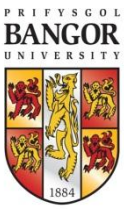
Wales Marine Non-native Species Inshore Monitoring Network

School of Ocean Sciences

Bangor University



Summary

This project aimed to examine the problem of detecting the arrival and spread of marine non-native species at coastal sites in Wales. Its remit was to examine a range of different methodologies which could be used to establish an 'Inshore Monitoring Network'. These methodologies were not designed to be exhaustive but were focused on establishing settlement panels in different coastal environments: intertidal aquaculture sites, marinas and sub-tidal fishing grounds (where crab and lobster post are deployed). The project also aimed to compare the results of these approaches with an offshore monitoring network established by Cefas and a Rapid Assessment Survey (RAS) approach at marinas.

The broad conclusions of the work are as follows:

Aquaculture sites

Establishing settlement panels, or using oyster shell as settlement units, over spring and summer periods at intertidal aquaculture sites (culture of *Crassostrea gigas*) in the Menai Strait, North Wales proved ineffective at detecting non-native species. Using deployments of 6 and 12 weeks, only two non-natives were detected, the barnacle *Austrominus modestus* and the solitary ascidian *Corella eumyota*. Such aquaculture sites provide structures (trestles and oyster bags) to deploy panels and are logistically easy to access but do not necessarily provide useful sites to detect a large range of non-natives, probably because of their intertidal environment. The logic of using aquaculture areas as monitoring sites is that aquaculture operations can lead to the import of non-natives. As such it is recommended that targeted monitoring of 'at risk' sites will be more effective than routine monitoring.

Use of crab/lobster pots

This approach can potentially allow the cost-effective deployment of sub-tidal settlement panels at a range of coastal locations through collaboration with fishers. Unfortunately we were unable to deploy panels through this means owing to problems in maintaining communication with collaborating fishers.

Marinas

Settlement panels in marinas are a cost-effective and efficient means of detecting non-native fauna (but not flora). Using this approach ten non-native species were detected overall in both marinas although no species new to Wales were found. Neither the site within a marina (visitor versus resident moorings), nor angle of deployment of panels appeared to affect the number of non-natives detected overall. However these factors did affect the abundance of various species. Thus use of both orientations and panels distributed throughout different environmental conditions within a marina are recommended approaches. It should be noted that 12 week deployments were better than 6 weeks and summer deployments were better than spring.

A comparison of panel deployment in marinas with panels on offshore buoys showed clear advantages, both logistically and in detection rates, of the inshore approach. Offshore deployment had very low detection rates of non-natives and there were significant problems in transportation of preserved panels.

A Rapid Assessment Survey (RAS) undertaken at Holyhead marina at the same time as panel deployments allowed comparison of the two approaches. They detected a similar range of non-native species, although two species *Undaria pinnatifida* and *Styela clava* were found with the RAS approach (but not panels), while *Botrylloides violaceous* and *Austrominius modestus* were found on panels, but not on the RAS. The RAS approach is quick and simple to implement but does rely on experts to visit field sites. The panel approach allows detection to be done in the laboratory, and hence in theory panels can be transported to a central location for expert ID.

FIGURES

TABLES

General Introduction

This report follows on from an interim report to NRW on October 2014. Here we report on the following deliverables:

- Attend at conference in Belgium on marine invasive non-native species
- Assessment of marina sites
- Assessment of aquaculture sites
- Assessment of crab/lobster pots
- Algae survey of monitoring sites
- Comparisons of the effectiveness of the in shore network with an offshore network established by Cefas
- Comparison of the effectiveness of different monitoring techniques (rapid assessment versus settlement panels)

1. Report arrival of non-native species to NRW

A total of 10 non-native species were recorded throughout the project (see Table 8.1 for non-native fauna and Table 6.1 for non-native algae). These are described below in detail with regard to a range of sampling approaches. However no non-natives were recorded at any of our sampling sites, (neither marina, nor aquaculture) which could be considered new arrivals to Wales.

2. Conference in Belgium on marine invasive non-native species

Dr Kate Griffith from the School of Ocean Science gave a talk on non-natives at the one day BENELUX conference on invasive species in Ghent University (2/04/14). This talk presented the report prepared for NRW and published in the journal Marine Policy:

Sambrook K, Holt RHF, Griffith KM, Roche RC, Newstead R, Wyn G, Jenkins SR 2014 Capacity, capability and cross-border challenges associated with marine eradication programmes in Europe: the attempted eradication of an invasive non-native ascidian, *Didemnum vexillum.* Marine Policy 48: 51–58

3. Marina sites

3.1 Marinas and non-native species

In recent years, marinas and ports (and their associated boat traffic) have been increasingly recognised as important pathways for the introduction of non-native species (Hutchings et al. 2002; Drake and Lodge, 2004; Panov et al. 2007; Keller et al. 2011). Vessel traffic acts as a vector in two main ways, through transport of non-natives in ballast water and through hull fouling. Some progress has been made in regulating these pathways; for example the International Maritimes Organisation (IMO), adopted the Convention for the Control and Management of Ships' Ballast Water and Sediments in February 2004. This type of biosecurity regulation is a positive start but is concerned exclusively with commercial ships. Recreational vessels are often neglected in terms of biosecurity but play an important role in the introduction of non-natives via bio fouling (Murray et al. 2011). An additional concern is that as they are not restricted by their size, leisure boats can have access to pristine areas such as Marine Protected Areas.

Over the last decade or so a large proportion of marine non-native research has focused on marinas because of their propensity to act as non-native hotspots. Artificial structures within marinas, such as floating pontoons, provide a distinct habitat (Glasby, 1999). The fully submersed shallow hard substrata on such pontoons hosts specific benthic communities and non-natives introduced to an area via boat bio-fouling are common colonists (Bax et al. 2002). Fouling of recreational vessels within such marinas and subsequent vessel movement among marinas can lead to rapid non-native spread; Canning-Clode et al. (2013) showed a positive correlation between increasing ship traffic and the number of non-natives species detected in marinas. There are numerous examples of non-natives transferred by recreation vessels. The invasive kelp, *Undaria pinnatifida,* is known to have arrived in the UK via recreational boats (Hay, 1990; Farrell & Fletcher, 2006; Murray et al. 2011) and the distribution of this species now has extended along most of the south coast of England, and it has now arrived in Wales. Similarly, the black striped mussel, *Mytilopsis* sp. was also introduced by recreational vessels in Darwin, Australia (Bax et al. 2002). Australian authorities have successfully eradicated this species from the marinas but at a cost of 2.2 million $AU, highlighting the financial implications of non-native introductions. More recently, the carpet sea squirt *Didemnum vexillum* has been introduced in Holyhead Marina, most likely by leisure boats from Ireland.

A science-based monitoring programme is vital to enhance early detection of non-natives (Sambrook et al. 2014) which is a prerequisite for a successful eradication programme (Myers et al. 2000, Wotton et al. 2004, Genovesi 2005). The aim of this project was to trial different methods for monitoring non-natives in marinas and to identify which method is the best approach for this type of survey. Standard PVC settlement panels were deployed during spring and summer 2014 in both marinas, and for varying periods (6 week versus 12 week deployment) to investigate how colonisation rates varied with season across Wales and the most appropriate deployment duration. Panels were deployed in two orientations, vertical and horizontal, to determine the most effective method for detecting non-natives. In addition panels were deployed in different parts of the marina, near resident and near visitor moorings, to test whether the arrival of new vessels and different characteristics of the marina environment affected non-native colonisation.

3.2 Methods

3.2.1 Study area

Milford Haven and Holyhead marinas were selected as trial sites, both representing active marinas with fully marine conditions and situated in south and north Wales respectively. The ease of access to Holyhead marina and the previous research done on non-natives there made this marina a particularly useful test site.

Milford Haven is located in south-west Wales where the Bristol Channel and St George's Channel meet (Nelson-Smith, 1967). The Milford Haven waterway is one of the largest estuaries in Wales and the biggest and deepest natural harbour in the UK. The Milford Haven marina is located in a non-tidal basin within the Waterway and is sheltered from the main harbour. This marina has a predominantly saline environment, but local freshwater input means conditions tend to be brackish after heavy rain. The marina consists of a basin dock with one lock entrance and has 340 berths (Fig. 3.1). Every boat has to pass through the lock to enter the marina and all traffic is controlled by the Pier Head staff. The water depth within the lock is a minimum of 3m (Spring tide). As Bax et al. (2002) has shown, this system of lock gate creates a novel sheltered environment with consistent conditions i.e no tidal range. The combination of high vessel flux and specific conditions create an opportunity for non-native species to invade the marina and to prosper. Although the marina has visitors coming from around the world, including a recent vessel from Japan, (Milford Haven marina staff pers comm to Bue), Milford Haven is rarely the first port of arrival in the UK.

Holyhead marina is situated in North West Anglesey. This marina has been operational since 2001 and has 300 berths (Fig. 3.2). It is located within the confines of Holyhead Port, which is protected by a 2km long breakwater. Holyhead marina is deeper than Milford Haven with a depth over 3 meters at low tide and, unlike Milford Haven, the marina at Holyhead is open to free exchange of water (i.e. not enclosed within a lock system). The nearby ferry port to Ireland is located within Holyhead harbour and the presence of numerous non-native species including the carpet sea squirt, *Didemnum vexillum*, the bryozoan, *Schizoporella japonica,* and the kelp, *Undaria pinnatifida,* make Holyhead a potentially useful monitoring site for non-native introductions.

These two marinas differ in terms of their geographic location, infrastructure and salinity but both have a high level of recreational activities and easy access to Ireland.

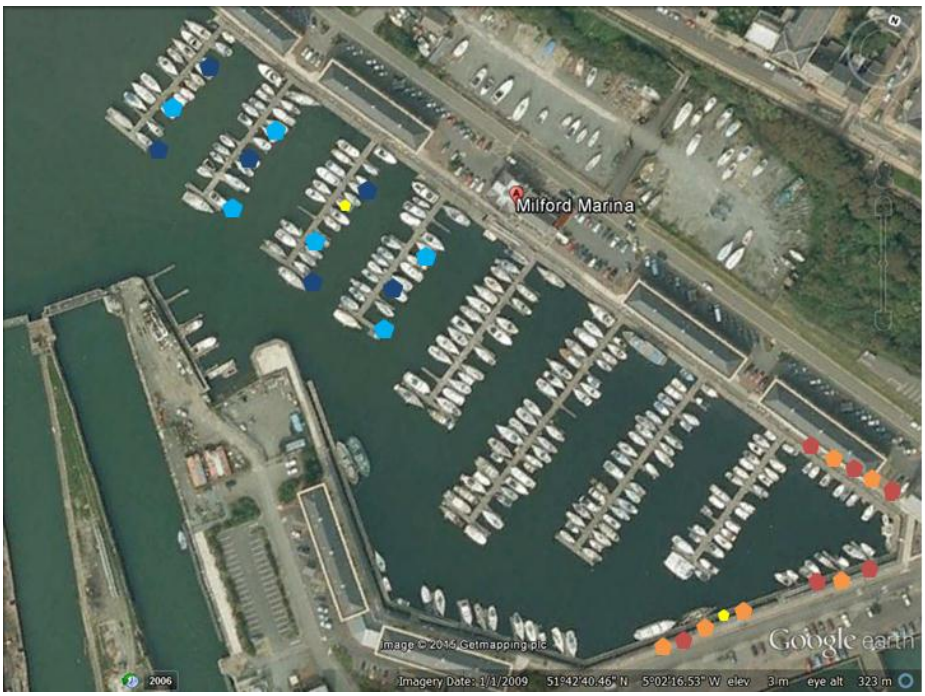


Figure 3.1 Milford Haven Marina showing location of settlement panel deployments. The red (6 weeks) and orange (12 weeks) circles represent the panels from the Resident area. The dark green (6 weeks) and pale green (12 weeks) circles are the panels from the Visitor area. Each circle include Vertical and horizontal panels. Yellow circles show where the temperature loggers were positioned (Source: www.earth.google.com).

Figure 3.2 Holyhead Marina showing location of settlement panel deployments. The red (6 weeks) and orange (12 weeks) circles represent the panels from the Resident area. The dark blue (6 weeks) and pale blue (12 weeks) circles are the panels from the Visitor area. Each circle include Vertical and horizontal panels. Yellow circles show where the temperature loggers were positioned (Source: www.earth.google.com).

3.2.2 Experimental design and implementation

Colonisation of PVC settlement panels (15cm x 15cm) was assessed over the spring and summer of 2014. The full survey design (implemented only in the summer) was based on assessing colonisation at distinct sites within each marina (a visitor and a resident area), over 2 different periods (6 and 12 weeks) and using vertical and horizontal panels (Fig. 3.3). In the spring the importance of panel orientation was only assessed at Holyhead. Each treatment combination was replicated 6 times (i.e. 6 panels).

Designated visitor berths (where visiting boats berth for a short period of time and there is generally quite frequent traffic) and resident areas (where boats berth for longer periods of time and traffic is generally less frequent) were identified at Holyhead and Milford Haven marinas (Fig. 3.1 and 3.2). In the spring 12 vertical panels (Fig. 3.4) (6 x 6 week and 6 x 12 week deployments) were deployed at each visitor and resident site in Milford Haven and Holyhead; panels were deployed at a depth of 1.5m and locations were separated by a minimum of 3 m (see Table 3.1 for deployment dates). In addition, a further 12 horizontal panels (6 x 6 week and 6 x 12 week deployments) were deployed at each site at Holyhead (Fig. 3.2). In the summer the full design (see Fig. 3.3 for details) was implemented over both marinas. The temperature was recorded at both marinas over the period of study (6th April 2014 to 8th October 2014) using TinyTag temperature loggers. Deployment and retrieval dates of the panels are given in Table 3.1.

Following collection, panels were photographed before being transported back to the laboratory for identification (Fig. 3.5 and 3.6). Here they were maintained in isolated water tanks with aeration. The identification of species is greatly improved when organisms are alive but this is limited by space and time. When this was not possible, organisms were relaxed in menthol crystal then preserved in 70% industrial methylated spirit (IMS). Panels were analysed under a dissecting microscope (x10 magnification). Organisms were identified to species level when possible and the percentage cover of each species was estimated.

3.2.3 Scrape samples

Scrape sampling consisted of removing samples of fouling organisms from the pontoon surface with a paint scraper and was undertaken to allow comparison of this technique with settlement panels in detecting non-natives. The samples were stored in seawater and transported to the laboratory for identification. Sub-tidal surfaces within the marina, which were near to locations where the suspended panels were deployed, were cleared at the start of the survey in April 2014 in order to compare colonisation of bared pontoon areas with PVC panels. After 6 weeks, the settlement on these surfaces was too low to be destructively sampled and this technique was abandoned. The technique was consequently modified for the following weeks and after 12 weeks of monitoring, these surfaces were video recorded. This technique however resulted in data of limited quality and the approach was not repeated in the summer.

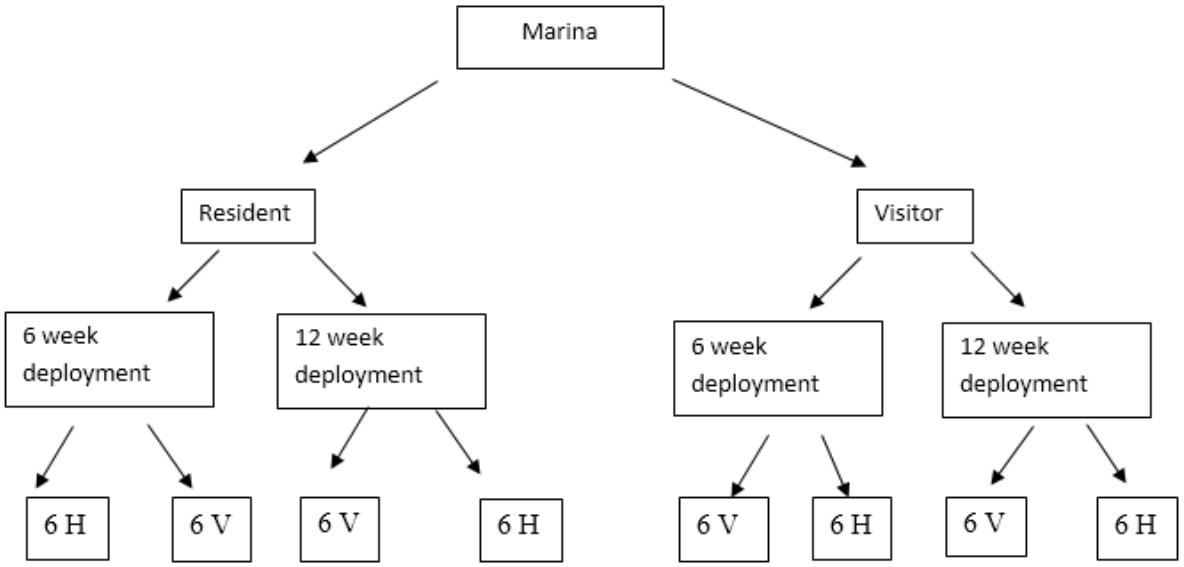


Figure 3.3 Full sampling design implemented at both Holyhead and Milford Haven in the summer showing the use of 'site' (Resident versus Visitor areas within the marina), length of deployment (6 week versus 12 week) and panel orientation (Vertical (V) versus Horizontal (H)). In the spring this design was implemented at Holyhead and a reduced design implemented at Milford Haven (without Horizontal panels).

Table 3.1*:* Timetable for deployment and collection of settlement panels at marina sites during 2014.

