The Influence of Marina Characteristics on Non-native Colonisation

A dissertation submitted in accordance with the requirements for the degree of Master of Science (MSc) in Marine Biology and submission to Aquatic Invasion Journal

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Abstract

Marinas are hot spots area for non-native species because the marina properties are likely to influence the distribution of non-natives. The different properties that impact NIS distribution include their closeness to the marina, salinity range, tidal fluctuations, depth, and ascidian presence, and so these elements influence the susceptibility to invasion. In marinas, transport vectors such as commercial shipping, recreational shipping, and aquaculture and fishery practices within enclosed marinas also have an important role in the distribution of non-native species from one region to another during the bioinvasion process. There has been an important increase in research studies on aquatic invasions over the last 20 years, but it can be difficult to specify the effects on non-native species distribution because the invasion process is affected by both environmental and biological factors. This can lead to a confusion when interpreting the data. Based on the field surveys and available literature, we conducted a study focusing on 10 non-native species of marine invertebrates in the UK. This study explains the distribution and ecology of five non-native species in Great Britain (*Styela clava* (Herdman, 1882), *Didemnum vexillum* (Kott, 2002), *Caprella mutica* (Schrin, 1935), *Crepidula fornicata* (Linnaeus, 1758), *Watersipora subtorquata* (d'Orbigny, 1852)) and the marina characteristics of five non-native species colonisation (*Austrominius modestus* (Darwin, 1854), *Ciona intestinalis* (Linnaeus, 1767), *Botrylloides violaceus* (Oka, 1927), *Tricellaria inopinata* (d'Hondt & Occhipinti Ambrogi, 1985), *Bugula neritina* (Linnaeus, 1758)) in Welsh coasts. The results showed that some of the variations in marina characteristics have affected the distribution of non-native species distribution. Variation among marinas in non-native species abundance, diversity and multivariate structure of the assemblages was very high. Within a marina, colonisation of tiles varied between locations at the entrance and within the marina but not in any consistent way. The effect of tile orientation was surprisingly low.

Keywords: non-native species, recreational boating, shipping, invasion, species diversity, marina characteristics, Welsh marinas.

Introduction

The distribution of non-native species

The identification of non-natives

Invasive non-native species (INNS) are species that have been introduced to an area outside their previous natural distribution and have an adverse environmental, economic or social impact on the new location (Corrales et al. 2020). In a marine environment, the rates of distribution of non-native species differ compared to the other species that are present, and this distribution happens rapidly. The non-natives are therefore generally called as 'invasive', and this term may also refer to a dominance (Eno 1997).

The invasion process

The stages of the invasion process include introduction, colonisation, and expansion phases. As the process mainly impacts non-native biodiversity, so it is referred to as bio-invasion (Corrales et al. 2020), and bioinvasion is determined according to the features of non-native species and the characteristics of the recipient community according to propagule pressure. The term 'propagule pressure' generally refers to the abundance or quantity of introduced species (propagule size) and the frequency that they arrive in the location (Ros et al. 2013). Invasive non-native species pass through all stages of the invasion process, from moving to a new environment to distribution after their establishment (Powell-Jennings and Callaway 2018).

According to Eno (1997), if a species successfully settles on a surface, it can then potentially invade the entire biotope(s), expanding to the full geographic range to which it accesses. In this case, an introduction success relies on the biological features of a species as well as the characteristics of the recipient environment; thus, the important factor for success is the presence of free niche availability (Chan and Briski 2017). However, a previous study by Carriker (1992) did not find that the biological characteristics of successful invaders (i.e. morphological, physiological, reproductive, genetic, behavioural) may be defined with any degree of reliability (Carlton et al. 1994). Instead, Carriker (1992) stated that an interaction between the physiological features of the species and the quality of the environment results in successful geographical dispersion (Carlton et al. 1994). For example, estuarine species only rarely invade oceanic habitats, and successful invasions in the opposite direction can be equally sparse. Yet successful invasion by estuarine species into other brackish waters is common, especially when they are at a similar latitude, as in the case of the most recent marine introductions to Britain (Carlton et al. 1994).

The effects of non-natives on the distribution of marine species

Non-native species pose a significant threat to the world's oceans (Willis et al. 2009) because they may have a negative effect on native species, through competition, predation or the transmission of viruses (Chan and Briski 2017). A non-native species can also affect the distribution of other non-native species because of a damaged ecosystem. In this case, a non-native species cannot spread around invasion areas, even though they are still considered as invaders (Orlando-Bonaca et al. 2019). For example, the introduction and distribution of invasive ascidians have attracted significant interest, especially over the last decade, because of both the widespread negative impacts on species and the ecological and economic harm caused to recipient ecosystems (Zhan et al. 2015). Once non-indigenous ascidians become established in new areas, they may overgrow and out-compete native species and ultimately become the dominant population. In this case, invasive ascidians diminish native species productivity and alter the ecology of invaded ecosystems (Zhan et al. 2015).

The extreme density of non-natives can have negative impacts on native and aquaculture species such as “cultured mussels”, through competition for space and food. For example, *Didemnum vexillum* (Kott, 2002), can replace mussels as the dominant species in fouling communities. This event is a major functional habitat change because mussels provide a year-round substrate for the settlement of other organisms (NEMESIS 2020); however, the ecological and economic impact of *D. vexillum* on the biology of sea scallops and fishing is yet unknown (CABI 2020). In addition, *D. vexillum* is a less dominant colonial ascidian and can grow on plates over established colonies of species (NEMESIS 2020). They grow on horizontal and vertical surfaces including intertidal, subtidal rocky surfaces and man-made substrates. The transport vectors for *D. vexillum* include broken leaves and by rafting in this way, *D. vexillum* can spread and colonise over long and short distances. These transportation events occur frequently, so these non-natives can lead to substantial effects on population dynamics (CABI 2020) and natural ecosystems by altering the local habitat (NEMESIS 2020).

The solitary ascidian *Styela clava* (Herdman, 1882), is a sessile filter feeder, an abundant species and often the dominant oyster fouling organisms in harbours. They are common on rocks and pylons and can reach a density of 500–1500 individuals per square meter (GISD 2020). Moreover, in English coasts, the growing population of *S. clava* is paralleled by a decrease in *Ciona intestinalis* because it excludes other organisms while also providing a secondary substrate for other fouling organisms (NEMESIS 2020).

Crepidula fornicata (Linnaeus, 1758), has been a very successful invader. It is a fouling organism with potentially important impacts on human structures and shellfish. They affect the growth of other bivalves by competing in a variety of ways as well as altering habitats in European waters (NEMESIS 2020). For example, attached limpets increase the hydrodynamic stress on mussels, and therefore the mussels shift energy resources to increase the production of byssus threads (NEMESIS 2020).

Watersipora subtorquata (d’Orbigny, 1852), can form very large colonies and have a considerable effect on pre-existing sessile communities through space occupancy, overgrowth interactions and their composition (GIS 2020). Their colonies provide non-toxic points of attachment for other organisms by allowing a diverse fouling community to develop which can adversely affect the speed and efficiency of ships. Their colonies often develop elevated leaf-like folds rising above the substrate and create additional space for colonisation by other organisms (NEMESIS 2020).

The ecological features of non-native species

<u>The characteristics of invaders in relation to invasion success</u>

The characteristics of invaders – fast growth rate, short generation times, high reproductive output, stress tolerance – serve an important role in determining the success of the invasion process in the area (Lenz et al. 2011). The adaptation of the species can influence their abundance and indicate the success of the invasion if they cannot successfully adapt to the new location due to their own characteristics (Osman and Whitlatch 2007).

Non-native species can distribute more widely on novel substrates than on adjacent natural surfaces in comparison to native species. A growth layer of some non-native species can serve to protect other non-natives from predation, showing that they are opportunistic species(Foster et al. 2016). Also, the invasion success of a non-native species in experimental fouling communities is dependent on the presence of large amounts of unoccupied space (CABI 2020). For example, the dense mats of *D. vexillum* are considered as physical barriers because the species influence the geochemical cycling of nutrients/elements and the circulation of dissolved oxygen, contributing to indirect shifts in benthic ecosystems (Zhan et al. 2015).

Compared with many marine species with long planktonic larval phases, the life history of ascidians provides a good opportunity to distinguish between artificial (passive) and natural (active) dispersal. Ascidians do not normally migrate far away, typically a few metres or less owing to their short-lived tadpole larvae (Zhan et al. 2015). These non-natives produce lecithrophic larvae that spend less than 24 hours in the water column, then settle on suitable habitats and metamorphose to adult colonies. This can allow local populations to build up and successfully occupy new habitats, and so become the dominant competitor (CABI 2020). The ascidian *S. clava* inhibits the settlement of co-occurring fouling sessile invertebrates through predation on planktonic larvae because of high filtering rates (NEMESIS 2020). It is not clear whether this causes the local extinction of many species. In marinas in the south-west of England, genetic differentiation is also detectable within some populations along 100-metre pontoons with very limited larval dispersal (GISD 2020). Also, ascidian species are mostly hermaphroditic, releasing both eggs and sperm for external fertilization, so they do not self-fertilize (GISD 2020).

Caprella mutica (Schrin, 1935), has a high reproductive capability at 4–20^{0}C. Their feeding methods include filtering small particles from the water, browsing on small filamentous algae, scraping tissue from large algae, scavenging, and predation. These feeding factors contribute to their invasion success (NEMESIS 2020). Lacking a free-swimming planktonic stage, *C. mutica* spends its entire life on the substrate surface. Consequently, these non-natives naturally have a limited dispersal potential, typically only a few kilometres per year, and do not have a planktonic larval stage. This characteristic is an impediment to natural global dispersal, supporting the theory that introductions are associated with human activity (GISD 2020), although the culture of Pacific Oysters (*Crassostrea gigas*) are likely vectors for the transport of *C. mutica* to different regions around the world (NEMESIS 2020).

Crepidula fornicata (Linnaeus, 1758), is well established on the southern coasts of England and Wales because their larvae can mostly move by themselves via water movement. Their larvae are also transported in ballast water when released into harbours or bays. Significantly, adults can attach to mobile species and ‘hitch’ a lift to new areas (GISD 2020). *C. fornicata* is also an unselective feeder and because of its occurrence near the marina bottom, often feeds on soft sediments (NEMESIS 2020).

Non-native species resistance to stress factors

The success of invasion in marine habitats is determined by the interaction between species-specific adaptations and site-specific environmental features (Lenz et al. 2011). Fluctuating habitats (i.e. intertidal habitats) are more likely to have successful invaders, as species growing in these environments are pre-adapted to variations in abiotic variables such as temperature, salinity, light intensity, and oxygen availability. Adaptation should be predestined by tolerating stressful situations during transport and after introduction to a new habitat (Lenz et al. 2011). Communities of non-native species are less stress-tolerant in their own area than in their introduced area. This difference can derive from another feature of successful invaders: the capacity to respond rapidly to new challenges (Lenz et al. 2011). As yet, however, it has not been possible to clarify how these factors on marine non-native species impact the primary mechanisms that enable new invasive species to become established (Foster et al. 2016).

Lenz et al. (2011) stated that non-native organisms do not migrate from volatile temperates to more secure subtropical/tropical environments. If a strong physiological tolerance is an important pre-adaptation for settlement in new habitats. Also, the variation between the non-native species and native species is not anticipated, but non-natives will be invasive elsewhere unless there are variations in stress resistance between the native and disruptive communities of the species (Lenz et al. 2011).

According to Lenz et al. (2011), a wide stress resistance of a species in its native range may suggest a good candidate for helping to identify characteristics of a possible invasive species. This may be predicted with organisms residing in highly fluctuating coastal environments, such as freshwater subtidal, intertidal and estuary. Such systems may be impacted by a variety of physical factors and related stressors: winds, hurricanes, tides, flooding, water movement, ice, strong sunlight and UV radiation (Lenz et al. 2011). These factors can change the salinity concentration in each marina, which in turn reduces the establishment of non-native species in areas with high freshwater input, as in the construction of new marinas such as near rivers (Foster et al. 2016). The abundance of non-native species declines when moving from upstream areas (lower salinity) to downstream areas (high salinity) in terms of physiological stress (Afonso et al. 2020). Higher survival rates under hypo-salinity, hypoxia or thermal stress suggest better tolerance. Changes in oxygen intake under hypoosmotic stress, however, requires further attention (Lenz et al. 2011). The frequency of the deviation from natural respiration through stress demonstrates the capacity of the body to endure hypo-salinity. The less severe the metabolic disturbance, the more the body can tolerate unfavourable circumstances and the lower the energetic debt (e.g. due to anaerobiosis) (Lenz et al. 2011).

