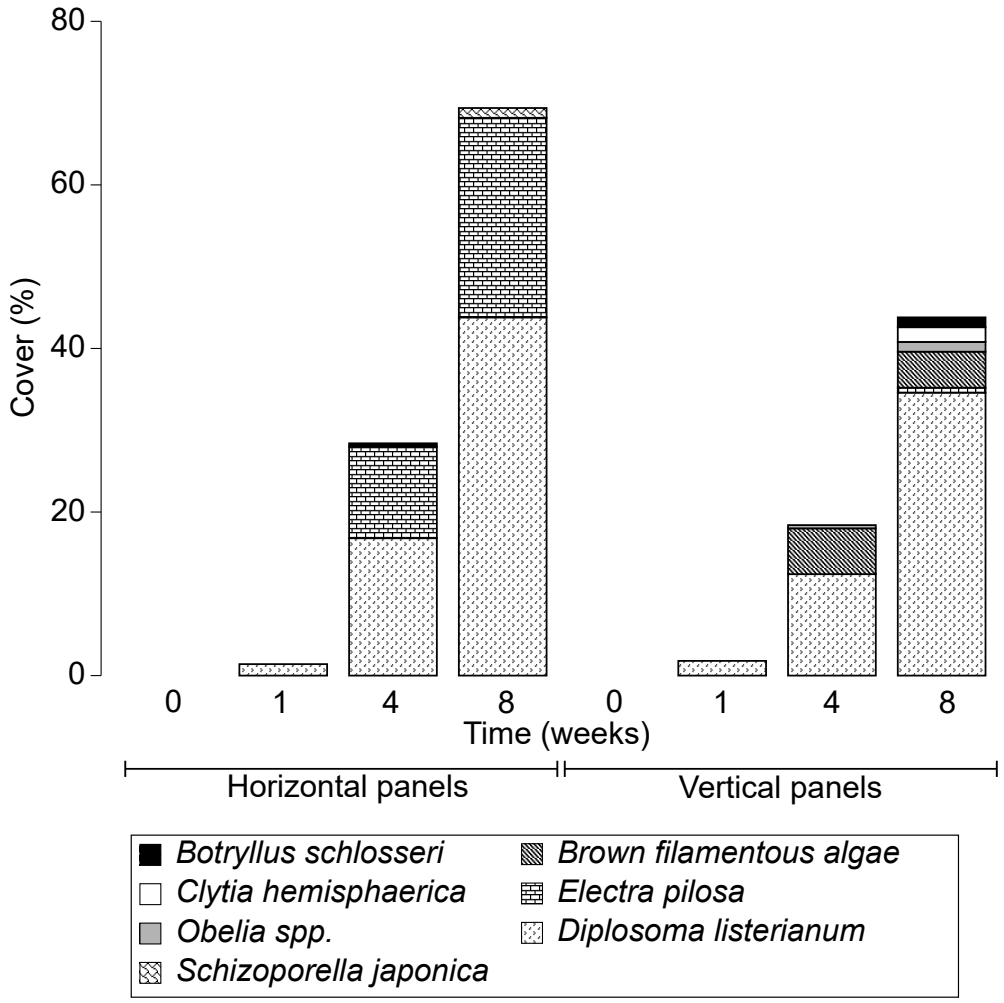

Figure C.9: *Stacked bar chart showing cover of different species on blank control panels set out at the beginning of the recovery period. Percentage cover of each species with more than 1% average cover is shown averaged for vertically and horizontally orientated panels.*

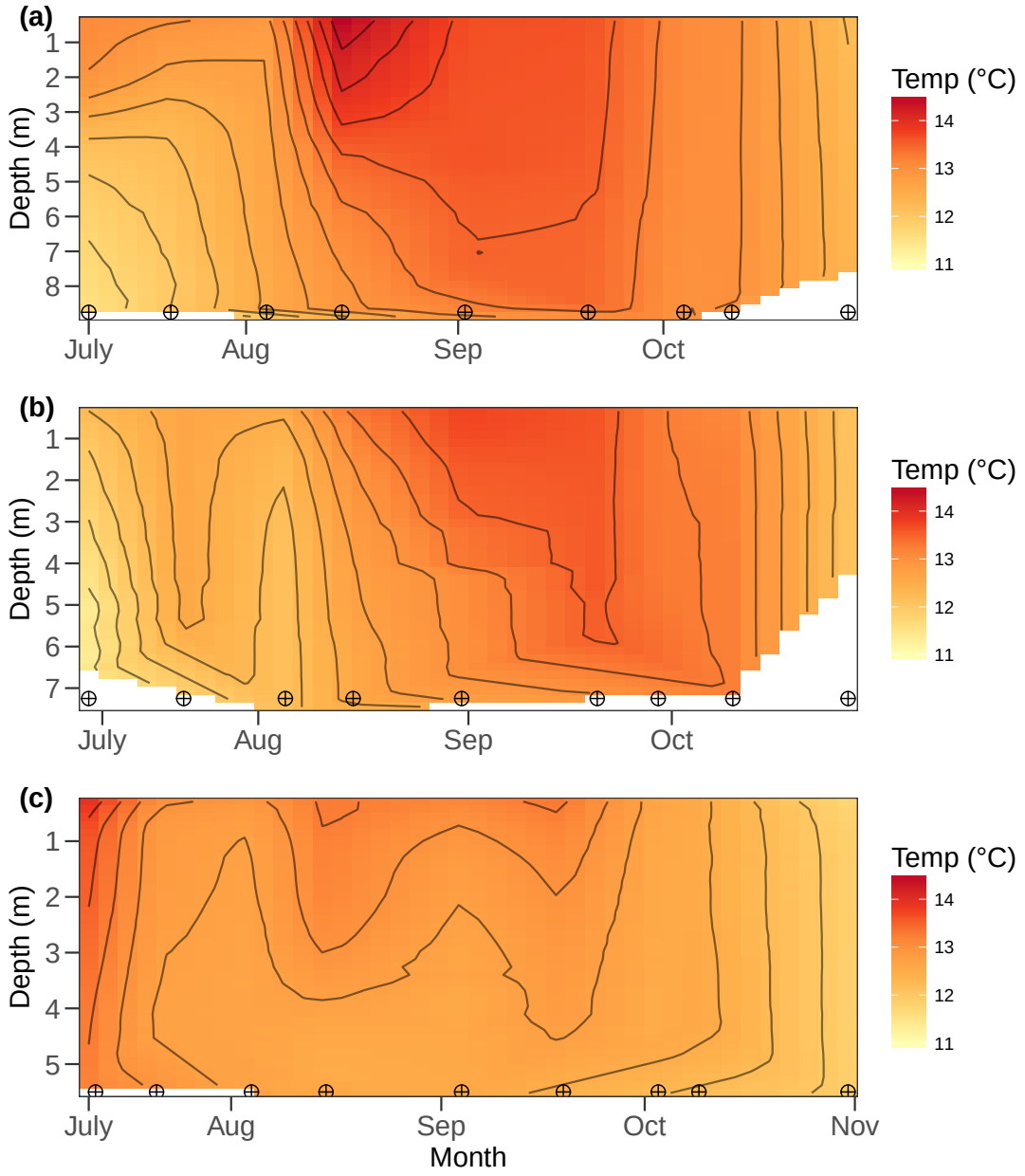

Figure C.10: *Figure showing the temperature depth profile of sites A (a), B (b), and C (c). Temperature data is interpolated from 8 CTD measurements taken every 2 weeks over 4 months (exact sample time is indicated by ⊕). Contour lines represent a 0.25 °C change in temperature.*

Table C.3: *Results of repeated-measures ANOVA on total percent cover of the biofouling community assemblage, excluding unidentified species. Analysis was performed using the PERMANOVA routine on a euclidean distance matrix.*

	Community assemblage						Native species					
Source	df	SS	MS	Pseudo-F	P(perm)	PERMDISP	df	SS	MS	Pseudo-F	P(perm)	PERMDISP
	Horizontal panels						Horizontal panels					
Salinity (Sa)	**3**	**5046.8**	**1682.3**	**4.9221**	**0.0051**		**3**	**4831.2**	**1610.4**	**3.404**	**0.0247**	
Exposure (Ex)	2	324.07	162.04	0.46752	0.6332		2	1069.8	534.91	1.1135	0.3332	
Time (Ti)	**3**	**13251**	**4417**	**42.253**	**0.0001**		**3**	**2717.4**	**905.79**	**7.9542**	**0.0002**	
SaxEx	**6**	**5818.5**	**969.76**	**2.7864**	**0.0242**	***	6	2316.2	386.03	0.79994	0.5754	
SaxTi	**9**	**7100.9**	**788.99**	**7.5474**	**0.0001**	**	**9**	**7264.6**	**807.17**	**7.0882**	**0.0001**	**n.s.**
ExxTi	6	500.32	83.386	0.79766	0.5836		6	432.65	72.109	0.63322	0.7008	
Panel(SaxEx)	**48**	**18669**	**388.94**	**3.7205**	**0.0001**	*	**48**	**26136**	**544.51**	**4.7815**	**0.0001**	*
SaxExxTi	18	3154.5	175.25	1.6764	0.0505		18	2786.5	154.81	1.3594	0.1658	
Residuals	118	12336	104.54				118	13437	113.88			
Total	213	71613					213	66279				
Pairwise comparison (Treatment)	Week 0: 0 ≠ 10* Week 1: 0 ≠ 17**; 0 ≠ 33**; 10 ≠ 17**; 10 ≠ 33** Week 4: 0 ≠ 10*; 0 ≠ 17**; 0 ≠ 33***; 10 ≠ 33***						Week 4: 0 ≠ 10***; 0 ≠ 17*; 0 ≠ 33** Week 8: 0 ≠ 10***; 0 ≠ 17***; 0 ≠ 33***					
	Vertical panels						Vertical panels					
Salinity (Sa)	3	4770.1	1590	0.48633	0.6963		3	2716.5	905.51	0.44224	0.7173	
Exposure (Ex)	2	1763.3	881.66	0.26991	0.7593		2	2649.9	1324.9	0.64761	0.5273	
Time (Ti)	**3**	**13108**	**4369.3**	**23.697**	**0.0001**	**n.s.**	3	975.85	325.28	1.1665	0.3197	
SaxEx	6	13007	2167.8	0.65768	0.6911		6	7373.9	1229	0.59575	0.7286	
SaxTi	9	3120.9	346.77	1.8806	0.0555		9	2023.6	224.85	0.80633	0.6071	
ExxTi	6	394.66	65.776	0.35673	0.9013		6	1274.1	212.34	0.76149	0.6091	
Panel(SaxEx)	**48**	**164820**	**3433.8**	**18.623**	**0.0001**	***	**48**	**102810**	**2141.8**	**7.6807**	**0.0001**	**
SaxExxTi	18	2964.5	164.7	0.8932	0.5847		18	4297.8	238.77	0.85624	0.6337	
Residuals	136	25077	184.39				136	37924	278.85			
Total	231	229380					231	162810				
Pairwise comparison (Salinity)												

	Non-native and cryptogenic species						Non-native species					
Source	df	SS	MS	Pseudo-F	P(perm)	PERMDISP	df	SS	MS	Pseudo-F	P(perm)	PERMDISP
	Horizontal panels											
Salinity (Sa)	**3**	**4500.9**	**1500.3**	**3.3734**	**0.0257**		3	18.432	6.144	0.60174	0.6505	
Exposure (Ex)	2	1036.1	518.07	1.1513	0.3213		2	9.6843	4.8421	0.47012	0.6561	
Time (Ti)	**3**	**5878.1**	**1959.4**	**10.512**	**0.0001**		**3**	**241.32**	**80.44**	**13.85**	**0.0001**	***
SaxEx	6	3600.6	600.1	1.329	0.2655		6	57.059	9.5098	0.9209	0.5009	
SaxTi	**9**	**13604**	**1511.5**	**8.1094**	**0.0001**	**	9	53.258	5.9176	1.0189	0.4393	
ExxTi	6	178.24	29.706	0.15938	0.9866		6	23.171	3.8619	0.66493	0.6903	
Panel(SaxEx)	**48**	**23813**	**496.1**	**2.6616**	**0.0001**	**n.s.**	**48**	**532.11**	**11.086**	**1.9087**	**0.0264**	**
SaxExxTi	18	5676	315.33	1.6918	0.052		18	86.344	4.7969	0.82591	0.6762	
Residuals	118	21994	186.39				118	685.34	5.808			
Total	213	80137					213	1718.2				
Pairwise comparison (Salinity)	Week 1: 0 ≠ 17*; 0 ≠ 33**; 10 ≠ 17**; 10 ≠ 33** Week 4: 10 ≠ 17**; 10 ≠ 33** Week 8: 0 ≠ 10**; 0 ≠ 17**; 0 ≠ 33***											
	Vertical panels						Vertical panels					
Salinity (Sa)	**3**	**5607.4**	**1869.1**	**3.4614**	**0.0239**		3	3.9829	1.3276	0.45313	0.7348	
Exposure (Ex)	2	745.91	372.95	0.69116	0.5022		2	2.7102	1.3551	0.46273	0.6505	
Time (Ti)	**3**	**7825**	**2608.3**	**20.304**	**0.0001**		**3**	**69.6**	**23.2**	**16.081**	**0.0001**	***
SaxEx	6	5089.7	848.28	1.5606	0.1815		6	6.0302	1.005	0.34153	0.9264	
SaxTi	**9**	**5751.1**	**639.01**	**4.9742**	**0.0001**	***	9	2.6051	0.28946	0.20063	0.9939	
ExxTi	6	1364.8	227.47	1.7707	0.1124		6	4.9259	0.82098	0.56904	0.7579	
Panel(SaxEx)	**48**	**26972**	**561.92**	**4.3741**	**0.0001**	***	**48**	**144.44**	**3.0091**	**2.0857**	**0.0047**	**
SaxExxTi	18	982.01	54.556	0.42468	0.9816		18	13.145	0.7303	0.50619	0.957	
Residuals	136	17471	128.47				136	196.21	1.4427			
Total	231	73254					231	445.89				
Pairwise comparison (Salinity)	Week 4: 0 ≠ 17***; 0 ≠ 33***; 10 ≠ 17**; 10 ≠ 33** Week 8: 10 ≠ 17*; 10 ≠ 33*											

*p<0.05, **p<0.01, ***p<0.001

The term TixPanel(SaxEx) was excluded from the model to account for the repeated-measures nature of the experimental design. P-values were obtained using 9,999 permutations of appropriate units. In the main model significant p-values (p<0.05) are presented in bold. Pairwise comparisons between ambient and freshwater treatments were carried out within the term Salinity*Time.

Table C.4: *Results of repeated-measures ANOVA on total species richness of the biofouling community assemblage, excluding unidentified species. Analysis was performed using the PERMANOVA routine on a euclidean distance matrix.*

	Community assemblage						Native species					
Source	df	SS	MS	Pseudo-F	P(perm)	PERMDISP	df	SS	MS	Pseudo-F	P(perm)	PERMDISP
				Horizontal panels						Horizontal panels		
Salinity (Sa)	3	43.537	14.512	1.4166	0.2554		3	33.192	11.064	1.5144	0.2267	
Exposure (Ex)	2	52.251	26.126	2.5118	0.095		2	24.264	12.132	1.6343	0.2044	
Time (Ti)	**3**	**47.626**	**15.875**	**6.2416**	**0.0005**		**3**	**36.948**	**12.316**	**8.1178**	**0.0002**	
SaxEx	6	123.34	20.557	1.9675	0.0952		6	65.66	10.943	1.4672	0.2148	
SaxTi	**9**	**63.34**	**7.0378**	**2.767**	**0.0061**	**n.s.**	**9**	**35.379**	**3.931**	**2.591**	**0.0087**	**n.s.**
ExxTi	6	7.8122	1.302	0.51191	0.8003		6	3.4413	0.57356	0.37804	0.8902	
Panel(SaxEx)	**48**	**565.24**	**11.776**	**4.6298**	**0.0001**	**n.s.**	**48**	**405.91**	**8.4564**	**5.5738**	**0.0001**	**n.s.**
SaxExxTi	18	29.946	1.6637	0.65409	0.8454		18	22.237	1.2354	0.81429	0.6867	
Residuals	118	300.13	2.5435				118	179.03	1.5172			
Total	213	1278.1					213	850.53				
Pairwise comparison (Treatment)	Week 1: 0 ≠ 17**; 10 ≠ 17* Week 8: 0 ≠ 10*; 10 ≠ 17*						Week 1: 0 ≠ 17* Week 4: 0 ≠ 33* Week 8: 10 ≠ 17*; 10 ≠ 33*					
				Vertical panels						Vertical panels		
Salinity (Sa)	3	25.422	8.4739	0.44376	0.7243		3	27.098	9.0328	0.91922	0.4411	
Exposure (Ex)	2	8.6412	4.3206	0.22644	0.7967		2	1.999	0.99952	0.10179	0.8973	
Time (Ti)	**3**	**277.47**	**92.489**	**34.194**	**0.0001**	**n.s.**	**3**	**180.88**	**60.292**	**33.927**	**0.0001**	
SaxEx	6	123.94	20.657	1.0738	0.3951		6	71.893	11.982	1.2108	0.3158	
SaxTi	9	45.962	5.1069	1.8881	0.0597		**9**	**35.623**	**3.9581**	**2.2273**	**0.0221**	**n.s.**
ExxTi	6	10.222	1.7037	0.6299	0.7008		6	5.3243	0.88738	0.49934	0.8089	
Panel(SaxEx)	**48**	**958.5**	**19.969**	**7.3828**	**0.0001**	***	**48**	**492.26**	**10.255**	**5.7709**	**0.0001**	**n.s.**
SaxExxTi	18	36.699	2.0388	0.75378	0.7466		18	16.298	0.90545	0.50951	0.9444	
Residuals	136	367.85	2.7048				136	241.69	1.7771			
Total	231	1864.3					231	1080.6				
Pairwise comparison (Salinity)							Week 1: 0 ≠ 17*; 0 ≠ 33* Week 4: 0 ≠ 17**; 17 ≠ 33*					

	Non-native and cryptogenic species						Non-native species					
Source	df	SS	MS	Pseudo-F	P(perm)	PERMDISP	df	SS	MS	Pseudo-F	P(perm)	PERMDISP
				Horizontal panels								
Salinity (Sa)	3	1.4884	0.49613	0.54153	0.6645		3	0.50324	0.16775	0.3132	0.8147	
Exposure (Ex)	2	4.5053	2.2526	2.4344	0.1048		2	0.92435	0.46218	0.85308	0.4171	
Time (Ti)	3	1.6746	0.55819	1.2052	0.309		**3**	**16.325**	**5.4417**	**23.547**	**0.0001**	***
SaxEx	6	9.7716	1.6286	1.7548	0.1321		6	3.2835	0.54725	1.0067	0.4339	
SaxTi	9	6.7275	0.7475	1.6139	0.1159		9	3.4685	0.38539	1.6677	0.1009	
ExxTi	6	2.6329	0.43882	0.94745	0.4672		6	0.44261	0.073768	0.31921	0.9219	
Panel(SaxEx)	**48**	**48.298**	**1.0062**	**2.1725**	**0.0003**	**n.s.**	**48**	**28.614**	**0.59612**	**2.5795**	**0.0002**	*
SaxExxTi	18	7.1502	0.39723	0.85767	0.6296		18	3.5172	0.1954	0.84552	0.641	
Residuals	118	54.652	0.46315				118	27.27	0.2311			
Total	213	135.91					213	86.304				
Pairwise comparison (Salinity)												
				Vertical panels						Vertical panels		
Salinity (Sa)	3	3.9354	1.3118	0.6389	0.5905		3	0.32898	0.10966	0.31951	0.8068	
Exposure (Ex)	2	3.2572	1.6286	0.79375	0.4584		2	0.5394	0.2697	0.78612	0.4528	
Time (Ti)	**3**	**19.061**	**6.3537**	**11.985**	**0.0001**	**n.s.**	**3**	**12.749**	**4.2497**	**20.966**	**0.0001**	***
SaxEx	6	7.6846	1.2808	0.6198	0.7109		6	0.30334	0.050556	0.14678	0.9883	
SaxTi	9	3.642	0.40467	0.76332	0.6419		9	0.84255	0.093617	0.46186	0.9004	
ExxTi	6	2.1756	0.3626	0.68396	0.6551		6	1.2558	0.20931	1.0326	0.409	
Panel(SaxEx)	**48**	**102.45**	**2.1344**	**4.026**	**0.0001**	**n.s.**	**48**	**16.833**	**0.35069**	**1.7301**	**0.0096**	***
SaxExxTi	18	11.316	0.62869	1.1859	0.2787		18	1.9461	0.10812	0.53341	0.9409	
Residuals	136	72.1	0.53015				136	27.567	0.2027			
Total	231	229.93					231	62.892				
Pairwise comparison (Salinity)												

*p<0.05, **p<0.01, ***p<0.001

The term TixPanel(SaxEx) was excluded from the model to account for the repeated-measures nature of the experimental design. P-values were obtained using 9,999 permutations of appropriate units. In the main model significant p-values (p<0.05) are presented in bold. Pairwise comparisons between ambient and freshwater treatments were carried out within the term Salinity*Time.