	Jan	**Feb**	**Mar**	**Apr**	**May**	**Jun**	**Jul**	**Aug**	**Sep**	**Oct**	**Nov**	**Dec**
6 weeks panels												
6 weeks deployment				✓			✓					
6 weeks collection					✓			✓				
6 weeks sample analysis					✓			✓				
12 weeks panels												
12 weeks deployment				✓			✓					
12 weeks collection							✓			✓		
12 weeks sample analysis							✓			✓		

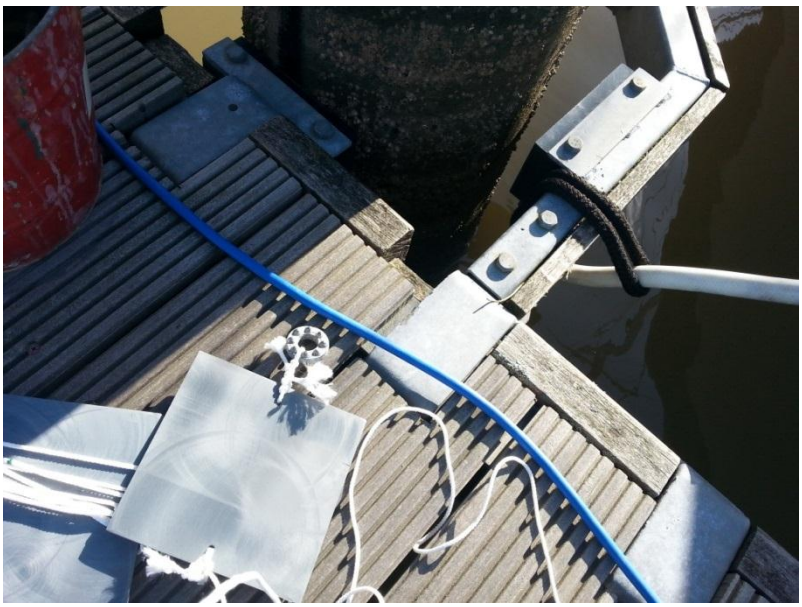


Figure 3.4 Vertical settlement panel with weight.

Figure 3.5 Vertical settlement panel collected on the 6th July 2014 after 12 weeks of immersion in the visitor site at Milford Haven marina.

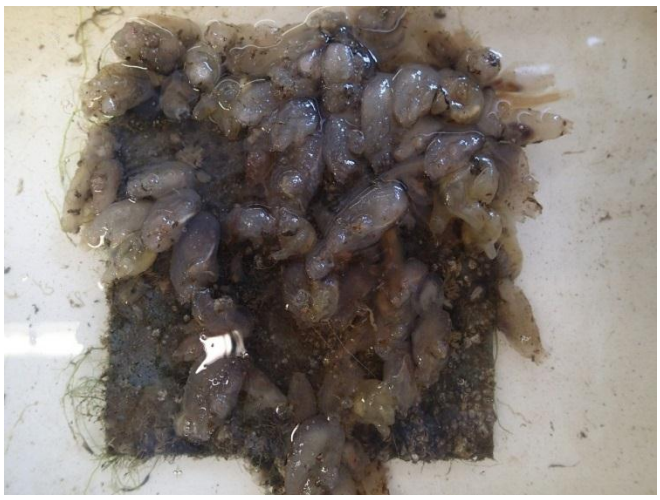


Figure 3.6 Vertical settlement panel ready for the identification in the laboratory at School of Ocean Sciences on (panels collected on the 6th July 2014 after 12 weeks of immersion in the visitor site at Milford Haven marina).

3.2.4 Data analysis

Although settlement panels were colonised in spring, the level of colonisation was far lower than in the summer. Preliminary analyses indicated little power to detect the effects of the principle factors of marina, site, deployment duration and panel orientation. For this reason only summer analyses are presented below.

Percentage cover (or density in the case of *Caprella mutica*) of dominant non-natives and mean number of non-natives per panel were analysed using a fully factorial 4-way ANOVA with duration, marina, site and angle as fixed factors (GMAV5, Underwood and Chapman 1989). Species that were analysed in this manner were those which were commonly found on settlement panels and included *Bugula neritina, Austrominius modestus, Tricellaria inopinata, Corella eumyota* and *Caprella mutica. Schizoporella japonica* appeared only in Holyhead marina and *Ficopomatus enigmaticus* only in Milford Haven marina; consequently, a 3-way ANOVA, omitting the factor 'marina; was used to analyse these species. Data were tested for homogeneity of variance using Cochran's test and where heterogeneity was observed data were transformed. Transformation failed to homogenise variances in a number of cases but the ANOVA was still undertaken; here care should be taken in interpreting significant results owing to an increased probability of Type I error. SNK tests were performed on significant interactions. All tests were made using the statistical software GMAV 5 for Windows (Underwood and Chapman 1989).

3.3 Results

3.3.1 Species Richness of non-native species

The pattern of species richness is described here first in terms of the total number of non-native species detected using each particular sampling methodology (then below in terms of mean richness utilising averages calculated across replicate panels).

A greater number of non-native species colonised the settlement panels at Holyhead marina compared to Milford Haven marina (Table 3.2). In terms of species, *Didemnum vexillum* was present only at Holyhead marina and *Ficopomatus enigmaticus* was found only at Milford Haven marina.

Overall, no clear patterns were found when comparing the number of non-native species at resident and visitor sites. Species numbers were similar and the same set of non-native species was found at visitor and resident sites within each marina (Table 3.2, 3.3, 3.4).

There was little difference in the number of non-natives detected using horizontal versus vertical panels although there was a slight tendency for horizontal panels to capture more non-native species; the number of colonised non-natives tended to be equal or higher on horizontal panels in comparison with vertical panels (except at the resident site at Milford Haven after 12 week deployment, see table 3.2 and 3.3). Colonisation of vertical and horizontal panels by non-natives consisted of the same subset of species at Holyhead marina. At Milford Haven marina, however, *Corella eumyota* was only found on vertical panels.

There was a clear difference in colonisation by non-natives between seasons as few species colonised the panels in spring (max = 6) compared to summer (max = 9). *D. vexillum* and *B. neritina* were not found in spring deployments.

Table 3.2 Total number of non-native species recorded using each of the different approaches/locations (H = Horizontal panel, V = Vertical panel).

	Milford Haven				**Holyhead**			
	Resident		Visitors		Resident		Visitors	
	H	**V**	**H**	**V**	**H**	**V**	**H**	**V**
Spring12 weeks	/	1	/	2	6	5	5	5
Summer 6 weeks	5	5	4	4	7	7	9	6
Summer 12 weeks	3	5	6	5	7	7	6	6

Table 3.3 List of non-native species recorded using each of the different approaches/locations (H = Horizontal panel, V = Vertical panel) at Milford Haven marina.

	Milford Haven			
	Resident		Visitors	
	H	**V**	**H**	**V**
Spring12 weeks	/	*A. modestus*	/	*A.modestus* *C. eumyota*
Summer 6 weeks	*T. inopinata* *F. enigmaticus* *B. neritina* *A. modestus* *C. mutica*	*F. enigmaticus* *B. neritina* *A. modestus* *B. violaceus* *C. mutica*	*T. inopinata* *F. enigmaticus* *B. neritina* *A. modestus*	*T. inopinata* *F. enigmaticus* *B. neritina* *A. modestus*
Summer 12 weeks	*F. enigmaticus* *B. neritina* *A. modestus*	*C. eumyota* *F. enigmaticus* *B. neritina* *A. modestus* *C. mutica*	*T. inopinata* *F. enigmaticus* *B. neritina* *A. modestus* *B. violaceus* *C. mutica*	*T. inopinata* *F. enigmaticus* *B. neritina* *A. modestus* *C. mutica*

Table 3.4 List of non-native species recorded using each of the different approaches/locations (H = Horizontal panel, V = Vertical panel) at Holyhead marina.

	Holyhead			
	Resident		Visitors	
	H	**V**	**H**	**V**
Spring12 weeks	*T. inopinata* *C. eumyota* *A. humilis* *S. japonica* *C. mutica* *A.modestus*	*T. inopinata* *A. humilis* *S. japonica* *C. mutica* *A.modestus*	*T. inopinata* *B. violaceus* *A. humilis* *S. japonica* *A.modestus*	*T. inopinata* *A. humilis* *S. japonica* *C. mutica* *A.modestus*
Summer 6 weeks	*T. inopinata* *S. japonica* *C. eumyota* *B. neritina* *A. modestus* *D. vexillum* *C. mutica*	*T. inopinata* *S. japonica* *C. eumyota* *B. neritina* *A. modestus* *D. vexillum* *C. mutica*	*T. inopinata* *S. japonica* *C. eumyota* *B. neritina* *A. modestus* *B. violaceus* *A. humilis* *D. vexillum* *C. mutica*	*T. inopinata* *S. japonica* *B. neritina* *A. modestus* *B. violaceus* *C. mutica*
Summer 12 weeks	*T. inopinata* *S. japonica* *C. eumyota* *B. neritina* *A. modestus* *B. violaceus* *A. humilis* *C. mutica*	*T. inopinata* *S. japonica* *C. eumyota* *B. neritina* *A. modestus* *A. humilis* *C. mutica*	*T. inopinata* *S. japonica* *C. eumyota* *B. neritina* *A. humilis* *C. mutica*	*T. inopinata* *S. japonica* *B. neritina* *B. violaceus* *A. humilis* *C. mutica*

Focusing on the mean number of non-native species utilises the variation observed among settlement panels and allows statistical analysis of pattern. The mean number of non-native species recorded across the survey area was higher in the summer compared to spring (Table 3.2, Fig. 3.7). Focusing on summer data only, the duration of panel deployment was shown to have a significant impact on the richness of non-natives recorded, but this effect only occurred for Holyhead marina (significant interaction of duration x marina: Table 3.5, Fig. 3.7). Species richness of non-natives at Holyhead was higher (mean: 5.38 SE ± 0.26) after 12 weeks of immersion than it was after 6 weeks (mean: 4.25 SE ± 0.28).

The marinas in which panels were deployed have a significant impact on the richness of non-natives recorded and this effect occurred for both sites (significant interaction of marina x site: Table 3.5, Fig. 3.7). Species richness of non-natives recorded at both sites was higher at Holyhead (mean: 5.04 SE ± 0.29) than at Milford Haven (mean: 3.33 SE ± 0.21). Another impact of marinas on the species richness of non-natives is shown but, this effect only took place for horizontal panels (significant interaction of marina x angle: Table 3.5, Fig. 3.7). Species richness of non-natives recorded on horizontal panels was higher at Holyhead (mean: 5.75 SE ± 0.20) than at Milford Haven (mean: 3.58 SE ± 0.22).

The angle of the settlement panels was revealed to have a significant impact on the richness of non-natives recorded, but this effect only took place for Holyhead. Species richness of non-natives at Holyhead was higher on horizontal panels (mean: 5.71 SE ± 0.20) than it was on vertical panels (mean: 3.88 SE ± 0.24).

Table 3.5 Analysis of Variance (ANOVA) comparing the species richness of settlement panels deployed at different angles at Holyhead and Milford Haven marinas for 6 and 12 weeks duration (Cochran's *C*-test, p = 0.2138).

Factor	df	MS	F	P
Duration	1	10.1400	12.39	0.0007
Marinas	1	39.0150	47.68	<0.0001
Site	1	0.0150	0.02	0.8926
Angle	1	23.2067	28.36	<0.0001
Duration X Marina	1	5.4150	6.62	0.0119
Duration X Site	1	2.2817	2.79	0.0989
Duration X Angle	1	0.0067	0.01	0.9283
Marina X Site	1	4.5067	5.51	0.0214
Marina X Angle	1	19.0817	23.32	<0.0001
Site X Angle	1	3.0817	3.77	0.0558
Duration X Marina X Site	1	0.0067	0.01	0.9283
Duration X Marina X Angle	1	0.4817	0.59	0.4452
Duration X Site X Angle	1	0.4817	0.59	0.4452
Marina X Site X Angle	1	0.1067	0.13	0.7190
Duration X Marina X Site X Angle	1	0.8067	0.99	0.3238
Residual	80	0.8183		

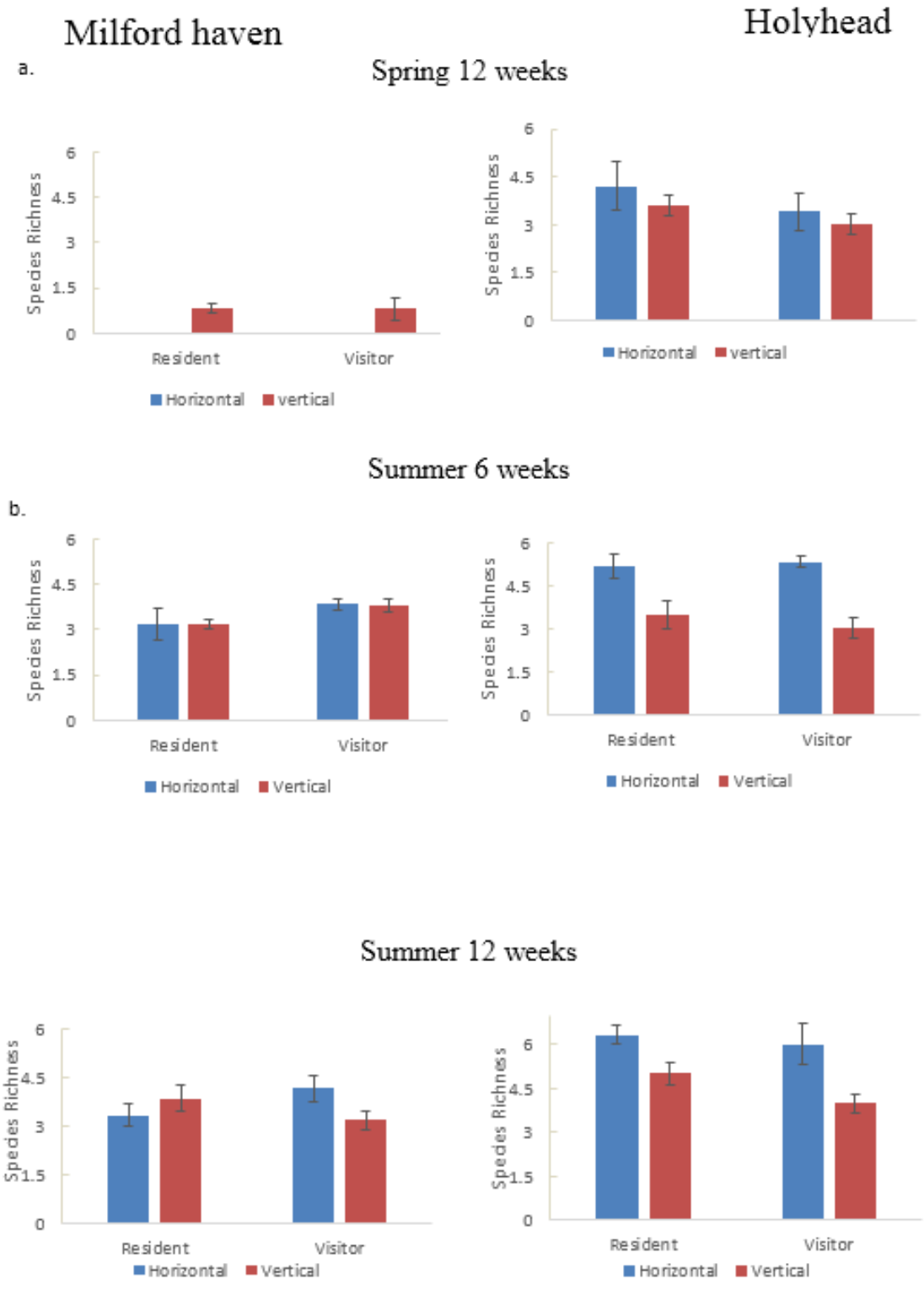


Figure 3.7 Mean (±SE, n = 6) species richness of Non-Native Species in Milford Haven (left) and Holyhead (right) marinas in 2014. Panels immersed for a duration of: (a) 12 weeks in spring. (b) 6 weeks in summer. (c). 12 weeks in summer.

3.3.2 Abundance of commonly found non-native species

Seven non-native species were found throughout our settlement panel survey which were sufficiently abundant to allow quantitative analysis. The patterns in abundance and the output from the 4 way ANOVAs are described in detail below for each species. However first we present a summary of the main findings (Table 3.6). As specified above these analyses focus only on summer data.

Table 3.6 Summary of analyses on the effect of 'marina', 'site', 'duration' and 'angle' on the colonisation of settlement panels deployed in summer 2014 by the seven most common non-native species. No entry indicates no significant effect. An entry indicates a significant effect of the factor on the abundance of the relevant non-native species. The direction of the effect is indicated and any modifying factors (i.e. indicating an interaction in the ANOVA) placed in parentheses below. MH = Milford Haven; Holy = Holyhead; Vert = Vertical; Hori = Horizontal; Vis = Visitors berth; Res = Resident berth; 12 = 12 weeks deployment; 6 = 6 week deployment.

	Bugula neritina	*Austrominius modestus*	*Tricellaria inopinata*	*Corella eumyota*	*Ficopomatus enigmaticus*	*Schizoporella japonica*	*Caprella mutica*
Marina	MH>Holy (Vis)	MH>Holy	Holy>MH	Holy>MH (12) Holy>MH (Hori)	MH>Holy (none at Holy)	Holy>MH (none at MH)	Holy>MH (Hori)
Site	Vis>Res (6) Vis>Res (MH)	Vis>Res (6) Vis>Res (MH)	Vis>Res (Holy)		Res>Vis (12)		
Duration	12>6 (Holy)		12>6 (Holy)	12>6 (Holy)	12>6 (Res)	12>6	
Angle		Vert>Hori (MH)	Hori>Vert (6) Hori>Vert (Holy)	Hori>Vert (Holy)			Hori>Vert (Holy)

There was no consistent effect of marina: three of the seven non-native species showed significant effects where abundances were greater for certain comparisons at Milford Haven, with four comparisons indicating greater abundances at Holyhead.