Another influential factor is broad tolerance to temperature changes as well as to salinity or freshwater (Foster et al. 2016). Since the species have been exposed to cold temperatures for a long time, their growth rate is inhibited and this restricts vertical colonisation of the species in areas with upwelling currents (Kaldy et al. 2015). According to Remane (1934), this comparison may be related to the so-called “paradox of brackish water”, because the lowest abundance of species is in areas with a higher freshwater effect (Afonso et al. 2020). For example, Ascidians are usually known as stenohaline species that do not tolerate large variations in atmospheric salinity. They have a broader range of tolerance than many other tunicates (Lenz et al. 2011). *S. clava* has a high tolerance of the changing environmental conditions such as salinity and temperature, so these solitary ascidians have a widespread distribution in the English Channel via ship fouling (CABI 2020). The non-native *D. vexillum* has slightly less mortality under mild (7 units) and extreme (14 units) hypo-salinity than the native *Diplosoma listerianum* (Lenz et al. 2011).

C. mutica has sufficient tolerance and flexibility of habitat, feeding, and life history to colonise much of the world’s waters, regardless of temperature (NEMESIS 2020), while *W. subtorquata* tolerates copper and mercury in antifouling paint so they can colonise hulls (NEMESIS 2020). Furthermore, their hard, encrusting colonies are tolerant to water current (GISD 2020).

The characteristics of the recipient community and niche availability

Non-natives occupy significant structural and functional areas within the invaded ecosystems (Ojaveer et al. 2018). According to Zhan et al. (2015), once the ascidian species is established, it often spreads locally and regionally through 'stepping-stone' introductions associated with a variety of human-mediated vectors, including movement. Ascidians are therefore a rather unique system to study how and to what extent human-mediated versus natural dispersion contributes to the geographical distribution of invasive species in habitats (Zhan et al. 2015). Ascidians such as *S. clava* and *D. vexillum* are long-term dominant resident ascidians in many harbours along the New England coast, but they do not spread more open coastal sites since communities with low-diversity have fewer competitors for any newly-settled recruits and juvenile life-stages. This indicates that their invasion success has been limited by competitors (e.g. predators) (Osman and Whitlatch 2007).

The increased biofouling complexity in habitats can allow for the introduction of additional species because this complexity can offer suitable habitats, food and sheltered niche areas (Ulman et al. 2019). However, if there is not enough environmental niche availability for colonization and there is greater biological resistance in the form of predators and competitors, the non-native species establishment, secondary distribution and colonisation may not be possible (Afonso et al. 2020).

The role of transport vectors on non-native species distribution

Transport vectors play a significant role in the distribution of non-natives (NEMESIS 2020). It has been shown that invasions are caused largely by marine traffic, by the fouling species transport on vessel hulls and through ballast water released into the area (Ulman et al. 2019). Aquaculture facilities are also essential vectors for movements of non-natives. For example, *D. vexillum* was introduced in the Gulf of Maine when Pacific oysters (*Crassostrea gigas*) were transported for aquaculture purposes (Zhan et al. 2015). Recreational traffic is another important vector for NIS in bigger locations where human activity is more prevalent (Afonso et al. 2020).

For example, *S. clava* has a well-adapted morphology with great attachment strength and streamlined body for transport on the hulls. *S. clava* is native to the Northwest Pacific shores of Asia and Russia (NEMESIS 2020). This species has probably been introduced to Great Britain (GB) from Korea as well as the seaboards of North America, Australia, and New Zealand (GISD 2020). They were first located in 1920 in Beverly, Massachusetts and Portsmouth, New Hampshire, and can now be found throughout southern New England, USA. They were first recorded in England in 1953 in Plymouth (NEMESIS 2020).

D. vexillum is native to eastern Japan and first recorded in marina in North Wales in autumn 2008 (GISD 2020). The specific vectors for introduction are largely unknown, though international shipping, local boat traffic and transport of aquaculture species are likely sources (CABI 2020). *C. mutica* is native to the Northwest Pacific. These caprellids have been introduced to the East (Delaware-Newfoundland) and West Coasts (California-Alaska) of North America, Europe (from Spain to Norway and Germany), and New Zealand (NEMESIS 2020). Although *C. mutica* have established their populations in the North Sea, on the west coast of Scotland, in the Irish Sea and the English Channel (GISD 2020), they are now known to inhabit much of the coast of North-western Europe, from Le Havre, France (on the English

Channel) to Helgoland, Germany (on the North Sea), including the Channel Coast of England, and the east and west coasts of Scotland and Ireland (CABI 2020).

C. fornicata is native from Point Escuminac, Canada, and can be found along the East Coast of America, down to the Caribbean (GISD 2020). In the English Channel, *C. fornicata* has spread from east to west. In European waters, they were first reported in Liverpool Bay, England, in 1872, but did not persist there (NEMESIS 2020). They were transported into Europe along with American oysters (*Crassostrea virginica*) dredged from oyster beds of Atlantic estuaries (CABI 2020). At the end of the nineteenth century, these non-natives were accidentally introduced in Europe where they found suitable conditions to settle and develop: free surfaces of sandy, coarse sediment, an optimal water temperature range, abundant suspended organic matter as food and no predators. These factors allow to rapid growth and reproductive success (CABI 2020). They were first recorded in Essex between 1887 and 1890. Expansion of their range is a possible response to a warming climate (NEMESIS 2020).

W. subtorquata is an encrusting bryozoan widely distributed around the world. These non-natives have been introduced to the Northeast Pacific, much of the coast of Australia, New Zealand, and the Atlantic coast of France. They were first reported from Australia in 1950 in Sydney Harbour (NEMESIS 2020). Their native range has not been determined, but they are becoming common in various regions worldwide on cool temperate coasts. They are distributed to GB from introduced populations in France. *W. subtorquata* was first detected in 2008 in marinas in Plymouth (Devon) and Poole (Dorset) (GB species formerly identified as *W. subtorquata*, reidentified by Vieira et al., 2014) and their transport to GB is likely to have taken place by recreational craft (GISD 2020). Their appearances in France are related to the culture of *C. gigas*) (NEMESIS 2020).

Hull fouling & Ballast water

This transport vector incorporates small boat activities (Minchin et al. 2006) and one pathway may involve other secondary vectors (Ulman et al. 2019). For example, hull fouling is as yet an unmanaged vector in the UK, so it poses a key risk for the introduction and secondary distribution of non-native species (Arenas et al. 2006a). The risk of invasion differs with the global shipping trend of vessels and the various coastal environments (Jägerbrand et al. 2019) (**Figure 1**). For remote dispersal, the hulls and sea chests of ships represent major vectors, primarily for transportation of juvenile and/or adult ascidians. In general, ballast water is not understood to be the key vector for remote dispersion, primarily owing to the limited survival period of free-swimming larvae, but the soil, ground and/or internal surfaces within ballast tanks can distribute harbour ascidians (Zhan et al. 2015). Therefore, over a period of three decades, the ballast water effect (via commercial shipping) has become widely understood to be a key vector of species introductions to coastal ecosystems. Despite enhanced global attempts to reduce the risk of ballast water-mediated invasions, ballast water remains a potent vector of non-native aquatic species introductions. This is particularly the case with intracoastal vessel traffic, whose features may minimise the viability and effectiveness of management of NIS by ballast water exchange (Darling et al. 2018).

Figure 1: Attachment of micro-organisms, including larvae of invertebrates such as ascidians, serpulids and barnacles through vessel hull fouling (Dafforn et al. 2011).

Marina habitats are affected by shipping and numerous other anthropogenic factors, such as air and land travel, manufacturing and climate change (Jägerbrand et al. 2019) (**Figure 2**). In the literature, the environmental effects of shipping on marine and nearshore ecosystems are categorised into three major groups: water discharges, physical effects and air pollution (Jägerbrand et al. 2019).

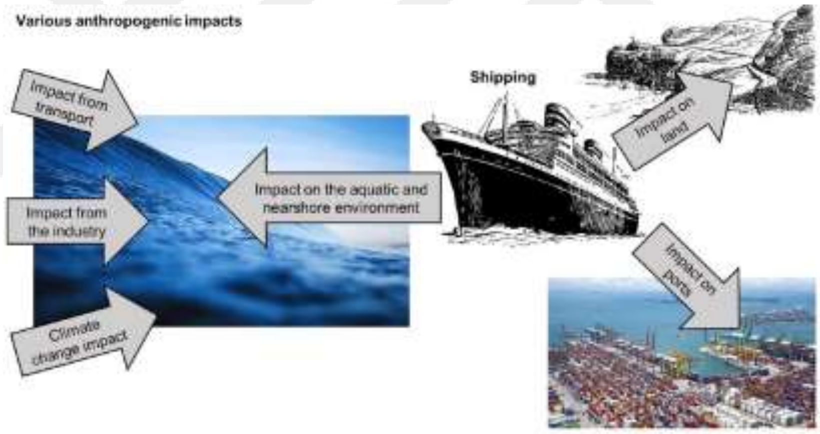


Figure 2. Overview of the environmental impact on the aquatic and nearshore environment, on land, and ports from shipping and other contributing anthropogenic impacts from, for example, the transport sector, the industrial sector, and climate change (Jägerbrand et al. 2019).

Marine vessels produce waves and tides that cause physical effects in marine ecosystem. Previous studies have revealed that ship waves are of differing height and length than the waves normally caused by wind, so can often cause vertical mixing and transfer of nutrients, and an intense process of eutrophication, potentially generating remote impacts. Wave energy also represents a physical threat for "oyster reefs", as well as for benthic invertebrates nesting in the shoreline area (Jägerbrand et al. 2019). The ballast water is used to stabilise vessels on the sea and contains suspended matter that can establish as sediment within the ballast tanks. The quantity of the sediment load becomes greater if the ballast water is loaded in shallow waters, rivers and estuaries. These physical factors mean that when a species is released into a new area, it does not automatically establish itself as a viable community (Jägerbrand et al. 2019).

Also, it is important that the sediments are removed from the ballast tanks, but this process interferes with the ship's activities (Maglić et al. 2019).

Ballast water exchange (BWE) is the most reliable method of reducing the abundance and species richness being transmitted from freshwater and low salinity ports, because of the large impact of the osmotic shock on residual freshwater coastal biota when exposed to the high salinity of open ocean water during and after the exchange (Darling et al. 2018). Limitations on the effectiveness of BWE can be exacerbated in the case of intracoastal vessel traffic, where regulations frequently permit the movement of unexchanged ballast, greatly raising the invasion risk by these voyages. This danger could be heightened in the future by increasing levels of ship traffic and warming patterns at higher latitudes, especially for dispersal-limited species (Darling et al. 2018).

Recreational boating

While recreational boats may not be a significant vector for the dispersal of non-native species over great distances, they may play an important role in successful introductions on a local scale (Ros et al. 2013). This means of non-native distribution can be successful due to short and relatively slow voyages undertaken by recreational craft (Foster et al. 2016), although recreational vessels are usually considered low-risk vectors for the same reason (Dafforn et al. 2009). They are connected with highly invaded systems as they occupy smaller marinas for non-natives distribution (Foster et al. 2016). These areas allow for the occurrence of introductory vectors such as marine ports (fouling and ballast water), marinas and aquaculture facilities in recreational areas (Afonso et al. 2020).

Crucially, Roy et al. (2012) state that non-native species occurring in Europe have begun to appear in high numbers since the 1700s, as a result of the rise in human migration. The variations in non-native species living in different locations depend on an increase in long-distance travel (Roy et al. 2012). In commercial shipping the expulsion of ship ballast water/sediments and hull fouling occur because ship travel is increasingly faster, leading to an increase in the survival rate of the species in ballast tanks (Ulman et al. 2019b). In the ballast tank, ballast water causes a significant osmotic shock impact owing to the exposure of high salinity ocean water, so some species remain in the ballast water tanks. This process is called Ballast Water Exchange (BWE) and is greater when connected with transit between marine ports (Gray et al. 2007). In addition, recreational shipping has important effects via hull-fouling, whereas aquaculture and fishery practices have a role in culture of species and transfer of material (Ojaveer et al. 2018).