Table C.5: *Results of permutational analysis of variance (PERMANOVA) on response of the whole biofouling assemblage. Analysis was performed on Bray-Curtis similarities calculated from untransformed and fourth-root transformed percentage cover data.*

	Community assemblage (Untransformed)						Community assemblage (4th root transformed)					
Source	df	SS	MS	Pseudo-F	P(perm)	PERMDISP	df	SS	MS	Pseudo-F	P(perm)	PERMDISP
				Horizontal panels						Horizontal panels		
Salinity (Sa)	**3**	**26285**	**8761.6**	**4.0484**	**0.0001**		**3**	**12880**	**4293.2**	**3.0864**	**0.0002**	
Exposure (Ex)	2	3160.3	1580.1	0.71914	0.699		2	2787.3	1393.6	0.9867	0.4473	
Time (Ti)	**3**	**39511**	**13170**	**24.558**	**0.0001**		**3**	**26325**	**8775**	**26.067**	**0.0001**	
SaxEx	6	16841	2806.9	1.2717	0.1669		6	10034	1672.3	1.1787	0.2211	
SaxTi	**9**	**30128**	**3347.5**	**6.242**	**0.0001**	*	**9**	**11690**	**1298.9**	**3.8585**	**0.0001**	**n.s.**
ExxTi	6	2815.9	469.31	0.8751	0.6402		6	2372.2	395.37	1.1745	0.2483	
Panel(SaxEx)	**48**	**119420**	**2487.8**	**4.639**	**0.0001**	**n.s.**	**48**	**76830**	**1600.6**	**4.7547**	**0.0001**	**
SaxExxTi	18	12684	704.66	1.314	0.0597		18	6208	344.89	1.0245	0.4227	
Residuals	118	63282	536.29				118	39723	336.64			
Total	213	327960					213	193690				
Pairwise comparison (Treatment)	Week 1: 0 $\neq$ 17**, 33**; 10 $\neq$ 17***, 33***						Week 1: 0 $\neq$ 17**, 33***; 10 $\neq$ 17***, 33***					
	Week 4: 0 $\neq$ 10**, 17**, 33***; 10 $\neq$ 17***, 33***						Week 4: 0 $\neq$ 17***,33***; 10 $\neq$ 17***, 33***					
	Week 8: 0 $\neq$ 10**, 17***, 33***; 10 $\neq$ 17**, 33**						Week 8: 0 $\neq$ 10*, 17***, 33**; 10 $\neq$ 17**, 33**					
				Vertical panels						Vertical panels		
Salinity (Sa)	3	23591	7863.6	1.5122	0.1398		3	17534	5844.7	1.577	0.1094	
Exposure (Ex)	2	4373.8	2186.9	0.42088	0.899		2	3857.1	1928.6	0.52079	0.8263	
Time (Ti)	**3**	**39874**	**13291**	**16.56**	**0.0001**		**3**	**25218**	**8405.9**	**18.058**	**0.0001**	
SaxEx	6	18738	3123	0.59619	0.9024		6	15680	2613.3	0.69982	0.8276	
SaxTi	**9**	**16776**	**1864**	**2.3224**	**0.0001**	**n.s.**	**9**	**10409**	**1156.6**	**2.4847**	**0.0001**	**n.s.**
ExxTi	6	3951	658.49	0.82044	0.7538		6	2691.9	448.66	0.96382	0.5335	
Panel(SaxEx)	**48**	**260860**	**5434.5**	**6.7711**	**0.0001**	***	**48**	**186180**	**3878.8**	**8.3326**	**0.0001**	***
SaxExxTi	18	10840	602.23	0.75035	0.9599		18	7129.3	396.07	0.85086	0.7759	
Residuals	136	109150	802.61				136	63308	465.5			
Total	231	491980					231	336600				
Pairwise comparison (Salinity)	Week 4: 0 $\neq$ 17**, 33**; 10 $\neq$ 17**, 33**						Week 4: 0 $\neq$ 17**, 33**					
	Week 8: 0 $\neq$ 17*, 33*; 10 $\neq$ 17*, 33*						Week 8: 0 $\neq$ 17**,33**; 10 $\neq$ 33*					

*p<0.05, **p<0.01, ***p<0.001

The term TixPanel(SaxEx) was excluded from the model to account for the repeated-measures nature of the experimental design. P-values were obtained using 9,999 permutations of appropriate units. In the main model significant p-values (p<0.05) are presented in bold. Pairwise comparisons between ambient and freshwater treatments were carried out within the term Salinity*Time.

Table C.6: *Species list for site A showing the percentage cover for each species averaged for freshwater and ambient treatments and for horizontally and vertically orientated panels.*

Taxon	Species	Horizontal		Vertical	
		Freshwater	Ambient	Freshwater	Ambient
Algae	*Algae (grouped)*	0 ± 0	0.006 ± 0.02	6.606 ± 7.96	10.14 ± 23.03
Annelida	*Hydroides norvegicus*	6 ± 2.45	1.011 ± 1.31	0.778 ± 0.83	0.21 ± 0.42
	Pomatoceros spp.	28 ± 9.45	1.361 ± 2.04	9 ± 5.07	5.705 ± 4.56
Bryozoa - encrusting	*Spirorbis (Spirorbis) spirorbis*	0.157 ± 0.37	0.006 ± 0.02	0.256 ± 0.42	0.12 ± 0.31
	Callopora dumerilli	0.007 ± 0.02	0.006 ± 0.02	0 ± 0	0 ± 0
	Celleporella hyalina	2.429 ± 1.4	0.356 ± 0.99	7.9 ± 7.98	0.53 ± 0.95
	Cryptosula pallasiana	0.157 ± 0.37	0 ± 0	0.572 ± 1	0.005 ± 0.02
	Electra pilosa	27.143 ± 8.97	0.911 ± 1.95	4.35 ± 4.59	1.71 ± 1.48
	Membraniporella nitida	0 ± 0	0 ± 0	0.006 ± 0.02	0 ± 0
	Schizoporella japonica	14.143 ± 3.89	0.033 ± 0.03	17.444 ± 11.91	1.61 ± 1.25
Bryozoa arborescent)	*Amathia gracilis*	0.721 ± 1.89	0 ± 0	0.006 ± 0.02	0 ± 0
	Bugula flabellata	2.286 ± 1.7	0.117 ± 0.33	0.117 ± 0.33	0.005 ± 0.02
	Bugula fulva	1.286 ± 1.38	0.339 ± 0.7	0.344 ± 0.7	0.9 ± 1.37
	Bugula simplex	0.293 ± 0.75	0 ± 0	0.239 ± 0.66	0 ± 0
	Bugula sp.	0.143 ± 0.38	0.006 ± 0.02	0.011 ± 0.02	0 ± 0
	Scruparia ambigua	0 ± 0	0 ± 0	0.117 ± 0.33	0 ± 0
	Tricellaria inopinata	12 ± 8.27	9.444 ± 4.28	5.444 ± 2.7	25.9 ± 17.3
Chordata - Tunicata (colonial)	*Applidium spp.*	0 ± 0	0.222 ± 0.67	0 ± 0	0.11 ± 0.31
	Botrylloides leachii	0 ± 0	0 ± 0	0 ± 0	0.3 ± 0.67
	Botrylloides sp.	0 ± 0	0 ± 0	0 ± 0	0.8 ± 1.4
	Botryllus schlosseri	9.864 ± 9.4	14.333 ± 7.52	12.228 ± 13.32	31.9 ± 12.1
	Diplosoma listerianum	25.714 ± 7.78	24.889 ± 14	19.778 ± 9.9	38 ± 15.85
Chordata - Tunicata (solitary)	*Ascidiella aspersa*	3.721 ± 8.52	77.333 ± 12	0.011 ± 0.02	9.8 ± 11.51
	Ascidiella scabra	2.721 ± 4.23	14.333 ± 11.47	0.578 ± 0.71	12.1 ± 13.42
	Ciona intestinalis	33.857 ± 10.16	5.339 ± 6.16	3.561 ± 5.02	0.9 ± 1.52
	Corella eumyota	0 ± 0	0.333 ± 1	0.006 ± 0.02	0.1 ± 0.32
	Dendrodoa grossularia	0 ± 0	0.444 ± 1.33	0 ± 0	0.2 ± 0.63
Cirripedia	*Austrominius modestus*	0.45 ± 0.51	0 ± 0	0.022 ± 0.03	0.005 ± 0.02
	Balanus crenatus	3 ± 2.31	0 ± 0	0.789 ± 1.08	0.01 ± 0.02
	Verruca stroemia	0 ± 0	0 ± 0	0.006 ± 0.02	0.005 ± 0.02
Cnidaria - Anthozoa	Tiny Anemone	5.143 ± 4.67	0.25 ± 0.66	0 ± 0	0 ± 0
Cnidaria - Hydrozoa	*Obelia spp.*	4.586 ± 6.02	0.9 ± 1.96	5.228 ± 7.71	0.815 ± 0.79
Mollusca - Bivalvia	*Anomia epphipium*	11.571 ± 3.36	0.028 ± 0.03	1.589 ± 3.93	0.125 ± 0.31
	Mytilus edulis	0.864 ± 0.89	0.261 ± 0.65	0.133 ± 0.33	0.335 ± 0.94
Porifera	*Leucosolenia complicata*	0.293 ± 0.75	0.006 ± 0.02	0.117 ± 0.33	0.605 ± 0.84
	Sycon ciliatum	0.007 ± 0.02	0.011 ± 0.02	0.028 ± 0.03	0.225 ± 0.41
	BLANK	4.143 ± 2.73	0.556 ± 0.73	22.778 ± 12.01	3.8 ± 4.37

Table C.7: *Species list for site B showing the percentage cover for each species averaged for freshwater and ambient treatments and for horizontally and vertically orientated panels.*

Phyla	Species	Mean percentage cover ± SD			
		Horizontal		Vertical	
		Freshwater	Ambient	Freshwater	Ambient
Algae	Algae (grouped)	0.81 ± 2.2	0.805 ± 1.24	38.08 ± 31.27	38.24 ± 32.36
Annelida	*Hydroides norvegicus*	0.23 ± 0.42	0.23 ± 0.41	0	0.005 ± 0.02
	Spirorbis (Spirorbis) spirorbis	0.01 ± 0.02	0	0	0
Bryozoa (encrusting)	*Amphiblestrum sp.*	0	0	0.005 ± 0.02	0
	Callopora sp.	0.105 ± 0.31	0.005 ± 0.02	0	0
	Celleporella hyalina	0.21 ± 0.42	0.31 ± 0.67	0.005 ± 0.02	0.005 ± 0.02
	Cryptosula pallasiana	0	0	0.005 ± 0.02	0
	Electra pilosa	15.8 ± 14.47	42.2 ± 14.5	1.505 ± 2.59	8.4 ± 9.83
	Leucosolenia complicata	0	0.01 ± 0.02	0.005 ± 0.02	0
	Scrupocellaria scruposa	0	0.005 ± 0.02	0	0.005 ± 0.02
Bryozoa (arborescent)	***Amathia gracilis***	0.01 ± 0.02	0.215 ± 0.41	5.005 ± 6.21	3.41 ± 5.67
	Pomatoceros spp.	4.51 ± 3.33	19.205 ± 8.69	1.7 ± 2.26	5.2 ± 3.82
Chordata - Tunicata (colonial)	*Botrylloides sp.*	0	0.5 ± 1.27	0	0
	Botrylloides leachii	0	0.6 ± 1.9	0	0
	Botryllus schlosseri	0.005 ± 0.02	0.1 ± 0.32	0	0
	Diplosoma listerianum	0.325 ± 0.47	0.31 ± 0.95	0.005 ± 0.02	0
	Didemnid (unconf.)	0	0	0.005 ± 0.02	0
	Applidium sp.	0.505 ± 1.58	0.01 ± 0.02	0	0.005 ± 0.02
Chordata - Tunicata (solitary)	*Ascidiella aspersa*	0.005 ± 0.02	1.5 ± 1.9	0.105 ± 0.31	0.8 ± 1.32
	Ascidiella scabra	0.42 ± 0.69	8.905 ± 6.82	0.305 ± 0.48	0.9 ± 1.85
	Ciona intestinalis	0.21 ± 0.42	0.32 ± 0.67	0	0.005 ± 0.02
	Clytia hemispherica	0.505 ± 0.97	0.515 ± 1.07	1.4 ± 2.46	1 ± 2
	Corella eumyota	0.03 ± 0.03	0.42 ± 0.69	0.025 ± 0.03	0.115 ± 0.31
Cirripedia	***Austrominius modestus***	0.015 ± 0.02	0.015 ± 0.02	0	0.3 ± 0.48
	Balanus crenatus	0.02 ± 0.03	0.12 ± 0.31	0.005 ± 0.02	0.01 ± 0.02
Cnidaria - Anthozoa	*Metridium dianthus*	0.505 ± 0.85	0.305 ± 0.48	0.11 ± 0.31	0.505 ± 0.97
	Tiny Anemone	0	0.41 ± 0.69	0	0.005 ± 0.02
Cnidaria - Hydrozoa	*Bougainvilla ramosa*	0.605 ± 1.26	1.21 ± 3.15	11.605 ± 16.72	16.81 ± 19.29
	Molgula sp.	0.215 ± 0.41	5.11 ± 11.08	0.01 ± 0.02	0.11 ± 0.31
	Obelia spp.	0.51 ± 1.09	6.31 ± 8.74	0.6 ± 1.33	2.01 ± 3.84
	Sarsia lovenii	0	0	0	0.3 ± 0.95
	Sarsia tubulosa	0	0	0	0.3 ± 0.95
	Turbularia indivisa	1.21 ± 3.45	4.3 ± 11.94	4.5 ± 7.89	5.41 ± 7.84
	Clava multicornis	0	0.005 ± 0.02	0	0.005 ± 0.02
Mollusca - Bivalvia	*Anomia epphipium*	22.2 ± 11.16	19.805 ± 14.15	2.505 ± 2.46	6 ± 4.74
	Mytilus edulis	0.02 ± 0.03	0.03 ± 0.03	0.03 ± 0.03	0.03 ± 0.03

Table C.8: *Species list for site C showing the percentage cover for each species averaged for freshwater and ambient treatments and for horizontally and vertically orientated panels.*

Phyla	Species	Mean percentage cover ± SD			
		Horizontal		Vertical	
		Freshwater	Ambient	Freshwater	Ambient
	Algae (grouped)	0.4 ± 0.97	0	23.605 ± 35.35	36.51 ± 21.85
Mollusca - Bivalvia	*Anomia epphipium*	0.82 ± 1.02	1.12 ± 1.18	0.13 ± 0.31	0.425 ± 0.83
Annelida	*Spirobranchus spp.*	4.8 ± 2.15	9.305 ± 4.8	1.105 ± 0.99	2.61 ± 3.53
	Spirorbis (Spirorbis) spirorbis	0.005 ± 0.02	0	0	0
Bryozoa (arborescent)	***Amathia gracilis***	29.2 ± 14.74	20.1 ± 18.71	19.4 ± 18.87	27.6 ± 14.33
	Celleporella hyalina	0.015 ± 0.02	0.02 ± 0.03	0.025 ± 0.03	0.21 ± 0.42
Bryozoa (encrusting)	*Cryptosula pallasiana*	1.41 ± 1.25	1.02 ± 1.23	0.335 ± 0.66	0.92 ± 1.08
	Membranipora membranaceae	9.205 ± 9.61	14.7 ± 10.76	0.015 ± 0.02	3.105 ± 4.35
Chordata - Tunicata (colonial)	*Diplosoma listerianum*	0.2 ± 0.63	0.1 ± 0.32	0	0
- Tunicata (solitary)	*Ciona intestinalis*	0.41 ± 0.69	0.015 ± 0.02	0	0.11 ± 0.31
Cirripedia	***Austrominius modestus***	9.1 ± 7.36	5.2 ± 3.49	9.81 ± 9.93	4.015 ± 5.63
	Balanus crenatus	1.41 ± 1.16	0.505 ± 0.52	0.915 ± 1.28	0.3 ± 0.95
Cnidaria - Anthozoa	***Cordylophora caspia***	35.7 ± 16.81	43.7 ± 10.93	36.6 ± 23.06	14.4 ± 9.03
Mollusca - Bivalvia	*Mytilus edulis*	2.715 ± 4.51	1.715 ± 1.62	1.72 ± 2.15	1.415 ± 1.49

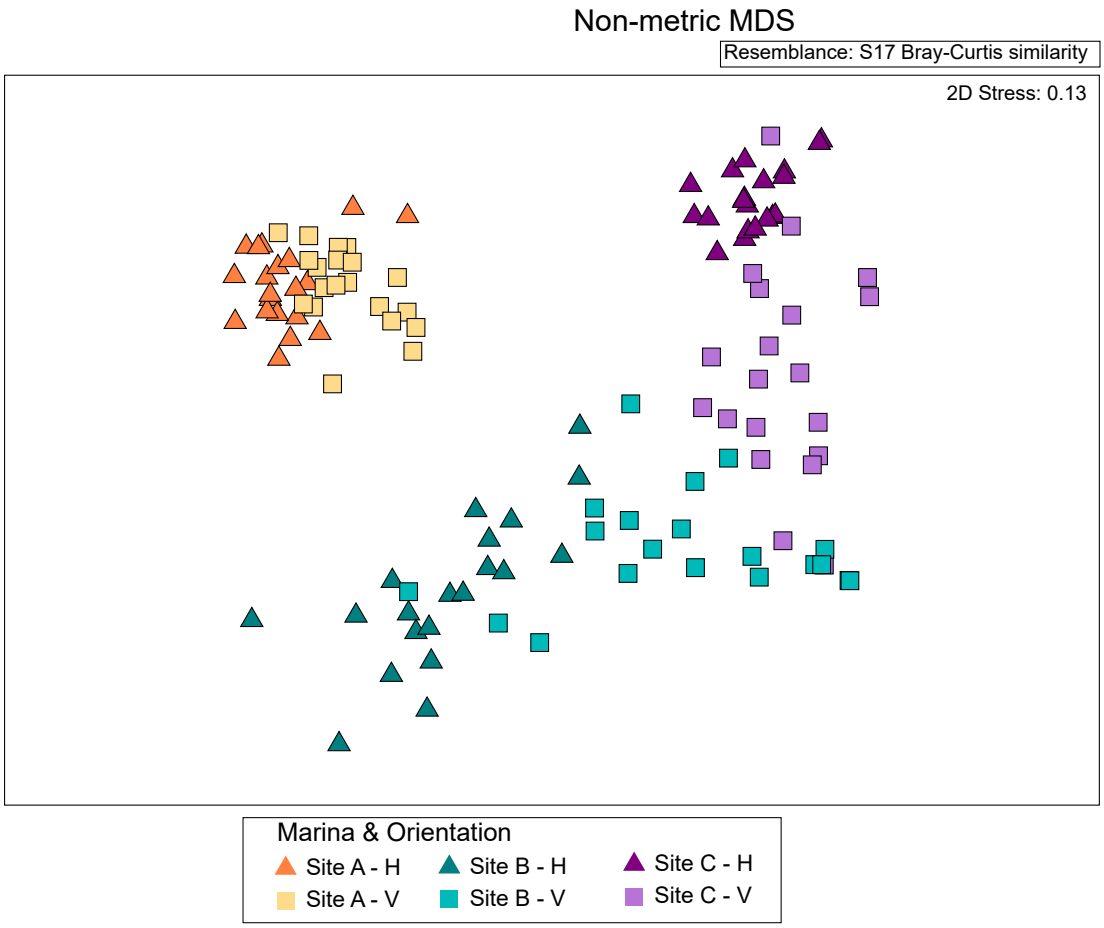

Figure C.11: *Figure showing nMDS ordination of panel communities from all three marinas in the freshwater immersion experiment based on untransformed percentage cover data and Bray-Curtis similarities from photos taken immediately after experimental treatment (Week = 0). Horizontal panel communities are shown by □, while vertical panel communities are represented by △.*