The effect of deployment site within the two marinas was significant for four of the non-native species. *B. neritina, A. modestus* and *T. inopinata* were more abundant on settlement panels deployed at visitor berths for certain comparisons, while *F. enigmaticus* was more abundant at the residents berth.

Five of the seven non-native species were more abundant on panels deployed for 12 weeks compared to 6 weeks. Only *C. mutica* and *A. modestus* didn't show this pattern.

The effect of panel angle was significant for four of the non-native species. *T. inopinata, C. eumyota* and *C. mutica* were more abundant on horizontal compared to vertical panels, although this pattern was only observed at Holyhead. *A. modestus* was more abundant on vertical panels (but only at Milford Haven).

Bugula neritina:

Focusing on summer data only, the duration of panel deployment was shown to have a significant impact on the cover of *B. neritina* recorded, but this effect only occurred for Holyhead marina (significant interaction of duration x marina: Table 3.7, Fig. 3.8). The cover of *B. neritina* at Holyhead was higher (mean: 14.7% SE ± 0.008) after 12 weeks of immersion than it was after 6 weeks (mean: 3.3% SE ± 0.020)

The marinas where panels were deployed were shown to have a significant effect on the cover of *B. neritina* recorded, but this effect only occurred at the visitor site (significant interaction of marina x site: Table 3.7, Fig. 3.8). The cover of *B. neritina* at the visitor site was higher (mean: 19.7% SE ± 0.023) at Milford Haven than it was at Holyhead (mean: 6.9% SE ± 0.016).

The site of the panel deployment had a significant impact on the cover of *B. neritina* recorded, but this effect only took place for the 6 weeks duration of panel deployment (significant interaction of duration x site: Table 3.7, Fig. 3.8). The cover of *B. neritina* after 6 weeks deployment was higher (mean: 11.8% SE ± 0.003) at the visitor site than the resident site (mean: 3.9% SE ± 0.008). In addition, the significant effect of the sites occurred at Milford Haven (significant interaction of marina x site: Table 3.7, Fig. 3.8). The cover of *B. neritina* at Milford Haven was higher (mean: 19.9% SE ±0.0228) at the visitor site than it was at the resident site (mean: 7.9% SE ± 0.002). No effect of angle was shown on the cover of *B. neritina* recorded.

Table 3.7. Analysis of Variance (ANOVA) comparing the mean cover of *Bugula neritina* on settlement panels at different angle between Holyhead and Milford Haven marinas for 6 and 12 weeks duration (Cochran's *C*-test, p = 0.1934).

Factor	df	MS	F	P
Duration	1	0.1266	19.15	<0.001
Marinas	1	0.0567	8.59	0.0044
Site	1	0.0357	5.41	0.0226
Angle	1	0.0195	2.95	0.0898
Duration X Marina	1	0.0401	6.07	0.0159
Duration X Site	1	0.0394	5.96	0.0169
Duration X Angle	1	0.0001	0.01	0.9163
Marina X Site	1	0.1588	24.02	<0.001
Marina X Angle	1	0.0161	2.44	0.1223
Site X Angle	1	0.0007	0.11	0.7431
Duration X Marina X Site	1	0.0044	0.67	0.4155
Duration X Marina X Angle	1	0.0173	2.61	0.1098
Duration X Site X Angle	1	0.0014	0.21	0.6471
Marina X Site X Angle	1	0.0076	1.15	0.2869
Duration X Marina X Site X Angle	1	0.0017	0.25	0.6187
Residual	80	0.0066		

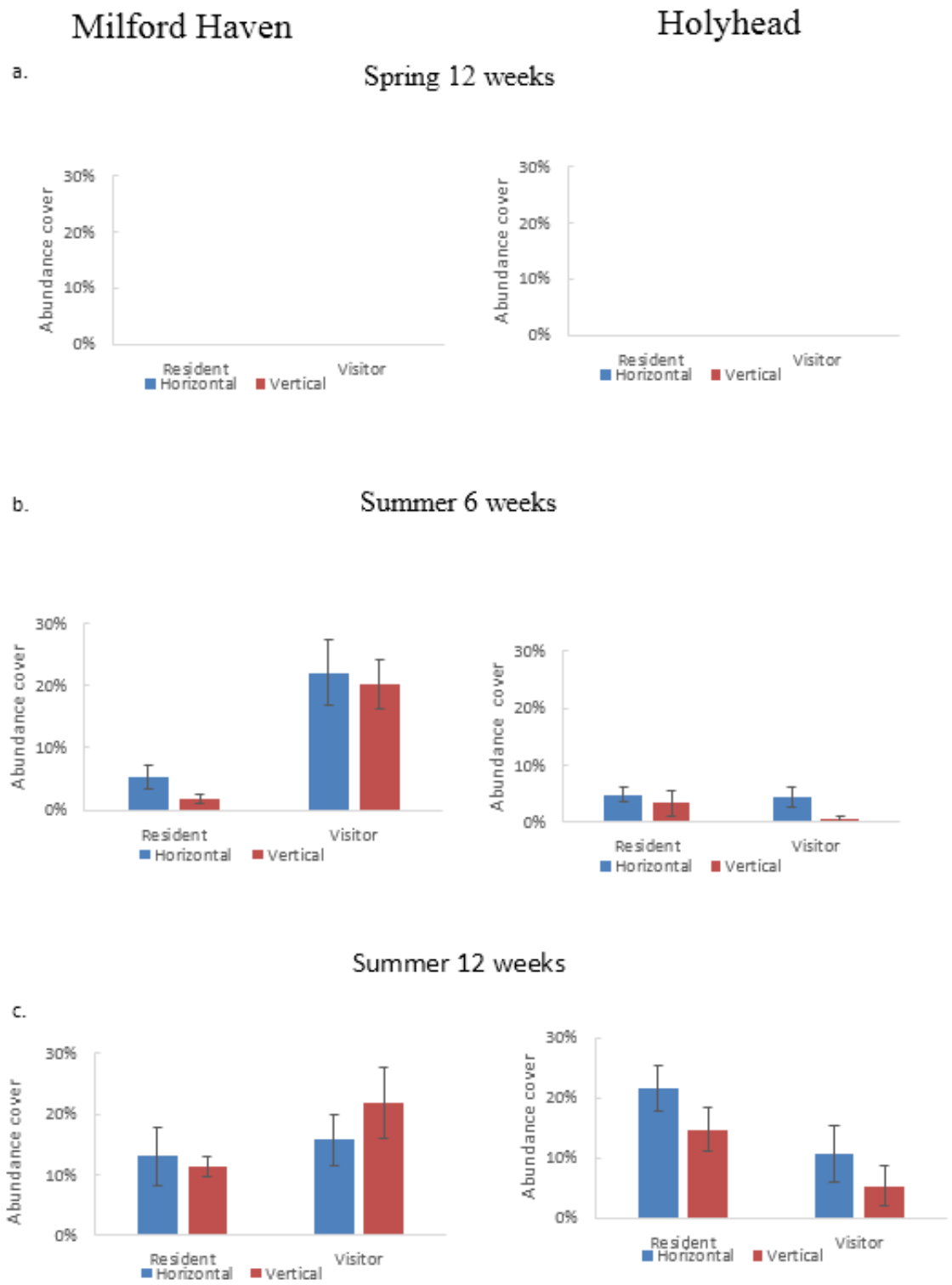


Figure 3.8 Mean (±SE, n = 6) percentage cover of *Bugula neritina* on settlement panels placed in Milford Haven (left) and Holyhead (right) marinas in 2014. Panels immersed for a duration of: (a) 12 weeks in spring. (b) 6 weeks in summer. (c) 12 weeks in summer.

Austrominius modestus:

Focusing on summer data only, the marinas in which panels were deployed had a significant impact on the cover of *A. modestus* recorded, and the effect is seen at any duration, sites and angles. Cover of *A. modestus* on panels at Milford Haven was higher (mean: 6.9% SE ± 0.011) than it was at Holyhead marina (mean: 0.2% SE ± 0.0004).

The angle of the deployment panels was shown to have a significant impact on the cover of *A. modestus* recorded, but the effect only occurred at Milford Haven (significant interaction of marina x angle, Table 3.8, Fig. 3.9) and at 6 weeks of deployment (significant interaction of duration x angle, Table 3.8, Fig. 3.9). The cover of *A. modestus* at Milford Haven was higher (mean: 9.9% SE ± 0.019) on vertical panels than it was on horizontal panels (mean: 4.1% SE ± 0.008). In addition, the cover of *A. modestus* at 6 weeks of deployment was higher (mean: 5.9% SE ± 0.017) on vertical panels than it was on horizontal panels (mean: 1.6% SE ± 0.005).

Overall there is no effect of sites on the cover of *A. modestus.* By focusing on the interactions, the sites were shown to have a significant impact on the cover of *A. modestus*, but this effect took place only at a duration of 6 weeks of deployment (significant interaction of duration x site, Table 3.8, Fig. 3.9) and at Milford Haven (significant interaction of marina x site, Table 3.8, Fig. 3.9). The cover of *A. modestus* at 6 weeks of deployment was higher (mean 6% SE ± 0.017) at the visitor site than it was at the resident site (mean 1.5% SE ± 0.005). In addition, the cover of *A. modestus* at Milford Haven was higher (mean 8.3 % SE ± 0.015) at the visitor site than it was at the resident site (mean: 5.5% SE ± 0.0.16).

Table 3.8 Analysis of Variance (ANOVA) comparing the mean cover of *Austrominius modestus* on settlement panels at different angle between Holyhead and Milford Haven marinas for 6 and 12 weeks duration. The comparison of sites was judged at significance level $p < 0.01$ as variances were heterogeneous (Cochran's *C*-test, $p < 0.05$).

Factor	df	MS	F	P
Duration	1	0.0003	0.26	0.6101
Marinas	1	0.1092	86.73	<0.001
Site	1	0.0042	3.37	0.0702
Angle	1	0.0195	15.48	0.0002
Duration X Marina	1	0.0002	0.16	0.6925
Duration X Site	1	0.0240	19.06	<0.001
Duration X Angle	1	0.0052	4.15	0.0450
Marina X Site	1	0.0051	4.03	0.0481
Marina X Angle	1	0.0195	15.48	0.0002
Site X Angle	1	0.0029	2.34	0.1299
Duration X Marina X Site	1	0.0215	17.11	0.0001
Duration X Marina X Angle	1	0.0049	3.92	0.0513
Duration X Site X Angle	1	0.0356	28.25	<0.001
Marina X Site X Angle	1	0.0029	2.34	0.1299
Duration X Marina X Site X Angle	1	0.0363	28.87	<0.001
Residual	80	0.0013		

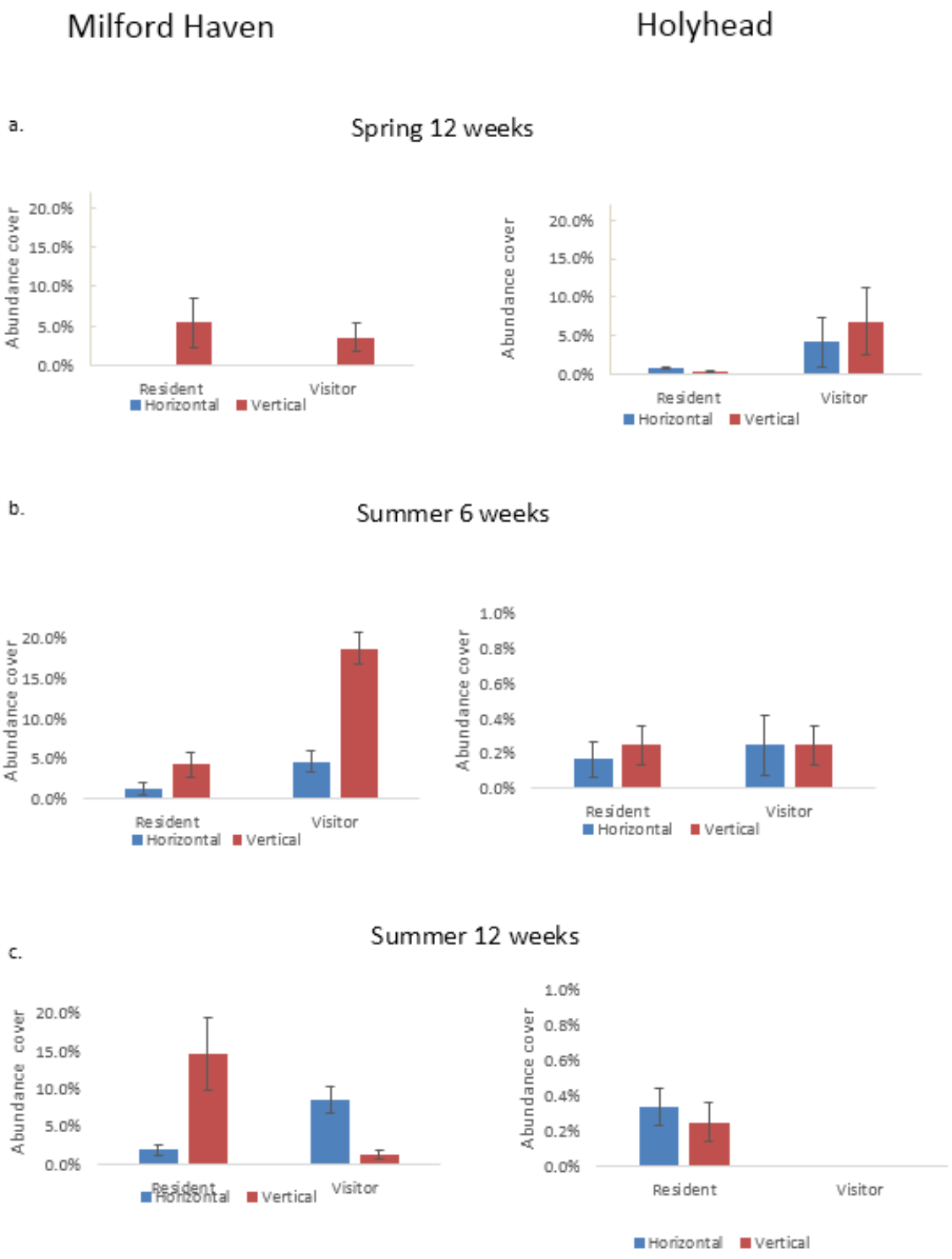


Figure 3.9 Mean (±SE, n = 6) percentage cover of *Austrominius modestus* on settlement panels placed in Milford Haven (left) and Holyhead (right) marinas in 2014. Panels immersed for a duration of: (a) 12 weeks in spring. (b) 6 weeks in summer. (c) 12 weeks in summer.

Tricellaria inopinata:

Focusing on summer data only, the duration of panel deployment was shown to have a significant impact on the cover of *T. inopinata*, but this effect only occurred for Holyhead marina (significant interaction of duration x marina: Table 3.9, Fig. 3.10). Cover of *T. inopinata* at Holyhead was higher (mean: 13.4% ± 0.016 value) after 12 weeks of immersion than it was after 6 weeks (mean: 8.6% ± 0.020).

The marinas in which panels were deployed were shown to have a significant impact on the cover of *T. inopinata*, and this effect occurred at both sites, angles and duration. Cover of *T. inopinata* was higher (mean: 11% SE ± 0.013) at Holyhead than it was at Milford Haven (mean: 0.4% SE ± 0.001).

The sites where the panels were deployed have a significant impact on the cover of *T. inopinata*, but this effect only took place at Holyhead marina (significant interaction of marina x site: Table 3.9, Fig. 3.10). Cover of *T. inopinata* at Holyhead was higher (mean: 14.7% SE ± 0.021) at the visitor site than it was at the resident site (mean: 7.3% SE ± 0.013).

The angle of the panel settlements was shown to have a significant impact on the cover of *T. inopinata*, but this effect only occurred at a duration of deployment of 6 weeks (significant interaction of duration x angle: Table 3.9, Fig. 3.10) and at Holyhead marina (significant interaction of marina x angle: Table 3.9, Fig. 3.10). Cover of *T. inopinata* on panels deployed for 6 weeks was higher (mean: 7.3% SE ± 0.021) on the horizontal panels than it was on the vertical panels (mean: 2% SE ± 0.008). Cover of *T. inopinata* on panels at Holyhead only was higher (mean: 13.9% SE ± 0.017) on the horizontal panels than it was on the vertical panels (mean: 8.1% SE ± 0.019).

Table 3.9 Analysis of Variance (ANOVA) comparing the mean cover of *Tricellaria inopinata* on settlement panels at different angle between Holyhead and Milford Haven marinas for 6 and 12 weeks duration. The comparison of sites was judged at significance level $p < 0.01$ as variances were heterogeneous (Cochran's *C*-test, $p < 0.05$).