Marina features as hotspots for non-native species distribution

With their sheltered habitat and high propagule pressure, marinas have become 'hotspots' for the distribution of non-natives (Ulman et al. 2019). Marinas are called as 'secured islands' for underwater non-native species and provide an entry-point for non-natives via recreational yachts or by means of a network of appropriate ecosystems (Ashton 2006). Marinas have diverse and complicated natural ecosystems, as well as offering a strong point of connectivity among marine systems. These systems have hydrographic boundaries at greater spatial scales than terrestrial ecosystems, and therefore the distribution of marine species is harder to evaluate than in coastal environments (Ros et al. 2013). In this case, the characteristics of marinas can be used to identify the influence of NIS prevalence (Foster et al. 2016).

Non-natural benthic surfaces (known as artificial substrates) such as floating docks, pilings and boat hulls have been identified as places for the first arrival and establishment of many introduced marine species, as distributed by anthropogenic vectors (Lambert 2019). For example, in all parts of its native and introduced range, *S. clava* is more frequently reported on anthropogenic structures than natural surfaces (NEMESIS 2020). Hard-bottom communities, especially within artificial hard substrates such as docks and pilings, allow to the colonisation of non-natives within bays and estuaries (Ros et al. 2013). Dock floats are especially favoured habitats because their motion provides rapid water exchange, and a fresh supply of food-laden water. Other colonised man-made structures include pilings, piers, aquaculture structures, and boat hulls. Since colonial ascidians such as *D. vexillum* produce lecithotrophic larvae that have an abbreviated planktonic stage, the likelihood of larvae surviving in ballast water is very low. Thus, the most likely vectors for transport are hull fouling, aquaculture or rafting (CABI 2020). Artificial structures are considered in all facets of marinas. In particular, non-natives in enclosed habitats are correlated with the artificial hard substrate, especially with fouling communities in harbours and marinas (Ulman et al. 2019).

The number of berths contributes to the higher NIS quantity as they increase vessel traffic in a marina (Ulman et al. 2019). Larger marina sizes also impact NIS distribution through transport vectors, as marina length becomes one of the most effective factors for NIS distribution in artificial environments over natural ecosystems (Orlando-Bonaca et al. 2019). Additionally, in marinas, the presence of floating pontoons has a significant role because NIS has larger distribution in shallower areas than in their deeper equivalents owing to their distance from the seafloor (Ulman et al. 2019). Pontoon length represents the availability of a hard infrastructure for NIS colonisation, but is also an indirect measurement of the size of marinas (Dafforn et al. 2009).

The primary aim of the study is to research the ecology and distribution of important non-natives in the UK and show how marina characteristics affect the diversity of non-native species in Welsh coasts. Key factors in this study are therefore marina identity; position within marina; orientation of tile; and the period for colonisation. This study evaluates each of these factors in order to analyse how they affect non-native species diversity and abundance. The study aim will be tested using the following hypotheses:

- Non-native species diversity increases with marina size;
- Non-native species diversity is greater within enclosed areas of the marina compared to marina entrances;
- Non-native species diversity is greater on horizontally oriented surfaces than vertical.

Materials and methods

Study area

The study was conducted on eight marinas in Wales: Pwllheli (Pwll), Holyhead (HH), Burry Port (BP), Deganwy (DG) and Milford Haven (MH), Swansea (SW), Neyland (NY), and Victoria Dock (VD) in August and September 2009 **(Figure 3).**

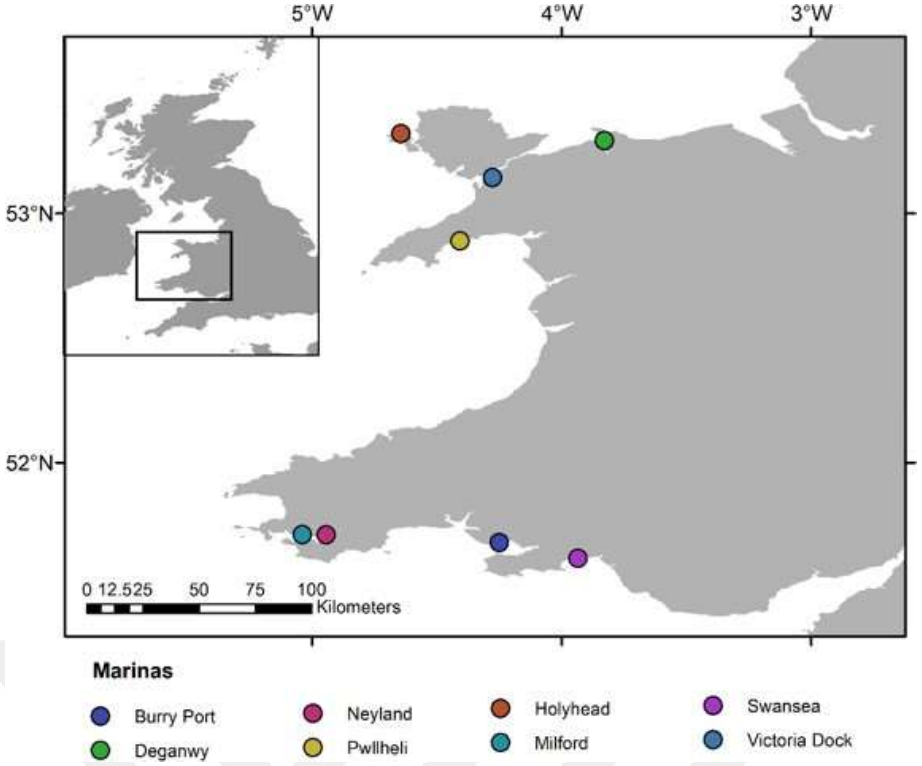


Figure 3. The map showing locations of marinas surveyed for non-native species in August and September 2009 (DiGSBS250K 2011).

Implementation of design

The study design consists of an ecological field experiment to assess how marina characteristics in eight marinas affect non-native colonisation. The study site has deployed settlement tiles at two positions in each marina: the inner tile, which is well within the marina and the outer tile at the marina entrance. An equal number of vertical and horizontal tiles was deployed at each site with half sampled at two weeks (very early colonisation) and half sampled at eight weeks (later colonisation) (**Figure 4**)

Figure 4. The produced diagram shows samplings for two weeks and eight weeks in eight marinas on the Welsh coast during the survey (N= panel numbers).

In this study, five panels were spaced in and outside each marina, with 60 samplings taken, totalling 300 samplings across all sites. The settlement tiles, which were made from black ‘Correx’ (approximately 4mm wide) (**Figure 5**) to provide a vertical and horizontal component, were deployed at a two-meter depth (**Figure 6**) using thin nylon cord tied to marina pontoons and weighted with a small fishing weight. After the tiles were removed, at two weeks and eight weeks, they were scored in the laboratory under a dissecting microscope using a grid to aid the determination of % cover (in colonial organisms) or the number of organisms (for solitary organisms). All recognisable organisms were identified to the lowest possible resolution. After identification, the organisms were characterised to taxa.

Figure 5. Black ‘Correx’ (approximately 4mm wide) and the photo of eight-week panel taken in the survey area.

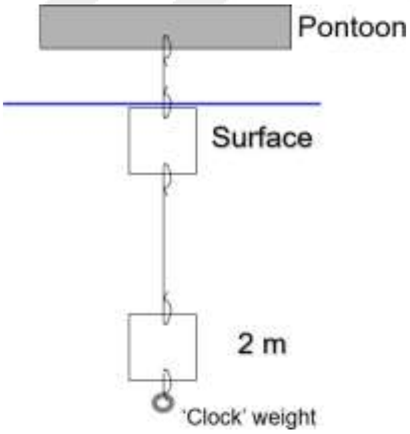


Figure 6. The photo showing the depth level (two-meter) for both colonisation period in the survey area.

Data analysis

The analysis was run separately for two-week data (very early colonisation) and eight-week data (later colonisation). Firstly, univariate analysis was conducted by using a combination of Excel and R studio to determine the effects of each marina position (inner, outer) and orientation (vertical, horizontal) among marinas on univariate response variables such as community diversity (Simpson's diversity index ($1-\lambda$) and Margalef species richness index (d)), native species and abundance of non-native *Austrominius modestus* (Darwin, 1854), *Ciona* *intestinalis* (Linnaeus, 1767), *Botrylloides violaceus* (Oka, 1927), *Bugula neritina* (Linnaeus, 1758), *Tricellaria inopinata* (d'Hondt & Occhipinti Ambrogi, 1985).

Secondly, raw data (late colonisation) was used and three-way mixed-model ANOVA which is fully factorial was run to examine an interaction effect between three independent variables (marina, position (inner/outer), orientation (vertical/horizontal)) on non-native species diversity, richness and abundance by calculating variance homogeneity. This allows for the application of a crossed model with marina, position in marina and orientation. Cochran's test was used to determine homogeneity (P-value<0.05=heterogeneity or P-value>0.05 =homogeneity). Then data was transformed to log-transformed data. If the result is not statistically significant, it is not necessary to follow up our results with a post hoc analysis. If there is a statistically significant result, a post hoc analysis was conducted.

Finally, a multivariate analysis was conducted. The biological similarity matrix (Bray-Curtis) was calculated using square root-transformed abundance data, and PERMANOVA Analysis (PERmutational Multivariate ANalysis Of VAriance) conducted using the same three-way model described above. Following PERMANOVA analysis, multi-dimension scaling (MDS) plots were created to explore significant interactions in each marina.

Results

Non-native species composition

In the survey, there were six species of solitary ascidian, eight species of colonial ascidians, two species of Mollusca, five species of worms, 13 species of Bryozoan, three species of Barnacle, four species of Hydroids, four species of Sponges, one species of Shrimp, one species of Algae and egg mass. The most abundant non-native species were *A. modestus* Darwin, 1854 (~ 70% of total abundance), followed by 26% of *C. intestinalis* Linnaeus, 1767, 0.64% of *T. inopinata* d'Hondt & Occhipinti Ambrogi, 1985, 0.3% of *B. violaceus* Oka, 1927 and 0.1% of *B. neritina* Linnaeus, 1758.

Diversity indices of benthic communities; Simpson's index (1-λ) and Margalef species richness index (d) in each marina; the results of Univariate analysis and Three-way mixed factorial model ANOVA have been shown respectively (**Figures 7,8, Table 1).** According to Cochran's test results, the data had heterogeneous variance (P<0.05). After log-transforming, Cochran's test showed no heterogeneity (P>0.05). There was a significant difference in Simpson diversity index among marinas (F (7,128) =17,34, P-value=0.0000), and significant interaction between marina and position (F (7,128) =3.63, P-value=0.0013). This means the effect of position varies among marinas. Investigating further using a Student–Newman–Keuls (SNK) post hoc test, there was no consistency in the difference between inner and outer in Simpson diversity (**Table 1**).

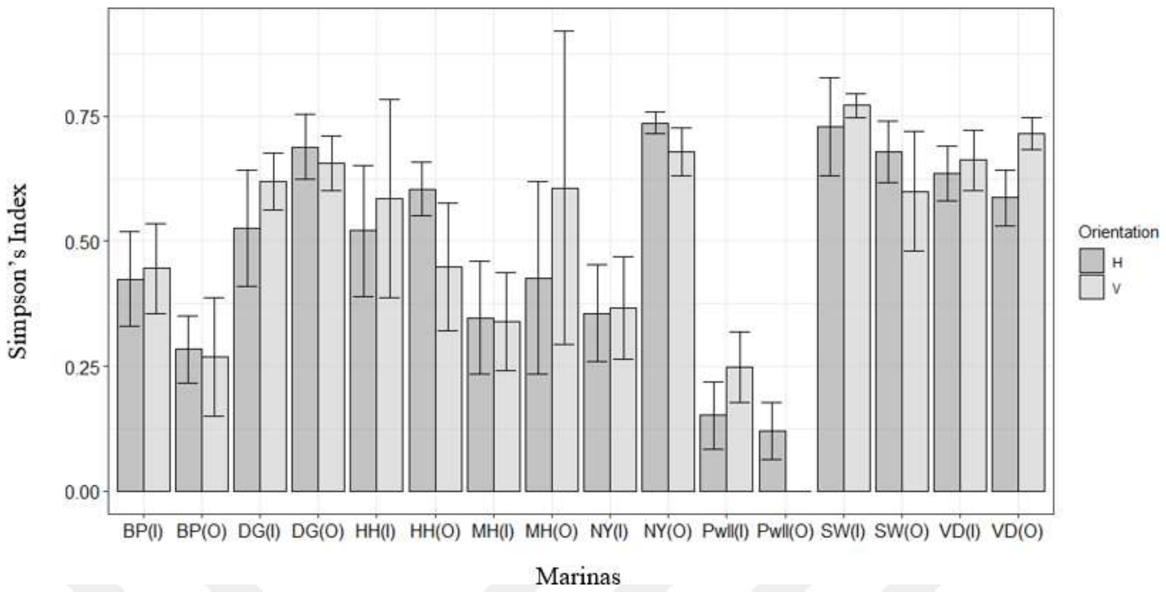


Figure 7. Simpson diversity in eight marinas (BP: Burry Port, DG: Deganwy, HH: Holyhead, MH: Milford Haven, NY: Neyland, Pwll: Pwlheli, SW: Swansea, VD: Victoria Dock) in two positions ((I): Inner,(O): outer) through two orientation tiles (H: horizontal, V: Vertical) in the survey area.