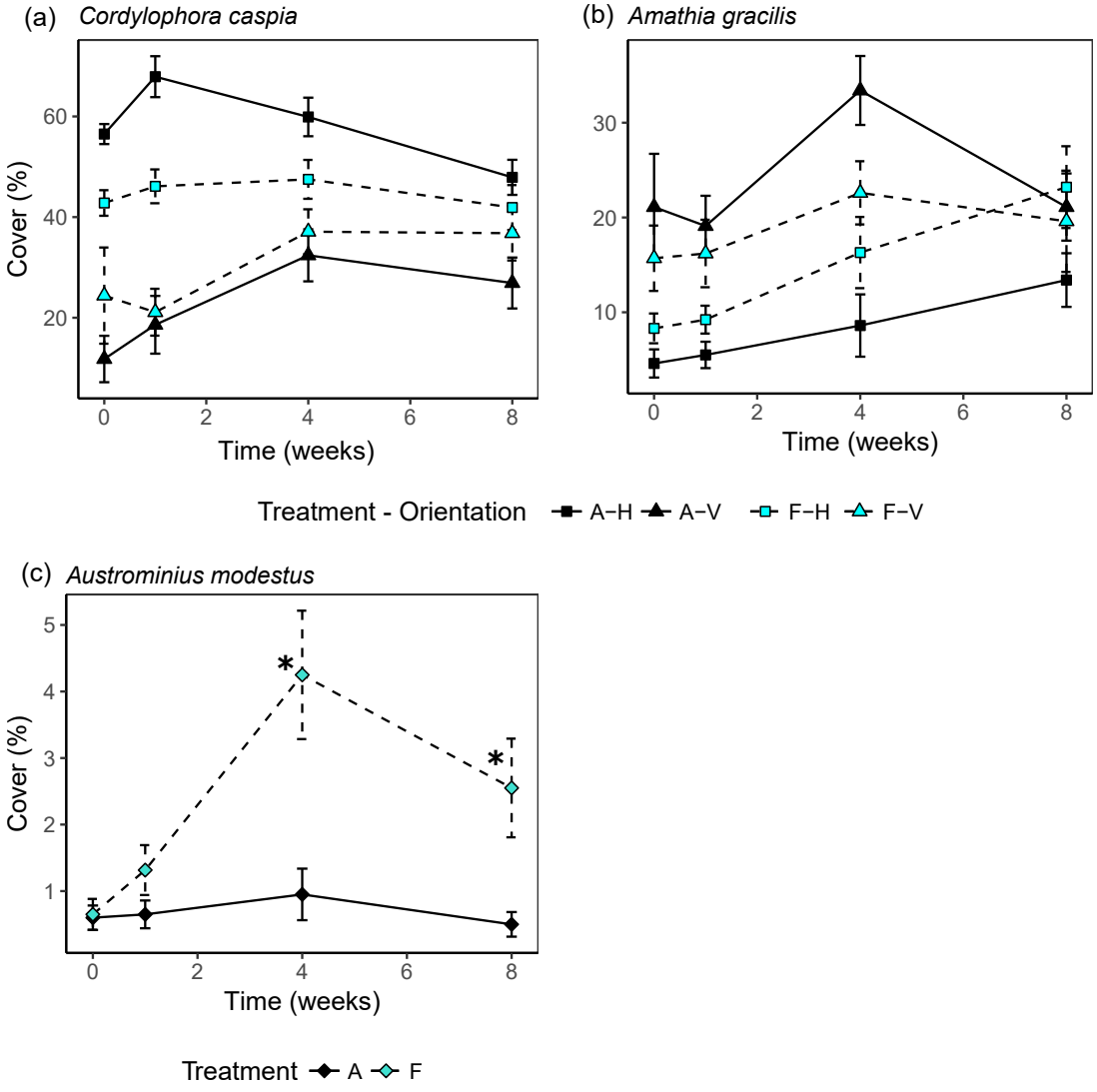

Figure C.12: *Figure showing treatment effect on mean percentage cover of Cordylophora caspia (a), Amathia gracilis (b) and Austrominius modestus (c) in site C. Treatment is classed as ambient seawater (A) or as freshwater (F), and orientation of panels in the field is shown as horizontal (H = △) or vertical (V = □). Cover is merged between panel orientations for Austrominius modestus as there was no significant difference in cover between horizontal and vertical panels. Significant pair-wise test results between treatments at each time point are indicated on graph (c): *p<0.05.*

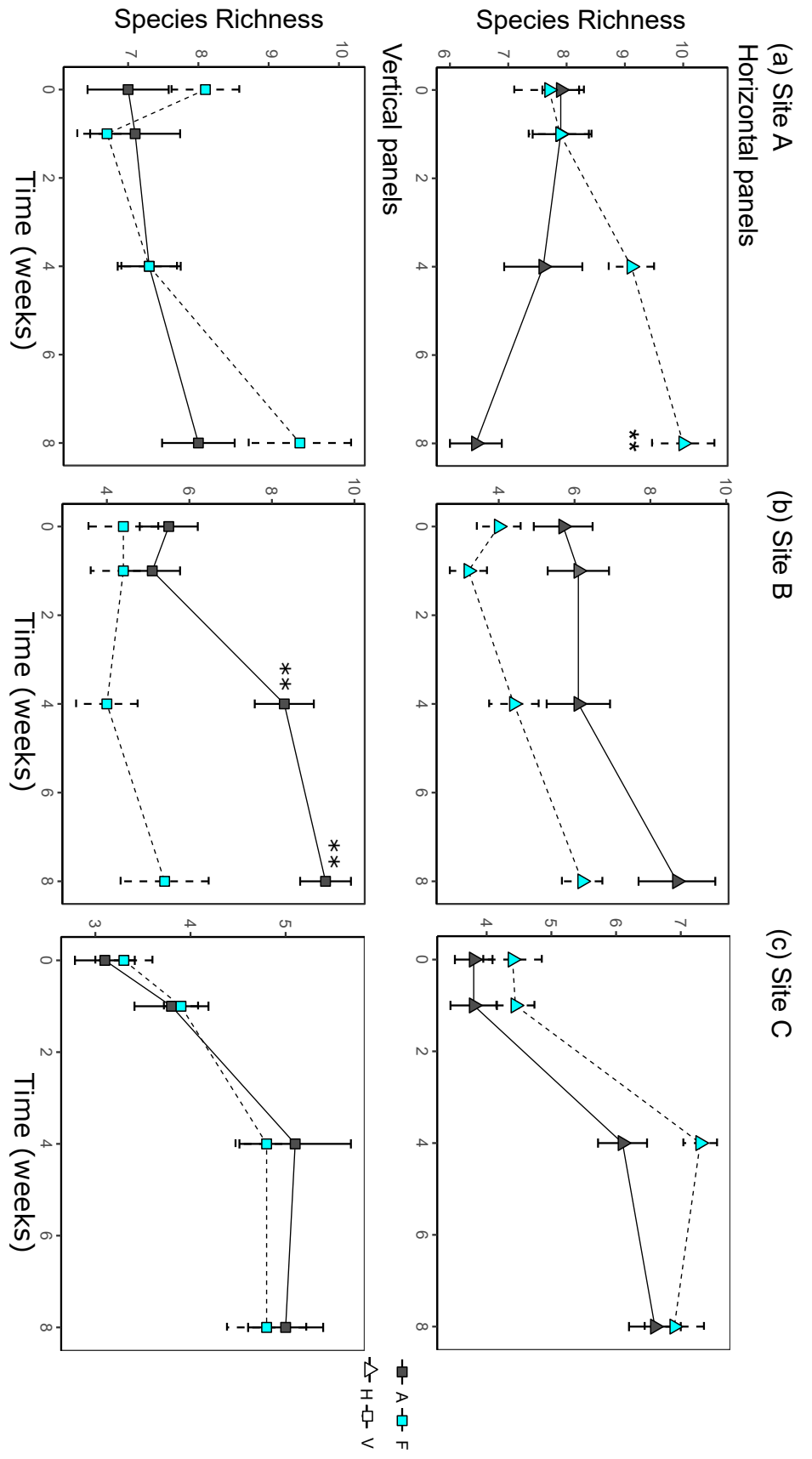

Figure C.13: *Line graph showing species richness on horizontal and vertical panels in site A (a), site B (b), and site C (c) . The mean (±SE) percentage cover on panels exposed to the freshwater (F) and ambient control (A) treatment is shown at 0,1,4 and 8 weeks post-treatment. Significant pair-wise test results between treatments at each time point are indicated on the graphs (*p<0.05, **p<0.01, ***p<0.001).*

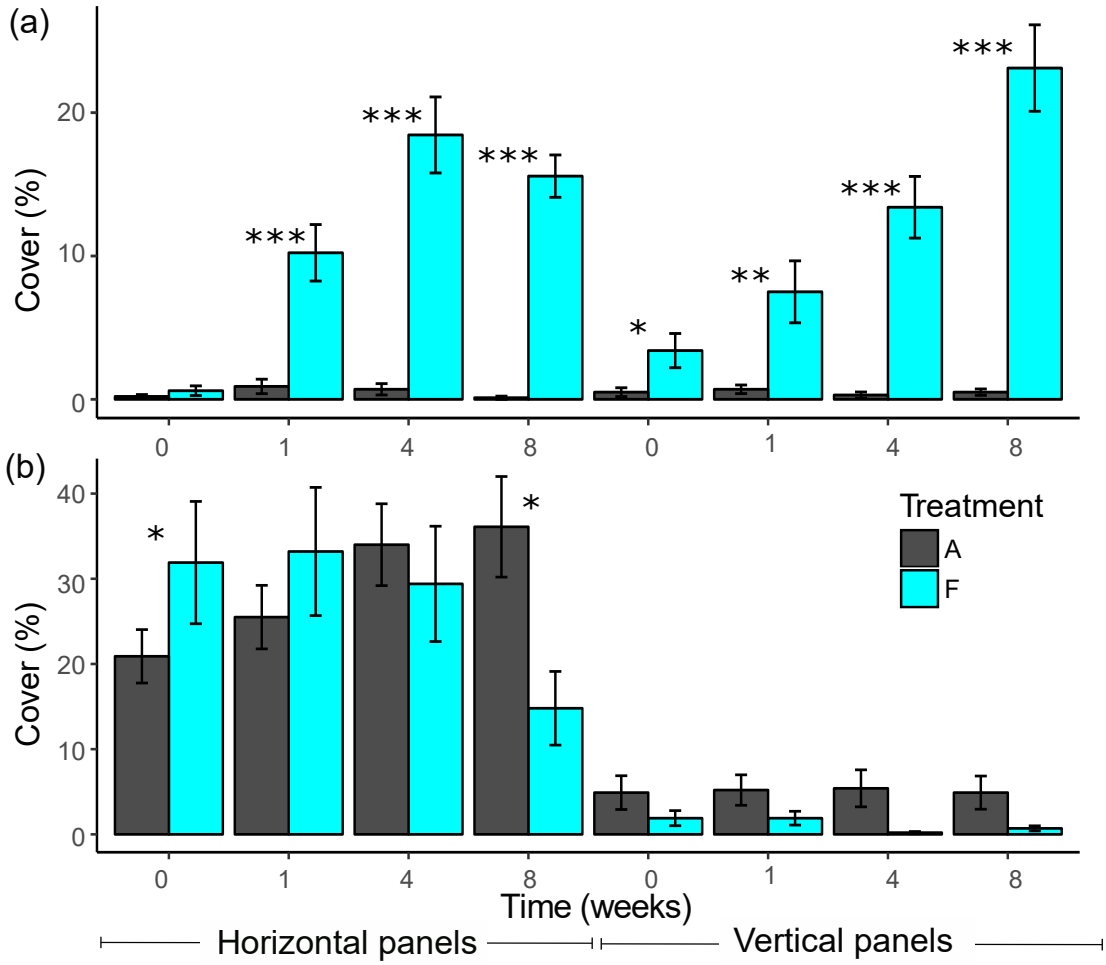

Figure C.14: *Bar chart showing mean (±SE) percentage cover of encrusting bryozoans in site A (a) and site B (b) on horizontally and vertically orientated panels. Cover is shown at each recovery monitoring time point at 0,1,4 and 8 weeks post-treatment. Significant results of pairwise t-tests between treatments at each time point are shown: *p<0.05, **p<0.01, ***p<0.001.*

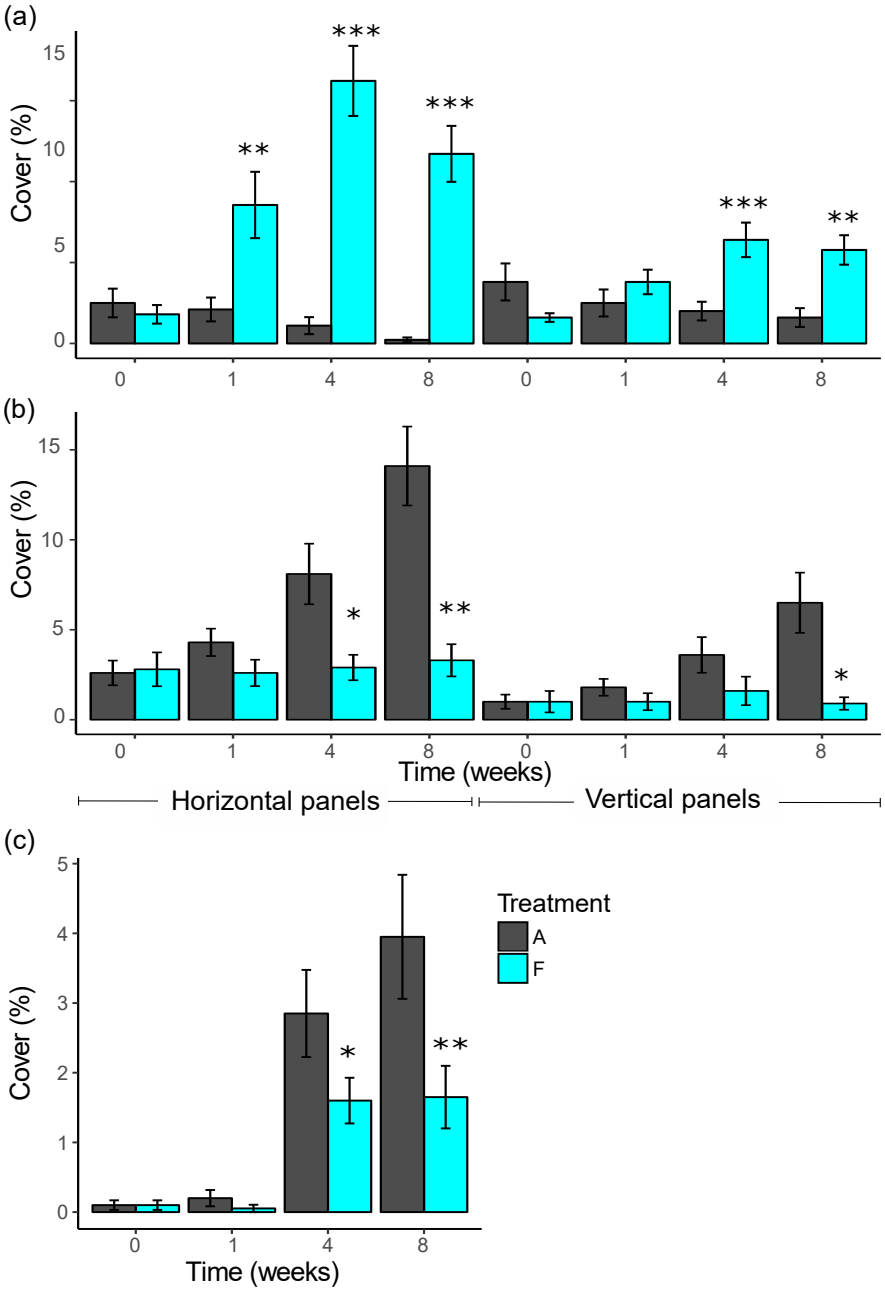

Figure C.15: *Bar chart showing mean (±SE) percentage cover of colonial ascidians in sites A (a), B (b) and C (c) on horizontally and vertically orientated panels. Cover is shown at each recovery monitoring time point at 0,1,4 and 8 weeks post-treatment. Cover is averaged over horizontal and vertical panels in site C as effect of treatment did not significantly interact with the effect of panel orientation. Significant results of pairwise t-tests between treatments at each time point are shown: *p<0.05, **p<0.01, ***p<0.001.*

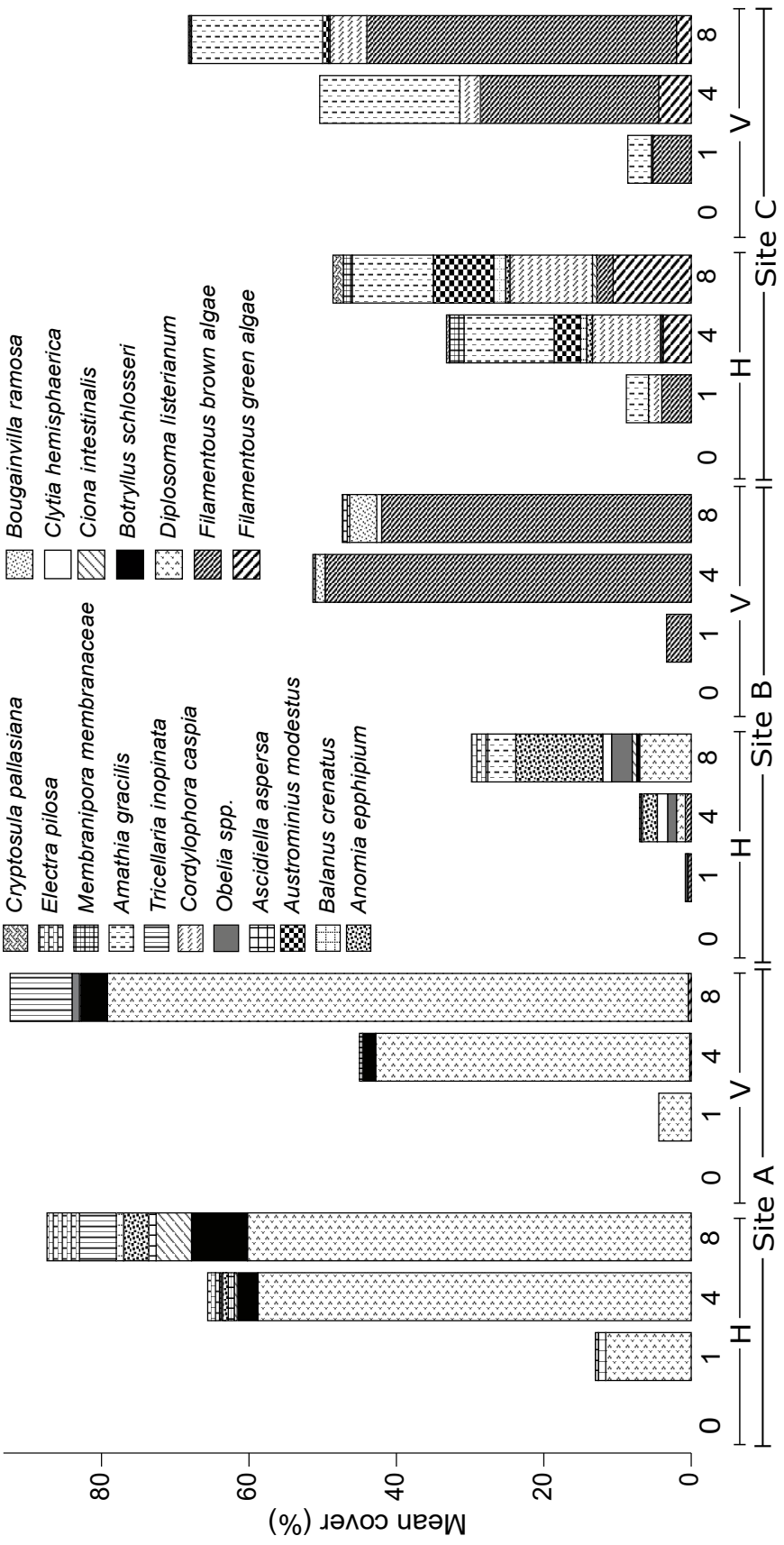

Figure C.16: *Stacked bar chart showing cover of different species on blank control panels set out at the beginning of the recovery period in sites A, B, and C. Percentage cover of each species with more than 1% average cover is shown averaged for vertically and horizontally orientated panels.*

Table C.9: *Results of repeated-measures ANOVA on total percent cover of the biofouling community assemblage in site A, excluding unidentified species. Analysis was performed using the PERMANOVA routine on a euclidean distance matrix.*

	Community assemblage						Native species					
Source	df	SS	MS	Pseudo-F	P(perm)	PERMDISP	df	SS	MS	Pseudo-F	P(perm)	PERMDISP
	Horizontal panels						Horizontal panels					
Treatment (Tr)	**1**	**6042.6**	**6042.6**	**229.95**	**0.0001**		**1**	**2705.4**	**2705.4**	**20.755**	**0.0002**	
Pontoon (Po)	2	201.57	100.79	3.8309	0.046		2	115.2	57.598	0.44237	0.6489	
Time (Ti)	**3**	**5661**	**1887**	**45.556**	**0.0001**		**3**	**5673.1**	**1891**	**31.251**	**0.0001**	
TrxPo	**2**	**261.69**	**130.84**	**4.9733**	**0.0249**	***	**2**	**982.55**	**491.28**	**3.7732**	**0.0472**	**n.s.**
TrxTi	**3**	**4998.6**	**1666.2**	**40.225**	**0.0001**	***	**3**	**2200.7**	**733.56**	**12.123**	**0.0001**	**n.s.**
PoxTi	6	352.69	58.782	1.4191	0.2388		6	715.17	119.2	1.9698	0.0933	
Panel(PoxTr)	14	354.99	25.356	0.61215	0.8346		**14**	**1884.3**	**134.6**	**2.2243**	**0.0264**	**n.s.**
TrxPoxTi	6	258.68	43.113	1.0408	0.4259		6	718.19	119.7	1.9781	0.093	
Residuals	36	1491.2	41.422				36	2178.4	60.511			
Total	73	20403					73	20484				
Pairwise comparison (Treatment)	Ambient ≠ freshwater: Weeks 1***, 4***, 8*						Ambient ≠ freshwater: Weeks 1*, 4***, 8*					
	Vertical panels						Vertical panels					
Treatment (Tr)	**1**	**15475**	**15475**	**47.617**	**0.0001**		**1**	**2599.8**	**2599.8**	**12.942**	**0.0035**	
Pontoon (Po)	2	44.491	22.246	0.06839	0.9397		2	251.15	125.58	0.62458	0.538	
Time (Ti)	**3**	**4266.7**	**1422.2**	**10.695**	**0.0001**		**3**	**1869**	**623**	**7.3835**	**0.0006**	
TrxPo	2	760.09	380.04	1.1684	0.3329		2	252.75	126.37	0.62856	0.5488	
TrxTi	**3**	**9709.3**	**3236.4**	**24.338**	**0.0001**	*	**3**	**2074.9**	**691.62**	**8.1968**	**0.0002**	*
PoxTi	6	790.48	131.75	0.99074	0.4495		6	355.87	59.311	0.70294	0.6448	
Panel(PoxTr)	**15**	**5074.8**	**338.32**	**2.5441**	**0.0108**	*	**15**	**3134.5**	**208.97**	**2.4766**	**0.0098**	*
TrxPoxTi	6	591.98	98.664	0.74195	0.6254		6	210.6	35.099	0.41598	0.8618	
Residuals	40	5319.2	132.98				40	3375.1	84.377			
Total	78	41235					78	14842				
Pairwise comparison (Treatment)	Ambient ≠ freshwater: Week 1***, 4***, 8**						Ambient ≠ freshwater: Week 4***, 8*					