Factor	df	MS	F	P
Duration	1	0.0111	4.23	0.0430
Marinas	1	0.2690	102.58	<0.0001
Site	1	0.0406	15.48	0.0002
Angle	1	0.0183	6.98	0.0099
Duration X Marina	1	0.0159	6.05	0.0161
Duration X Site	1	0.0002	0.06	0.8085
Duration X Angle	1	0.0151	5.74	0.0189
Marina X Site	1	0.0271	10.32	0.0019
Marina X Angle	1	0.0224	8.56	0.0045
Site X Angle	1	0.0017	0.65	0.4231
Duration X Marina X Site	1	0.0010	0.37	0.5463
Duration X Marina X Angle	1	0.0161	6.15	0.0153
Duration X Site X Angle	1	0.0087	3.30	0.0729
Marina X Site X Angle	1	0.0006	0.23	0.6309
Duration X Marina X Site X Angle	1	0.0099	3.77	0.0558
Residual	80	0.0026		

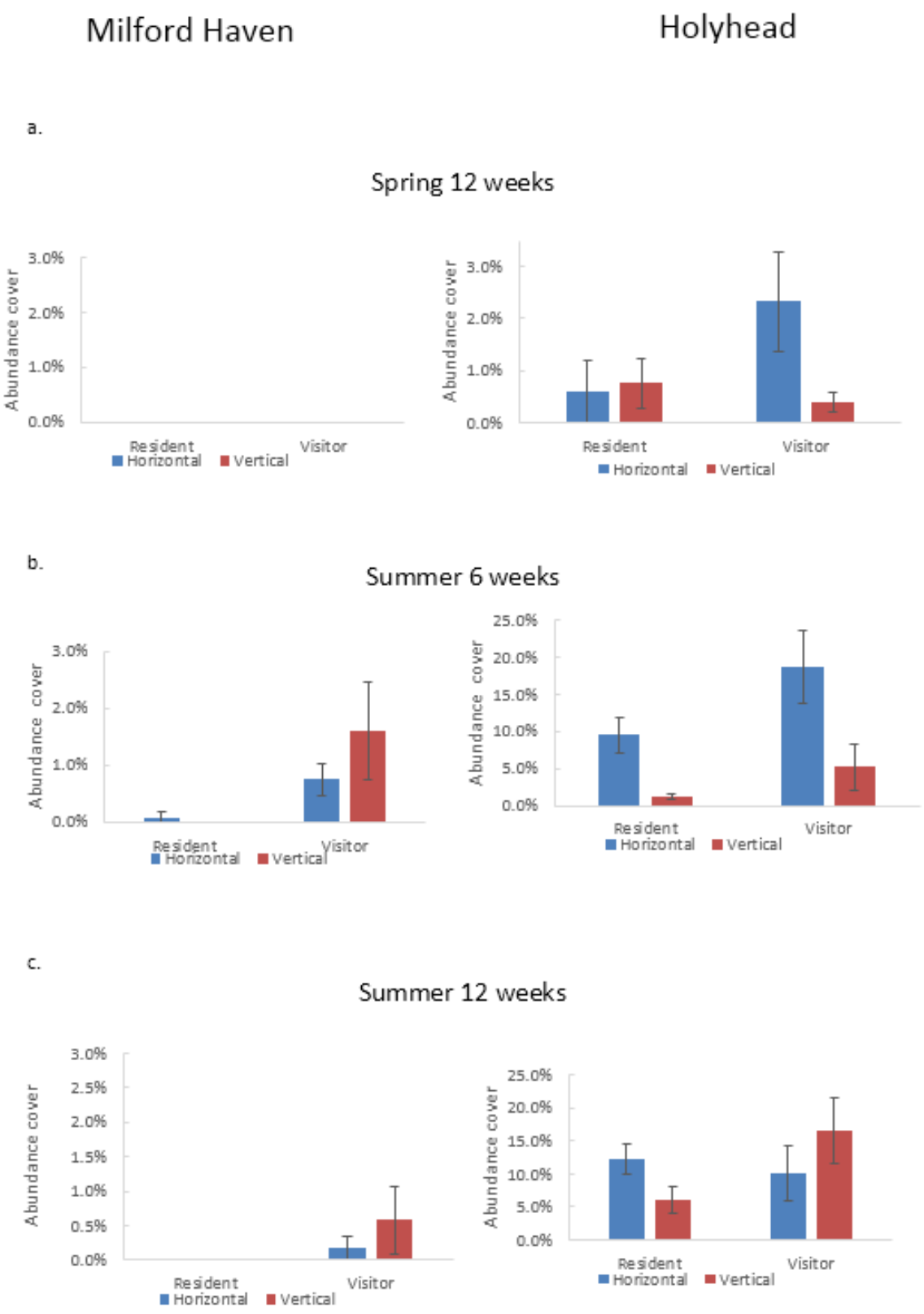


Figure 3.10 Mean (±SE, n = 6) percentage cover of *Tricellaria inopinata* on settlement panels placed in Milford Haven (left) and Holyhead (right) marinas in 2014. Panels immersed for a duration of: (a) 12 weeks in spring. (b) 6 weeks in summer. (c) 12 weeks in summer.

Corella eumyota:

Focusing on summer data only, the duration of panel deployment was shown to have a significant impact on the cover of *C. eumyota* recorded, but this effect occurred only at Holyhead marina (significant interaction of duration x marina: Table 3.10, Fig. 3.11). Cover of *C. eumyota* at Holyhead was higher (mean: 2.3% SE ± 0.009) after 12 weeks of immersion than it was after 6 weeks (mean: 0.2% SE ± 0.001).

The marinas in which panels were deployed appeared to have a significant impact on the cover of *C. eumyota*, but this effect happened only at 12 weeks of panel deployment (significant interaction of duration x marina: Table 3.10, Fig. 3.11) and for horizontal panels (significant interaction of marina x angle: Table 3.10, Fig. 3.11). The reason may be due to the low recorded abundance at 6 weeks of panel deployment. Cover of *C. eumyota* at 12 weeks was higher (mean: 2.3% SE ± 0.009) at Holyhead than it was at Milford Haven (mean: 0.6% SE ± 0.001). In addition, cover of *C. eumyota* recorded on horizontal panels was higher (mean: 2.1% SE ± 0.008) at Holyhead than it was at Milford Haven (mean: <0% SE ± <0).

There is no overall effect of angle on the cover of *C. eumyota.* Focusing on interactions, angle, however, was shown to have a significant impact on the cover of *C. eumyota*, but this effect was seen only at Holyhead marina (significant interaction of marina x angle: Table 3.10, Fig. 3.11). Cover of *C. eumyota* at Holyhead was higher (mean: 2.1% SE ± 0.008) on horizontal panels than it was on vertical panels (mean: 0.4% SE ± 0.003).

Table 3.10 Analysis of Variance (ANOVA) comparing the mean cover of *Corella eumyota:* (Cochran's test p<0.01) on settlement panels at different angle between Holyhead and Milford Haven marinas for 6 and 12 weeks duration. The comparison of sites was judged at significance level $p < 0.01$ as variances were heterogeneous (Cochran's *C*-test, $p < 0.05$).

Factor	df	MS	F	P
Duration	1	0.0027	7.07	0.0095
Marinas	1	0.0035	9.14	0.0034
Site	1	0.0011	2.89	0.0931
Angle	1	0.0015	3.94	0.0506
Duration X Marina	1	0.0024	6.27	0.0143
Duration X Site	1	0.0009	2.23	0.1397
Duration X Angle	1	0.0011	2.80	0.0981
Marina X Site	1	0.0009	2.38	0.1266
Marina X Angle	1	0.0018	4.58	0.0354
Site X Angle	1	0.0001	0.26	0.6143
Duration X Marina X Site	1	0.0007	1.79	0.1853
Duration X Marina X Angle	1	0.0013	3.35	0.0711
Duration X Site X Angle	1	0.0001	0.26	0.6143
Marina X Site X Angle	1	0.0002	0.44	0.5101
Duration X Marina X Site X Angle	1	0.0002	0.11	0.5101
Residual	80	0.0004		

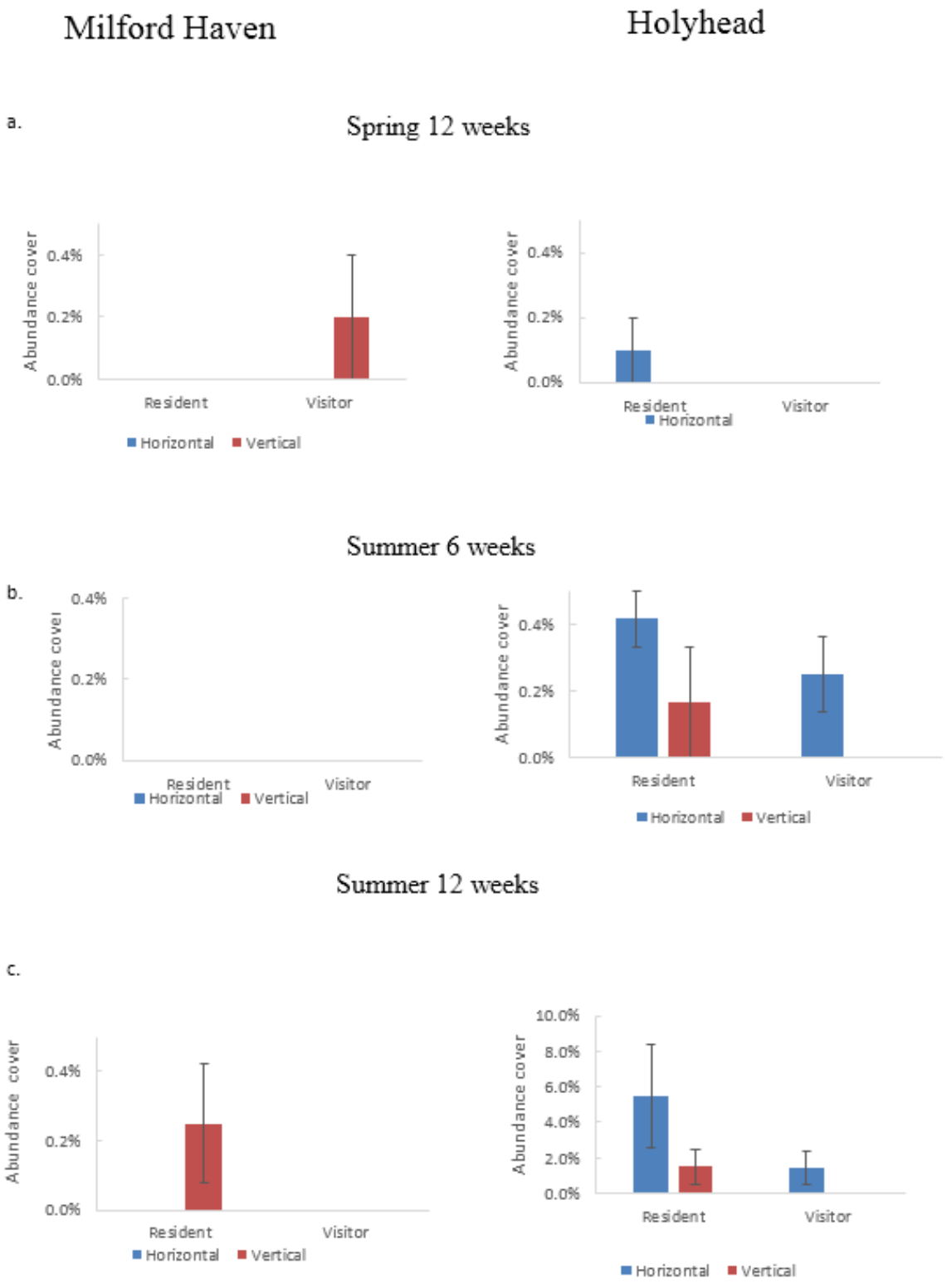


Figure 3.11 Mean (±SE, n = 6) percentage cover of *Corella eumyota* on settlement panels placed in Milford Haven (left) and Holyhead (right) marinas in 2014. Panels immersed for a duration of: (a) 12 weeks in spring. (b) 6 weeks in summer. (c) 12 weeks in summer.

Ficopomatus enigmaticus:

F. enigmaticus has not been found in Holyhead. Focusing on summer and Milford Haven data only, the duration of deployment was shown to have a significant impact on the cover of *F. enigmaticus* recorded, but the effect occurred only at the resident site (significant interaction of duration x site: Table 3.11, Fig. 3.12). Cover of *F. enigmaticus* at the resident site was higher (mean: 16.3% SE ± 0.029) after 12 weeks of deployment than it was after 6 weeks (mean: 5.7% SE ± 0.016).

The sites where the panels were deployed appeared to have an impact on the cover of *F. enigmaticus,* but the effect occurred only at 12 weeks of deployment (significant interaction of duration x site: Table 3.11, Fig. 3.12). Cover of *F. enigmaticus* at 12 weeks of deployment was higher (mean: 16.3% SE ± 0.029) at resident site than it was at visitor site (mean: 5.3% SE ± 0.014).

No effect of angle was shown.

Table 3.11 Analysis of Variance (ANOVA) comparing the mean cover of *Ficopomatus enigmaticus* on settlement panels at different angle for 6 and 12 weeks duration (Cochran's *C*-test, p = 0.1934).

Factor	df	MS	F	P
Duration	1	0.0445	9.31	0.0040
Site	1	0.0508	10.63	0.0023
Angle	1	0.0019	0.39	0.5374
Duration X Site	1	0.0251	5.25	0.0272
Duration X Angle	1	0.0016	0.34	0.5650
Site X Angle	1	0.0091	1.91	0.1747
Duration X Site X Angle	1	0.0040	0.84	0.3660
Residual	40	0.0048		

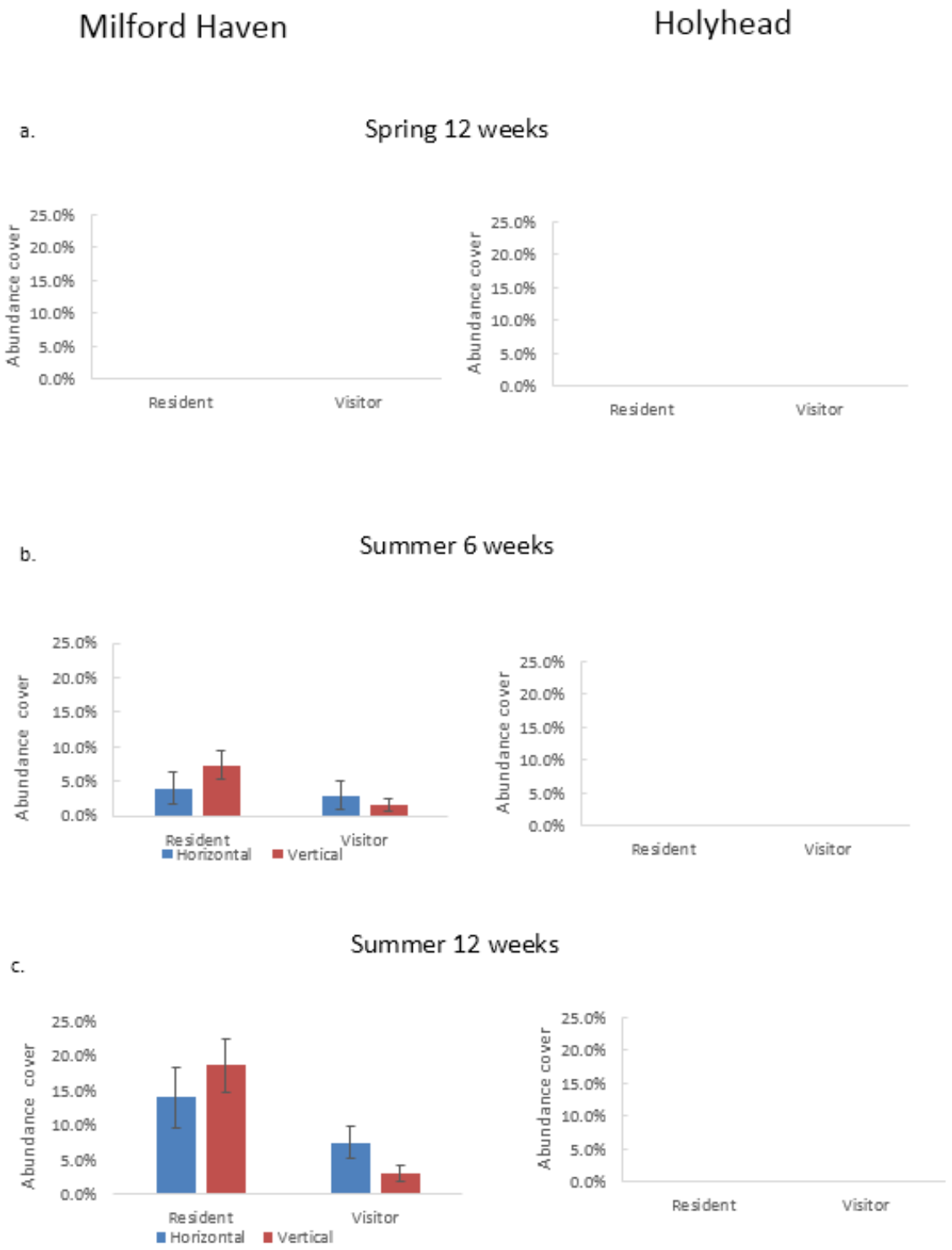


Figure 3.12 Mean (±SE, n = 6) percentage cover of *Ficopomatus enigmaticus* on settlement panels placed in Milford Haven (left) and Holyhead (right) marinas in 2014. Panels immersed for a duration of: (a) 12 weeks in spring. (b) 6 weeks in summer. (c) 12 weeks in summer.

Schizoporella japonica:

S. japonica was not found in Milford Haven marina. Focusing on summer and Holyhead data only, the duration of deployment was shown to have a significant impact on the cover of *S. japonica* (Significant duration: Table 3.12, Fig. 3.13). Cover of *S. japonica* was higher after 12 weeks (mean: 0.3% SE ± 0.001) than it was after 6 weeks (mean: 0.9% SE ± 0.002).