Table 1. The results of Three-way Mixed Model ANOVA showing differences in Simpson diversity among responsible variables (level of significance P-value < 0.05).

Three-way Mixed Model ANOVA
Analysis of Variance and Covariance

Source	df	Mean Squares(MS)	F-value	P-value
Marina	7	0.3592	17.34	0.0000
Position	1	0.0038	0.05	0.8285
Orientation	1	0.0021	0.35	0.5723
Marina*Position	7	0.0752	3.63	0.0013
Marina*Orientation	7	0.0059	0.28	0.9591
Position*Orientation	1	0.0273	1.93	0.2075
Marina*Position*Orientation	7	0.0142	0.68	0.6853
RES	128	0.0207		
TOTAL	159			

For species richness, according to Cochran's test results, the data had homogeneous variance (P>0.05). After log-transforming, the result of Cochran's test did not change. There was a significant difference in species richness among marinas (F(7,128) =32.49,P-value=0.0000), and a significant interaction between marina and position (F(7, 128) =7.35, P-value=0.0000). This means the effect of position varies among marinas. Investigating further using an SNK post hoc test, there was no consistency in the difference between inner and outer in species richness (**Table 2**).

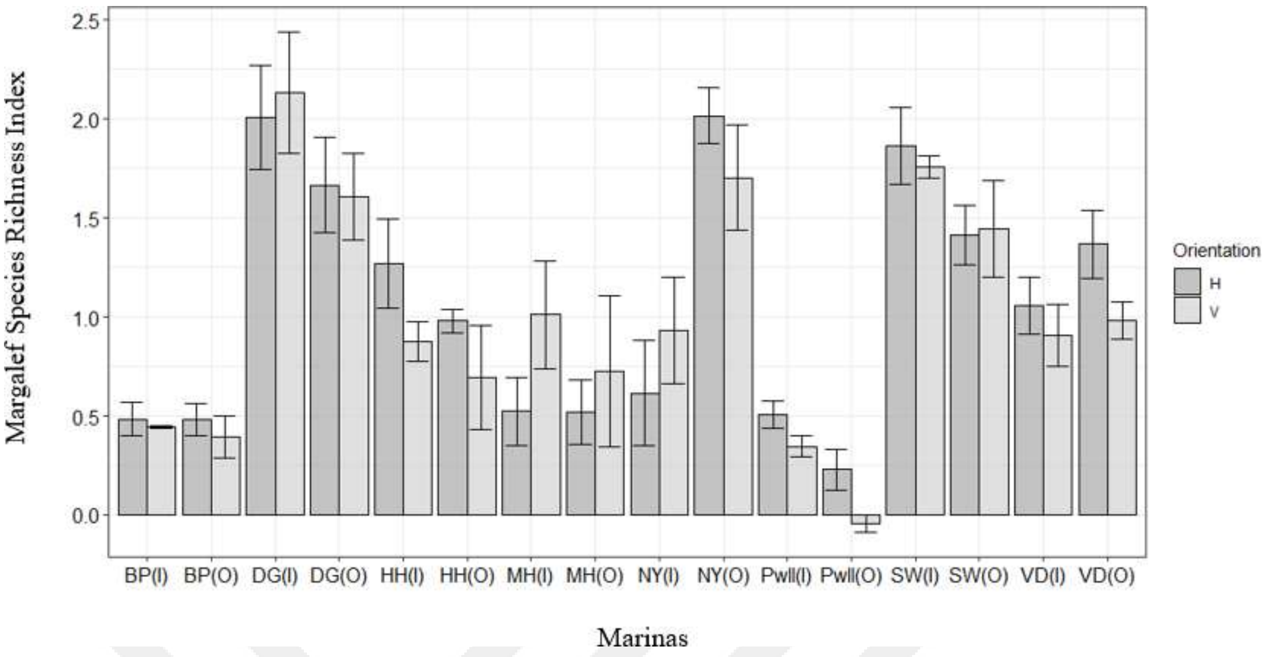


Figure 8. Species richness in eight marinas (BP: Burry Port, DG: Deganwy, HH: Holyhead, MH: Milford Haven, NY: Neyland, Pwll: Pwlheli, SW: Swansea, VD: Victoria Dock) in two positions ((I): Inner,(O): outer) through two orientation tiles (H: horizontal, V: Vertical) in the survey area.

Table 2. The results of Three-way Mixed Model ANOVA showing differences in species richness among responsible variables (level of significance P-value < 0.05).

Three-way Mixed Model ANOVA
Analysis of Variance and Covariance

Source	df	Mean Squares (MS)	F-value	P-value
Marina	7	104.4679	32.49	0.0000
Position	1	0.9000	0.04	0.8508
Orientation	1	3.6000	0.85	0.3876
Marina*Position	7	23.6286	7.35	0.0000
Marina*Orientation	7	4.2429	1.32	0.2462
Position*Orientation	1	4.2250	2.62	0.1494
Marina*Position*Orientation	7	1.6107	0.50	0.8324
RES	128	3.2156		
TOTAL	159			

The univariate test for the selected five discriminating species showed there was a significant difference in the abundance of these non-natives (P-value <0.05) according to marina position in orientation for both very early colonisation and later colonisation. The very early colonisation of *A. modestus* Darwin, 1854 was more vertical within HH marina, whereas there is more later colonisation of *A. modestus* in vertical orientation in enclosed NY marina. This means that as colonisation period increases, *A. modestus* has more preferred to vertically distribute in enclosed HH marinas (**Figures 9A, 9B**).

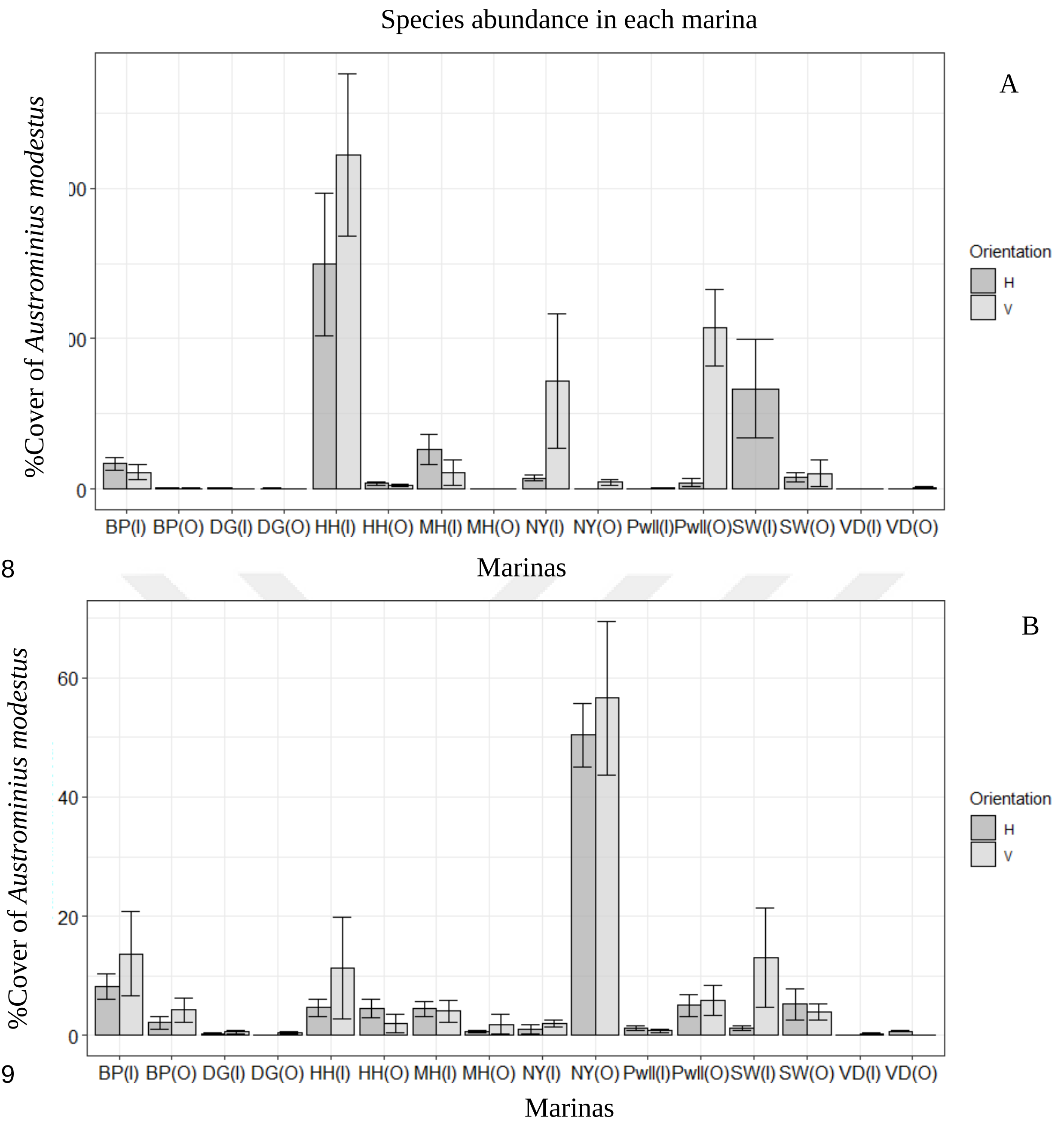


Figure 9. The percentage cover of *Austrominius modestus* abundance collected from eight marinas in two-meter depth (BP: BurryPort, DG: Deganwy, HH: Holyhead, MH: Milford Haven, NY: Neyland, Pwll: Pwlheli, SW: Swansea, VD: Victoria Dock) in two positions ((I): Inner,(O): outer) through two orientation tiles (H: horizontal, V: Vertical) at the end of two weeks (A) and eight weeks (B).

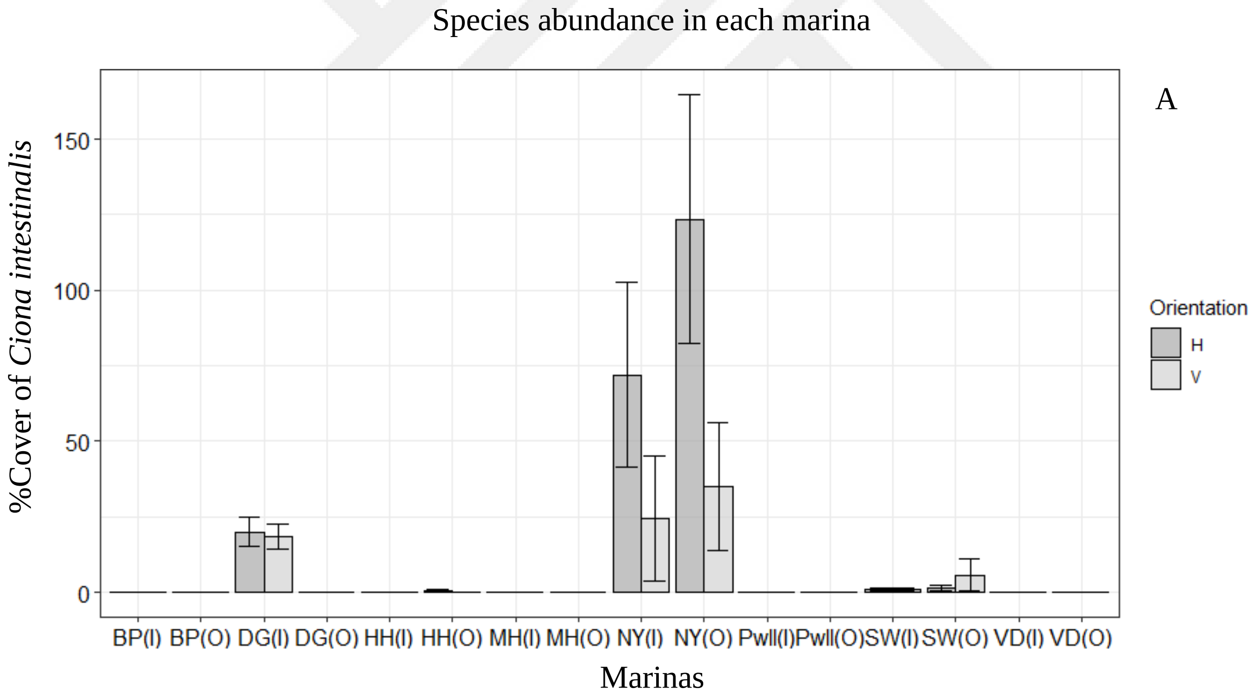
According to Cochran’s test results, the eight-week data had heterogeneous variance (P<0.05). After log-transforming, Cochran’s test showed no heterogeneity (P>0.05). This shows a significant difference in the abundance of *A. modestus* among marinas (F(7,128) =23.50,P-value=0.0000), and a significant interaction between marina and position (F(7,128) =21.10,P-value=0.0000). Therefore, the effect of position varies among marinas, but there was no consistency in the difference between inner and outer in the abundance of *A. modestus* (**Figure 9B**, **Table 3**).