	Non-native species						Non-native and cryptogenic species					
Source	df	SS	MS	Pseudo-F	P(perm)	PERMDISP	df	SS	MS	Pseudo-F	P(perm)	PERMDISP
	Horizontal panels						Horizontal panels					
Treatment (Tr)	1	107.72	107.72	1.484	0.2347		**1**	**818.77**	**818.77**	**9.9825**	**0.0075**	
Pontoon (Po)	2	391.35	195.67	2.7006	0.1029		2	535.86	267.93	3.269	0.0695	
Time (Ti)	**3**	**410.62**	**136.87**	**19.904**	**0.0001**		**3**	**9436.5**	**3145.5**	**58.479**	**0.0001**	
TrxPo	2	327.55	163.77	2.2603	0.1382		2	453.67	226.84	2.7676	0.0999	
TrxTi	**3**	**346.41**	**115.47**	**16.791**	**0.0001**	**n.s.**	**3**	**1104.9**	**368.28**	**6.8469**	**0.0007**	**n.s.**
PoxTi	6	57.408	9.568	1.3913	0.2424		6	422.52	70.42	1.3092	0.2739	
Panel(PoxTr)	**14**	**1072.3**	**76.589**	**11.137**	**0.0001**	**n.s.**	14	1172.3	83.738	1.5568	0.1463	
TrxPoxTi	6	48.878	8.1463	1.1846	0.3354		6	298.17	49.695	0.92391	0.4866	
Residuals	36	247.57	6.8768				36	1936.4	53.788			
Total	73	2869.9					73	19942				
Pairwise comparison (Treatment)	Ambient ≠ freshwater: Weeks 8*						Ambient ≠ freshwater: Weeks 1**, 4***					
	Vertical panels						Vertical panels					
Treatment (Tr)	1	86.439	86.439	0.6119	0.4471		**1**	**5085.2**	**5085.2**	**14.991**	**0.001**	
Pontoon (Po)	2	903.5	451.75	3.1944	0.0705		2	63.23	31.615	0.093139	0.9132	
Time (Ti)	**3**	**2617.9**	**872.64**	**23.054**	**0.0001**		**3**	**9580.4**	**3193.5**	**16.549**	**0.0001**	
TrxPo	**2**	**1429.3**	**714.63**	**5.0532**	**0.0195**	**	2	1550.4	775.18	2.2837	0.1376	
TrxTi	3	280.39	93.463	2.4692	0.0771		**3**	**3864.4**	**1288.1**	**6.6755**	**0.0015**	**n.s.**
PoxTi	6	150.6	25.1	0.66312	0.6737		6	473.37	78.895	0.40885	0.8693	
Panel(PoxTr)	**15**	**2226.7**	**148.44**	**3.9217**	**0.0005**	**n.s.**	15	5240.6	349.37	1.8105	0.0676	
TrxPoxTi	6	170.59	28.432	0.75115	0.6079		6	932.31	155.38	0.80524	0.577	
Residuals	40	1514.1	37.852				40	7718.6	192.97			
Total	78	10233					78	34020				
Pairwise comparison (Treatment)							Ambient ≠ freshwater: Weeks 1**, 4***					

*p<0.05, **p<0.01, ***p<0.001

The term TixPanel(TrxPo) was excluded from the model to account for the repeated-measures nature of the experimental design. P-values were obtained using 9,999 permutations of appropriate units. In the main model significant p-values (p<0.05) are presented in bold. Pairwise comparisons between ambient and freshwater treatments were carried out within the term Treatment*Time.

Table C.10: *Results of repeated-measures ANOVA on total percent cover of the biofouling community assemblage in site B, excluding unidentified species. Analysis was performed using the PERMANOVA routine on a euclidean distance matrix.*

	Community assemblage						Native species					
Source	df	SS	MS	Pseudo-F	P(perm)	PERMDISP	df	SS	MS	Pseudo-F	P(perm)	PERMDISP
				Horizontal panels						Horizontal panels		
Treatment (Tr)	**1**	**11740**	**11740**	**22.776**	**0.0003**		**1**	**5947**	**5947**	**17.752**	**0.0017**	
Pontoon (Po)	**2**	**14068**	**7033.8**	**13.646**	**0.0006**	**	**2**	**12938**	**6469.1**	**19.311**	**0.0003**	
Time (Ti)	**3**	**4865.9**	**1622**	**40.147**	**0.0001**		**3**	**4857.1**	**1619**	**24.181**	**0.0001**	
TrxPo	2	2880.3	1440.2	2.794	0.0929		**2**	**3145.7**	**1572.8**	**4.695**	**0.0284**	**
TrxTi	**3**	**4665.7**	**1555.2**	**38.495**	**0.0001**	**n.s.**	**3**	**4852.7**	**1617.6**	**24.159**	**0.0001**	**n.s.**
PoxTi	6	326.86	54.477	1.3484	0.2557		6	568.86	94.81	1.416	0.2363	
Panel(PoxTr)	**14**	**7216.4**	**515.46**	**12.759**	**0.0001**	*	**14**	**4690**	**335**	**5.0034**	**0.0001**	**n.s.**
TrxPoxTi	6	284.67	47.445	1.1744	0.3498		6	273.81	45.635	0.68157	0.6556	
Residuals	42	1696.8	40.4				42	2812.1	66.955			
Total	79	45542					79	39081				
Pairwise comparison (Treatment)	Ambient ≠ freshwater: Weeks 1*, 4***, 8***						Ambient ≠ freshwater: Weeks 4***, 8***					
				Vertical panels						Vertical panels		
Treatment (Tr)	1	202.4	202.4	0.18986	0.6751		1	158.25	158.25	0.15073	0.7016	
Pontoon (Po)	2	180.81	90.407	0.084807	0.9217		2	267.32	133.66	0.12731	0.8794	
Time (Ti)	**3**	**4649.3**	**1549.8**	**4.4619**	**0.0074**	**n.s.**	**3**	**5249.4**	**1749.8**	**5.1282**	**0.0047**	**n.s.**
TrxPo	2	3083	1541.5	1.446	0.2638		2	3305	1652.5	1.5739	0.234	
TrxTi	3	1245	415	1.1948	0.324		3	1536.6	512.19	1.5011	0.2223	
PoxTi	6	3224.4	537.4	1.5472	0.1796		6	3379.7	563.28	1.6508	0.1515	
Panel(PoxTr)	**14**	**14924**	**1066**	**3.0692**	**0.0027**	**n.s.**	**14**	**14699**	**1049.9**	**3.077**	**0.0021**	**n.s.**
TrxPoxTi	6	2141.3	356.89	1.0275	0.4216		6	2588.7	431.45	1.2645	0.2948	
Residuals	42	14588	347.33				42	14331	341.21			
Total	79	45347					79	46150				
Pairwise comparison (Treatment)												

	Non-native and cryptogenic species					
Source	df	SS	MS	Pseudo-F	P(perm)	PERMDISP
			Horizontal panels			
Treatment (Tr)	**1**	**347.76**	**347.76**	**4.9659**	**0.0447**	***
Pontoon (Po)	2	92.327	46.163	0.6592	0.5433	
Time (Ti)	3	43.14	14.38	1.0854	0.3709	
TrxPo	2	117.46	58.731	0.83865	0.4604	
TrxTi	3	91.928	30.643	2.313	0.0909	
PoxTi	6	73.602	12.267	0.92595	0.4922	
Panel(PoxTr)	**14**	**980.42**	**70.03**	**5.2861**	**0.0001**	***
TrxPoxTi	6	87.217	14.536	1.0972	0.3757	
Residuals	42	556.42	13.248			
Total	79	2433.8				
Pairwise comparison (Treatment)						
			Vertical panels			
Treatment (Tr)	1	2.2436	2.2436	0.026974	0.8704	
Pontoon (Po)	2	78.343	39.172	0.47094	0.6328	
Time (Ti)	3	157.06	52.354	2.0125	0.1279	
TrxPo	2	236.92	118.46	1.4242	0.2691	
TrxTi	3	66.985	22.328	0.85831	0.4746	
PoxTi	6	215.37	35.894	1.3798	0.2494	
Panel(PoxTr)	**14**	**1164.5**	**83.178**	**3.1974**	**0.002**	*
TrxPoxTi	6	109.92	18.32	0.70425	0.6458	
Residuals	42	1092.6	26.014			
Total	79	94344				
Pairwise comparison (Treatment)						

*p<0.05, **p<0.01, ***p<0.001

The term TixPanel(TrxPo) was excluded from the model to account for the repeated-measures nature of the experimental design. P-values were obtained using 9,999 permutations of appropriate units. In the main model significant p-values (p<0.05) are presented in bold. Pairwise comparisons between ambient and freshwater treatments were carried out within the term Treatment*Time

Table C.11: *Results of repeated-measures ANOVA on total percent cover of the biofouling community assemblage in site C, excluding unidentified species. Analysis was performed using the PERMANOVA routine on a euclidean distance matrix.*

	Community assemblage						Native species					
Source	df	SS	MS	Pseudo-F	P(perm)	PERMDISP	df	SS	MS	Pseudo-F	P(perm)	PERMDISP
	Horizontal panels						Horizontal panels					
Treatment (Tr)	**1**	**716.07**	**716.07**	**5.017**	**0.0434**		1	65.845	65.845	0.69548	0.4113	
Pontoon (Po)	2	210.5	105.25	0.7365	0.4914		2	105.99	52.995	0.5589	0.5929	
Time (Ti)	**3**	**6784.3**	**2261.4**	**46.255**	**0.0001**		**3**	**3459.1**	**1153**	**62.204**	**0.0001**	*
TrxPo	2	30.18	15.09	0.10559	0.9039		2	349.16	174.58	1.8412	0.1939	
TrxTi	**3**	**539.8**	**179.93**	**3.6803**	**0.0197**	**n.s.**	3	106.48	35.493	1.9148	0.1386	
PoxTi	6	414.89	69.149	1.4143	0.2343		6	158.23	26.372	1.4227	0.232	
Panel(PoxTr)	**14**	**2072.5**	**148.04**	**3.0279**	**0.0037**	**n.s.**	**14**	**1385.7**	**98.982**	**5.3399**	**0.0001**	*
TrxPoxTi	6	227.15	37.859	0.77436	0.5868		6	154.64	25.773	1.3904	0.2447	
Residuals	41	2004.5	48.891				41	759.99	18.536			
Total	78	16139					78	7621				
Pairwise comparison (Treatment)	Ambient ≠ freshwater: Week 0*, 1*											
	Vertical panels						Vertical panels					
Treatment (Tr)	1	20.144	20.144	0.045971	0.8337		1	189.74	189.74	0.22839	0.64	
Pontoon (Po)	2	2039.2	1019.6	2.3269	0.1324		2	4380	2190	2.6361	0.1035	
Time (Ti)	**3**	**7309.9**	**2436.6**	**30.792**	**0.0001**	**	**3**	**4151**	**1383.7**	**5.7973**	**0.0018**	*
TrxPo	2	360.12	180.06	0.41091	0.6771		2	3577	1788.5	2.1529	0.1531	
TrxTi	3	118.6	39.534	0.4996	0.6765		3	398.38	132.79	0.55639	0.6485	
PoxTi	6	997.35	166.22	2.1006	0.0718		6	2153.8	358.96	1.504	0.1959	
Panel(PoxTr)	**14**	**6134.7**	**438.19**	**5.5376**	**0.0001**	**n.s.**	**14**	**11631**	**830.75**	**3.4807**	**0.001**	**n.s.**
TrxPoxTi	6	525.04	87.506	1.1058	0.3834		6	1478.9	246.48	1.0327	0.4102	
Residuals	42	3323.5	79.131				42	10024	238.67			
Total	79	22096					79	37386				
Pairwise comparison (Treatment)												

	Non-native and cryptogenic species					
Source	df	SS	MS	Pseudo-F	P(perm)	PERMDISP
	Horizontal panels					
Treatment (Tr)	1	347.63	347.63	1.5046	0.2472	
Pontoon (Po)	2	294.67	147.33	0.63678	0.5377	
Time (Ti)	**3**	**1064.4**	**354.8**	**5.9232**	**0.0017**	
TrxPo	2	174.25	87.125	0.37656	0.6956	
TrxTi	**3**	**1123.3**	**374.44**	**6.2511**	**0.0022**	**n.s.**
PoxTi	6	694.8	115.8	1.9332	0.0967	
Panel(PoxTr)	**14**	**3370.2**	**240.73**	**4.0188**	**0.0002**	*
TrxPoxTi	6	418.05	69.675	1.1632	0.3374	
Residuals	41	2455.9	59.899			
Total	78	11340				
Pairwise comparison (Treatment)	Ambient ≠ freshwater: Week 0*, 1*					
	Vertical panels					
Treatment (Tr)	1	86.234	86.234	0.23106	0.6387	
Pontoon (Po)	2	847.94	423.97	1.136	0.3464	
Time (Ti)	**3**	**11287**	**3762.3**	**11.727**	**0.0001**	*
TrxPo	2	2046.1	1023.1	2.7413	0.1022	
TrxTi	3	441.59	147.2	0.4588	0.7136	
PoxTi	6	1153.6	192.26	0.59926	0.7147	
Panel(PoxTr)	14	5224.9	373.21	1.1632	0.3263	
TrxPoxTi	6	2397.1	399.52	1.2453	0.3023	
Residuals	42	13475	320.83			
Total	79	36436				
Pairwise comparison (Treatment)						

*p<0.05, **p<0.01, ***p<0.001
The term TixPanel(TrxPo) was excluded from the model to account for the repeated-measures nature of the experimental design. P-values were obtained using 9,999 permutations of appropriate units. In the main model significant p-values (p<0.05) are presented in bold. Pairwise comparisons between ambient and freshwater treatments were carried out within the term Treatment*Time.

Table C.12: *Results of repeated-measures ANOVA on total species richness of the biofouling community assemblage in site A, excluding unidentified species. Analysis was performed using the PERMANOVA routine on a euclidean distance matrix.*

	Community assemblage						Native species					
Source	df	SS	MS	Pseudo-F	P(perm)	PERMDISP	df	SS	MS	Pseudo-F	P(perm)	PERMDISP
	Horizontal panels						Horizontal panels					
Treatment (Tr)	**1**	**24.653**	**24.653**	**7.0071**	**0.0223**		1	5.2941	5.2941	2.6876	0.1223	
Pontoon (Po)	2	1.9363	0.96813	0.27538	0.762		2	0.30059	0.15029	0.076295	0.9228	
Time (Ti)	3	4.7345	1.5782	0.71932	0.5429		3	0.29464	0.098214	0.04898	0.9852	
TrxPo	2	1.8147	0.90734	0.25809	0.7725		2	4.2782	2.1391	1.0859	0.3541	
TrxTi	**3**	**28.492**	**9.4974**	**4.3289**	**0.0106**	**n.s.**	3	9.3699	3.1233	1.5576	0.215	
PoxTi	6	10.141	1.6902	0.77041	0.5983		6	6.1445	1.0241	0.51072	0.8009	
Panel(PoxTr)	14	50.385	3.5989	1.6404	0.1178		14	27.547	1.9677	0.98129	0.4798	
TrxPoxTi	6	8.8482	1.4747	0.67217	0.6738		6	5.2029	0.86715	0.43246	0.8544	
Residuals	36	78.982	2.1939				36	72.186	2.0052			
Total	73	224					73	139.09				
Pairwise comparison (Treatment)	Ambient ≠ freshwater: Weeks 8**											
	Vertical panels						Vertical panels					
Treatment (Tr)	1	0.65161	0.65161	0.13839	0.7086		1	0.19084	0.19084	0.070566	0.7881	
Pontoon (Po)	2	23.951	11.975	2.5412	0.1127		2	18.937	9.4687	3.4993	0.0596	
Time (Ti)	**3**	**24.734**	**8.2447**	**4.1328**	**0.0121**	**n.s.**	3	11.785	3.9283	2.2916	0.0897	
TrxPo	2	5.2428	2.6214	0.55627	0.5853		2	11.55	5.7752	2.1343	0.1577	
TrxTi	3	7.1255	2.3752	1.1906	0.322		3	3.5866	1.1955	0.69741	0.5601	
PoxTi	6	14.193	2.3655	1.1857	0.3351		6	9.4894	1.5816	0.92261	0.4926	
Panel(PoxTr)	**15**	**73.451**	**4.8968**	**2.4546**	**0.0123**	**n.s.**	15	41.597	2.7731	1.6177	0.1148	
TrxPoxTi	6	1.375	0.22917	0.11487	0.9948		6	2.1754	0.36257	0.21151	0.9711	
Residuals	40	79.799	1.995				40	68.569	1.7142			
Total	73	224					78	173.75				
Pairwise comparison (Treatment)												

	Non-native species						Non-native and cryptogenic species					
Source	df	SS	MS	Pseudo-F	P(perm)	PERMDISP	df	SS	MS	Pseudo-F	P(perm)	PERMDISP
	Horizontal panels						Horizontal panels					
Treatment (Tr)	1	6.6614	6.6614	62.615	0.0001		**1**	**1.6335**	**1.6335**	**5.2471**	**0.0382**	
Pontoon (Po)	2	1.1802	0.5901	5.5508	0.0155		2	1.3188	0.65938	2.1204	0.1582	
Time (Ti)	**3**	**4.3699**	**1.4566**	**21.452**	**0.0001**		**3**	**2.3151**	**0.77168**	**5.3783**	**0.0058**	
TrxPo	**2**	**1.1802**	**0.5901**	**5.5508**	**0.0174**	***	2	1.2646	0.63231	2.0333	0.1809	
TrxTi	**3**	**4.3699**	**1.4566**	**21.452**	**0.0001**	***	**3**	**4.4087**	**1.4696**	**10.242**	**0.0002**	**
PoxTi	6	0.73785	0.12297	1.8111	0.1248		6	1.1231	0.18718	1.3046	0.2832	
Panel(PoxTr)	14	1.5222	0.10873	1.6013	0.1263		**14**	**4.5014**	**0.32153**	**2.2409**	**0.0263**	**
TrxPoxTi	6	0.73785	0.12297	1.8111	0.1255		6	1.1229	0.18716	1.3044	0.2769	
Residuals	36	2.4444	0.067901				36	5.1653	0.14348			
Total	73	25.041					73	23.405				
Pairwise comparison (Treatment)	Ambient ≠ freshwater: Weeks 1*, 4***, 8***						Ambient ≠ freshwater: Weeks 4***, 8**					
	Vertical panels						Vertical panels					
Treatment (Tr)	**1**	**4.8543**	**4.8543**	**15.215**	**0.0029**	***	**1**	**4.9806**	**4.9806**	**7.9029**	**0.0142**	**n.s.**
Pontoon (Po)	2	1.228	0.614	1.9235	0.1838		2	1.2893	0.64463	1.022	0.3896	
Time (Ti)	3	1.1268	0.3756	1.818	0.16		**3**	**4.8888**	**1.6296**	**5.4541**	**0.0024**	**n.s.**
TrxPo	2	0.48598	0.24299	0.76122	0.4904		2	0.52289	0.26145	0.41452	0.6897	
TrxTi	3	0.21216	0.070719	0.34231	0.7991		3	1.5643	0.52144	1.7452	0.1715	
PoxTi	6	0.64833	0.10806	0.52303	0.7874		6	0.4159	0.069317	0.232	0.9661	
Panel(PoxTr)	15	4.9028	0.32685	1.5821	0.1257		**15**	**9.7986**	**0.65324**	**2.1863**	**0.026**	**n.s.**
TrxPoxTi	6	0.5986	0.099766	0.4829	0.8195		6	0.95548	0.15925	0.53298	0.7825	
Residuals	40	8.2639	0.2066				40	11.951	0.29878			
Total	78	25.367					78	38.709				
Pairwise comparison (Treatment)												

*p<0.05, **p<0.01, ***p<0.001

The term TixPanel(TrxPo) was excluded from the model to account for the repeated-measures nature of the experimental design. P-values were obtained using 9,999 permutations of appropriate units. In the main model significant p-values (p<0.05) are presented in bold. Pairwise comparisons between ambient and freshwater treatments were carried out within the term Treatment*Time.