Table 3.12 Analysis of Variance (ANOVA) comparing the mean cover of *Schizoporella japonica* (Cochran's test $p<0.01$) on settlement panels at different angles for 6 and 12 weeks duration.

Factor	df	MS	F	P
Duration	1	0.0004	5.42	0.0251
Site	1	0.0001	0.71	0.4048
Angle	1	<0.0001	0.12	0.7306
Duration X Site	1	<0.0001	0.12	0.7306
Duration X Angle	1	0.0002	2.26	0.1409
Site X Angle	1	0.0001	1.01	0.3200
Duration X Site X Angle	1	0.0002	2.26	0.1409
Residual	40	0.0001		

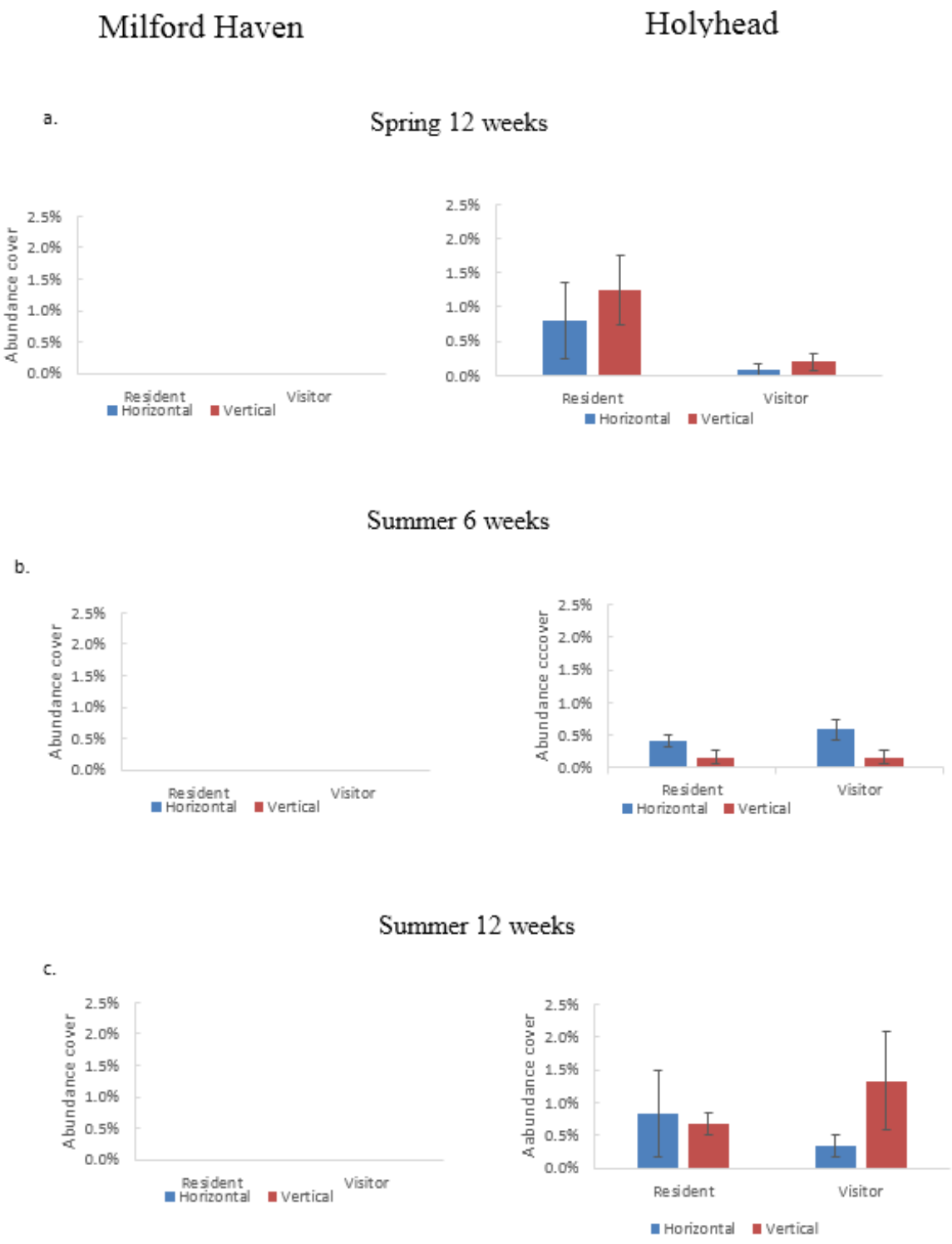


Figure 3.13 Mean (±SE, n = 6) percentage cover of *Schizoporella japonica* on settlement panels placed in Milford Haven (left) and Holyhead (right) marinas in 2014. Panels immersed for a duration of: (a) 12 weeks in spring. (b) 6 weeks in summer. (c) 12 weeks in summer.

Caprella mutica:

Focusing on summer data only, the angle of the settlement panels was shown to have a significant impact on the cover of *C. mutica* recorded, but this effect occurred only at Holyhead marina (significant interaction of marina x angle: Table 3.13, Fig. 3.14). Cover of *C. mutica* on horizontal panel was higher at Holyhead (mean: 7.1 SE ± 1.087) than it was at Milford Haven (mean: 0.333 SE ± 0.214). In addition cover of *C. mutica* at Holyhead was higher on horizontal panels (mean: 7.1 SE ± 1.087) than it was on vertical panels (mean: 1.8 SE ± 0.477). In addition,

Table 3.13 Analysis of Variance (ANOVA) comparing the mean cover of *Caprella mutica* (Cochran's test $p<0.01$) on settlement panels at different angles for 6 and 12 weeks duration.

Factor	df	MS	F	P
Duration	1	20.3965	2.16	0.1451
Marinas	1	385.0007	40.86	<0.0001
Site	1	1.9694	0.21	0.6488
Angle	1	141.9850	15.07	0.0002
Duration X Marina	1	15.2402	1.62	0.2071
Duration X Site	1	16.8757	1.79	0.1846
Duration X Angle	1	0.2871	0.03	0.8619
Marina X Site	1	2.5840	0.27	0.6019
Marina X Angle	1	118.7038	12.60	0.0006
Site X Angle	1	5.1569	0.55	0.4616
Duration X Marina X Site	1	0.5475	0.06	0.8101
Duration X Marina X Angle	1	15.6413	1.66	0.2013
Duration X Site X Angle	1	8.9121	0.95	0.3337
Marina X Site X Angle	1	6.3809	0.68	0.4130
Duration X Marina X Site X Angle	80	9.4215		
Residual	80			

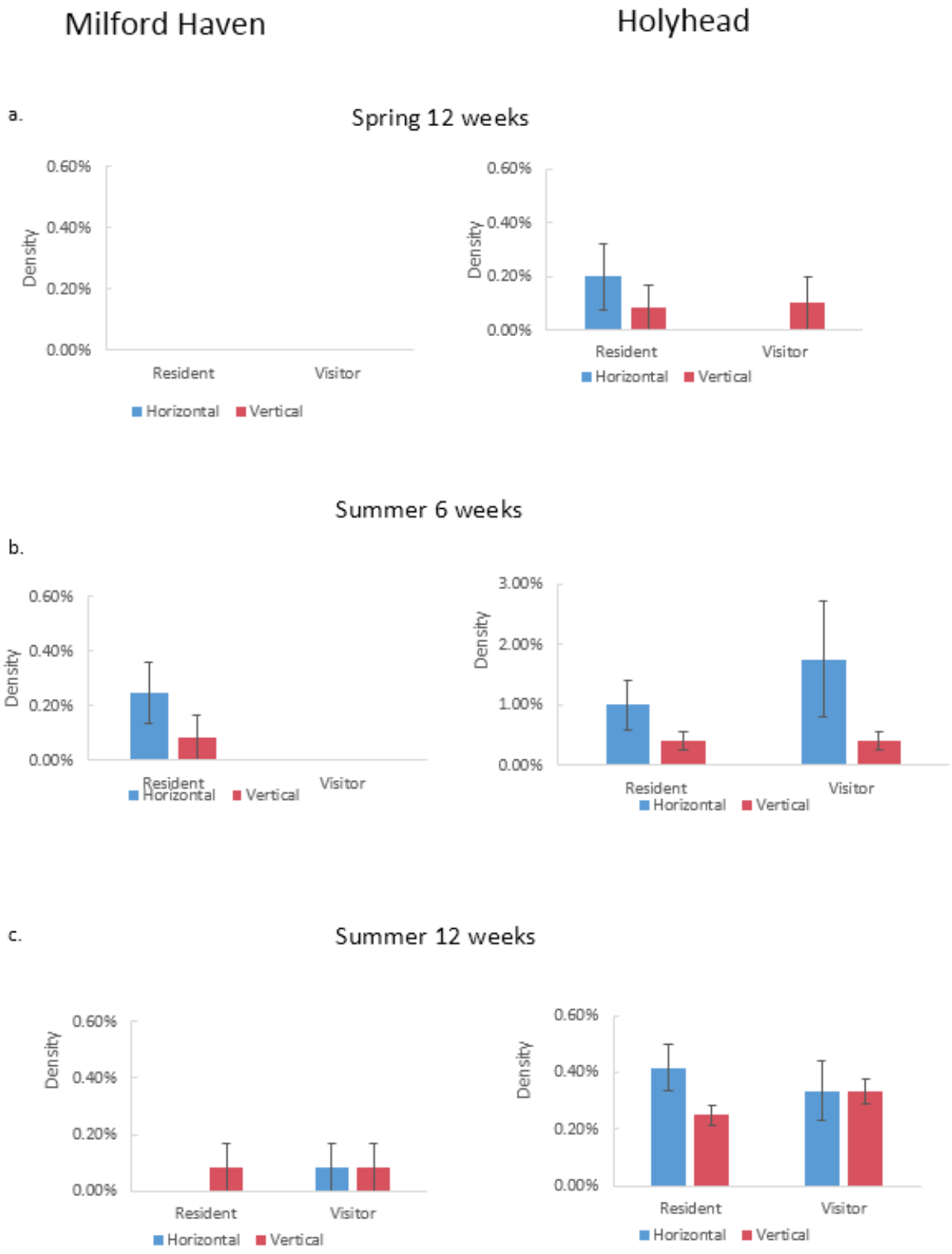


Figure 3.14 Mean (±SE, n = 6) density of *Caprella mutica* on settlement panels placed in Milford Haven (left) and Holyhead (right) marinas in 2014. Panels immersed for a duration of: (a) 12 weeks in spring. (b) 6 weeks in summer. (c) 12 weeks in summer.

Asterocarpa humilis

Asterocarpa humilis was only found at Holyhead marina. Owing to its low abundance, formal statistical analyses were not conducted. There was negligible cover found after 6 weeks and up to a maximum mean cover of 1% in the summer and 1.5% in the spring (Fig. 3.15).

Botrylloides violaceus

Botrylloides violaceus was found at both Holyhead and Milford Haven, in both spring and summer sampling but showed very low abundance with no discernible pattern among treatments (Fig. 3.16).

Didemnum vexillum

Didemnum vexillum was only found at Holyhead. It was found at very low abundance only in the summer 6 week deployment (Fig. 3.17).

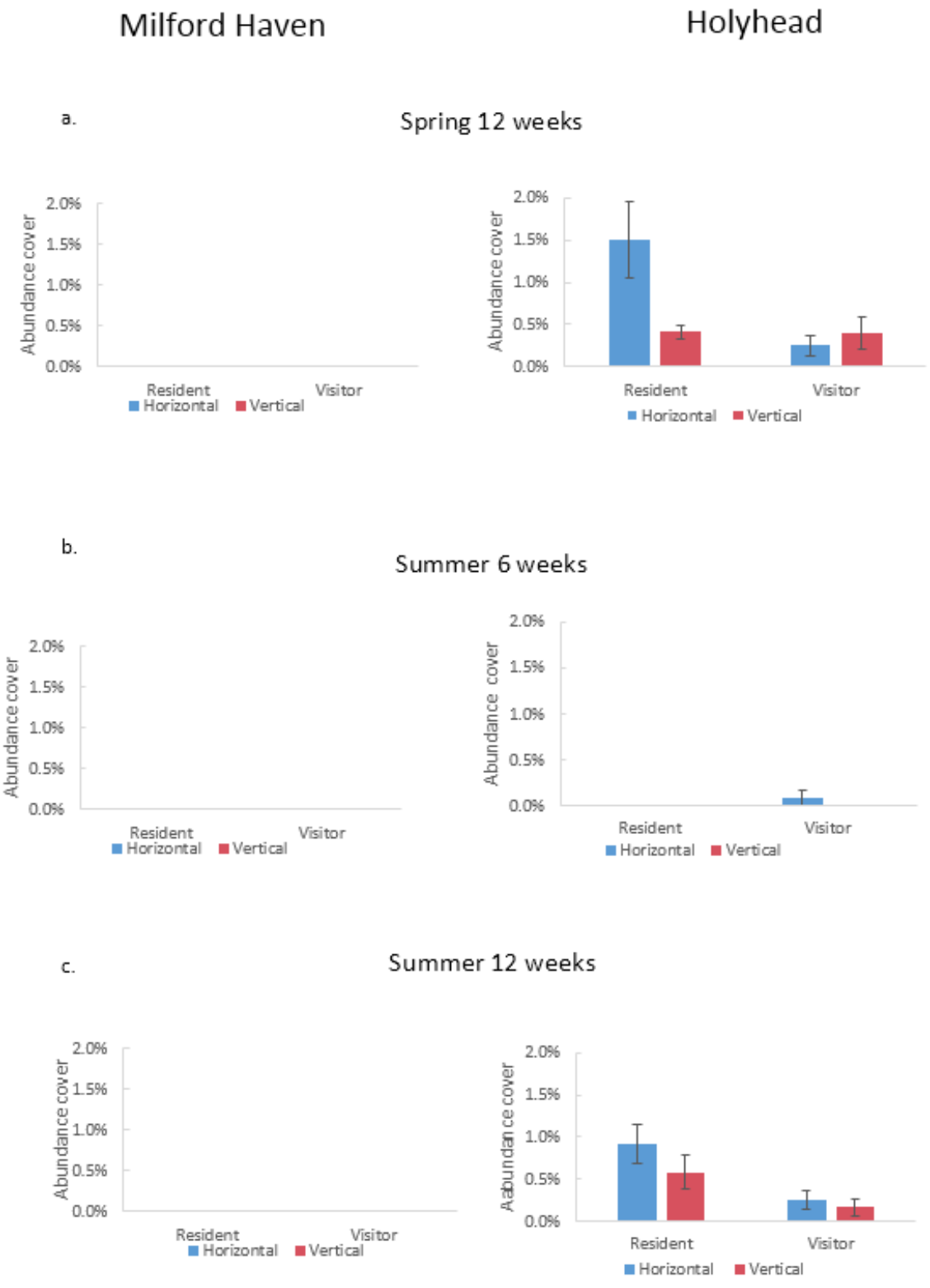


Figure 3.15 Mean (±SE, n = 6) percentage cover of *Asterocarpa humilis* on settlement panels placed in Milford Haven (left) and Holyhead (right) marinas in 2014. Panels immersed for a duration of: (a) 12 weeks in spring. (b) 6 weeks in summer. (c) 12 weeks in summer.

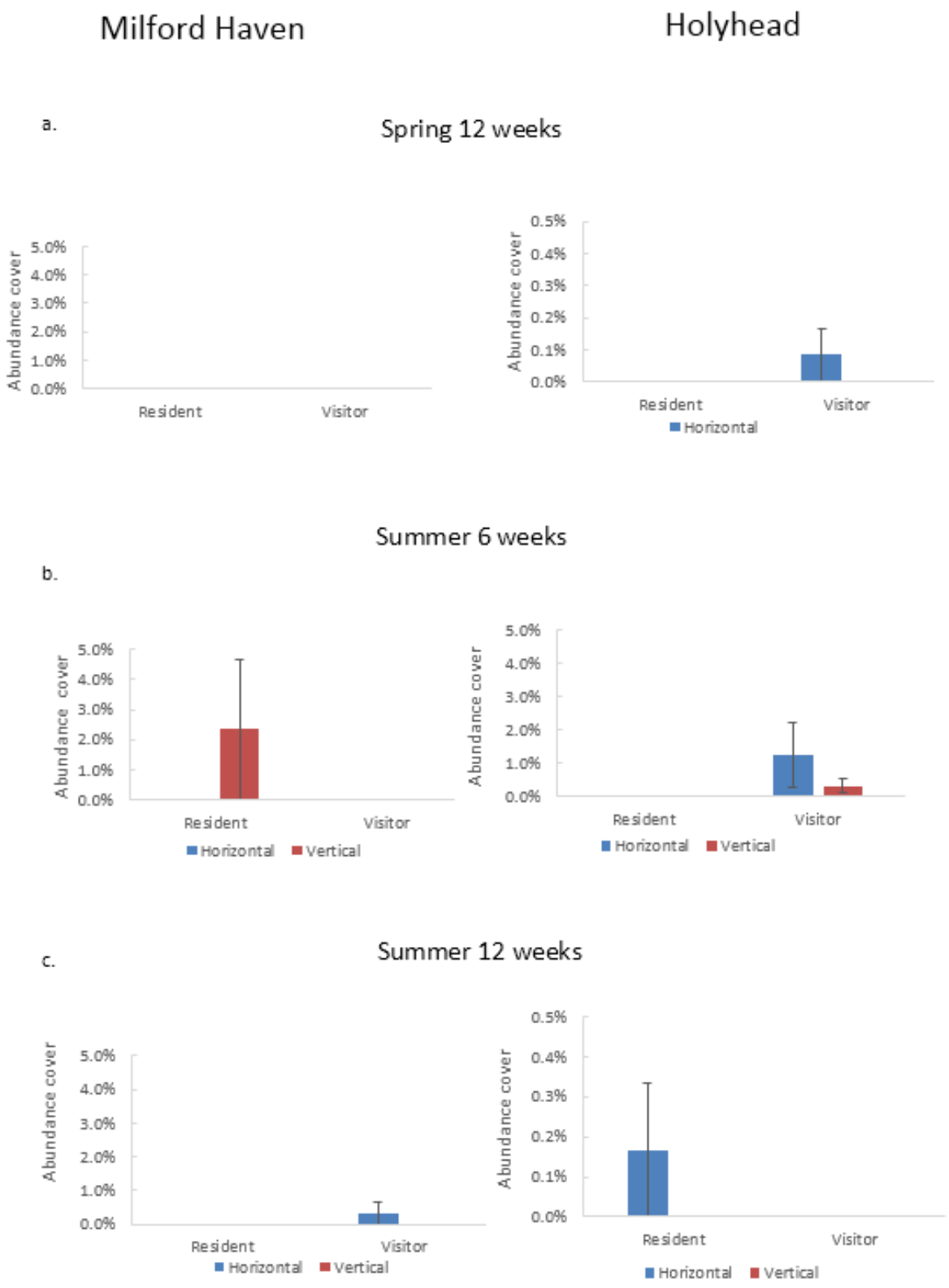


Figure 3.16 Mean (±SE, n = 6) percentage cover of *Botrylloides violaceus* on settlement panels placed in Milford Haven (left) and Holyhead (right) marinas in 2014. Panels immersed for a duration of: (a) 12 weeks in spring. (b) 6 weeks in summer. (c) 12 weeks in summer.