Table 3. The results of Three-way Mixed Model ANOVA showing differences in *Austrominius modestus* abundance among responsible variables (level of significance P-value < 0.05).

Three-way Mixed Model ANOVA
Analysis of Variance and Covariance

Source	df	Mean Squares (MS)	F-value	P-value
Marina	7	10.7054	23.50	0.0000
Position	1	1.7871	0.19	0.6793
Orientation	1	1.1082	2.50	0.1577
Marina*Position	7	9.6149	21.10	0.0000
Marina*Orientation	7	0.4428	0.97	0.4547
Position*Orientation	1	1.1314	1.69	0.2348
Marina*Position*Orientation	7	0.6695	1.47	0.1838
RES	128	0.4556		
TOTAL	159			

In the very early colonisation, *C. intestinalis* Linnaeus, 1767 has more abundance in horizontal orientation in enclosed NY marina. The abundance of *C. intestinalis* decreased both within and enclosed NY marina in horizontal orientation in later colonisation, while the distribution of *C. intestinalis* increased vertically in enclosed DG marina in the later colonisation. This means that as colonisation period increases, these non-natives have more preferred to distribute vertically enclosed DG marina (**Figures 10A, 10B**).



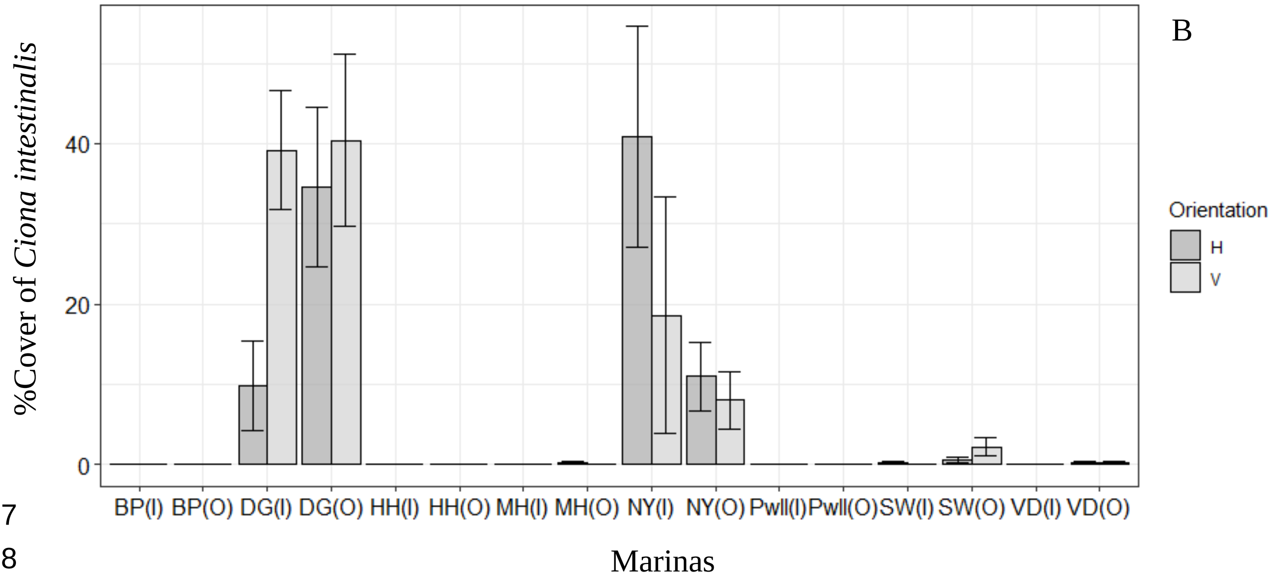


Figure 10. The percentage cover of *Ciona intestinalis* abundance collected from eight marinas in two-meter depth (BP: Burry Port, DG: Deganwy, HH: Holyhead, MH: Milford Haven, NY: Neyland, Pwll: Pwlheli, SW: Swansea, VD: Victoria Dock) in two positions ((I): Inner,(O): outer) through two orientation tiles (H: horizontal, V: Vertical) at the end of two-weeks (A) and eight-weeks (B).

According to Cochran's test results, the eight-week data had heterogeneous variance (P>0.05). After log-transforming, the result of Cochran's test did not change. This shows a significant difference in the abundance of *C. intestinalis* among marinas (F(7,128)=32.30,P-value=0.0000). There was also a significant interaction between marina and position as well as marina and orientation (F(7,128) =2.76,P-value=0.0286),(F(7,128) =0.97,P-value=0.0105). Therefore, the effect of position and orientation varies among marinas. SNK exploration of these interactions shows that there were some differences between positions, but the direction of difference was not consistent (**Figure 10B, Table 4**).

Table 4. The results of Three-way Mixed Model ANOVA showing differences in *Ciona intestinalis* abundance among responsible variables (level of significance P-value < 0.05).

Three-way Mixed Model ANOVA
Analysis of Variance and Covariance

Source	df	Mean Squares(MS)	F-value	P-value
Marina	7	2978.0023	39.30	0.0000
Position	1	4.1924	0.02	0.8819
Orientation	1	1.7060	2.33	0.9306
Marina*Position	7	176.4683	2.76	0.0286
Marina*Orientation	7	209.3304	0.97	0.0105
Position*Orientation	1	6.0000	0.05	0.8312
Marina*Position*Orientation	7	122.5614	1.62	0.1361
RES	128	75.7792		
TOTAL	159			

In the very early colonisation, *B. violaceus* Oka, 1927 has more abundant in enclosed DG marina in horizontal orientation, whereas the abundance of *B. violaceus* increased in horizontal orientation within SW marina in the later colonisation period. Thus, as the colonisation period increases, *B. violaceus* has preferred to horizontally distribute within SW marina (**Figures 11A, 11B**).

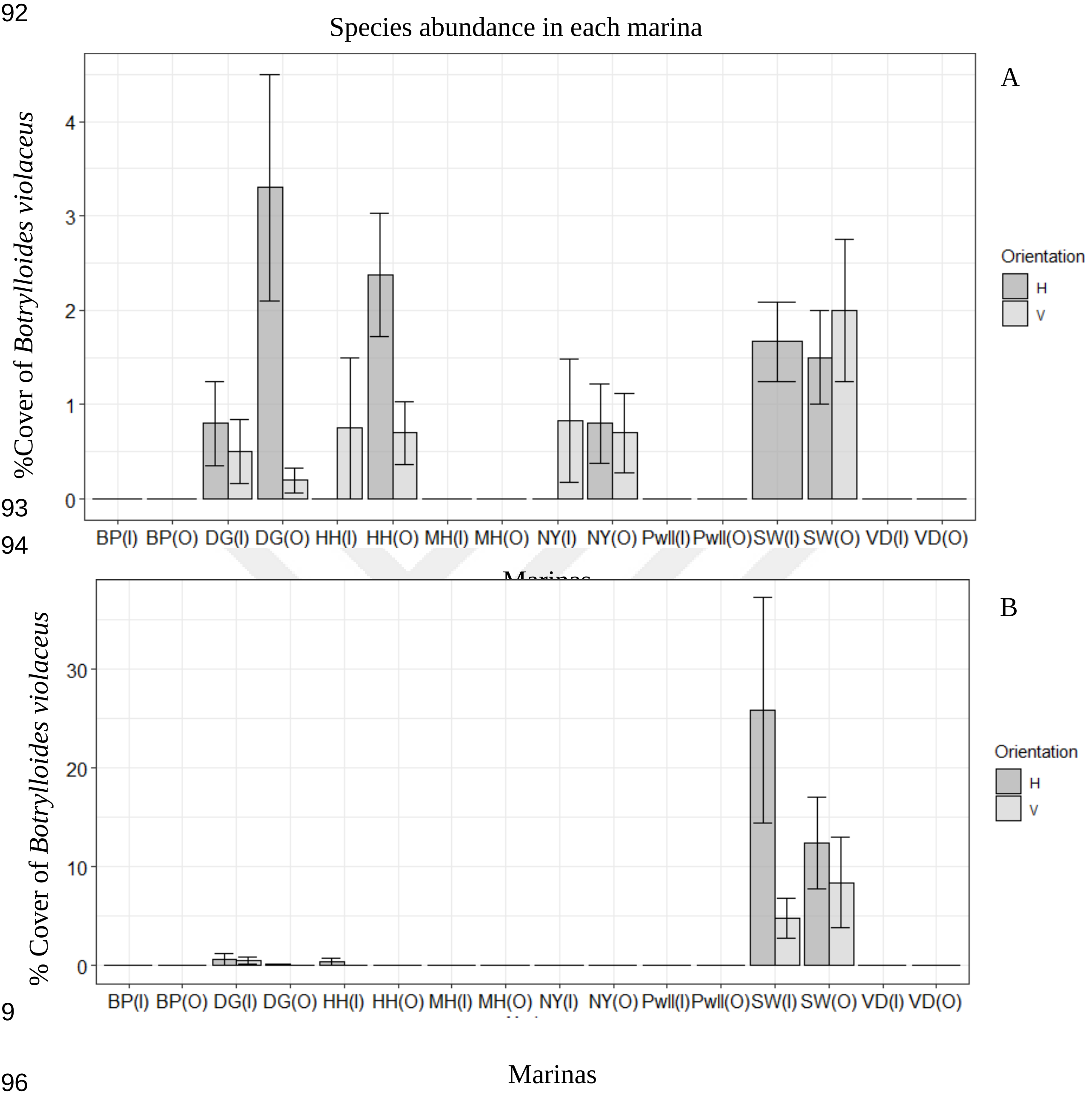


Figure 11. The percentage cover of *Botrylloides violaceus* abundance collected from eight marinas in two-meter depth (BP: BurryPort, DG: Deganwy, HH: Holyhead, MH: Milford Haven, NY: Neyland, Pwll: Pwlheli, SW: Swansea, VD: VictoriaDock) in two positions ((I): Inner,(O): outer) through two orientation tiles (H: horizontal, V: Vertical) at the end of two weeks (A) and eight weeks (B).