Table C.13: *Results of repeated-measures ANOVA on total species richness of the biofouling assemblage in site B, excluding unidentified species. Analysis was performed using the PERMANOVA routine on a euclidean distance matrix.*

	Community assemblage						Native species					
Source	df	SS	MS	Pseudo-F	P(perm)	PERMDISP	df	SS	MS	Pseudo-F	P(perm)	PERMDISP
				Horizontal panels						Horizontal panels		
Treatment (Tr)	**1**	**115.34**	**115.34**	**7.2841**	**0.018**	**n.s.**	**1**	**45.833**	**45.833**	**4.5833**	**0.0499**	**n.s.**
Pontoon (Po)	2	55.415	27.707	1.7498	0.2137		2	29.379	14.689	1.4689	0.2622	
Time (Ti)	**3**	**97.647**	**32.549**	**15.524**	**0.0001**	**n.s.**	**3**	**94.598**	**31.533**	**20.427**	**0.0001**	**n.s.**
TrxPo	2	4.1603	2.0802	0.13137	0.8805		2	0.55901	0.2795	0.02795	0.9728	
TrxTi	3	5.1468	1.7156	0.81822	0.4992		3	8.2955	2.7652	1.7913	0.1608	
PoxTi	6	2.7232	0.45387	0.21647	0.9722		6	2.9513	0.49189	0.31865	0.9245	
Panel(PoxTr)	**14**	**221.69**	**15.835**	**7.5522**	**0.0001**	**n.s.**	**14**	**140**	**10**	**6.4781**	**0.0001**	**n.s.**
TrxPoxTi	6	9.423	1.5705	0.74903	0.6252		6	8.0942	1.349	0.87392	0.5257	
Residuals	42	88.062	2.0967				42	64.833	1.5437			
Total	79	585.8					79	389.69				
Pairwise comparison (Treatment)												
				Vertical panels						Vertical panels		
Treatment (Tr)	**1**	**101.35**	**101.35**	**6.4837**	**0.0229**		**1**	**89.806**	**89.806**	**8.1448**	**0.0129**	
Pontoon (Po)	2	39.403	19.702	1.2604	0.3138		2	27.723	13.862	1.2572	0.3113	
Time (Ti)	**3**	**72.368**	**24.123**	**9.2889**	**0.0002**		**3**	**80.844**	**26.948**	**12.641**	**0.0001**	
TrxPo	2	31.563	15.781	1.0096	0.383		2	29.157	14.578	1.3222	0.2921	
TrxTi	**3**	**41.555**	**13.852**	**5.3338**	**0.0036**	**n.s.**	**3**	**23.893**	**7.9642**	**3.736**	**0.0175**	**n.s.**
PoxTi	6	15.394	2.5656	0.98795	0.4477		6	13.66	2.2766	1.068	0.3921	
Panel(PoxTr)	**14**	**218.85**	**15.632**	**6.0194**	**0.0001**	***	**14**	**154.37**	**11.026**	**5.1724**	**0.0003**	***
TrxPoxTi	6	10.568	1.7613	0.67823	0.6659		6	11.194	1.8656	0.87517	0.5198	
Residuals	42	109.07	2.5969				42	89.533	2.1317			
Total	79	702.8					79	568.2				
Pairwise comparison (Treatment)	Ambient ≠ freshwater: Weeks 4**, 8**						Ambient ≠ freshwater: Weeks 4**, 8**					

	Non-native and cryptogenic species					
Source	df	SS	MS	Pseudo-F	P(perm)	PERMDISP
			Horizontal panels			
Treatment (Tr)	1	5.6146	5.6146	4.3022	0.0591	
Pontoon (Po)	2	1.9072	0.95361	0.7307	0.4933	
Time (Ti)	3	1.2301	0.41004	0.97596	0.4202	
TrxPo	2	1.2675	0.63373	0.4856	0.6081	
TrxTi	**3**	**4.0256**	**1.3419**	**3.1938**	**0.0355**	**
PoxTi	6	1.7786	0.29643	0.70556	0.6416	
Panel(PoxTr)	**14**	**18.271**	**1.3051**	**3.1063**	**0.0018**	**n.s.**
TrxPoxTi	6	1.3749	0.22915	0.5454	0.7682	
Residuals	42	17.646	0.42014			
Total	79	51.95				
Pairwise comparison (Treatment)	Ambient ≠ freshwater: Week 1**					
			Vertical panels			
Treatment (Tr)	1	0.46282	0.46282	0.61417	0.4427	
Pontoon (Po)	2	1.804	0.90199	1.197	0.3245	
Time (Ti)	3	1.403	0.46766	1.9874	0.1304	
TrxPo	2	1.5252	0.76261	1.012	0.3881	
TrxTi	**3**	**2.5027**	**0.83424**	**3.5451**	**0.0234**	*
PoxTi	6	0.94263	0.1571	0.66763	0.6736	
Panel(PoxTr)	**14**	**10.55**	**0.75357**	**3.2024**	**0.0009**	**
TrxPoxTi	6	3.1683	0.52805	2.244	0.0586	
Residuals	42	9.8833	0.23532			
Total	79	33.8				
Pairwise comparison (Treatment)	Ambient ≠ freshwater: Week 4**					

*p<0.05, **p<0.01, ***p<0.001

The term TixPanel(TrxPo) was excluded from the model to account for the repeated-measures nature of the experimental design. P-values were obtained using 9,999 permutations of appropriate units. In the main model significant p-values (p<0.05) are presented in bold. Pairwise comparisons between ambient and freshwater treatments were carried out within the term Treatment*Time.

Table C.14: *Results of repeated-measures ANOVA on total species richness of the biofouling community assemblage in site C, excluding unidentified species. Analysis was performed using the PERMANOVA routine on a euclidean distance matrix.*

	Community assemblage						Native species					
Source	df	SS	MS	Pseudo-F	P(perm)	PERMDISP	df	SS	MS	Pseudo-F	P(perm)	PERMDISP
				Horizontal panels						Horizontal panels		
Treatment (Tr)	1	9.1363	9.1363	3.4882	0.0891		1	2.0762	2.0762	1.2278	0.2809	
Pontoon (Po)	2	2.1789	1.0894	0.41544	0.667		2	1.7037	0.85187	0.50323	0.6145	
Time (Ti)	**3**	**100.97**	**33.657**	**35.497**	**0.0001**	**n.s.**	**3**	**93.3**	**31.1**	**41.887**	**0.0001**	
TrxPo	2	4.7659	2.383	0.9087	0.4272		2	0.59229	0.29614	0.17494	0.8453	
TrxTi	3	2.4171	0.80569	0.84973	0.4749		3	3.1768	1.0589	1.4262	0.2502	
PoxTi	6	9.391	1.5652	1.6507	0.1578		**6**	**10.904**	**1.8174**	**2.4477**	**0.0413**	**n.s.**
Panel(PoxTr)	**14**	**37.992**	**2.7137**	**2.862**	**0.0044**	*	**14**	**24.425**	**1.7446**	**2.3498**	**0.018**	**
TrxPoxTi	6	4.8181	0.80302	0.84692	0.5435		6	5.4323	0.90538	1.2194	0.3068	
Residuals	41	38.875	0.94817				41	30.442	0.74248			
Total	78	243.37					78	204.68				
Pairwise comparison (Treatment)												
				Vertical panels						Vertical panels		
Treatment (Tr)	1	0.69839	0.69839	0.22132	0.6464		1	3.0533	3.0533	1.6037	0.2285	
Pontoon (Po)	2	5.4455	2.7227	0.86281	0.434		2	1.0677	0.53386	0.28041	0.7528	
Time (Ti)	**3**	**40.118**	**13.373**	**13.403**	**0.0001**	*	**3**	**24.695**	**8.2315**	**9.1958**	**0.0003**	**n.s.**
TrxPo	2	0.78284	0.39142	0.12404	0.8879		2	4.1513	2.0757	1.0902	0.3675	
TrxTi	3	1.5605	0.52016	0.52135	0.6645		3	1.584	0.52799	0.58985	0.6196	
PoxTi	6	3.7478	0.62463	0.62606	0.705		6	4.4847	0.74745	0.83501	0.5458	
Panel(PoxTr)	**14**	**44.179**	**3.1557**	**3.1629**	**0.0021**	**	**14**	**26.654**	**1.9039**	**2.1269**	**0.0319**	***
TrxPoxTi	6	6.0396	1.0066	1.0089	0.4296		6	3.5105	0.58509	0.65363	0.6859	
Residuals	42	41.904	0.99772				42	37.596	0.89514			
Total	79	147.95					79	110.99				
Pairwise comparison (Treatment)												

	Non-native and cryptogenic species					
Source	df	SS	MS	Pseudo-F	P(perm)	PERMDISP
			Horizontal panels			
Treatment (Tr)	**1**	**2.5019**	**2.5019**	**7.0374**	**0.0194**	**n.s.**
Pontoon (Po)	2	0.089303	0.044652	0.12548	0.8879	
Time (Ti)	3	0.35049	0.11683	0.65171	0.5784	
TrxPo	2	2.0654	1.0327	2.9021	0.0869	
TrxTi	3	0.97301	0.32434	1.8092	0.1659	
PoxTi	6	1.6955	0.28258	1.5763	0.178	
Panel(PoxTr)	**14**	**5.1167**	**0.36548**	**2.0387**	**0.0381**	***
TrxPoxTi	6	1.7349	0.28915	1.6129	0.1711	
Residuals	41	7.35	0.17927			
Total	78	21.747				
Pairwise comparison (Treatment)						
			Vertical panels			
Treatment (Tr)	1	0.83115	0.83115	1.0297	0.3224	
Pontoon (Po)	2	2.431	1.2155	1.5059	0.2487	
Time (Ti)	**3**	**3.1303**	**1.0434**	**4.7123**	**0.0066**	**n.s.**
TrxPo	2	3.39	1.695	2.1	0.1494	
TrxTi	3	0.089344	0.029781	0.1345	0.939	
PoxTi	6	0.82918	0.1382	0.62411	0.7121	
Panel(PoxTr)	**14**	**11.3**	**0.80714**	**3.6452**	**0.0012**	**
TrxPoxTi	6	1.4933	0.24889	1.124	0.3567	
Residuals	42	9.3	0.22143			
Total	79	32.488				
Pairwise comparison (Treatment)						

*p<0.05, **p<0.01, ***p<0.001

The term TixPanel(TrxPo) was excluded from the model to account for the repeated-measures nature of the experimental design. P-values were obtained using 9,999 permutations of appropriate units. In the main model significant p-values (p<0.05) are presented in bold. Pairwise comparisons between ambient and freshwater treatments were carried out within the term Treatment*Time.

Table C.15: *Results of permutational analysis of variance (PERMANOVA) on the response of the biofouling assemblage in site A. Analysis was performed on Bray-Curtis similarities calculated from untransformed and fourth-root transformed data.*

	Community assemblage (untransformed)						Community assemblage (4th root transformed)					
Source	df	SS	MS	Pseudo-F	P(perm)	PERMDISP	df	SS	MS	Pseudo-F	P(perm)	PERMDISP
				Horizontal panels						Horizontal panels		
Treatment (Tr)	**1**	**29268**	**29268**	**28.198**	**0.0001**		**1**	**17920**	**17920**	**24.948**	**0.0001**	
Pontoon (Po)	2	4020.2	2010.1	1.9395	0.0681		2	1494.4	747.22	1.0418	0.3892	
Time (Ti)	**3**	**37213**	**12404**	**47.594**	**0.0001**		**3**	**11406**	**3801.9**	**18.849**	**0.0001**	
TrxPo	2	1533.1	766.54	0.73963	0.6732		2	1280.5	640.26	0.89266	0.4948	
TrxTi	**3**	**21179**	**7059.6**	**27.087**	**0.0001**	n.s.	**3**	**8521.3**	**2840.4**	**14.082**	**0.0001**	**
PoxTi	6	2260.1	376.69	1.4453	0.1201		6	1298.5	216.41	1.0729	0.3995	
Panel(PoxTr)	**14**	**15194**	**1085.3**	**4.1641**	**0.0001**	n.s.	**14**	**10496**	**749.74**	**3.7169**	**0.0001**	*
TrxPoxTi	6	1198.6	199.77	0.7665	0.7534		6	1293.4	215.57	1.0687	0.4153	
Residuals	36	9382.6	260.63				36	7261.6	201.71			
Total	73	137250					73	65209				
Pairwise comparison (Treatment)	Ambient ≠ freshwater: Weeks 1***, 4***, 8***						Ambient ≠ freshwater: Weeks 1***, 4***, 8***					
				Vertical panels						Vertical panels		
Treatment (Tr)	**1**	**28330**	**28330**	**20.314**	**0.0001**		**1**	**15655**	**15655**	**15.288**	**0.0001**	
Pontoon (Po)	2	5952	2976	2.1317	0.0264		**2**	**3983.8**	**1991.9**	**1.9432**	**0.0373**	
Time (Ti)	**3**	**23160**	**7719.9**	**18.073**	**0.0001**		**3**	**5605.9**	**1868.6**	**6.6737**	**0.0001**	
TrxPo	**2**	**7072.7**	**3536.3**	**2.5331**	**0.0146**	***	**2**	**5660.8**	**2830.4**	**2.7611**	**0.01**	n.s.
TrxTi	**3**	**19320**	**6440**	**15.076**	**0.0001**	**	**3**	**5893.4**	**1964.5**	**7.0159**	**0.0001**	n.s.
PoxTi	6	2250	374.99	0.87787	0.6094		6	1855.7	309.29	1.1046	0.3695	
Panel(PoxTr)	**15**	**21926**	**1461.8**	**3.422**	**0.0001**	n.s.	**15**	**16134**	**1075.6**	**3.8415**	**0.0001**	n.s.
TrxPoxTi	6	2850.6	475.11	1.1122	0.3596		6	1101.6	183.59	0.65569	0.8444	
Residuals	40	17086	427.16				40	11200	280			
Total	78	131310					78	69826				
Pairwise comparison (Treatment)	Ambient ≠ freshwater: Weeks 1**, 4***, 8***						Ambient ≠ freshwater: Weeks 0**, 1**, 4***, 8***					

*p<0.05, **p<0.01, ***p<0.001

The term TixPanel(TrxPo) was excluded from the model to account for the repeated-measures nature of the experimental design. P-values were obtained using 9,999 permutations of appropriate units. In the main model significant p-values (p<0.05) are presented in bold. Pairwise comparisons between ambient and freshwater treatments were carried out within the term Treatment*Time.

Table C.16: *Results of permutational analysis of variance (PERMANOVA) on response of the whole biofouling assemblage in site B. Analysis was performed on Bray-Curtis similarities calculated on untransformed and fourth-root transformed data.*

	Community assemblage (untransformed)						Community assemblage (4th root transformed)					
Source	df	SS	MS	Pseudo-F	P(perm)	PERMDISP	df	SS	MS	Pseudo-F	P(perm)	PERMDISP
				Horizontal panels						Horizontal panels		
Treatment (Tr)	**1**	**11537.0**	**11537**	**8.605**	**0.0005**		**1**	**7580.3**	**7580.3**	**4.1767**	**0.0112**	
Pontoon (Po)	**2**	**18701.0**	**9350.6**	**6.9741**	**0.0002**		**2**	**7926.7**	**3963.3**	**2.1838**	**0.0354**	
Time (Ti)	**3**	**11721.0**	**3907.1**	**17.067**	**0.0001**		**3**	**11502**	**3834.1**	**12.762**	**0.0001**	
TrxPo	**2**	**5681.4**	**2840.7**	**2.1187**	**0.0333**	***	2	4762.2	2381.1	1.312	0.2444	
TrxTi	**3**	**7586.3**	**2528.8**	**11.046**	**0.0001**	n.s.	**3**	**3364.2**	**1121.4**	**3.7325**	**0.0001**	n.s.
PoxTi	6	940.4	156.73	0.68463	0.8166		6	1439.7	239.94	0.79863	0.7188	
Panel(PoxTr)	**14**	**18771.0**	**1340.8**	**5.8568**	**0.0001**	n.s.	**14**	**25409**	**1814.9**	**6.0408**	**0.0001**	n.s.
TrxPoxTi	6	1698.6	283.11	1.2367	0.2242		6	1547.6	257.94	0.85852	0.6551	
Residuals	42	9614.8	228.92				42	12619	300.44			
Total	79	84671.0					79	75220				
Pairwise comparison (Treatment)	Ambient ≠ freshwater: Weeks 0*, 1**, 4**, 8***						Ambient ≠ freshwater: Weeks 1**, 4**, 8***					
				Vertical panels						Vertical panels		
Treatment (Tr)	1	6107.1	6107.1	1.9551	0.1329		**1**	**7001.6**	**7001.6**	**2.8625**	**0.0304**	n.s.
Pontoon (Po)	2	4820.6	2410.3	0.77161	0.6127		2	7843.7	3921.8	1.6034	0.1056	
Time (Ti)	**3**	**12685.0**	**4228.4**	**4.7719**	**0.0001**		**3**	**9243.2**	**3081.1**	**5.7197**	**0.0001**	n.s.
TrxPo	2	9332.1	4666.1	1.4938	0.1904		2	7215.7	3607.8	1.475	0.1732	
TrxTi	**3**	**5721.5**	**1907.2**	**2.1523**	**0.0423**	*	3	2430.3	810.11	1.5039	0.1327	
PoxTi	6	8157.7	1359.6	1.5344	0.1058		6	4381.4	730.24	1.3556	0.1357	
Panel(PoxTr)	**14**	**43732.0**	**3123.7**	**3.5252**	**0.0001**	n.s.	**14**	**34243**	**2445.9**	**4.5406**	**0.0001**	n.s.
TrxPoxTi	6	4712.2	785.36	0.8863	0.5864		6	3221.4	536.91	0.99671	0.4836	
Residuals	42	37217.0	886.12				42	22624	538.68			
Total	79	133010.0					79	101690				
Pairwise comparison (Treatment)	Ambient ≠ freshwater: Weeks 4***											

*p<0.05, **p<0.01, ***p<0.001

The term TixPanel(TrxPo) was excluded from the model to account for the repeated-measures nature of the experimental design. P-values were obtained using 9,999 permutations of appropriate units. In the main model significant p-values (p<0.05) are presented in bold. Pairwise comparisons between ambient and freshwater treatments were carried out within the term Treatment*Time.

Table C.17: *Results of permutational analysis of variance (PERMANOVA) on response of the whole biofouling assemblage in site C. Analysis was performed on Bray-Curtis similarities calculated on untransformed and fourth root transformed data.*

	Community assemblage (untransformed)						Community assemblage (4th root transformed)					
Source	df	SS	MS	Pseudo-F	P(perm)	PERMDISP	df	SS	MS	Pseudo-F	P(perm)	PERMDISP
				Horizontal panels						Horizontal panels		
Treatment (Tr)	**1**	**1692.0**	**1692**	**3.2519**	**0.0313**		1	761.48	761.48	1.0287	0.3896	
Pontoon (Po)	**2**	**4283.1**	**2141.6**	**4.1104**	**0.0023**		2	2345.7	1172.8	1.5821	0.163	
Time (Ti)	**3**	**9641.8**	**3213.9**	**22.676**	**0.0001**		**3**	**9769**	**3256.3**	**22.02**	**0.0001**	
TrxPo	2	1439.6	719.81	1.3816	0.218		2	1325.9	662.93	0.89424	0.517	
TrxTi	**3**	**1219.1**	**406.35**	**2.8671**	**0.0055**	**	3	**1546.3**	**515.45**	**3.4855**	**0.0032**	n.s.
PoxTi	**6**	**1843.6**	**307.27**	**2.168**	**0.0095**		6	1637.6	272.94	1.8456	0.0628	
Panel(PoxTr)	**14**	**7583.8**	**541.7**	**3.8221**	**0.0001**	n.s.	**14**	**10832**	**773.7**	**5.2318**	**0.0001**	***
TrxPoxTi	**6**	**1599.3**	**266.54**	**1.8806**	**0.0261**	n.s.	6	821.55	136.92	0.92589	0.5352	
Residuals	41	5810.9	141.73				41	6063.2	147.88			
Total	78	41611.0					78	38800				
Pairwise comparison (Treatment)	Ambient ≠ freshwater: Weeks 0*						Ambient ≠ freshwater: Weeks 0*, 1*					
				Vertical panels						Vertical panels		
Treatment (Tr)	1	934.5	934.5	0.5125	0.6703		1	943.27	943.27	1.047	0.3701	
Pontoon (Po)	2	8395.7	4197.9	2.3022	0.0714		2	3559.8	1779.9	1.9757	0.0888	
Time (Ti)	**3**	**14571.0**	**4857**	**9.7029**	**0.0001**	***	**3**	**4726.6**	**1575.5**	**6.1931**	**0.0001**	
TrxPo	2	5035.5	2517.7	1.3808	0.261		2	1887.7	943.84	1.0477	0.4037	
TrxTi	3	949.4	316.48	0.63223	0.7516		3	231.91	77.304	0.30387	0.9457	
PoxTi	6	4914.2	819.04	1.6362	0.0684		6	2767	461.16	1.8127	0.0355	n.s.
Panel(PoxTr)	**14**	**25528.0**	**1823.4**	**3.6426**	**0.0001**	**	**14**	**12612**	**900.89**	**3.5412**	**0.0001**	***
TrxPoxTi	6	3280.3	546.72	1.0922	0.382		6	1679.7	279.95	1.1004	0.3684	
Residuals	42	21024.0	500.57				42	10685	254.4			
Total	79	84783.0					79	39038				
Pairwise comparison (Treatment)												

*p<0.05, **p<0.01, ***p<0.001

The term TixPanel(TrxPo) was excluded from the model to account for the repeated-measures nature of the experimental design. P-values were obtained using 9,999 permutations of appropriate units. In the main model significant p-values (p<0.05) are presented in bold. Pairwise comparisons between ambient and freshwater treatments were carried out within the term Treatment*Time.