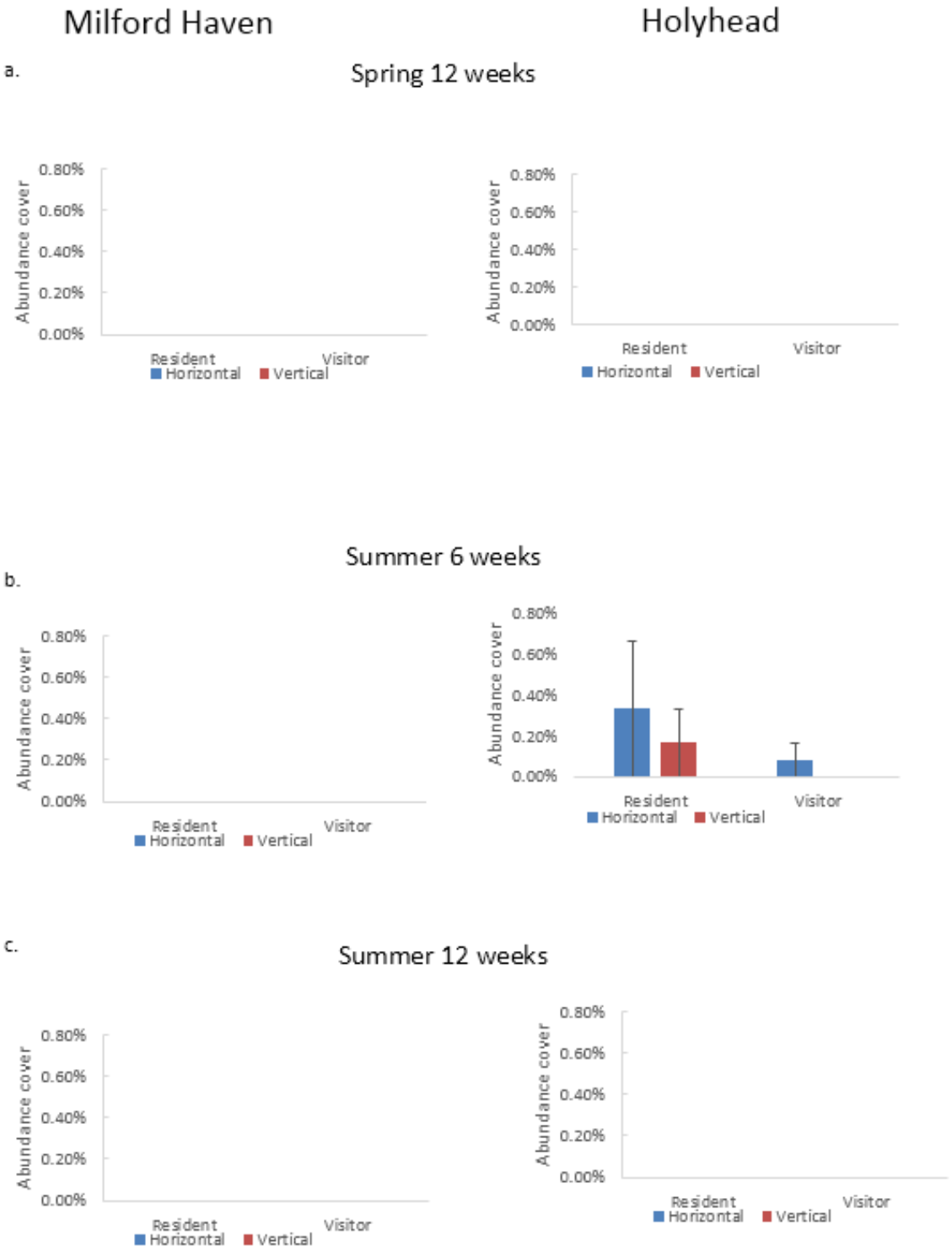


Figure 3.17 Mean (±SE, n = 6) percentage cover of *Didemnum vexillum* on settlement panels placed in Milford Haven (left) and Holyhead (right) marinas in 2014. Panels immersed for a duration of: (a) 12 weeks in spring. (b) 6 weeks in summer. (c) 12 weeks in summer.

3.3.3 Temperature

The temperature logger from Holyhead marina was not found and thus no temperature data are available for this marina. In contrast, Milford Haven's water temperature have been recorded and show no significant difference between resident and visitor sites in Milford Haven marina (T-test: t = 1.0409, p=0.298). The average temperature during the monitoring in spring (08/04- 06/07) was 14.33°C (SE ± 0.054) with a minimum of 10.43°C and maximum of 18.50°C. The average temperature during the monitoring in summer (06/07–07/10) was 18.10°C (SE ± 0.007) with a minimum of 15.76°C and maximum of 20.53°C. Overall mean temperature from April to October 2014 in Milford Haven marina was 16.25 °C.

3.4 Discussion

This study examined a number of monitoring techniques that may determine the efficacy of non-native detection within a marina. We examined the impact of location within marina (visitor vs resident) over two different sampling periods (spring vs summer and 6 weeks vs 12 weeks) and investigated whether there is a difference between the sampling potential of vertical and horizontal panels. It seemed that horizontal panels were the more efficient technique for detecting non-native species as average species richness was greater on panels of this orientation. However, vertical panels were still effective and recorded the same suite of species. When considering the site treatment (resident versus visitor) the location of the panels within the marina had no effect on the overall number of non-native species detected. Seasonality was clearly an important factor in detecting non-natives as a far greater number of non-natives in summer compared to spring. For example, some non-natives were present on panels exclusively in summer (i.e. *D. vexillum* and *B. neritina*).

In terms of species abundance, the effect of panel angle was significant for four of the non-native species recorded; the abundance of *T. inopinata, C. eumyota* and *C. mutica* was greater on horizontal panels than vertical panels, indicating that panel orientation can result in important variation of community assemblages. This pattern may be explained by environmental conditions within the marina such as light and shade (Connwell, 1999; Glasby, 2000). The preference of sessile organisms to colonise horizontal or vertical surface has been shown in several studies (Glasby, 2000; Connell, 1999; Schmidt, 1982) and was also observable in the colonisation patterns of native species recorded in this study. For example, the native spirorbis polychaete was consistently more abundant on vertical panels than horizontal ones and *Watersipora subtorquata* was more abundant on horizontal than vertical surface, as was found by (Glasby, 2000; Connell, 1999). In the present study, the colonisation by *C. eumyota* was greater on horizontal panels, which is an agreement with findings reported by Glasby (2000) where solitary ascidians were more abundant on the horizontal underside of panels (2000). Therefore, panel orientation should be considered in future monitoring surveys.

When the impact of monitoring techniques was assessed on an individual species basis, the effect of deployment site within the marinas had a significant impact on four of the non-native species; *B. neritina, A. modestus* and *T. inopinata* were more abundant on settlement panels deployed at visitors berths, while *F. enigmaticus* was more abundant on panels at the residents berth. Few studies have compared panels placed outside and inside the marinas (Turner et al. 1997, Webb & Keough, 2000) so general conclusions about the effects of location on fouling assemblages are difficult to make. These differences may not be attributable to the "location" as such, but may have more to do with the hydrodynamics or salinity fluctuations in those locations.

Results indicated that colonisation of non-natives was higher after a 12 week deployment than after 6 weeks. Similarly, species richness of non-natives was greater after 12 week deployment than it was after 6 weeks. Therefore longer-term deployment of panels would be more effective for the purposes of monitoring non-natives.

In the current study, the presence and abundance of certain non-native species did differ among marinas. Such differences may be due to the characteristics of the marina which may influence the type of non-native species that can survive there; Holyhead marina is an open saline marina, whilst Milford Haven marina is an enclosed basin that experiences freshwater influx. The water temperature varied greatly across seasons in Milford Haven marina with more than 5 degrees difference between spring and

summer. Being a non-tidal basin, the water temperature is increasing faster than an open area creating a new habitat with specific environmental conditions.

The various different methodologies used within this study had little effect on the actual number of species detected (except deployment season and duration). However, the deployment locations and the panel orientation did have impacts on the abundance of different non-natives. It could be argued that a technique which increases the abundance of particular non-natives may maximise the ability to detect that species. Thus, in conclusion, panels deployed at different orientations and with a wide distribution in the marina would probably maximise the ability to detect the maximal range of non-native species.

4. Aquaculture sites

4.1 Aquaculture and non-native species

Aquaculture activity continues to increase and is one of the major marine pathways for introduction of non-natives (Minchin 2006; Ruis et al. 2011). There are numerous examples of aquaculture practices leading to the introduction of unwanted species either through deliberate introduction (for example the Pacific oyster, *Crassostrea gigas*) or through non-native 'hitch-hiking' on aquaculture products (Minchin 2006; Ruis et al. 2011). Although aquaculture is regulated and management practices have improved over the years, the risk of invasion is still present.

Natural habitats are modified by aquaculture activities. For example, a comparative study of the habitat value of shellfish aquaculture gear and natural habitats showed that aquaculture provides a greater habitat value than non-vegetated seabed (Dealteris et al. 2004). Indeed, this work indicated that aquaculture gear provided substrate for sessile invertebrates and the abundance of organism per m^2 on the gear was higher than that on the seabed (Dealteris et al. 2004). With the resurgence of aquaculture activities, greater numbers of artificial structures are being created with new environmental conditions and this may increase the risk of the establishment of non-native species (Ruis et al. 2011).

There is potential within Wales for aquaculture activities to lead to non-native colonisation. Wales is the UK leader for seabed mussel production and accounted for 8,996.0 tonnes of the total 26,021.3 tonnes of mussel harvested in UK in 2012 (Cefas, 2015). The Menai Strait East Order area, which consists of four companies based in Bangor, produces 7-10,000 tonnes of mussels per year. The juvenile mussels or seeds cultured are imported from different locations, making the potential risk of non-native species introduction higher. This was illustrated in 2006 when the non-native slipper limpet (*Crepidula fornicate*) was accidentally introduced into the Menai Strait with a consignment of mussel spat.

Here we focus on two oyster farms along the Menai Strait as aquaculture sites which could potentially be a focus for arrival of non-natives. The intertidal nature of the sites and the aquaculture structures make sampling logistically possible. Sampling was conducted over the spring and summer of 2014 to assess the effectiveness of non-native detection using settlement panels and oyster shell as substrata.

4.2 Trial aquaculture sites in Wales

4.2.1 Study area

Two oyster farms in the Menai Strait were chosen to determine the effectiveness of using aquaculture sites to monitor for the arrival of non-native species. These farms were the Menai Oysters & Mussels Ltd and Plas Menai both of which are situated in the Menai Strait, within the Menai Strait and Conwy Bay Special Area of Conservation. Both farms use the bag culture technique in which the non-native Pacific Oyster, *Crassostrea gigas*, is farmed. Large mesh bags containing oyster spat are attached to trestle tables in the intertidal zone. This type of culture requires intensive labour since to produce efficient oyster stocks, the bags must be thinned out regularly as the oysters grow in size. The oyster production for Menai Oysters & Mussels Ltd is located away from the mussel beds to prevent mussel settlement within the oyster bags. Menai Oysters & Mussels Ltd produce 10 to 12 tonnes annually. The stock is sourced from Morecambe Bay.

4.2.2 Pilot study

Prior to the full-scale monitoring trial, a one-month pilot from 20th October to 17th November 2013 was conducted at the site of Menai Oysters & Mussels Ltd. This study provided valuable information for the full-scale study. For example low stocking densities in experimental oyster bags led to movement and possible abrasion. Thus higher stock densities of oyster shells *(*35 *Crassostrea gigas* per bag*)* were used in the full scale study.

4.2.3 Experimental design and implementation

PVC settlement panels (10cm x 10cm) and oyster shell were used as substrata to assess colonisation by non-native species. These were deployed over the spring and summer 2014 at 2 locations, Menai Oysters & Mussels Ltd and Plas Menai (Fig. 4.1). Three distinct sites within the low shore at each location were used (i.e. trestle table, 1, 2 and 3) and sampling undertaken over 2 different periods (4 and 12 weeks) (Fig. 4.2). (Initially high shore sites were used as well but colonisation was minimal and thus this approach was abandoned and only low shore data will be presented.) At each site (trestle table) an oyster bag was used to deploy the settlement units (made up of oyster shell and PVC settlement panels). Each bag contained 35 oyster shells and had 10 PVC settlement panels attached facing down to the seabed (Fig. 4.3 and 4.4). 5 PVC settlement panels and 5 oyster shells were sampled at each of the two sampling periods (4 and 12 weeks).

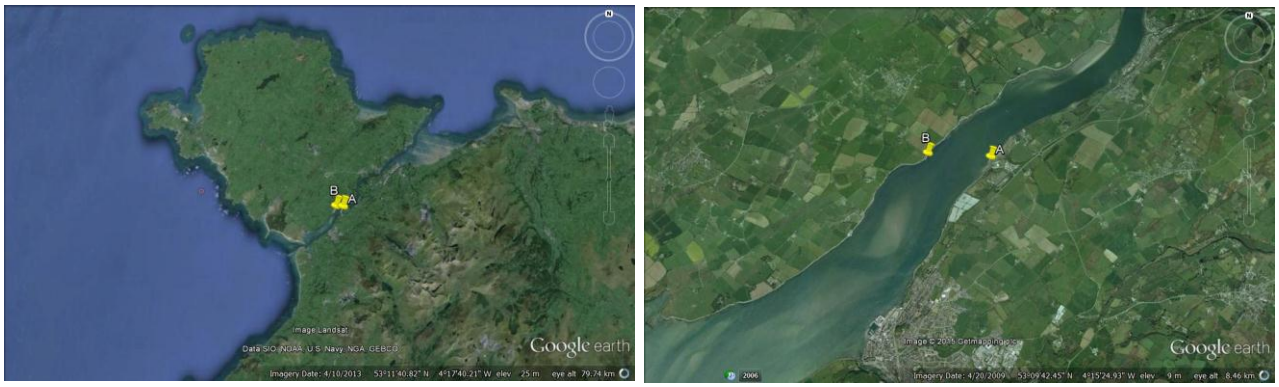


Figure 4.1 Oyster farms chosen for the experiment, Plas Menai (A) and Menai Oysters & Mussels Ltd (B) (source: Google earth).

Detailed procedures

Experimental oyster bags were prepared by placing 35 *Crassostrea gigas shells* (25 of which were convex) in a 14mm diamond mesh bag (0.5m x 1m). Oyster shell size was standardised based on the grading system employed by the aquaculture operation for determining oysters ready for commercial sale. Prior to deployment, oyster shells were immersed in freshwater for a minimum of 3 days then air-dried outside in order to kill any marine organisms. After this decontamination process, shells were cleaned using a wire brush and scraper to remove any remaining epibiota. Oyster bags were sealed using joncs. 10 PVC settlement panels (5 for 4 week and 5 for 12 week deployments) were attached to the underside of the mesh bag using cable ties with the roughened side of the panel facing down (Fig 4.3).

Codes were etched onto the smooth side (back) of the panels, for identification of site position on return to the laboratory. A settlement panel was placed on the top of the bag to identify the location and to ensure the bag was not moved during the day-to-day oyster farm operational activities.

Following collection, panels were photographed before being transported back to the laboratory for identification (Fig. 4.5 and 4.6). Five convex oyster shells were also selected from each oyster bag in spring and transported back to the laboratory. Secure storage racks were used for transporting panels and oyster shells were placed into a sealable plastic bag containing details on location, taking care not to disturb any of the epibiota on the shell. In the lab the panels and oysters were maintained in water tank with airline (without water flow). When this was not possible, organisms were relaxed in menthol crystal then preserved in 70% industrial methylated spirit (IMS). Panels and oyster shells were analysed under a dissecting microscope (x10 magnification). Organisms were identified to species level when possible and the percentage cover of each species was estimated. Length and width of each oyster shells were also recorded.