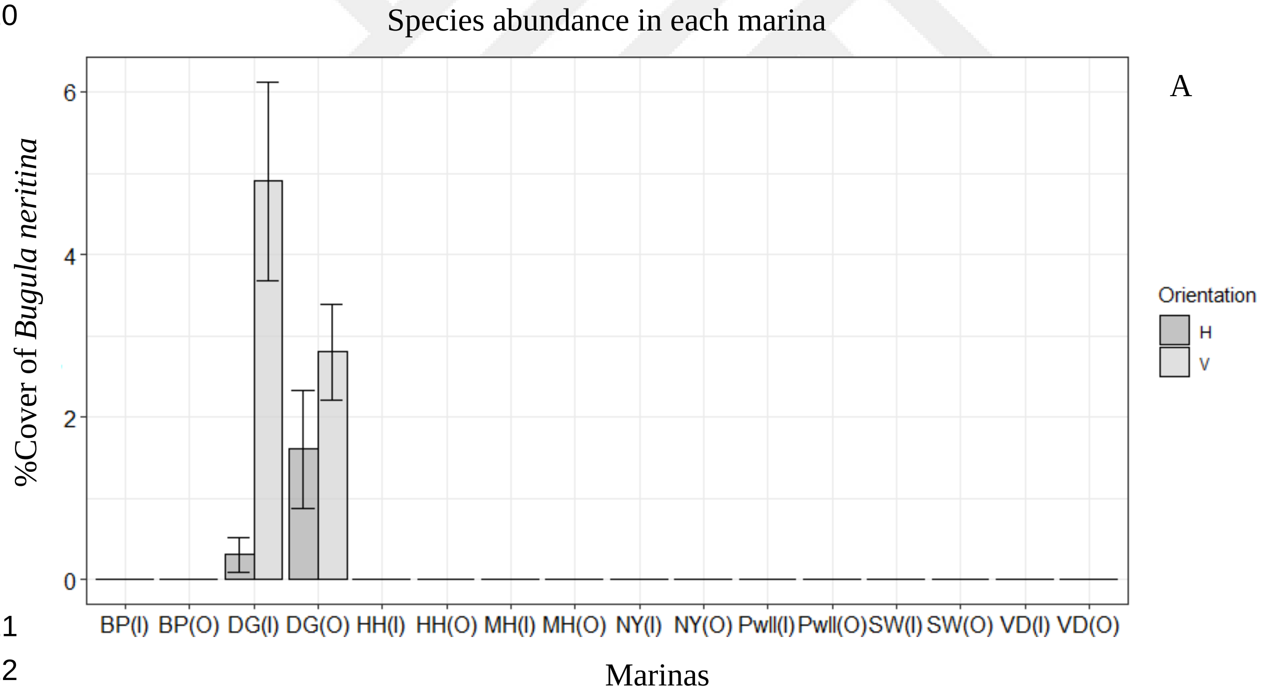
According to Cochran's test results, the eight-week data had heterogeneous variance (P>0.05). After log-transforming, the result of Cochran's test did not change. This shows a significant difference in the abundance of *B. violaceus* among marinas (F(7,128) =34.79, P-value=0.0000). There was also a significant interaction between marina and orientation (F(7,128) =3.97, P-value=0.0006), meaning the effect of orientation varies among marinas. SNK exploration of this interaction shows that at the only marina (SW) where *B. violaceus* was abundant, there was significantly more *B. violaceus* on horizontal surfaces than vertical (**Figure 11B, Table 5**).

Table 5. The results of Three-way Mixed Model ANOVA showing differences in *Botrylloides violaceus* abundance among responsible variables (level of significance P-value < 0.05).

Three-way Mixed Model ANOVA
Analysis of Variance and Covariance

Source	df	Mean Squares(MS)	F-value	P-value
Marina	7	753.8307	34.79	0.0000
Position	1	33.2952	2.44	0.1626
Orientation	1	103.2190	1.20	0.3098
Marina*Position	7	13.6703	0.63	0.7297
Marina*Orientation	7	86.1147	3.97	0.0006
Position*Orientation	1	26.6753	1.04	0.3423
Marina*Position*Orientation	7	25.7125	1.19	0.3151
RES	128	21.6704		
TOTAL	159			

In the early colonisation, *B. neritina* Linnaeus, 1758 is the only species within DG marina in vertical orientation, whereas the abundance of *B. neritina* increased in horizontal orientation in enclosed DG marina in the later colonisation period. This means that as the colonisation period increased, *B. neritina* preferred to horizontally spread in enclosed DG marina (**Figures 12A, 12B**).



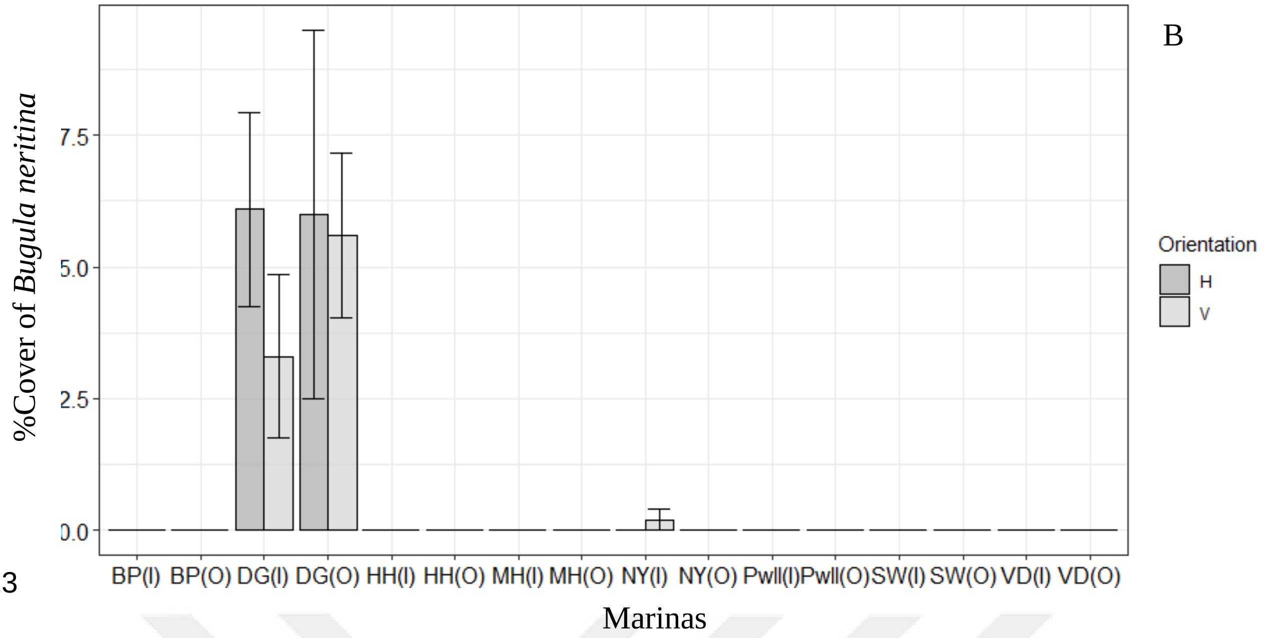


Figure 12. The percentage cover of *Bugula neritina* abundance collected from eight marinas in two-meter depth (BP: BurryPort, DG: Deganwy, HH: Holyhead, MH: Milford Haven, NY: Neyland, Pwll: Pwlheli, SW: Swansea, VD: Victoria Dock) in two positions ((I): Inner,(O): outer) through two orientation tiles (H: horizontal, V: Vertical) at the end of two weeks (A) and eight weeks (B).

According to Cochran's test results, the eight-week data had heterogeneous variance (P>0.05). After log-transforming, the result of Cochran's test did not change. This shows a significant difference in the abundance of *B. neritina* among marinas (F(7,128)=73.51,P-value=0.0000), but there was no significant interaction between marina and orientation or marina and position. This means the effects of orientation and position do not vary among marinas. However, SNK exploration of this interaction shows the only marina (DG) where *B.neritina* was abundant on vertical orientation rather than horizontal (**Figure 12B, Table 6**).

Table 6. The results of Three-way Mixed Model ANOVA showing differences in *Bugula neritina* abundance among responsible variables (level of significance P-value < 0.05).

Three-way Mixed Model ANOVA
Analysis of Variance and Covariance

Source	df	Mean Squares(MS)	F-value	P-value
Marina	7	361.0389	73.51	0.0000
Position	1	0.6725	0.34	0.5796
Orientation	1	0.8140	0.37	0.8972
Marina*Position	7	1.9935	0.41	0.7297
Marina*Orientation	7	2.2203	0.45	0.8672
Position*Orientation	1	1.9203	0.50	0.5017
Marina*Position*Orientation	7	3.8280	0.78	0.6057
RES	128	4.9114		
TOTAL	159			

T. inopinata d'Hondt & Occhipinti Ambrogi, 1985 has more abundance in horizontal orientation in enclosed DG marina for both colonisation periods. As colonisation period increases, the distribution of *T. inopinata* has horizontally increased in enclosed NY marina and vertically in enclosed SW marina although their abundance has decreased both within and enclosed other marinas. Even so, these non-native species have preferred to vertically distribute outside marinas (**Figures 13A, 13B**).

Species abundance in each marina

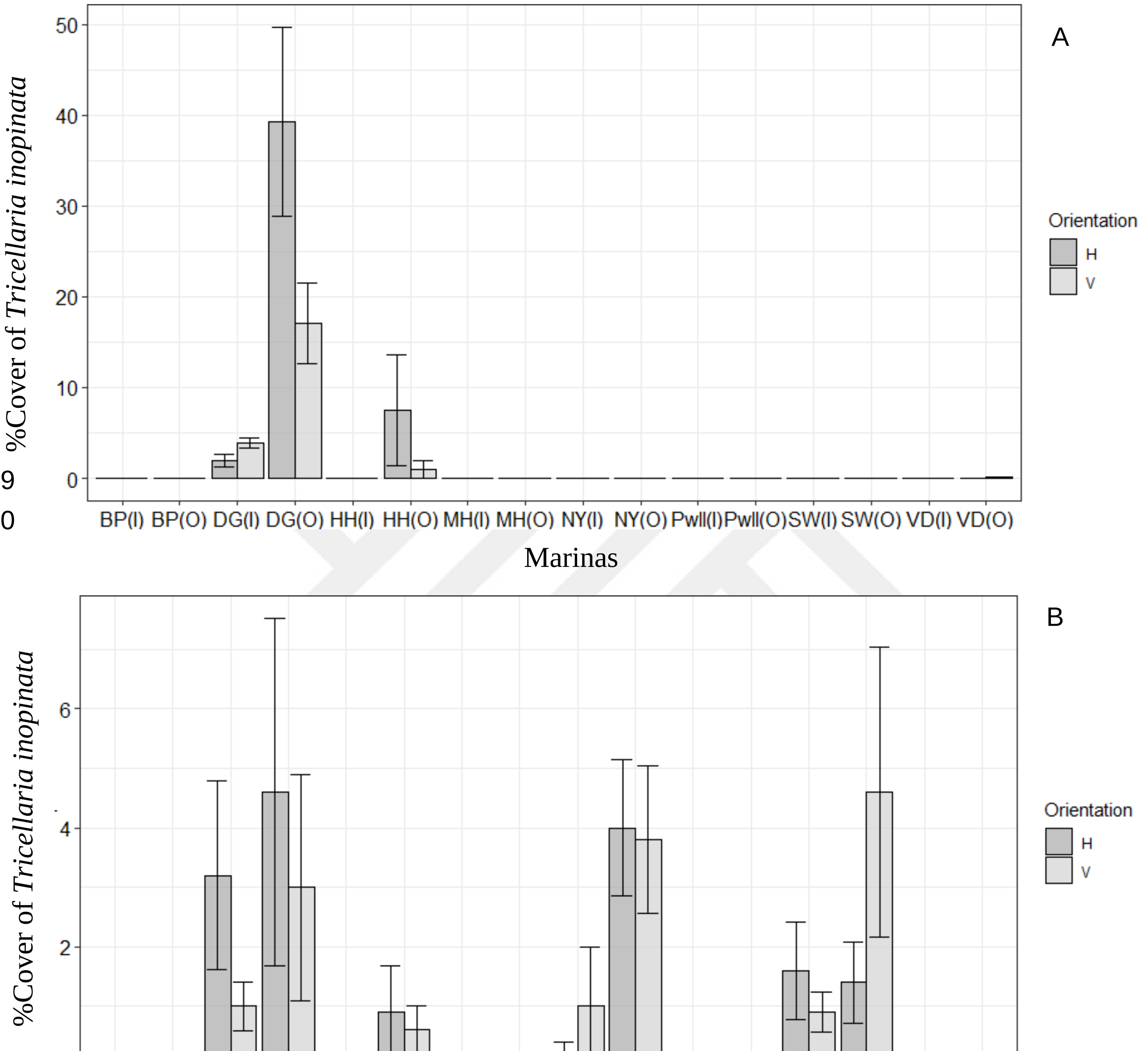


Figure 13. The percentage cover of *Tricellaria inopinata* abundance collected from eight marinas in two-meter depth (BP: BurryPort, DG: Deganwy, HH: Holyhead, MH: Milford Haven, NY: Neyland, Pwll: Pwlheli, SW: Swansea, VD: Victoria Dock) in two positions ((I): Inner,(O): outer) through two orientation tiles (H: horizontal, V: Vertical) at the end of two weeks (A) and eight weeks (B).

According to Cochran's test results, the eight-week data had heterogeneous variance (P>0.05). After log-transforming, the result of Cochran's test did not change. This shows a significant difference in the abundance of *T. inopinata* among marinas (F(7,128) =16.82, P-value=0.0000). There was also a significant interaction between marina and position (F(7,128)=3.13, P-value=0.0044), meaning the effect of position varies among marinas. SNK exploration of this interaction shows that there were some differences between positions, but the direction of difference was not consistent (**Figure 13B, Table 7**).