Table C.18: *PERMANOVA results of individual NNS and cryptogenic species in Site A*

Site A

Source	df	SS	MS	Pseudo-F	P(perm)	perms
		Diplosoma listerianum				
Treatment (Tr)	**1**	**4954.7**	**4954.7**	**23.464**	**0.0001**	**9850**
Orientation (Or)	**1**	**2339.6**	**2339.6**	**11.08**	**0.0032**	**9839**
Pontoon (Po)	2	1017.8	508.91	2.4115	0.1141	9951
Time (Ti)	**3**	**32873**	**10958**	**105.17**	**0.0001**	**9957**
TrxOr	1	250.95	250.95	1.1884	0.2813	9807
TrxPo	2	550.38	275.19	1.304	0.2859	9955
TrxTi	**3**	**2025.1**	**675.02**	**6.4783**	**0.0011**	**9952**
OrxPo	2	326.64	163.32	0.7739	0.4561	9951
OrxTi	3	367.04	122.35	1.1742	0.3261	9946
PoxTi	6	465.53	77.588	0.74464	0.6137	9934
TrxOrxPo	2	212.27	106.14	0.50293	0.608	9952
TrxOrxTi	3	483.54	161.18	1.5469	0.2097	9955
TrxPoxTi	6	568.88	94.813	0.90995	0.4915	9937
OrxPoxTi	6	429.13	71.522	0.68642	0.6626	9939
Pa(OrxTrxPo)	**29**	**6329.4**	**218.26**	**2.0947**	**0.0059**	**9916**
TrxOrxPoxTi	6	180.97	30.161	0.28946	0.9465	9934
Res	76	7918.9	104.2			
Total	152	67006				

Source	df	SS	MS	Pseudo-F	P(perm)	perms
		Schizoporella japonica				
Tr	**1**	**706.47**	**706.47**	**39.904**	**0.0001**	**9834**
Or	**1**	**119.92**	**119.92**	**6.7734**	**0.0121**	**9838**
Po	2	31.408	15.704	0.88766	0.4364	9958
Ti	**3**	**770.84**	**256.95**	**36.485**	**0.0001**	**9951**
TrxOr	1	70.302	70.302	3.971	0.0532	9822
TrxPo	2	25.298	12.649	0.71497	0.5147	9957
TrxTi	**3**	**778.44**	**259.48**	**36.845**	**0.0001**	**9956**
OrxPo	2	49.181	24.591	1.39	0.2763	9953
OrxTi	**3**	**110.76**	**36.92**	**5.2424**	**0.0046**	**9953**
PoxTi	6	7.2666	1.2111	0.17197	0.978	9945
TrxOrxPo	2	53.079	26.539	1.5001	0.2523	9957
TrxOrxTi	**3**	**111.55**	**37.183**	**5.2797**	**0.0051**	**9950**
TrxPoxTi	6	5.2948	0.88246	0.1253	0.9892	9947
OrxPoxTi	6	13.797	2.2995	0.32652	0.9104	9956
Pa(OrxTrxPo)	**29**	**533.93**	**18.411**	**2.6143**	**0.0155**	**9885**
TrxOrxPoxTi	6	14.741	2.4568	0.34886	0.8963	9944
Res	76	535.24	7.0426			
Total	152	4346				

Source	df	SS	MS	Pseudo-F	P(perm)	perms
		Tricellaria inopinata				
Tr	**1**	**674.8**	**674.8**	**7.3814**	**0.0103**	**9852**
Or	1	3.2402	3.2402	0.035443	0.8515	9839
Po	**2**	**1513.3**	**756.64**	**8.2845**	**0.0025**	**9945**
Ti	**3**	**481.3**	**160.43**	**9.9069**	**0.0001**	**9952**
TrxOr	**1**	**479.95**	**479.95**	**5.2499**	**0.027**	**9841**
TrxPo	2	114.47	57.233	0.62666	0.5415	9955
TrxTi	**3**	**730.75**	**243.58**	**15.042**	**0.0001**	**9949**
OrxPo	2	172.16	86.078	0.94248	0.4017	9945
OrxTi	3	110.12	36.707	2.2667	0.0897	9959
PoxTi	6	41.628	6.9379	0.42843	0.8561	9934
TrxOrxPo	**2**	**958.49**	**479.25**	**5.2473**	**0.0112**	**9955**
TrxOrxTi	**3**	**223.66**	**74.555**	**4.6038**	**0.0063**	**9957**
TrxPoxTi	6	96.478	16.08	0.99294	0.4318	9949
OrxPoxTi	6	212.75	35.458	2.1896	0.0536	9944
Pa(OrxTrxPo)	**29**	**2795.9**	**96.41**	**5.9535**	**0.0001**	**9898**
TrxOrxPoxTi	6	63.225	10.538	0.65071	0.6867	9950
Res	76	1230.7	16.194	0.34886	0.8963	9944
Total	152	11894	7.0426			
Total	152	4346				

Table C.19: *PERMANOVA results of individual cryptogenic species in Site B*

Site B

Source	df	SS	MS	Pseudo-F	P(perm)	perms
		Amathia gracilis				
Tr	1	28.451	28.451	0.57718	0.4592	9832
Or	1	39.395	39.395	0.79918	0.3745	9827
Po	2	65.89	32.945	0.66834	0.5277	9944
Ti	3	46.157	15.386	0.89276	0.4507	9950
TrxOr	1	158.12	158.12	3.2076	0.0872	9836
TrxPo	2	128.55	64.274	1.3039	0.2885	9946
TrxTi	3	117.98	39.328	2.282	0.09	9952
OrxPo	2	109.49	54.746	1.1106	0.3488	9952
OrxTi	3	86.46	28.82	1.6723	0.1772	9949
PoxTi	6	216.11	36.018	2.0899	0.0693	9954
TrxOrxPo	2	76.217	38.109	0.77309	0.4794	9952
TrxOrxTi	3	39.74	13.247	0.76864	0.5159	9945
TrxPoxTi	6	59.657	9.9428	0.57693	0.7574	9942
OrxPoxTi	6	61.386	10.231	0.59365	0.734	9936
Pa(OrxTrxPo)	**28**	**1380.2**	**49.294**	**2.8603**	**0.0001**	**9910**
TrxOrxPoxTi	6	148.91	24.818	1.4401	0.2043	9929
Res	84	1447.7	17.234			
Total	159	4268.5				

Source	df	SS	MS	Pseudo-F	P(perm)	perms
		Jassa sp.				
Tr	1	79.718	79.718	3.1052	0.0743	9881
Or	1	1.5014	1.5014	0.058482	0.858	9884
Po	2	5.1006	2.5503	0.099338	0.9396	9962
Ti	3	31.434	10.478	2.4036	0.0709	9957
TrxOr	1	0.023789	0.023789	0.0009266	0.9844	9882
TrxPo	2	0.96973	0.48487	0.018886	0.9899	9955
TrxTi	3	28.621	9.5404	2.1885	0.0924	9958
OrxPo	2	47.934	23.967	0.93355	0.4381	9942
OrxTi	3	9.4139	3.138	0.71981	0.5574	9959
PoxTi	6	10.269	1.7115	0.3926	0.8775	9936
TrxOrxPo	2	52.507	26.254	1.0226	0.4049	9952
TrxOrxTi	3	12.827	4.2756	0.98078	0.4131	9951
TrxPoxTi	6	10.069	1.6782	0.38496	0.8865	9942
OrxPoxTi	6	36.283	6.0471	1.3871	0.2295	9947
Pa(OrxTrxPo)	**28**	**718.84**	**25.673**	**5.8891**	**0.0001**	**9890**
TrxOrxPoxTi	6	21.051	3.5086	0.80483	0.5762	9944
Res	84	366.19	4.3594			
Total	159	1443.9				

Table C.20: *PERMANOVA results of individual NNS and cryptogenic species in site C*

Site C

Source	df	SS	MS	Pseudo-F	P(perm)	perms
			Amathia gracilis			
Treatment (Tr)	1	0.35986	0.35986	0.0024572	0.9608	9829
Orientation (Or)	**1**	**4713.3**	**4713.3**	**32.183**	**0.0001**	**9839**
Pontoon (Po)	2	3198.5	1599.2	10.911	0.0005	9956
Time (Ti)	**3**	**2063.3**	**687.77**	**10.139**	**0.0001**	**9951**
TrxOr	1	432.53	432.53	2.9534	0.0942	9849
TrxPo	2	485.03	242.51	1.6546	0.2095	9954
TrxTi	3	287.49	95.831	1.4127	0.2456	9943
OrxPo	2	353.08	176.54	1.2044	0.3091	9950
OrxTi	3	483.64	161.21	2.3765	0.0755	9949
PoxTi	**6**	**1762.7**	**293.78**	**4.3309**	**0.001**	**9927**
TrxOrxPo	2	19.173	9.5867	0.065406	0.9385	9945
TrxOrxTi	3	68.224	22.741	0.33524	0.8012	9940
TrxPoxTi	6	796.24	132.71	1.9563	0.0861	9943
OrxPoxTi	6	344.41	57.402	0.8462	0.5294	9946
Pa(OrxTrxPo)	**28**	**4167**	**148.82**	**2.1938**	**0.0039**	**9899**
TrxOrxPoxTi	6	431.26	71.877	1.0596	0.3803	9946
Res	83	5630.3	67.835			
Total	158	25990				

Source	df	SS	MS	Pseudo-F	P(perm)	perms
			Austrominius modestus			
Tr	**1**	**54.251**	**54.251**	**4.5526**	**0.0418**	**9846**
Or	1	8.5031	8.5031	0.71356	0.4081	9842
Po	2	33.273	16.637	1.3945	0.2748	9955
Ti	**3**	**74.155**	**24.718**	**9.1573**	**0.0001**	**9951**
TrxOr	1	0.17734	0.17734	0.014882	0.8996	9831
TrxPo	2	20.4	10.2	0.85497	0.4372	9955
TrxTi	**3**	**60.289**	**20.096**	**7.445**	**0.0006**	**9951**
OrxPo	2	13.789	6.8947	0.57792	0.5698	9944
OrxTi	3	12.314	4.1047	1.5207	0.2166	9945
PoxTi	6	37.681	6.2802	2.3266	0.0467	9952
TrxOrxPo	2	3.0837	1.5419	0.12924	0.8789	9944
TrxOrxTi	3	8.9493	2.9831	1.1051	0.349	9954
TrxPoxTi	6	17.456	2.9094	1.0778	0.3762	9952
OrxPoxTi	6	11.266	1.8776	0.69559	0.6403	9951
Pa(OrxTrxPo)	**28**	**341.43**	**12.194**	**4.5174**	**0.0001**	**9913**
TrxOrxPoxTi	6	12.806	2.1344	0.79071	0.5651	9950
Res	83	224.04	2.6993			
Total	158	975.06				

			Cordylophora caspia			
Tr	1	200.47	200.47	0.45694	0.5008	9830
Or	**1**	**25526**	**25526**	**58.181**	**0.0001**	**9855**
Po	**2**	**4298.1**	**2149.1**	**4.8931**	**0.0149**	**9945**
Ti	**3**	**1930.6**	**643.53**	**4.9972**	**0.0043**	**9946**
TrxOr	1	1708.4	1708.4	3.8939	0.0607	9852
TrxPo	2	1933.8	966.9	2.2015	0.1263	9946
TrxTi	3	362.69	120.9	0.93879	0.4296	9956
OrxPo	2	240.02	120.01	0.27324	0.7573	9951
OrxTi	**3**	**3047**	**1015.7**	**7.8868**	**0.0002**	**9950**
PoxTi	6	743.94	123.99	0.96282	0.4488	9932
TrxOrxPo	2	1530.6	765.29	1.7425	0.1976	9957
TrxOrxTi	3	339.4	113.13	0.87851	0.446	9944
TrxPoxTi	6	1338.5	223.08	1.7323	0.1332	9944
OrxPoxTi	6	455.23	75.872	0.58916	0.7282	9949
Pa(OrxTrxPo)	**28**	**12546**	**448.06**	**3.4793**	**0.0001**	**9912**
TrxOrxPoxTi	6	98.406	16.401	0.12736	0.9903	9944
Res	83	10689	128.78	0.34886	0.8963	9944
Total	158	69815	7.0426			
Total	152	4346				

Table C.21: *Table showing the results of RM-ANOVA on laboratory analysis of panels.*

Source of variation	df	SS	MS	Pseudo-F	P(perm)	PERMDISP
			Cover (%)			
Marina (Ma)	2	90962	45481	67.239	0.0001	
Treatment (Tr)	1	6223.4	6223.4	9.2007	0.0029	
Orientation (Or)	1	11627	11627	17.189	0.0001	
MaxTr	2	12146	6072.8	8.9779	0.0004	
MaxOr	2	15589	7794.4	11.523	0.0001	
TrxOr	1	1496	1496	2.2117	0.1404	
MaxTrxOr	2	21155	10578	15.638	0.0001	**
Residuals	103	69670	676.41	1.6723	0.1772	
Total	114	216590	36.018	2.0899	0.0693	
Pairwise comparison (Treatment)	Site A: (H) A≠F**, (V) A≠F*** Site B: (H) A≠F***, (V) A=F Site C: (H&V) A=F					

Source of variation	df	SS	MS	Pseudo-F	P(perm)	PERMDISP
			Species richness			
Marina (Ma)	2	1142.7	571.35	65.638	0.0001	
Treatment (Tr)	1	14.124	14.124	1.6225	0.2037	
Orientation (Or)	1	47.06	47.06	5.4064	0.0224	
MaxTr	2	200.42	100.21	11.513	0.0001	***
MaxOr	2	67.117	33.559	3.8553	0.0232	***
TrxOr	1	12.398	12.398	1.4243	0.2366	
MaxTrxOr	2	13.906	6.9532	0.79879	0.4548	
Residuals	103	896.57	8.7046	0.71981	0.5574	
Total	114	2339.2	1.7115	0.3926	0.8775	
Pairwise comparison (Treatment)	Site A: (H) A≠F***, (V) A=F Site B: (H&V) A=F Site C: (H&V) A=F					

Table C.22: *Table showing the results of PERMANOVA on BC 4th root transformed of laboratory data*

Source of variation	df	SS	MS	Pseudo-F	P(perm)	PERMDISP
Marina (Ma)	**2**	**132470**	**66235**	**125.28**	**0.0001**	
Treatment (Tr)	**1**	**6035.7**	**6035.7**	**11.416**	**0.0001**	
Orientation (Or)	**1**	**17726**	**17726**	**33.528**	**0.0001**	
MaxTr	**2**	**9856.6**	**4928.3**	**9.3216**	**0.0001**	
MaxOr	**2**	**3392.9**	**1696.4**	**3.2088**	**0.0002**	
TrxOr	1	926.89	926.89	1.7532	0.0961	
MaxTrxOr	**2**	**2094.7**	**1047.4**	**1.981**	**0.0194**	***
Res	103	54455	528.69	1.6723	0.1772	
Total	114	228240	36.018	2.0899	0.0693	
Pairwise comparison (Treatment)	Site A: (H) A≠F***, (V) A≠F*** Site B: (H) A≠F***, (V) A=F Site C: (H) A=F, (V) A ≠ F*					

D - Authored Articles

D.1 Foster et al. (2016) Marine Biology 163:173

Foster V, **Giesler RJ**, Wilson AMW, Nall CR, Cook EJ (2016) Identifying the physical features of marina infrastructure associated with the presence of non-native species in the UK. Marine Biology 163: 173. https://doi.org/10.1007/s00227-016-2941-8

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INVASIVE SPECIES - ORIGINAL PAPER

Identifying the physical features of marina infrastructure associated with the presence of non-native species in the UK

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Abstract Marine invasive non-native species (NNS) are one of the greatest threats to global marine biodiversity, causing significant economic and social impacts. Marinas are increasingly recognised as key reservoirs for invasive NNS. They provide submersed artificial habitat that unintentionally supports the establishment of NNS introduced from visiting recreational vessels. While ballast water and shipping vectors have been well documented, the role of recreational vessels in spreading NNS has been relatively poorly studied. Identification of the main physical features found within marinas, which relate to the presence of NNS, is important to inform the development of effective biosecurity measures and prevent further spread. Towards this aim, physical features that could influence the presence of NNS were assessed for marinas throughout the UK in July 2013. Thirty-three marine and brackish NNS have been recorded in UK marinas, and of the 88 marinas studied in detail, 83 contained between 1 and 13 NNS. Significant differences in freshwater input, marina entrance width and seawall length were associated with the presence of NNS. Additionally, questionnaires were distributed to marina managers and recreational vessel owners to understand current biosecurity practices and attitudes to recreational vessel biosecurity. The main barriers to biosecurity compliance were cited as cost and time. Further work identifying easily distinguished features of marinas could be used as a proxy to assess risk of invasion.

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Introduction

Biological invasions by non-native species (NNS) are generally accepted to be one of the greatest threats to biodiversity worldwide (CBD 1992). Invasive NNS can cause significant economic and social impacts and are estimated to cause global damage amounting to $120 billion annually (Pimentel et al. 2005). Biosecurity measures including quarantine, customs and legislative controls have long been in place for terrestrial pest species (Cook et al. 2016). During the last decade, marine invasive NNS have received increased attention from both scientists and policy makers (Genovesi et al. 2014; Hulme 2009, 2015); yet both ecological knowledge and biosecurity practices to address marine invasive NNS still lag behind those of terrestrial species (Williams and Grosholz 2008; Cook et al. 2016).

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To date, commercial vessels have been the primary focus for marine NNS biosecurity measures, due to long voyages across biogeographic boundaries and the ability of organisms to be transported by ballast water (Briski et al. 2013; Seebens et al. 2013). Recreational vessels were generally considered low-risk vectors due to shorter voyages, frequent cleaning regimes and high home port fidelity (Ashton et al. 2006a). However, recreational boating has now been identified as a significant vector for the introduction and spread of NNS, especially at more local scales, allowing the secondary spread of these species away from sites of first introduction (Boos et al. 2011; Clarke Murray et al. 2011; Ashton et al. 2014; Zabin et al. 2014). Recreational vessels can transport invasive NNS via hull fouling, internal fouling in pipes, in ballast, bilge or anchor-well water, and in inlets leading off the hull (Darbyson et al. 2009; Acosta and Forrest 2009). The short, relatively slow voyages typical of recreational vessels make successful spread more likely, as there is a higher probability that fouling species will survive (Coutts et al. 2010; Clarke Murray et al. 2011). Furthermore, they are more likely to be able to colonise the receiving site, given that the environmental and climatic conditions are likely to be similar to the source habitat (Minchin 2006; Coutts et al. 2010).

Measures to control marine invasive NNS once they have become established can be costly and time-consuming (Hulme 2009). It is, therefore, key to target high-risk entry points and manage the critical pathways in order to eradicate the initial introduction before species are able to establish and spread (Katsanevakis et al. 2013; Williams et al. 2013). Control of the secondary spread of established marine NNS is also crucial, as local and incremental expansion in range will ultimately determine the extent of the economic, social and environmental impact of an NNS (Ashton et al. 2006b; Forrest et al. 2009).

Another emerging dimension of NNS spread is through the rapid expansion of artificial, novel habitats, which are expanding in scope, scale and distribution throughout a diversity of intertidal and subtidal marine settings, as a result of increased coastal populations, trade, tourism and the exploitation of natural resources (Mineur et al. 2012). In Europe, it has been estimated that 22,000 km^2 of the coastal zone has been 'hardened' by artificial surfaces, and in some areas more than 50 % of the coastline has been modified (Airoldi and Beck 2007; Dafforn et al. 2015a). NNS are highly opportunistic and are more likely to occur on these novel substrates than on adjacent natural surfaces compared with native species (Connell 2001; Dafforn et al. 2012; Airoldi et al. 2015). Artificial structures are particularly prevalent in harbours and marinas (Rivero et al. 2013), and these locations are subjected to high propagule pressure from NNS due to the volume of traffic concentrated in a specific area and the high occurrence of NNS in ballast water, sea chests and on vessel hulls (Clark and Johnston 2009; Mineur et al. 2012).