Deployment for the spring sampling was made on the 15th and 16th April 2014 and for the summer sampling on 17th and 18th of July. In both spring and summer, collection of panels and oysters for sampling was made 4 weeks (16th and 18th of May-Spring; 14th of August-Summer), and 12 weeks later (14th of July-spring; 13th and 14th of October-summer). Owing to low settlement on the panels of spring deployment, only data collected in summer as been used in analyses.

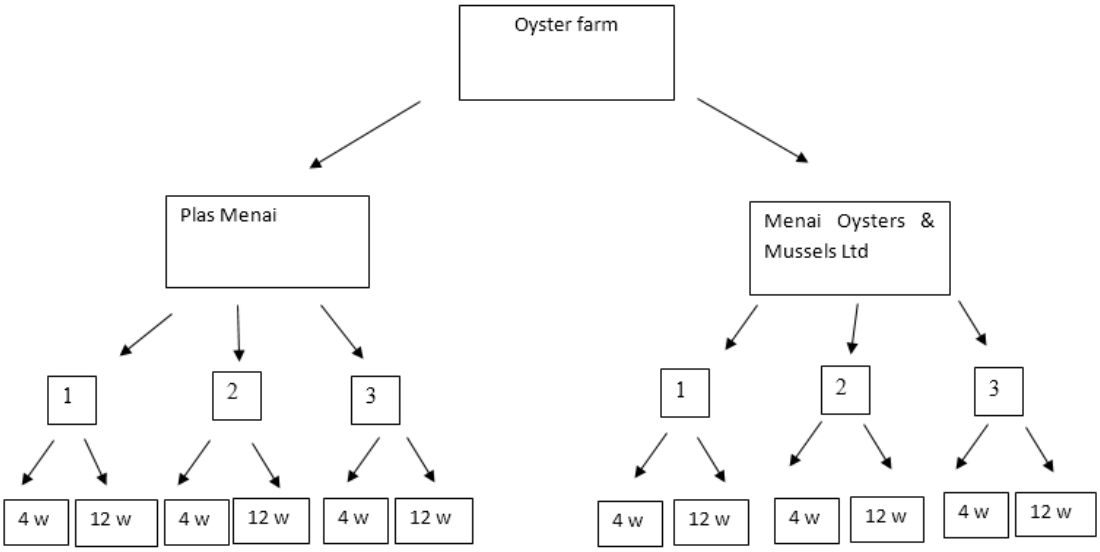


Figure 4.2 Sampling design for the inshore monitoring trial in aquaculture site in summer 2014. Each site (1, 2 and 3) consisted of an oyster bag with 10 panels attached (n = 5 for 4 weeks, n = 5 for 12 weeks) and filled with 35 *Crassostrea gigas* shells.

Figure 4.3 PVC settlement panels (n = 5 for 4 weeks, n = 5 for 12 weeks) on oyster bags filled with 35 Crassostrea gigas oyster shells. Once attached, the panels were facing down the trestle table.

Figure 4.4 Experimental oyster bags at the oyster farm showing low and high shore positions.

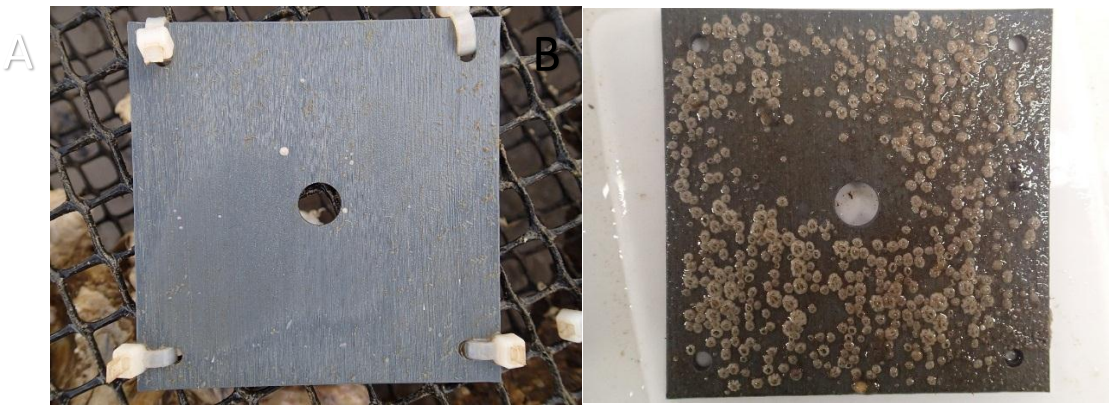


Figure 4.5 Settlement panels collected in spring 2014 at low shore location of Mania Oysters & Mussels Ltd. A. after 4 weeks. B. after 12 weeks.

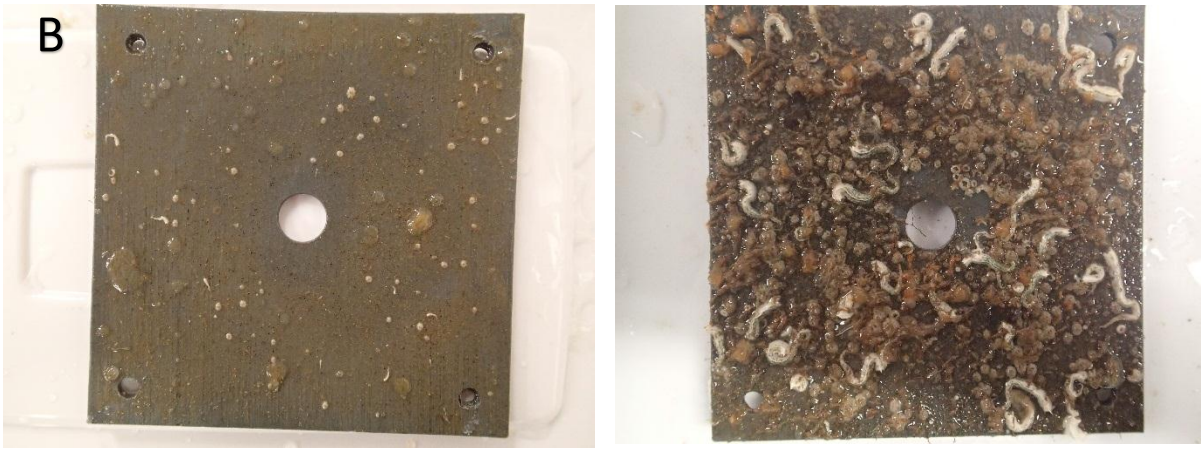


Figure 4.6 Settlement panels collected in summer 2014 at low shore location of Menai Oysters & Mussels Ltd. A. after 4 weeks. B. after 12 weeks.

4.2.4 Data analysis

Dominant non-native species were analysed in a three-way ANOVA with duration, location and site as factors (GMAV5, Underwood and Chapman 1989). The non-natives of interest were the barnacle *Austrominius modestus* and the ascidian *Corella eumyota*. The abundance of *C. eumyota* was recorded as count data while *A. modestus* was recorded as percentage cover. When the outcome from the Cochran test was significant and no transformation possible, the analysis of variance was still completed as the sample size was large enough, making the analysis robust from the start (N > 30; Underwood 1981, 1997). When differences among treatments were found, SNK tests were performed to determine the significance of difference between factors.

4.3 Results

Only 2 non-native species, the barnacle *Austrominius modestus* and the ascidian *Corella eumyota* were found on the settlement panels and oyster shells.

4.3.1 Abundance of commonly found non-native species

Austrominius modestus

Focusing on summer data, the duration of the deployment was shown to have an impact on the abundance of *A. modestus* recorded at both locations (significant interaction of duration x location, Table 4.1, Fig. 4.7). Cover of *A. modestus* recorded at Plas Menai was higher (mean: 44.6% ± 0.035) at 12 weeks of deployment than 4 weeks (mean: 14.9% ± 0.027). At Menai Oysters & Mussels Ltd, cover of *A. modestus* was higher (mean: 16.4% ± 0.024) at 12 weeks of deployment than 4 weeks (mean: 7.1% ± 0.009).

In addition, the location of the deployment appeared to have an impact on the abundance of *A. modestus* at both duration (significant interaction of duration x location, Table 4.1, Fig. 4.7). Cover of *A. modestus* recorded at 4 weeks was higher (mean: 14.9% ± 0.027) at Plas Menai than at Menai Oysters & Mussels Ltd (mean: 7.1% ± 0.009). Cover of *A. modestus* recorded at 12 weeks was higher (mean: 44.6% ± 0.024) at Plas Menai than at Menai Oysters & Mussels Ltd (mean: 16.4% ± 0.024).

Table 4.1 Analysis of Variance (ANOVA) comparing the abundance of *Austrominius modestus* on the settlement panels at different sites between Menai Oysters & Mussels Ltd and Plas Menai for 4 and 12 weeks duration (Cochran's *C*-test, p = 0.2795).

Factor	df	MS	F	P
Duration	1	0.5683	55.49	<0.001
Location	1	0.4852	47.38	<0.001
Site	2	0.0032	0.31	0.7354
Duration X Location	1	0.1577	15.39	0.0003
Duration X Site	2	0.0021	0.20	0.8173
Location X Site	2	0.0110	1.07	0.3510
Duration X Location X site	2	0.0074	0.72	0.4908
Residual	48	0.0102		

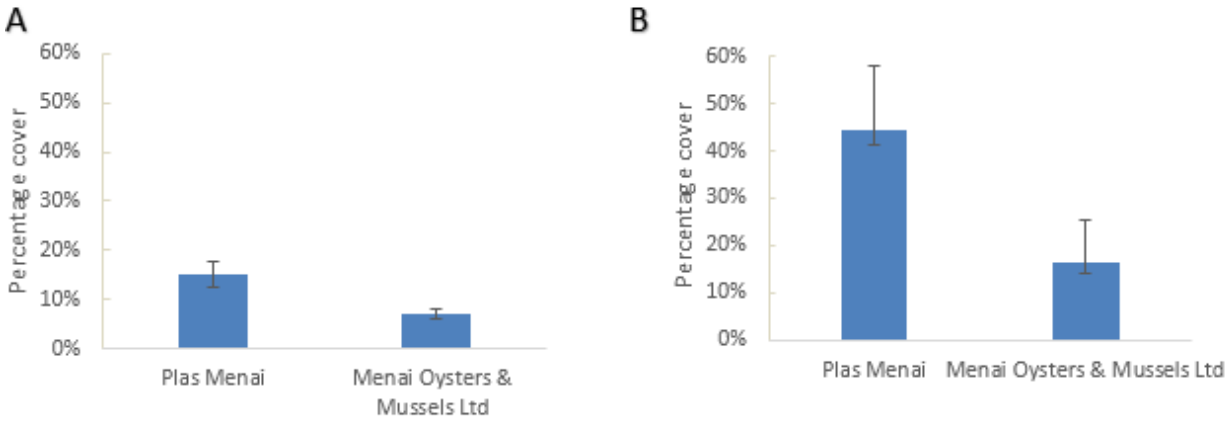


Figure 4.7 Mean percentage cover of *Austrominius modestus* at two oyster farms in North Wales during summer 2014. A. after 4 weeks. B. after 12 weeks.

Corella eumyota:

Focusing on summer data, the location of the panel's deployment was shown to have an impact on the density of *C. eumyota*, but this effect occurred only at the far left site of the oyster farm's trestles (significant interaction of location x site: Table 4.5, Figure 4.10). Density of *C. eumyota* recorded on panels attached on low shore trestle table at L1 of the oyster farm was higher at Menai Oysters & Mussels Ltd location (mean: 12 ± 2.4) than it was at Plas Menai (mean: 0 ± 0).

The site of deployment (1, 2, and 3) was revealed to have a significant impact on the density of *C. eumyota*, but this effect happened only at Menai Oysters & Mussels Ltd location (significant interaction of location x site: Table 4.2, Fig. 4.8). Density of *C. eumyota* recorded on panels at Menai Oysters & Mussels Ltd location was higher (mean: 12.0 ± 2.4) at LB1 than it was at LB2 (mean: 1.0 ± 0.795). In addition, density of *C. eumyota* recorded at Menai Oysters & Mussels Ltd location was higher (mean: 12.0 ± 2.4) on LB1 than it was at LB3 (mean: 2.3 ± 0.93). Density of *C. eumyota* at LB3 was not significantly different that it was at LB2.

Duration did not have an impact on the density of *C. eumyota*.

Table 4.2 Analysis of Variance (ANOVA) comparing the abundance of *Corella eumyota* on the settlement panels at different sites between Menai Oysters & Mussels Ltd and Plas Menai for 4 and 12 weeks duration. The comparison of location was judged at significance level $p < 0.01$ as variances were heterogeneous (Cochran's *C*-test, $p < 0.05$).

Factor	df	MS	F	P
Duration	1	21.6000	1.77	0.1902
Location	1	385.0667	31.48	<0.001
Site	2	19.2667	14.65	<0.001
Duration X Location	1	24.0667	1.97	0.1672
Duration X Site	2	7.4000	0.60	0.5502
Location X Site	2	182.0667	14.88	<0.001
Duration X Location X site	2	6.6667	0.54	0.5834
Residual	48	12.2333		

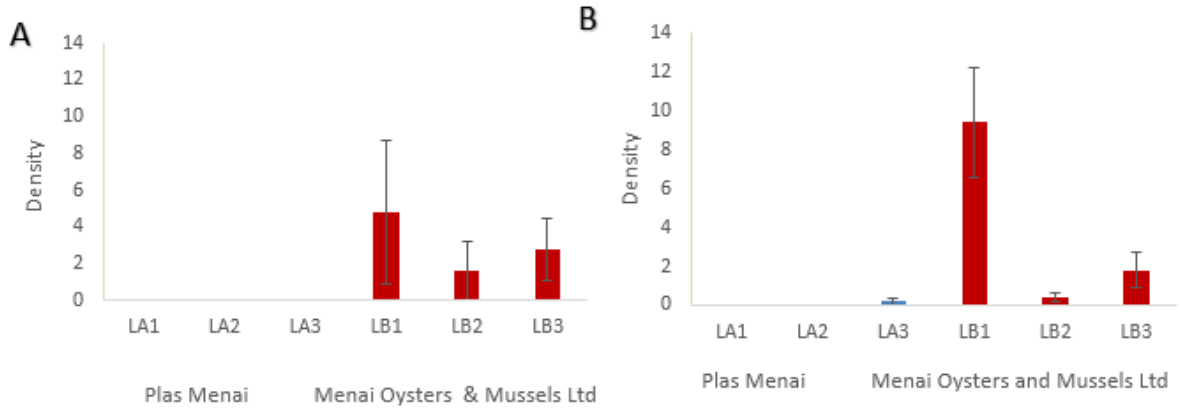


Figure 4.8 Density of *Corella eumyota* at two oyster farms in North Wales during summer 2014. A. after 4 weeks. B. after 12 weeks.

4.4 Discussion

Only 2 non-natives were recorded during the survey (i.e. *Corella eumyota* and *Austrominius modestus*).

Despite the low number of non-natives detected there are lessons learnt from the sampling procedures examined. The clear difference in abundance between the seasons, with limited colonisation during spring, clearly indicates sampling should take place in summer months. Indeed, the low recruitment in spring made statistical analysis difficult to carry out. Additionally, sampling carried out across shore heights clearly indicated sampling effort should be concentrated mainly on the low shore.

It was interesting to see that the abundance of non-natives was significantly higher in a productive oyster farm. If the bag with settlement panels was deployed on an empty trestle table, the presence of non-natives declined. In fact, the oyster farm at Plas Menai was not as active as Menai Oysters & Mussels Ltd. This could explain the reduced settlement of *C. eumyota* at Plas Menai. It is also important to note that *A. modestus* was a dominant species at Plas Menai and occupied most of the available space on the settlement panels. This may have prevented the settlement of other species and may explain the significant difference in abundance of *C. eumyota*.

In term of method trialling, the oyster shell method was interesting but a long time was required to process the samples. An oyster shell is rough with tiny crevices creating more surface to settle and differ greatly in size, making hard to achieve a standardised analysis. In addition, less *C. eumyota* were found on the shell compared to the panels. Empty oyster shells may have lost their biogenic engineering qualities value. As Smyth and Roberts (2010) found out, living oyster shells show higher species diversity than non-living hard substrata.

No algae colonised panels and only few filamentous algae were present on the oyster shells. This might be due to lack of direct sunlight as panels are positioned facing the ground. However this approach is clearly inappropriate for sampling algae.

It is important to note the presence of a polyclinid ascidian during the summer period, which looks like *Aplidium glabrum*. *Aplidium glabrum* is a northern species and its distribution in the UK is limited to the North of Scotland (Millar, 1966). This polyclinid ascidian was recorded at Holyhead marina, Port Dinorwic (Y Felinheli) and Victoria Dock (Caernarfon) during the rapid assessment survey carried out by MBA (Comprehensive Reassessment of Non-Native Species in Welsh marinas, January 2015). Researchers at the MBA consider that a rapid southern extension of *Aplidium glabrum* is unlikely and speculate on the observed species being an *Aplidium* species, not yet identified formally which is not native to the UK. Further investigation is needed.

5. Crab/lobster pots sites

5.1 Crab/lobster pots and non-natives species

A potentially convenient means of deploying and retrieving coastal settlement panels sub-tidally is through collaboration with crab and lobster fishers. To assess the potential use of crab/lobster fisheries as monitoring sites within an Inshore Monitoring Network a small-scale trial was attempted.