Table 7. The results of Three-way Mixed Model ANOVA showing differences in *Tricellaria inopinata* abundance among responsible variables (level of significance P-value < 0.05).

Three-way Mixed Model ANOVA Analysis of Variance and Covariance				
Source	df	Mean Squares(MS)	F-value	P-value
Marina	7	232.7167	16.82	0.0000
Position	1	153.1174	3.54	0.1019
Orientation	1	0.8928	0.08	0.7912
Marina*Position	7	43.2406	3.13	0.0044
Marina*Orientation	7	11.8000	0.85	0.5457
Position*Orientation	1	3.1887	0.43	0.5317
Marina*Position*Orientation	7	7.3684	0.53	0.8084
RES	128	13.8324		
TOTAL	159			

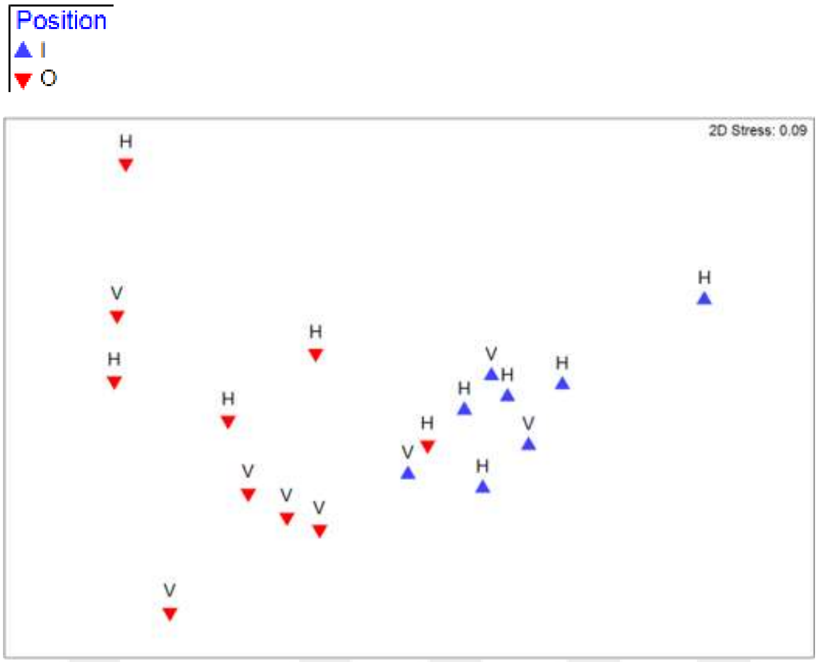
For Multivariate analysis, the results of community structure analysis undertaken using PRIMER and the PERMANOVA based on Bray-Curtis similarity for the abundance of dominant species is shown through three responsible factors (Marina-random; Position- fixed; Orientation–fixed) (**Table 8)**, and the produced multi-dimension plots (MDS) are presented **(Figure 14)**.

Table 8. PERMANOVA table of results.

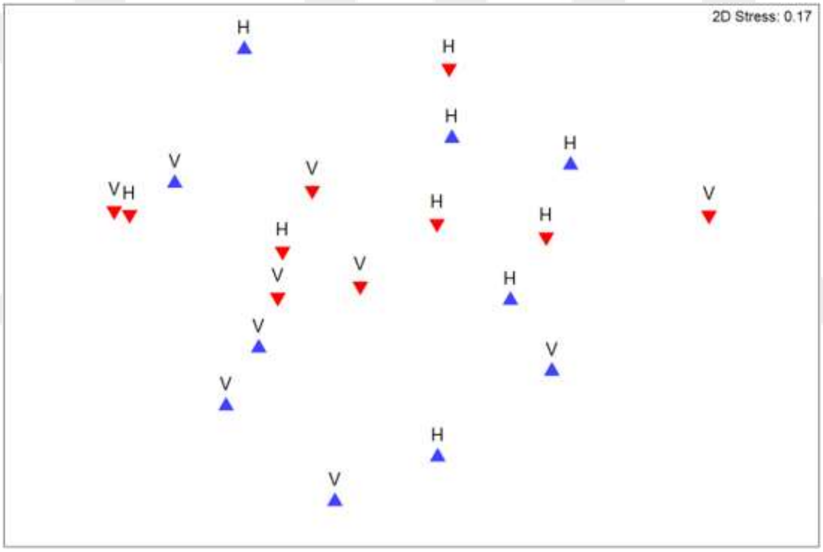
PERMANOVA results				
Source	df	Mean Squares(MS)	Pseudo-F	P(perm)
Marina	7	38557	42.262	0.001
Position	1	5989.6	0.85975	0.579
Orientation	1	872.91	0.72952	0.569
Marina*Position	7	6997.6	7.67	0.001
Marina*Orientation	7	1198	1.3131	0.099
Position*Orientation	1	686.42	0.68154	0.612
Marina*Position*Orientation	7	1007.6	1.1045	0.31
RES	120	912.33		
TOTAL	151			

In Table 8, there is a significant effect of marina and significant interaction between marina and position (P<0.05). Then, MDS plots were created individually for each marina to explore significant interactions. These MDS plots demonstrate that there is a clear effect of position for some marinas (VD, BP, and NY). This effect is less clear through an examination of MDS’s for orientation **(Figure 13)**.