The concept of ecological engineering has emerged in recent years as an attempt to integrate 'ecological, economic and social needs into the design of man-made ecosystems' (Firth et al. 2014), addressing the concurrent but competing drivers of essential coastal infrastructure and need for habitat restoration (Dafforn et al. 2015b). There have been a number of studies which attempt to alter the design of artificial structures to enhance native biodiversity (Dafforn et al. 2015a; Firth et al. 2014). However, there is a pressing need to more specifically design infrastructure to prevent the establishment of NNS. Similar to the growing trend of environmentally responsible 'green build' structures on land, developers could embrace similar design and build concepts into coastal marine infrastructure (Dafforn et al. 2015b), incorporating biosecurity considerations which could potentially reduce NNS establishment.

To reduce the likelihood of NNS occurrence in marinas, there is a need for research to identify the relationships between the physical features of marina infrastructure and the presence of NNS (Airoldi et al. 2015). Additionally, this information needs to be integrated into policy and practices related to coastal marine infrastructure development (Wilson et al. 2015). This study aims to identify specific features of UK marinas which might influence the establishment of NNS, and draws on information from marina operators and users to provide recommendations for a more holistic approach to biosecurity across the recreational boating industry.

Methods

Identifying marinas with non-native species

The contact details for 213 marinas and harbours around the UK were compiled from the Practical Boat Owners online marina guide (Practical Boat Owner 2013). This was expanded to 239 marinas using information from data sets listed below.

A list of brackish and marine NNS recorded in UK marine or coastal habitats was compiled based on published reviews (Eno et al. 1997; Arenas et al. 2006; Minchin et al. 2013), literature searches and unpublished field studies. Non-native fish species and microorganisms were excluded from the list. A total of eight existing inventories with information on the presence and/or absence of NNS in one or more of the 239 marinas were reviewed and the data for each marina compiled. These included published inventories and papers by Beveridge et al. (2011), Holt and Cordingley (2011), Arenas et al. (2006) and Ashton et al. (2006a), Minchin and Nunn (2013), Nall et al. (2015), and

unpublished records from Scottish marina surveys conducted by Cook (2013, unpubl.). Any species identified by these data sets were then searched for using the NBN Gateway (National Biodiversity Network 2011), in order to locate additional survey records.

Marina location and construction information

The location and construction details of the marinas, identified as having data on NNS presence, were then investigated with virtual site surveys using the 'Path' measuring tool in Google Earth (version 7.1) (Google 2013). The accuracy of aerial and satellite images, which were taken before 2008, was checked with marina websites and online marina guides where available.

Measurements and details were taken of six specific marina features; (i) entrance width (m) at the narrowest point between structures that enclosed the marina. Marina entrances were grouped into three categories; (1) <30 m (enclosed), (2) >30 m (semi-enclosed) and (3) not surrounded by structures (open). (ii) Distance (m) to a freshwater source from the mouth of the marina entrance for enclosed or semi-enclosed marinas and from the midpoint of the marina pontoons for open marinas. A freshwater source was defined as any river or stream identifiable on Google Earth and did not take account of size or flow or include other possible sources, such as storm drains. The measurements were grouped into <20, 20–1000 and >1000 m from a freshwater source. (iii) Total pontoon length (m) was measured for each marina and classified as either <450, 450–1100 or >1100 m. Finger pontoons allowing access to individual vessels were excluded from this for ease of measurement. This exclusion results in underestimations of total pontoon length for some marinas, especially large marinas such as Bangor, Co. Down, where there are a high number of secondary finger pontoons. (iv) Total length (m) of seawalls, vertical structures made of concrete or stone within the marina, was measured and classified as <200, 200–550 or >550 m. (v) Total length (m) of all man-made sloping, boulder structures (i.e. rubble breakwaters) within the marina confines, was measured and classified as either <25, 25–250 or >250 m. (vi) The presence or absence of swing moorings in the marinas was recorded. When marinas had no easily defined boundaries, then moorings were considered to be within the marina vicinity, if they were within 100 m of a pontoon.

Statistical analysis

A generalised linear model (GLM) with a quasi-Poisson distribution (data were over-dispersed, un-banded count data with a poisson dispersion factor of 2.38) and a log link function was used to analyse the relationship between marina features and NNS richness. Continuous covariates were grouped into classes to prevent outlier influence. Variance inflation factor (VIF) values confirmed that there was no collinearity among these (Zuur et al. 2010). An automated variable selection technique was used, and the relative quality of the models was compared using the quasi-Akaike information criterion (QAIC). The model with the lowest QAIC was considered the best quality, containing only the covariates that explained the variation in NNS counts. The deviance was used to assess the final model fit.

To identify the marina features associated with the presence of each of the five most common NNS (those that occurred in the most marinas), a GLM with a binomial distribution and a logit link function was constructed. Covariates were the same as the quasi-Poisson model described above, with VIF values used to assess collinearity among covariates. Binomial model selection used stepwise automated variable selection with the relative quality of the model assessed using the Akaike information criterion (AIC). The model fit was assessed using the R^2 value to assess to what extent the model could explain the variance of the data.

All data were analysed using the software R version 3.0.1 (R Core Team 2013) in the integrated development environment RStudio, version 0.97.551 (RStudio Team 2012). The models were fitted using the function 'GLM' with model selection completed using the 'dredge' function in the R package MuMIn for quasi-Poisson models (Barton 2013), and the function 'step' for binary models (RStudio Team 2012). The 'vif' function in the R package 'car' was used to calculate the VIF values for all models (Fox and Weisberg 2011).

Marina operator and recreational sailor surveys

A survey designed to gather further information on marina design and marina management practices was sent to all marinas and harbours on the contact list compiled from the Practical Boat Owners marina guide (Practical Boat Owner 2013). Marina operators were asked a series of questions on marina use, dominant construction materials and fouling species, and on best management practices (BMP) for cleaning of submerged structures. Recreational sailors were invited to participate in a separate, online survey designed to investigate boating practices and attitudes towards hull-cleaning practices. The recreational sailor survey was disseminated via a public internet forum, the Yachting and Boating World forum (Yachting and Boating World 2013), and on the Practical Boat Owners online news feed (Hodgetts 2013). Both surveys were electronic, constructed using SurveyMonkey (2013) and included both multiple choice and open-ended questions (see Online Resource 1). Both surveys were left open for

a period of 42 days during June and July 2013. Responses were tabulated and answers to open-ended questions grouped into themes for analysis.

Results

Non-native species in UK marinas

A review of brackish and marine NNS recorded in UK marine or coastal habitats resulted in a species list of 105 species (Online Resource 2). This list includes species for which there is only a single record, and which may not be established in the UK. A discussion of the criteria for classification of species as non-native and established is provided in Minchin et al. (2013) for the majority of species listed. Of these, 105 NNS known to occur in UK waters, 31 % of these species ($n = 33$) were found to occur in UK marinas. Of the 239 UK marinas identified in our analysis in July 2013, only 88 (35 %) had data on the presence/absence of NNS (Fig. 1).

The five most commonly occurring species in the UK marinas surveyed were the barnacle *Austromininus modesus*, the ascidians *Styela clava* and *Corella eumyota*, the bryozoan *Tricellaria inopinata* and the macroalga *Undaria pinnatifida*. An additional six species, which were included in rapid assessment survey target lists (*Asparagopsis armata*, *Anotrichium furcellatum*, *Bonnemaisonia hamifera*, *Crassostrea gigas*, *Diadumene lineata* and *Eriochier sinensis*), were reported as absent from the marinas surveyed for them (Table 1).

Of the 88 marinas with data on NNS, the maximum number of NNS recorded in a single marina was 13. Marinas on the south coast of England and in Northern Ireland typically had the greatest number of NNS. Over 92 % of marinas with NNS data had between 1 and 8 NNS (Fig. 2), and 49 % of the marinas contained 1–2 NNS. Scotland and Wales had no more than seven different NNS reported in one marina, while the majority of marinas in these areas contained five or less NNS (Fig. 1). Only five marinas (6 %) surveyed for NNS were not found to have any of the listed NNS species present.

Marina features and non-native species

Four out of the six marina features were included in the final fitted model for NNS counts (Table 2). Moorings and breakwaters were the two features excluded from this model, as neither explained any of the variation in the NNS counts. The probability of NNS presence in a marina increased significantly ($\alpha = 0.05$) as the distance from a freshwater source increased, $\beta = 0.90$, $p < 0.001$ (Fig. 3a). The probability of a semi-enclosed marina containing NNS was significantly higher than an open marina or an enclosed marina, $\beta = 0.60$, $p < 0.05$ (Fig. 3b). There was also a significantly greater probability of a marina containing NNS with 200–550 m of seawalls compared with those with <200 or >550 m, $\beta = 0.49$, $p < 0.05$ (Fig. 3c). Pontoon length was not significant in predicting the number of NNS in a marina, but it appeared to explain some of the variance in the data and so was retained in the model (Fig. 3d). This model was considered a 'good fit' and explains a significant amount of variability in the data, as indicated by the deviance, Model $\chi^2(8) = 56.44$, $p < 0.001$.

Influence of marina features on commonly occurring non-native species

The final binomial GLM models for the five most commonly occurring NNS indicated that different marina features are important for determining whether certain species will be present or absent (Table 3).

The ascidian *S. clava* occurred in 13 % of the marinas ($n = 32$). The final model for this species found that there was a highly significant probability of *S. clava* being present in a marina as seawall length increased ($\beta = 3.31$, $p < 0.01$), total pontoon length exceeded 1100 m ($\beta = 2.87$, $p < 0.01$) and where marinas were classified as 'open' compared with 'enclosed' ($\beta = 2.24$, $p < 0.05$). The final model explained 53 % of the variability in the presence of *S. clava* ($R^2 = 0.53$).

Tricellaria inopinata was found in 11 % marinas ($n = 25$). There was a significantly greater probability of *T. inopinata* being present in semi-enclosed compared with enclosed marinas ($\beta = 4.25$, $p < 0.05$). There was a significantly greater probability of this species being present in marinas where the boulder breakwaters were 25 m or less compared with between 25 and 250 m long ($\beta = -4.39$, $p < 0.05$). Although the presence of moorings and the distance of the marina from freshwater were not significant, they were retained as they explained some of the variance in the data. The full model explained 65 % of the variability in the presence of *T. inopinata* ($R^2 = 0.65$).

Corella eumyota occurred in 9 % UK marinas ($n = 22$). There is a significantly greater probability of *C. eumyota* being present when the boulder breakwaters are <25 m compared with between 25 and 250 m long ($\beta = -1.73$, $p < 0.05$). The probability of *C. eumyota* being present is also significantly greater when the pontoon length is <450 m compared with between 450 and 1100 m ($\beta = -2.18$, $p < 0.05$). The full model only explains 25 % of the variability in the presence of *C. eumyota* ($R^2 = 0.25$).

In the case of the barnacle *Austrominius modestus*, which was ranked as most common NNS, occurring in

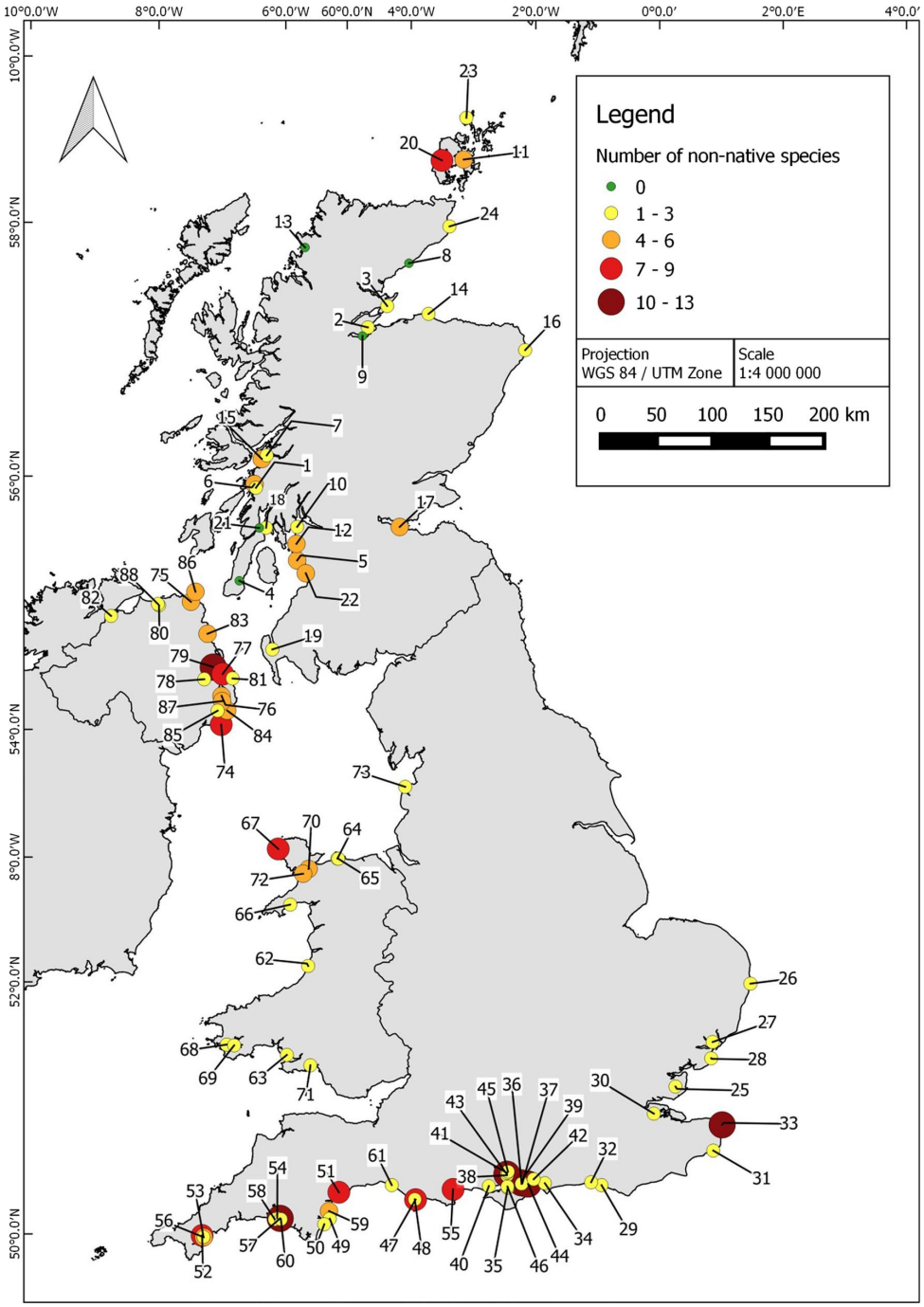

Fig. 1 Map of the UK showing distribution of marinas which have been surveyed for non-native species. The total number of different non-native species is represented by the size and *colour of the circle*. The reference numbers for each marina are in Online Resource 3

Table 1 Non-native species for which marina surveys have been conducted, including the number of marinas each species occurred in by region, as well as the total number of marinas across the UK from which each species was recorded

Phylum	Species	Scotland	Wales	England	Northern Ireland	UK total
Annelida	*Ficopomatus enigmaticus*	0	2	3	0	5
	Hydroides ezoensis	0	0	1	0	1
Arthropoda	*Amphibalanus improvisus*	0	0	0	1	1
	Austrominius modestus	**7**	**9**	**11**	**8**	**35**
	Caprella mutica	12	0	1	6	19
	Eriocheir sinensis	0	0	0	0	0
	Gammarus tigrinus	0	1	1	2	4
	Monocorophium acherusicum	0	0	0	1	1
	Monocorophium sextonae	0	0	0	2	2
	Monocorophium insidiosum	0	0	0	6	6
Bryozoa	*Bugulina fulva*	0	0	0	2	2
	Bugula neritina	0	2	11	1	14
	Bugulina simplex	1	0	2	5	8
	Schizoporella japonica	3	0	0	0	3
	Tricellaria inopinata	**11**	**0**	**9**	**5**	**25**
	Watersipora subtorquata	0	0	0	1	1
Chordata	*Aplidium glabrum*	0	0	1	3	4
	Botrylloides violaceus	2	0	5	1	8
	Corella eumyota	**4**	**3**	**5**	**10**	**22**
	Didemnum vexillum	1	0	0	1	2
	Perophora japonica	0	0	2	0	2
	Styela clava	**2**	**4**	**23**	**3**	**32**
Cnidaria	*Cordylophora caspia*	0	0	0	3	3
	Diadumene lineata	0	0	0	0	0
Mollusca	*Crassostrea gigas*	0	0	0	0	0
	Crepidula fornicata	0	0	8	0	8
	Potamopyrgus antipodarum	0	0	0	1	1
Chlorophyta	*Codium fragile* ssp. *fragile*	8	0	7	0	15
Ochrophyta	*Colpomenia peregrina*	0	1	1	5	7
	Sargassum muticum	3	2	6	4	15
	Undaria pinnatifida	**0**	**0**	**22**	**1**	**23**
Rhodophyta	*Anotrichium furcellatum*	0	0	0	0	0
	Asparagopsis armata	0	0	0	0	0
	Bonnemaisonia hamifera	0	0	0	0	0
	Grateloupia subpectinata	0	0	2	0	2
	Grateloupia turuturu	0	0	6	0	6
	Dasysiphonia japonica	4	0	0	0	4
	Neosiphonia harveyi	0	0	9	0	9
	Solieria chordalis	0	0	1	0	1
Total number of species		12	8	22	22	33

The five most common species are highlighted in bold

15 % of the marinas ($n = 35$) and the Japanese kelp *U. pinnatifida* which was recorded in 10 % of the marinas ($n = 23$), it appears that none of the features can be used as indicators for the presence of these species.

Marina operator survey

Of the 213 marinas contacted, 40 marina operators completed the survey in full (18.8 % response rate). The

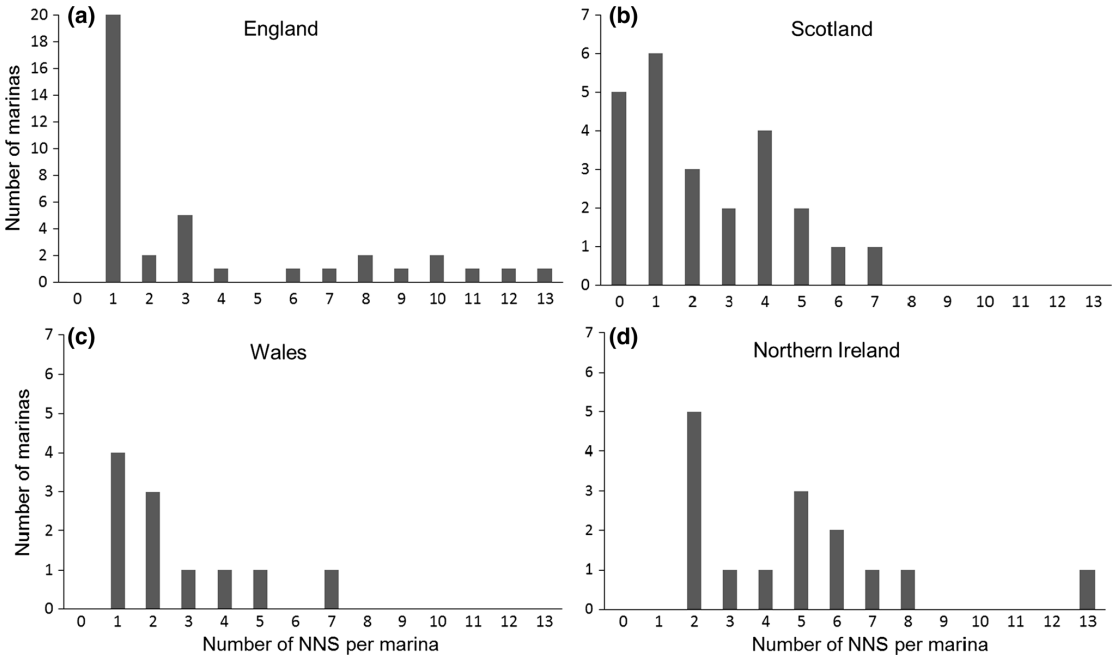

Fig. 2 *Bar charts* showing frequency distribution of marina NNS counts for each region of the UK. *Each graph* shows the number of marinas in which NNS surveys were carried out plotted against the number of NNS detected per marina. **a** England ($n = 38$), **b** Scotland ($n = 24$), **c** Wales ($n = 11$) and **d** Northern Ireland ($n = 15$). Note that scales along the *y* axis are not the same for each region. Scotland is the only region with documented absences of NNS ($n = 5$) where NNS were not found during targeted surveys

Table 2 GLM output for the non-native species count with key marina features as covariables

Coefficients	β (SE)	95 % CI for odds ratio		
		Lower	Odds ratio	Upper
Intercept	−0.09 (0.35)			
Fresh 20–1000	0.54 (0.27)	1.00	1.71	2.94
Fresh >1000	0.90 (0.24)***	1.56	2.46	3.97
Entrance >30	0.60 (0.25)*	1.13	1.83	2.99
Entrance open	0.27 (0.24)	0.83	1.31	2.13
Seawall 200–550	0.49 (0.21)*	1.08	1.63	2.51
Seawall >550	0.07 (0.27)	0.63	1.07	1.80
Pontoon 450–1100	0.07 (0.25)	0.64	1.07	1.76
Pontoon >1100	0.41 (0.23)	0.97	1.51	2.36

Includes beta (β), standard error (SE), odds ratios and upper and lower 95 % confidence intervals (CI) for odds ratio for each coefficient

Model $\chi^2(8) = 56.44$, $p < 0.001$

Significance codes: *** 0.001; ** 0.01; * 0.05

majority of the boat traffic occurring in 93 % of the marinas was recorded as recreational, with only three marinas reporting that both recreational and commercial traffic were equally prevalent. Ninety-five per cent of the marinas reported that their traffic was primarily from the UK, with only two reports of European traffic and one of worldwide traffic (beyond UK and European waters).

Marina operators were asked whether they were aware of NNS within their marina. Twenty-five per cent of marina operators stated that they did have NNS present ($n = 10$), 32.5 % stated they did not ($n = 13$), and 42.5 % were unsure ($n = 17$). Of all 40 of the respondents, 18 marinas were known to contain NNS; however, only three of these responded that they were aware of NNS in their marina, while nine stated they were unsure. Six marinas stated they were not aware of any NNS in their marinas, including one in which nine NNS have been recorded.

Thirty-nine respondents gave information regarding BMP employed for cleaning structures below the water line. Twenty-six per cent of the respondents confirmed they had BMPs for structure cleaning, while the majority did not (54 %). Twenty-one per cent of marina operators who responded to the questionnaire were unsure whether they had BMPs. Of the marina operators that did have BMPs for cleaning underwater structures, e.g. floats, pontoons and slipways, 30 % scraped off fouling organisms, 10 % dry docked pontoons, and 10 % washed down

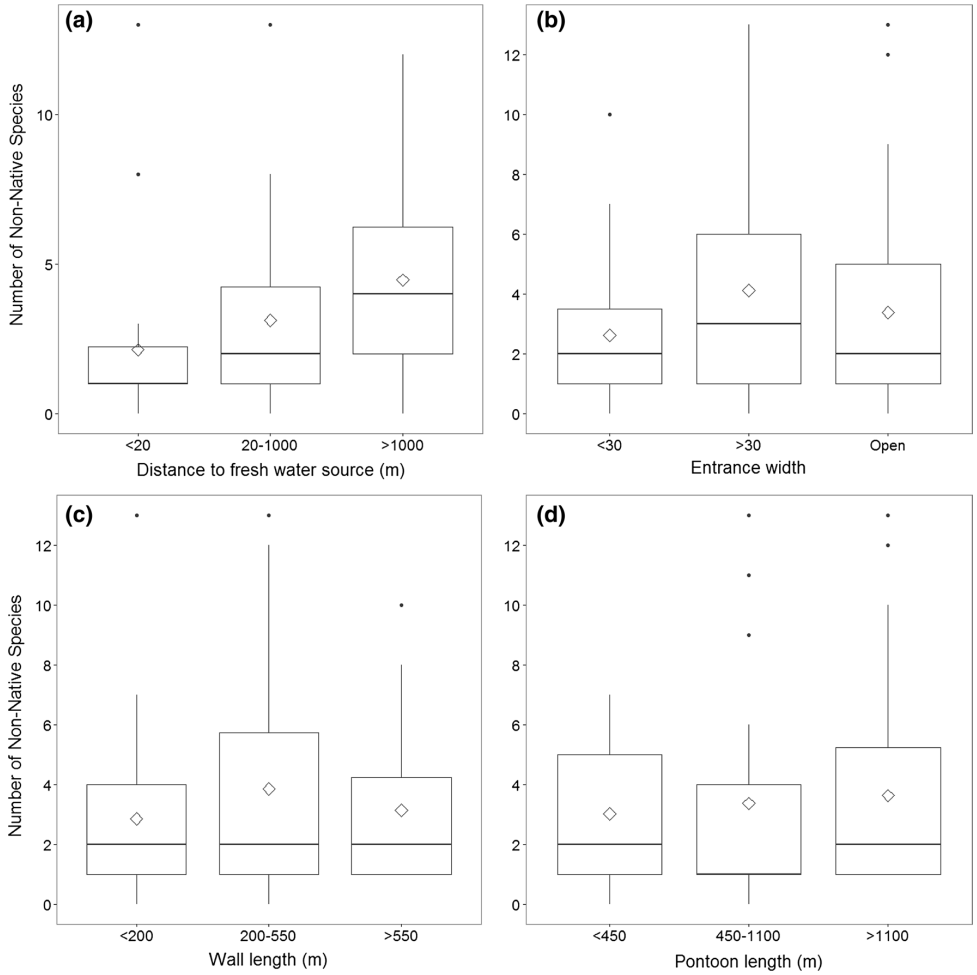

Fig. 3 *Box plot* showing of non-native species per marina in relation to marina features retained in GLM model. **a** Distance of marina from a fresh water source [<20 m ($n = 28$), 20–1000 m ($n = 24$), >1000 m ($n = 36$)]. **b** Marina entrance width [<30 m ($n = 27$), >30 m ($n = 25$) or open ($n = 36$)]. **c** Seawall length [<200 m ($n = 30$), 200–550 m ($n = 20$)]. **d** Pontoon length [<450 m ($n = 31$), 450–1100 m ($n = 21$), >1100 ($n = 36$)]. *Box plots* show median values (*solid horizontal line*), mean values (*open diamonds*), 50th percentile values (*box outline*), ±1.5 of the interquartile values (*whiskers*) and outlier values (*black circles*)

pontoons periodically. Forty per cent of respondents with BMPs stated that they used filters or similar devices to collect and dispose of debris from their onshore boat-washing facilities.

Recreational sailor survey

There were 105 responses to the online survey, of which 100 were fully completed and used in subsequent analysis. All responses were from recreational sailors in UK waters, with ten respondents also sailing on a commercial basis.

Eighty-nine respondents owned their own vessels. Eighty per cent of owners sailed most frequently within UK waters, 16 % travelled primarily within European waters and 4 % travelled worldwide. Over 50 % of boat owners applied antifouling as frequently as required by the paint manufacturer with no other fouling removal in between applications. Thirty-eight per cent additionally scrubbed vessel hulls to remove fouling between antifouling paint applications. Fewer than 8 % of owners did not use antifouling paint on the hulls of their boats. During the spring and summer sailing season, most owners sailed their boat

Table 3 Outputs of binomial GLMs for each of the five most common non-native species, with rank indicating the most common species (1–5), coefficients of the key marina features, beta (β) and standard error (SE)

Species	Rank	Coefficients	β (SE)	z value	R^2
A. modestus	1	Intercept	0.51 (0.28)	1.85	0
S. clava	2	Intercept	−3.58 (1.14)**	−3.13	0.53
		Seawall 200–550	2.91 (1.04)**	2.81	
		Seawall >550	3.31 (1.17)**	2.82	
		Pontoon 450–1100	−0.21 (0.96)	−0.22	
		Pontoon >1100	2.87 (1.11)**	2.59	
		Entrance >30	1.38 (0.96)	1.44	
		Entrance open	2.24 (1.11)*	2.02	
T. inopinata	3	Intercept	−1.81 (1.64)	−1.11	0.65
		Freshwater 20–1000	−0.09 (1.54)	−0.06	
		Freshwater >1000	3.22 (1.75)	1.84	
		Breakwater 25–250	−4.39 (1.74)*	−2.52	
		Breakwater >250	−0.97 (1.68)	−0.58	
		Mooring	3.62 (1.93)	1.89	
		Entrance >30	4.25 (1.84)*	2.30	
		Entrance open	2.63 (1.61)	1.64	
U. pinnatifida	4	Intercept	−17.57 (2284.10)	−0.01	0.58
		Breakwater 25–250	0.97 (1.48)	0.65	
		Breakwater >250	−1.89 (1.06)	−1.78	
		Pontoon 450–1100	17.59 (2284.10)	0.01	
		Pontoon >1100	19.86 (2284.10)	0.01	
C. eumyota	5	Intercept	1.12 (0.58)	1.94	0.25
		Breakwater 25–250	−1.73 (0.85)*	−2.04	
		Breakwater >250	−0.27 (0.88)	−0.31	
		Pontoon 450–1100	−2.18 (1.01)*	−2.16	
		Pontoon >1100	−0.65 (0.79)	−0.83	

Significance codes: *** 0.001; ** 0.01; * 0.05

regularly each month (62 %), while 10 % sailed less than once per month. The average residency time in a marina, other than the home mooring, was commonly 24 h–3 days.

Ninety per cent of respondents stated they knew about NNS. Participants were then asked whether they would be more likely to store their boat at a marina with 'green credentials' demonstrating that the marina actively participated in controlling the introduction and growth of NNS. Forty-one per cent of respondents stated that they probably, or definitely, would. Respondents were also asked their opinion about being encouraged, or being required by law to clean the hull of their boat before leaving a UK marina known to be a hot spot for NNS. There were 93 responses to this question, and answers were classified as falling into three categories: (1) against, (2) undecided or (3) supportive. Sixty-three per cent of respondents were against the hull cleaning, with the majority stating that the main reason would be the associated costs of hauling out and removing fouling. Other reasons cited for opposition to mandatory hull cleaning included the addition time it would require, the lack of facilities and the impractical nature of doing this numerous times a year. Ten per cent of respondents also raised the point that recreational boaters should not be penalised for a problem that they believe to be primarily created by the commercial shipping industry. Of the 26 % of respondents who supported the concept, over 25 % raised concerns about the cost and time required for hull cleaning.

Discussion

Non-native species records in UK marinas

This study identified 33 NNS recorded in UK marinas. A high proportion of UK marinas where surveys had been conducted had one or more NNS (94 %), and 45 % had three or more. This aligns with other studies which suggest that man-made artificial environments within marinas are highly suitable for the establishment of NNS (Ruiz

et al. 2000; Airoldi et al. 2015; Dafforn et al. 2015a). Of the 105 NNS recorded in UK waters in 2013, 31 % were recorded in marinas. A similar percentage of known NNS (34 %) was recorded by Pederson et al. (2003) for 20 marinas along the north-eastern coast of the USA. Data on the abundance of NNS and native species community composition in each marina were not available for analysis in this study, thus making it hard to detect any impact by the NNS; however, studies have previously found that introduced and cryptogenic species are frequently more abundant than native species in marinas and harbours (López-Legentil et al. 2015).

Overall, the number of NNS per marina varied between regions of the UK, with marinas located on the south coast of England typically having the greatest number of NNS (Fig. 1). Initial records of new NNS are often from sites in the English Channel, reflecting the high volume of international and recreational traffic and proximity to European sites compared to elsewhere in the UK (Minchin et al. 2013). Lower sea-surface temperature, current patterns and less vessel activity may be factors in explaining the lower numbers of NNS in Scottish marinas relative to high numbers of NNS found elsewhere in the UK (Nall et al. 2015). The majority of recreational vessel movement is within UK waters, with 80 % of vessel owners predominantly travelling within the UK. Nevertheless, vessels do not need to have arrived from different biogeographic regions to facilitate movement of NNS. Studies have shown recreational vessels can act vectors for the secondary spread of NNS within a region and can connect highly invaded systems with smaller marinas (Minchin 2006; Zabin et al. 2014).

Of the 239 marinas listed in the UK, only 37 % had been surveyed for NNS. Survey effort has not been evenly distributed across the UK, with less than a quarter of English marinas surveyed, compared with over 66 % for Scotland, and 85 and 94 % for Wales and Ireland, respectively. This may, in part, be explained by the higher number of marinas in England. However, there remain areas which have a paucity of data as the majority of studies focus on the south coast, although more recent surveys have extended into under-surveyed areas along the east coast of England (Bishop et al. 2015). The patchiness of survey effort, and the high numbers of NNS present in all surveyed marinas, underpins the need for coordinated, regular long-term monitoring of harbours and marinas across the UK. A potential catalyst is the EU Marine Strategy Framework Directive, which requires the development of a monitoring programme to address the targets developed for NNS, although currently this is still under review in the UK (DEFRA 2014).

The data used in the present study most likely underestimate the presence and distribution of NNS in UK marinas. Many of the original surveys collated in the present study used targeted rapid assessment surveys (RAS) to search for NNS in marinas. RAS are limited to a survey of the top 0.5 m of the water column, missing species which favour deeper water and soft substrates (Ashton et al. 2006a). However, the use of targeted RAS for NNS monitoring has been shown to be time and cost effective in comparison with alternative methods such as extensive baseline surveys or analysis of photograph and scrape samples (Cook et al. 2015). Future surveys of marinas and harbours should attempt to supplement RAS with periodic surveys of the water column and sea floor. Regular monitoring, particularly at 'hot spot' sites for NNS establishment, will enable early detection of NNS, increasing the likelihood of a successful control programme.

Physical features affecting NNS establishment

Marinas located within 20 m of a freshwater source had significantly fewer NNS than those sited over 1 km away. Similar patterns of reduced NNS numbers in marinas with high freshwater input have been observed in the north of Scotland (Nall et al. 2015) and in the USA (Ruiz et al. 2009). Certain NNS have a broad tolerance to variation in temperature and salinity, and there are a number of brackish NNS (Paavola et al. 2005; Gollasch 2006). However, few species will be capable of surviving the transition from fully marine conditions during transfer on hulls of recreational vessels to very low salinity in marinas with a high volume of freshwater (Boos et al. 2011; David et al. 2013). Proximity to a freshwater source was used as an indirect measure of salinity in this study. It is clear that variation in the salinity of the water column may occur in each marina dependent on rainfall and tidal conditions (Bax et al. 2002). Further research, therefore, is required to assess the specific salinity patterns over an extended period in individual marinas to help validate the results of this study. Construction of new marinas in areas of high freshwater input, such as rivers or river mouths, however, would reduce the likelihood of establishment of marine NNS, although care would still be needed to minimise the introduction of brackish and/or freshwater species from other pathways (Kelly et al. 2013).

The results also found that marinas with a medium length of seawall and those with a semi-enclosed entrance had significantly more NNS. Similar trends have been observed for fouling species in other regions, with higher recruitment of species in partially enclosed compared with open marinas (Floerl and Inglis 2003). Enclosed and partially enclosed marinas have complex circulation patterns, which can result in higher water residency and limited dispersal of planktonic larvae, effectively increasing propagule pressure and the likelihood of settlement of NNS larvae (Floerl and Inglis 2003). Reduced tidal flushing also

prevents the dispersal of pollutants and sediment, lowering water quality and increasing physical and chemical disturbance of fouling communities (Clark and Johnston 2005; Rivero et al. 2013). Water quality in fully enclosed marinas may be so low that recruitment success of native and non-native fouling species is reduced.

Pontoon length and seawall length represent the availability of hard infrastructure for colonisation by NNS but are also indirect measures of the size of marinas. Size of a marina and corresponding level of vessel activity have been shown to correspond to the likelihood of NNS arriving (Ricciardi 2006; Floerl et al. 2009; Nall et al. 2015). Responses from marina operators suggested the majority of fouling was observed attached to pontoon floats, in agreement with previous work showing a greater prevalence of NNS on floating as opposed to fixed structures (Dafforn et al. 2009b). Nall et al. (2015) also found a greater number of fouling NNS were found in harbours with floating structures. In the results of the GLM model examining marina features, pontoon length did explain some of the variability in the model but was not a significant factor on its own, possibly as a result of interaction with confounding factors such as vessel activity which may also contribute to likelihood of NNS arrival (Ricciardi 2006; Nall et al. 2015).

The physical features assessed in this study are by no means comprehensive, and since this analysis was carried out, other features influencing NNS establishment have been identified. Additional features of marinas identified as influencing NNS prevalence include: underlying habitat (sandy vs. rocky) (Airoldi et al. 2015), harbour type (recreational vs. fishing or commercial use) (López-Legentil et al. 2015), maintenance regime (Airoldi and Bulleri 2011) and levels of disturbance (Dafforn et al. 2009a; Crooks et al. 2011). Of the marinas used in this study, nearly all (93 %) of marina traffic was recreational, suggesting harbour type is unlikely to be a confounding factor in our analysis. Maintenance of marina infrastructure could constitute a disturbance to fouling communities and has been shown to favour NNS establishment (Airoldi and Bulleri 2011). High levels of disturbance are frequently associated with NNS establishment in both terrestrial and marine systems (Davis et al. 2000; Bulleri and Airoldi 2005; Britton-Simmons and Abbott 2008). Marinas frequently have higher levels of disturbance arising from high levels of vessel activity, changed water circulation and chemistry, and high levels of pollution and freshwater run-off (Arenas et al. 2006; Bax et al. 2002; Rivero et al. 2013). However, accurately characterising levels of disturbance requires much more detailed knowledge of the environmental dynamics and operational practices than was feasible for this study.

In addition, geographic distance between marinas was identified by López-Legentil et al. (2015) as significantly related to differences in ascidian communities in Catalan marinas, but was found to be non-significantly related to similarity between marinas in the USA at 1–200 km scales by Lord et al. (2015). The same study identified sea-surface temperature and proximity to areas with high volumes of cargo shipping as influencing variability in NNS richness at large scales (Lord et al. 2015). A study around San Francisco bay and nearby marinas showed smaller fishing and recreational vessels are important in connecting highly invaded ports with smaller marinas and are capable of transferring NNS between them (Zabin et al. 2014). The proximity of marinas in the present study with the highest numbers of NNS (10+) to areas with international shipping warrants further investigation to determine whether the same is true of UK vessels.

The present study focused specifically on fouling NNS and did not consider the relationship between chosen marina features and the native biofouling community, or interactions between native and NNS. Limitations in the availability of data characterising both native and NNS in each marina meant that this was not feasible for a study of this scale. The features identified in this study as influencing NNS, freshwater input and degree of enclosure, will also affect native species dynamics (Floerl and Inglis 2003; David et al. 2013). Characterisation of both native and NNS within marinas with different environmental and physical features is an important next step and may also allow for analysis of impact of NNS establishment.

The five most common NNS identified were all sessile epibenthic species with a relatively short planktonic phase in their life cycle. Recruitment of taxa for which larvae settle quickly has been found to be increased within marinas, while settlement of species with longer planktonic phases is reduced or absent (Rivero et al. 2013). Marina features most correlated with the presence of individual NNS differed across the five most common NNS. This suggests that individual life history characteristics and environmental tolerances may play an important role in predicting which species will establish at a particular location; however, identifying what these are for each individual species is likely to be a complex and time-consuming process. Instead, further analysis of the features identified in this study and others that affect general patterns of NNS settlement may allow for characterisation of the risk of NNS establishment in different marinas. Supplementary analysis of the features identified here and in other studies would allow for guidance to be developed on the features of marinas that can be manipulated to reduce invasion by marine NNS.

Current biosecurity practices and attitudes

Our survey of recreational sailors indicated that knowledge of NNS is not uncommon, with over 90 % stating that

they knew about NNS. Previous invasive species eradication programmes worldwide have relied on the assistance and awareness of stakeholders (Bax et al. 2002; Holt and Cordingley 2011). However, participants in the survey constitute only a small subset of the wider recreational boating community and the use of the forum to invite survey participants favoured responses from those actively interested in, or antagonistic to, issues of invasive species in marinas. The response of boat owners to proposed changes in boating behaviour demonstrates that there are still barriers, specifically those of cost and time, to the adoption of biosecurity methods. These need to be overcome if the role of recreational vessels as vectors of secondary spread is to be reduced.

Modifications to the design of existing marinas could aid in increasing uptake of current biosecurity recommendations. Novel solutions to reduce biofouling are being trialled, such as rotating pontoon floats (Holt and Cordingley 2011) and in-water encapsulation devices which could reduce costs of treatment to control NNS (Roche et al. 2015). Meanwhile, incorporation of adequate haul out space and methods to prevent removed biofouling from re-entering the water are some of the basic methods that should be considered in any marina development. Awareness of marina operators on the presence of NNS within their own marinas was low, with few respondents correctly identifying whether or not NNS were present. Additionally, just over a quarter of marina operators who responded to the question stated that they had BMPs for the cleaning of underwater structures. As these structures may represent significant reservoirs of NNS, this demonstrates a need for much clearer guidance for marina operators on biosecurity planning for the marina itself (Payne et al. 2014), not just the vessels that use it.

The development of marinas constitutes a significant change to the near-shore environment (Rivero et al. 2013) and results in a site with high potential for increased establishment of NNS. The high prevalence of NNS found within marinas suggests that incorporating biosecurity measures into marina design and operation might greatly reduce secondary spread of NNS by the recreational sailing sector (Cook et al. 2016). While there may be some associated costs with changes to project development processes and operation, over 40 % of sailors questioned indicated that they would preferentially use marinas which offered increased biosecurity. Furthermore, approaching biosecurity from a more holistic, ecosystem- and site-based perspective, in which marina designers, operators and users are actively engaged in marina design and management processes may help lessen the perception that recreational boaters are being overly tasked with the responsibility of preventing NNS movement.

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Compliance with ethical standards

Conflict of interest All authors declare they have no conflict of interest.

Animal rights No animals were used in the production of this paper.

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