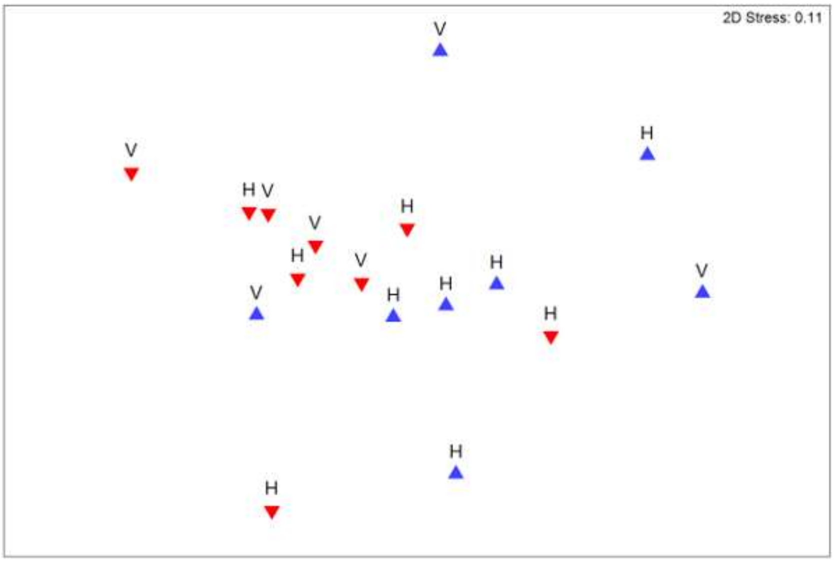
BP



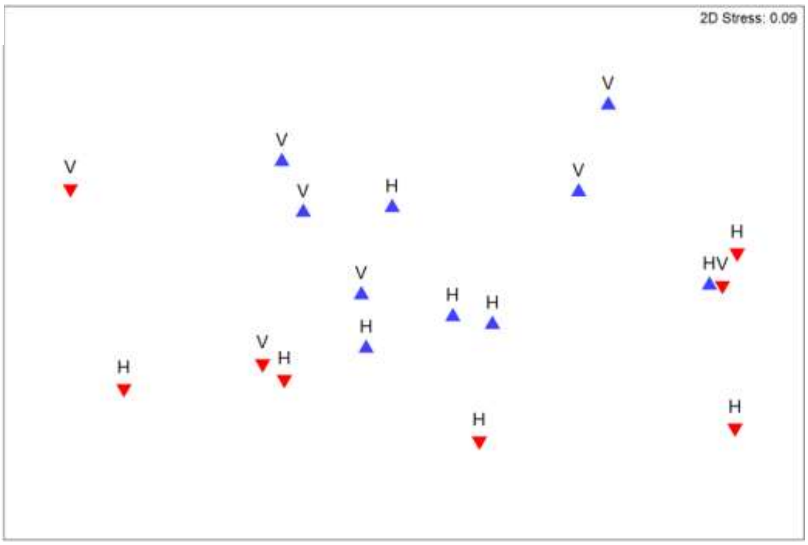
DG



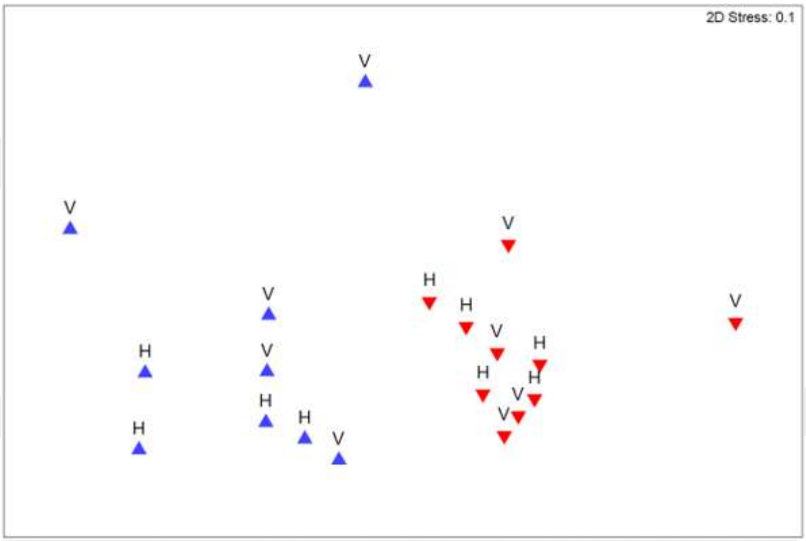
HH



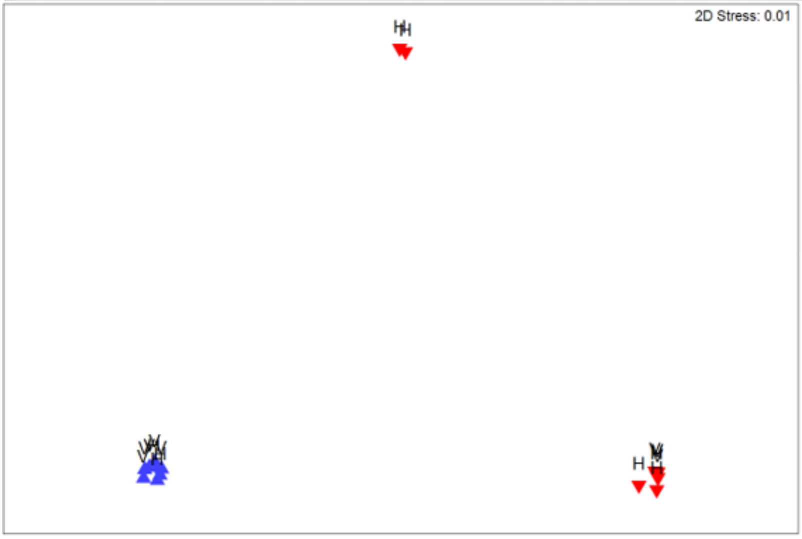
MH



NY



Pwll



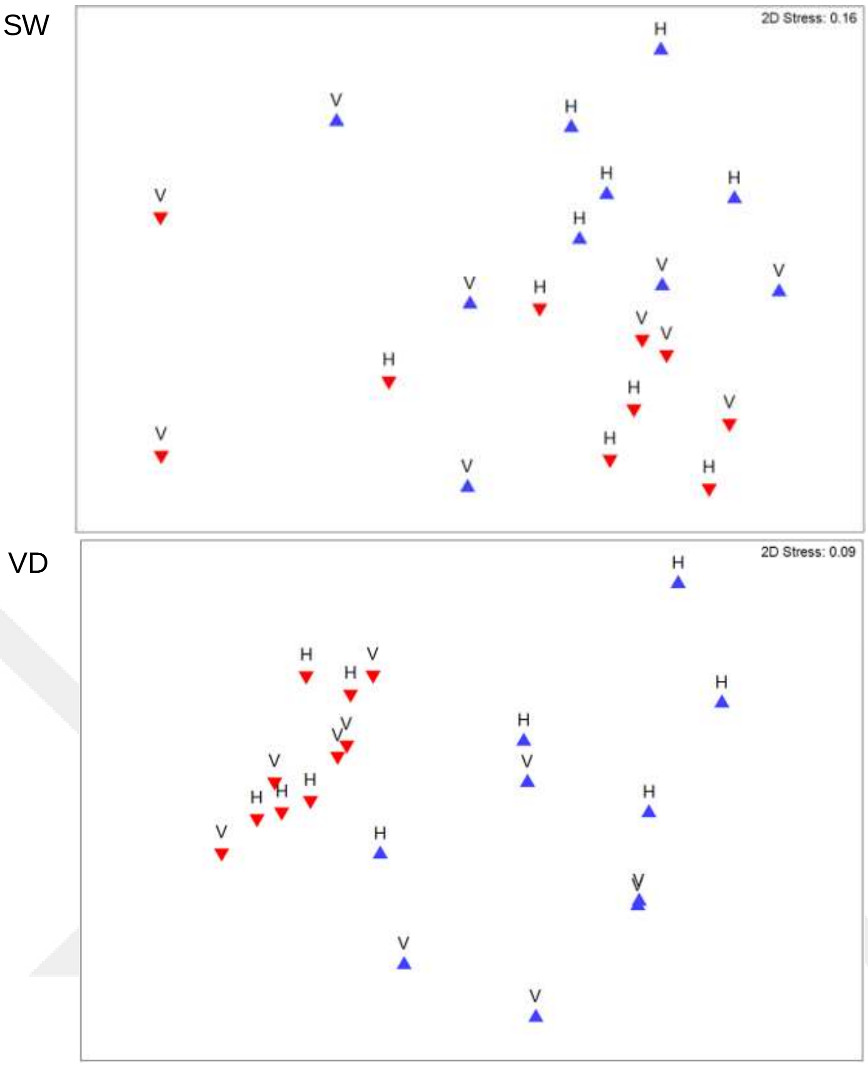


Figure 14. The significant interaction between fixed factors (orientation and position) and random factor (marina) in eight marinas (BP: BurryPort, DG: Deganwy, HH: Holyhead, MH: Milford Haven, NY: Neyland, Pwll: Pwlheli, SW: Swansea, VD: Victoria Dock, in two positions ((I): Inner,(O): outer) through two orientation tiles (H: horizontal, V: Vertical) at the end of eight weeks).

Discussion

How do non-natives distribute in the UK?

The clarification of non-natives is a crucial pre-requisite step to addressing several fundamental questions in species invasion biology, such as: who are invaders? Where are they coming from? What are the effects they cause in the invaded habitats? (Zhan et al. 2015). This study has mentioned how the species number, their biological and physiological characteristics, and the quality of recipient environment have an important role in the introduction success, changing the propagule size and frequency. Also, the distribution of non-native or other species affects the other non-native distribution, damaging the ecosystem because the species compete for space for food and habitat changes. However, novel substrates or attaching to other species protects non-natives. Still, there can be stress factors like predators, depth levels, salinity and temperature concentration, tolerance capacity and competitors.

Researchers also observed that macrobenthic populations exhibit spatial variations along a gradient of size. Arrighetti and Penchaszadeh (2010) proposed that sediment characteristics played a key role in the spatial dynamics of macrobenthic assemblies. Ivan et al. (2009) observed that food consistency was a structuring element in the macrobenthic population. Previous work has shown that aquaculture has led to the spatial structures of macrobenthos assemblages (Edgar et al. 2005). The adaptability of biological matter is one of its most remarkable and important characteristics, described as the willingness to deal with unexpected changes in the environment (Conrad and Calow 1986). In this study, potential prospects for colonisation of unstable ecosystems are different. The proportion of species have appeared in only a few stations such as VD, BP, and NY. Additionally, competition, predation, parasitism and symbiotic relations between macrobenthos may also impact the spatial patterns of macrobenthic assemblies. Recognizing major environmental variables that form the distribution of macrobenthic assemblies is not an easy task, as they always vary between space and may represent various interactive factors (Lu 2005). The relationship between the spatial patterns of the macrobenthic assemblies and the environmental variables is directly linked to the type of data selected in local conditions (Arrighetti and Penchaszadeh 2010).

Reproductivity has also played an important role. A high level of reproductive success increases the species distribution. In this case, a long planktonic larval phase is a good opportunity for invasion. Also, feeding methods increase invasion success. For example, sessile filter feeders spread easily in marinas. The presence of the Pacific oyster (*C. gigas*) can cause an increase in the non-native species distribution (NEMESIS 2020). The existence of several human-mediated vectors, as well as unique biological and/or genetic characteristics, environmental changes, and global climate changes can facilitate non-natives to conquer obstacles at various stages and successfully invade new environments. This occurrence depends on habitat types (e.g. sandy, rocky), harbour type (recreational, fishing or commercial use), and maintenance regime (Foster et al. 2016). Also, the absence of benthic predators or stable water currents can provide higher non-native species performance in marinas (Afonso et al. 2020).

How is the non-native species diversity affected by marina characteristics?

The study found there was a significant difference in the abundance of five discriminating species among marinas. Non-native species variations depend on some factors. Firstly, a key factor is a closeness to marina. In marina, the closeness depends on the salinity range and in general marinas which are fully saline are subject to infrequent salinity excursions and harbour more non-native species than brackish water sites or those subject to regular fluctuations e.g. in an estuary (Foster et al. 2016). Holyhead and Bury Port are marina sites, so are more susceptible to invasion more than Deganwy, Victoria Dock, Pwllheli Haven, and Swansea which are brackish water sites. Neyland is both a marina and brackish water site(Wood et al. 2015).

Secondly, the bigger marinas increase the risk of invasion by spreading locally, nationally and internationally, so they have high numbers of non-native species, and are easily accessible (Foster et al. 2016). In this study, the number of non-natives was the highest in Holyhead marina, likely a consequence of its large size and high boat traffic (Wood et al.2015).

Thirdly, there is the factor of larval retention within more enclosed marinas which may lead to larger populations of NIS (Wood et al. 2015). Larval dispersal and recruitment are likely to occur when water temperatures increase and may result in the spread of non-natives from the marina into the wider harbour area (Arenas et al. 2006b). As the temperature decreases, metabolic activity decreases (Kaldy et al. 2015). Thus, as the water temperature increases, the development of species increases. This allows upper intertidal colonisation and cold stratification, and increased metabolic activity that may have a significant impact on the species colonisation ability (Kaldy et al. 2015). For example, the temperature range in Holyhead Marina is between 5°C and 22°C throughout the year, so it is expected that regressed colonies increase in size during the spring and summer when water temperatures reach above 8–12°C and become favourable for asexual growth. The presence of non-native species in Holyhead Marina and their absence in the wider harbour area suggests that they were introduced into the region on the hulls of one or more infected recreational vessels (Arenas et al. 2006b). Swansea Marina and Victoria Dock are superabundant and there is a severe fouling nuisance on yacht hulls, pontoons and ropes (Wood et al. 2015).

The larval dispersal influences recruiting activities among marinas, so ‘open niche’ areas suffer from the impacts of invasions (Ulman et al. 2019). In this study, there were significant interactions between marina and position for *A. modestus* Darwin, 1854 and *T. inopinata* d'Hondt & Occhipinti Ambrogi, 1985 and significant interactions between marina and position as well as orientation effect for *C. intestinalis* Linnaeus, 1767. *A. modestus* is the most frequently recorded species from marinas around the UK, especially in habitats subjected to fluctuating salinity. *T. inopinata* is a frequently recorded species on primary hard substrates (Wood et al. 2015). *C. intestinalis* grows in the shallower depths (Bishop et al. 2015). According to Darling et al. (2018), through BWE, the diversity of open ocean species increase, resulting in an overall change in population composition (Darling et al. 2018). While in many cases this leads to dramatic declines in propagule pressure at recipient ports, several studies have suggested that the efficacy can vary widely depending on the vessel’s route, travel duration, biotic composition, and environmental conditions (Darling et al. 2018). In this case, fouling rates change widely between environments, being strongly affected by factors such as local productivity, and the structure and nature of available habitats (Johnson and Shanks 2003).

Another factor is depth. Shallow water sites may dry out during low tides; they are also susceptible to greater temperature fluctuations during summer and winter, which may kill off some species. Deeper waters can provide refuges from low salinity events, as when the waters are often highly stratified with the freshwater forming a surface layer over a higher-salinity lower base layer (Wood et al. 2015). This means that narrow areas with low salinity have more niche availability because of the freshwater effect, so non-natives are more vulnerable to colonisation in shallow areas (Afonso et al. 2020). NNS may survive at depth on ropes, chains and pilings and then recolonise rapidly on surface structures at a later date (Wood et al. 2015). In this study, Holyhead, Swansea, and Milford Haven marinas have more non-native species abundance because of the increasing temperature fluctuations with depth. Especially in Swansea Marina, there was a significant interaction between marina and orientation for *B. violaceus*. The depth also affects artificial light presence. Dafforn et al. (2009) stated that marine vessels lead to biological effects of artificial light in marine ecosystems because of decreasing light with depth. These effects are generally on species orientation, reproduction, recruitment between shallow and deep plates, and predation. Another factor is contaminant concentration. Marina vessels increase contaminants as well as the recruitment of species (Johnson and Shanks 2003).

In terms of the depth levels, freshwater layers can persist on the surface after heavy rainfall, which is an advantage for *B. neritina* Linnaeus, 1758 during settlement and transport on a boat and contributes to the spread of invaders on the hulls of ships (Dafforn et al. 2009). In this study, there was no significant interaction between marina and position as well as orientation effect for *B. neritina*. According to Jagerbrand et al. (2019), this outcome can also arise because of unsuitable habitats to invade a marina. In a previous study by Ulman et al. (2019), it was stated that most non-natives do not invade artificial habitats in the marinas when the surrounding habitats are not suitable for colonisation due to limited circulation and/or larval scattering regimes. The high invasion success has been reported in disturbed ecosystems and communities with low species diversity because these disturbed habitats have high and frequent inputs of non-natives in harbours and marinas (Riera et al. 2018). This integration increases the invasion success, and thus the colonisation risk increases in the established communities (Riera et al. 2018). In contrast, in polluted marine habitats, propagule pressure restricts non-native settlement compared to other parameters such as abiotic factors (pollutants) or environmental disturbance, so it is interesting to note that under natural conditions, these disturbed habitats and propagule pressures are often related with each other in terms of non-native diversity (Riera et al. 2018). According to Arenas et al. (2006b), the present distribution of *B. neritina* is of interest because during the 1950s and 1960s this taxon was known in the UK, particularly in artificially warmed locations, notably the Swansea Docks. Finally, the presence of any ascidian species, native or non-native affects a wide range of fouling organisms (Wood et al. 2015).

Conclusion

Overall, the number of NIS per marina varies among marinas in the UK, especially with marinas situated on the south coast of England where there is the greatest NIS number. The introduction of new NIS are often from sites in the English Channel owing to the high volume of international and recreational traffic and closeness to European sites compared to elsewhere in the UK (Foster et al. 2016). During settlement, environmental factors affect the non-native species distribution, then invasion accelerates (Riera et al. 2018). Reliable evidence on the distribution of species and whether they have successfully occupied certain environments is quite difficult to access, since invasions might have gone unrecognised or unreported(Lenz et al. 2011). In addition, marina characteristics such as orientation and position within an enclosed marina lead to non-native colonisation in the area (Ulman et al. 2019). Yet, at present, there can be limited methodological tools as well as insufficient information about invasive species, their invading processes (Corrales et al. 2020), and habitat effect on marine invasions (Ros et al. 2013). The lack of basic data and understanding of the environmental nature of the variations of the assemblages of marine ecosystems restricts the implementation of conservation strategies in marine ecosystems under some circumstances, and a strategy for the management and conservation of marine ecosystems is therefore needed (Arrighetti and Penchaszadeh 2010).

The research field has a major spatial variability owing to geological, hydrodynamic and anthropogenic practises. Biotic variables should be viewed in the sense of macrobenthic distribution studies. Relationships between the spatial attempts of macrobenthic assemblages and the environmental variables of biological parameters need to be considered in certain ways, such that the relationships have proved to shape the distribution of macrobenthic abundance, complexity and multivariate composition of assemblages. In this survey, the settlement panels were used for detection and monitoring of NIS. This method can be a major problem because identifying many of the species is difficult before they are fully developed and it is challenging to distinguish the NIS species from other, often closely related, native species. Therefore, it is recommended that this method should not be used to collect samples in future surveys.

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