5.1.1 Methods

Following contact with the Welsh Fisherman's Association it was agreed that a representative would distribute settlement panels among local fishermen and advise them of the survey protocol. The survey was designed to deploy one pot at each of 5 five sites around Wales (Porth Colmon; Rhoscolyn; Swansea; Aberystwyth; Pwllheli) (Fig. 5.1). The lobster pots (Fig. 5.2) assigned for survey purposes were modified by securing five pre-roughened PVC panels onto the external frame of the pot using cable ties (ensuring that the rough side of the panel was facing outwards). Fishermen were requested to deploy their pot using the techniques utilised during their normal fishing practice and deploy using a set-and-forget approach, where they were asked not to check the pot and only retrieve the pot after a period of 8 weeks.

Figure 5.1 Location of lobster pot sites.

Unfortunately there was a breakdown in communication from the representative from the Welsh Fisherman's Association and the pots were not collected at the designated times. In addition, despite repeated requests, no information had been passed back to the project about the participating fishermen, so the whereabouts of the lobster pots remained unknown and direct communication with the fishermen could not be initiated.

In February a different representative from WFA made contact with us and he offered to locate the panels and make contact with the fishermen. He reported back that 4 pots had been lost in the winter storms and only the survey pot from Rhoscolyn remained intact. Due to adverse weather since our communication this fishermen has yet to retrieve his pot. Another fisherman reported back that he has

repeatedly observed a lot of growth on the plastic opening of his lobster pots, a sample of one of these will be collected and their suitability for monitoring purposes will be assessed. The use of the pot-openings may be a novel approach that could be easily utilised for monitoring non-natives.

Figure 5.2 The type of lobster pot planned to be used in non-native monitoring

6. Algae survey of monitoring

6.1. Introduction

This work was commissioned by the School of Ocean Sciences, Bangor University as part of the Natural Resources Wales project "Wales Marine Non-Native Species Inshore Monitoring Network".

A visit was made to study the seaweeds of Holyhead Marina pontoons by Francis Bunker (FB) and Mathilde Bue (MB) on 30th September 2014.

6.2.Methods

Seaweeds from five different locations around the marina were studied:

1. Visitor pontoon exposed
2. Visitor pontoon sheltered
3. Visitor pontoon side near entrance exposed
4. Resident pontoon inside middle (both sides)
5. Resident pontoon inside second pontoon

MB had been involved with similar studies looking for animals on the pontoons and directed FB to the study locations. Timed 30 minute searches were undertaken at each study location. where FB entered the water dressed in a dry suit and snorkelled to look for different species of seaweed and estimated their abundance on a six point SACFOR abundance scale (Hiscock, 1996). MB directed notes of the species identified and abundances estimated. Specimens that could not be reliably identified in the field were taken for later examination.

Some photographs were taken using an Olympus TG-3 waterproof camera.

Species noted and their abundance at each study location were entered into an Excel spreadsheet and the names and authorities matched with the WoRMS online database (www.marinespecies.org).

6.3.Results

A total of 43 different seaweeds were encountered during the survey, including four non-native species which are considered briefly below (Table 6.1). The complete results are supplied separately in appendix C.

Table 6.1. An inventory of seaweeds collected from Holyhead Marina - 30th September 2014. O= Occasional; R = Rare

Scientific Name	Visitor Exposed	Visitor Sheltered	Visitor exposed (side near entrance)	Resident inside middle (both sides)	Resident inside middle second position
Antithamnionella ternifolia	R			R	
Colpomenia peregrina				R	O
Neosiphonia harveyi		R	R	O	O
Undaria pinnatifida		O	R		

Antithamnionella ternifolia

This filamentous red seaweed was encountered at study sites 1 and 3. It is a widely distributed in the British Isles, is thought to have been introduced from Australia and was first encountered at Plymouth in 1906 (Maggs & Hommersand, 1993).

Colpomenia peregrina

This brown seaweed was present in the sheltered study sites 4 and 5. *Colpomenia peregrina* was introduced in 1907 from France into Cornwall and Dorset (Cotton, 1908) via the oyster *Crassostrea virginica*. It occurs naturally in the Pacific Ocean.

Neosiphonia harveyi

This filamentous species was present at stations 2, 3, 4 and 5.

It was first recorded from France as *Polysiphonia insidiosa* around 1832 and then in England in 1923 (Maggs & Hommersand, 1993). It is thought to have been introduced via oysters from the Pacific coast of America but probably originated in Japan (Eno *et al.*, 1997).

Undaria pinnatifida

This kelp was present at two stations, 2 and 3.

Commonly known as wakame, this edible seaweed was first found in the Solent in 2003 and is indigenous to temperate regions of Japan, China and Korea (Oakley, 2007).

6.4. Discussion

It should be noted that the in-water visibility was not good on the study day. This compromised finding species to some degree and a fresh wind and slightly choppy sea state made working at the exposed locations a little difficult.

Snorkelling proved a good method for collecting the seaweeds and it is doubtful whether so many would have been recorded simply by reaching over the edge of the pontoons.

The author was in a team that undertook a survey of the intertidal and subtidal survey of benthic communities around Holyhead Harbour in 2009. During this survey the non-native *Heterosiphonia* japonica was found to be plentiful. It is worth noting that the present survey looking only at marina pontoons and will most likely not have studied all the seaweeds present in Holyhead Marina.

7. Comparisons of the effectiveness of the in shore network with an offshore network established by Cefas

In parallel with the assessment of different approaches to establishing an inshore monitoring network for non-native species in Wales, a separate assessment of the effectiveness of an offshore network was undertaken by Cefas (with subcontracting work by the MBA). This offshore network utilised the Cefas SmartBuoy network around the coast of the UK. A total of 5 buoys were used for the study (Fig. 7.1). These buoys potentially offer a cost-effective solution to establishing an offshore monitoring network because non-native sampling may be combined with regular servicing of the buoys. Sampling was undertaken every 2-4 months over the period January 2014 to February 2015.

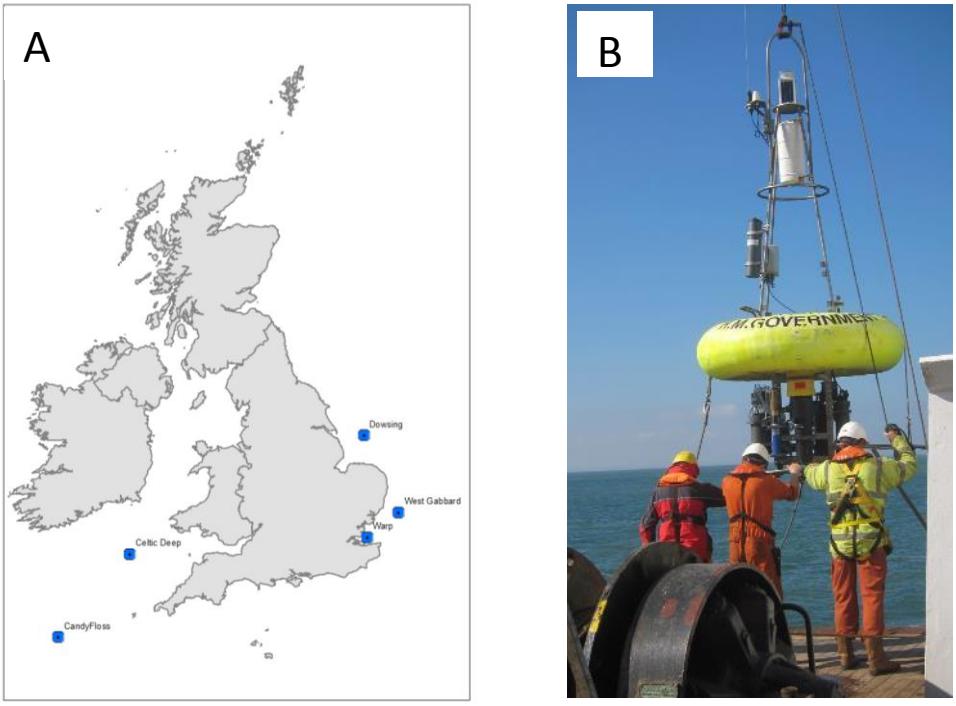


Figure 7.1 Location of buoys used in the off-shore monitoring trials (map created by Cefas). B. Deployment of replacement SmartBuoy, Warp, May 2014 (Image: NRW). Source Cefas-MBA report C5995

A preliminary report by Cefas/MBA addresses the following points:

i) Determine the suitability of scrapes vs. installation of settlement panels.

ii) Determine the suitability of horizontal versus vertical panels

iii) Trial techniques used in preservation of samples during transport.

iv) Trial the effectiveness of analysing for a hit list of species vs. complete analysis.

v) Determine the most effective number of replicates required on each buoy.

vi) Determine the timing for the most effective recording i.e. time of year where detection is optimised vs. risk of establishment.

Although the work undertaken by Cefas/MBA was targeted at the monitoring of non-natives, the scarcity of non-natives during the survey (see below) meant that the report focuses predominantly on how different methods impact on colonisation by **any** fouling organism.

7.1 Summary of the findings/conclusions of the Cefas/MBA study on offshore sampling

7.1.1. Logistical and methodological challenges of using an offshore network of buoys

Much of the Cefas/MBA report details the many logistical and methodological challenges faced in adding non-native sampling to an ongoing servicing schedule of five SmartBuoys around the coast of England and Wales. These practical challenges which generally relate to the preservation, labelling and transport of specimens can be summarised in bullet form below. (The detail of these issues is given in the Cefas/MBA report and hence will not be repeated here).

- Use of formalin versus other approaches (freezing, drying, alcohol)
- Use of buffered formalin
- Use of appropriate sample containers
- Appropriate labelling of containers
- Provision of appropriately trained personnel to undertake the biotic sampling at sea

7.1.2 Detection of non-natives

Only two non-native species were detected over the period of sampling - the bay barnacle (*Amphibalanus improvisus*) and the New Zealand Barnacle (*Austrominius modestus*). The lack of any number of non-natives and the (presumed) low abundance means that the different sampling strategies (scrape versus panels; vertical versus horizontal panels) and the different seasons of sampling are not considered with respect to non-natives.

7.1.3 Sampling methodologies with respect to the diversity of fouling organisms

The different sampling methodologies used on offshore buoys are considered with respect to the 'cumulative number of taxa' detected on buoys. Our approach on developing an inshore network has solely focused on non-natives and not on fouling organisms per se. Hence a comparison between inshore and offshore with regard to native fouling organisms will not be made.

7.2 Discussion of the relative merits of using an inshore versus an offshore network

The relative merits of developing an inshore versus and offshore network are considered in table 7.1.

Table 7.1 Issues to consider in comparing the efficacy of an inshore and offshore network to detect non-native species

	Offshore	Inshore
Logistical challenges	There are considerable challenges in developing appropriate sampling and transportation procedures. On the face of it these would not seem insuperable but the challenges of fitting into an offshore programme of work aboard a large research vessel and ensuring appropriate personnel are on hand is an issue. Preservation of samples and transportation is a particular issue.	Marinas provide accessible submersed surfaces in a sheltered environment while intertidal aquaculture sites are generally easy to access. There are limitations imposed by transporting samples (which may at times mean that the same kind of challenges of preservation are face as at offshore sites) but it is often possible to transport fresh samples to the laboratory for immediate identification. Thus in general logistical challenges are much reduced.
Replication	Use of a limited number of buoys (each with limited surface area) leads to restricted ability to obtain replicate samples	Use of marinas or intertidal aquaculture sites provides extensive appropriate surfaces where settlement panels can be deployed. Replicate samples can easily be achieved thus maximising chances of detecting non-natives.
Colonisation by non-natives	The biota which colonised panels attached to the buoys and the biota obtained from scraped samples was surprisingly limited in abundance and species richness. Colonisation by non-natives was limited to two species -the barnacles *Austrominius modestus* and *Amphibalanus improvus*.	Deployment of settlement panels in marinas resulted in the sampling of 9 non-native species in Holyhead and 7 in Milford Haven. Aquaculture sites were less successful with only two species detected. No non-natives new to Wales were recorded.
Algal sampling	Sampling of algae (whilst remote from the laboratory) within a programme ostensibly designed to sample animals is an issue which requires consideration because of complications with preservation	While no non-native algae were recorded from deployment of settlement surfaces in either marinas or aquaculture sites, it was demonstrated that with expert in situ sampling it was reasonably easy to detect algae non-natives at the marina site at Holyhead.

The offshore sampling developed by Cefas was surprisingly problematic and did not result in the detection of a large number of non-native species (although it did sample one species – *Amphibalanus improvisus*- which was not detected by the inshore sampling). The logic behind the offshore sampling programme was that it may provide early warning of the arrival of non-natives with minimal cost (i.e. through taking advantage of an ongoing programme of buoy maintenance). No details of cost are provided in the Cefas/MBA report and hence it is not possible to compare cost effectiveness of sampling. However ultimately few non-natives were detected. Although some of the logistical issues outlined in the Cefas/MBA report could have led to limited ability to detect non-natives, it seems that even if these were rectified, colonisation of offshore buoys is unlikely to lead to any advantage over an inshore system. For example, similar work by SAMS (Scottish Association for Marine Science) has also found that the study of offshore buoys may not yield much results in terms of non-native detection, when sampling offshore buoys in Scotland they detected a total of two non-native species (*Corella eumyota* and *Styela clava*) (Cook, 2015 *pers. comm*.). With far less effort and (assumed) cost inshore sampling of marinas (whether by RAS or settlement panels) leads to greater detection rates of non-natives.

8. Comparison of effectiveness of different monitoring techniques (rapid assessment versus settlement panels)

8.1 Comparison of Rapid Assessment Surveys (RAS) and Settlement Panels

In recent years, a technique known as Rapid Assessment Survey (RAS) has become a popular method for detecting non-native species in areas such as ports and marinas (e.g. Arenas *et al*. 2006; Minchin *et al*. 2006). RAS surveys are conducted over a finite period of time (typically 1-2 hours) by a team of taxonomic experts capable of identifying the majority of target non-native species in-situ. Within a RAS, artificial structures such as pontoons, fenders, ropes and buoys are a primary focus of study as these types of structures are usually the first to be colonised by non-native species. This is a particularly popular technique for monitoring non-natives, as the community assemblages on the targeted structures are always submerged but readily accessible at any state of the tide, making them ideal for cost-effective surveillance of non-native species.

In order to compare the effectiveness of different approaches to monitoring non-native species, the data collected from marinas in this report was compared to RAS data collected by the Marine Biological Association (MBA). The MBA conducted a number of surveys around Wales during June 2014 that included Holyhead and Milford Haven marinas. Their surveys coincided well with the study period for this report and were conducted whilst summer settlement panels for Inshore Non-Native Species network were in situ (see section 3).

8.1.1 Methods for RAS

A priority target list of 33 non-native species was created based on species previously identified in other UK marina environments and those deemed as likely to arrive via horizon scanning (see MBA, 2015 for more details on selection of target species). All surveys were conducted using the standard protocol developed by the MBA and will be described briefly here (for further details of survey techniques and forms used within the survey please refer to the report by MBA, 2015). Within the RAS, areas with available pontoons areas were divided equally between three survey groups, who worked independently for one hour to survey their respective areas for non-native species. In addition to inspecting pontoon structures, numerous submerged artificial substrates such as hanging ropes, fenders, etc., and natural substrates, such as kelps, were pulled up and examined for the presence of non-native species. For each non-native species encountered an estimate of abundance was made on a point scale at the end of the observation period; 0 – not observed; 1 – Rare to Occasional; 2 – Frequent to Common and 3 – Abundant to Superabundant. Hooks and scrapers were used if necessary to access material for inspection. At the end of the hour, the staff gathered to compare notes, specimens and to summarise their joint observations on a standard form. It should be noted that although a target list was created, any non-native species not on the list were also recorded if they were encountered. Kate Griffith (SOS) was a participant in some of these surveys and can contribute some observations.

8.1.2. Detection of non-natives using RAS and settlement panels techniques

A comparison between techniques suggested that neither technique has a greater potential for detecting the number of non-native species within a marina (Table 16); a maximum of nine species were detected at the Holyhead using both RAS and settlement panel techniques during the summer period. For Milford Haven marina, the RAS technique did record a greater number of species, with RAS recording eight non-native species and the settlement panels detecting a maximum of seven non-natives.

Table 2 The presence and abundance of target non-native species recorded using settlement panels (SP) deployed for 6 weeks (6wk), 12 weeks (12wk) and RAS within Holyhead and Milford Haven marinas in Wales. SP assessed using SACFOR (S = Super Abundant, A = Abundant, C = Common, F = Frequent, O = Occasional, R = Rare). RAS assessed via point score (0 = Absent; 1 = Occasional; 2 = Common; 3 = Abundant.

NON-NATIVE SPECIES	**Holyhead**			**Milford Haven**		
	SP 6wk	**SP 12wk**	**RAS**	**SP 6wk**	**SP 12wk**	**RAS**
Corella eumyota	R	R	1	-	R	1
Styela clava	-	-	1	-	-	1
Botrylloides violaceus	R	R	0	R	R	0
Didemnum vexillum	R	-	1		-	0
Asterocarpa humilis	R	R	1	-	-	1
Ficopomatus enigmaticus	-	-	0	R	F	2
Bulgula neritina	R	F	1	R	F	1
Tricellaria inopinata	R	F	2	R	R	1
Schizoporella japonica	R	R	3	-	-	0
Caprella mutica	R	R	1	R	R	0
Austrominius modestus	R	R	0	O	O	2
Undaria pinnatifida	-	-	1	-	-	0
Bugula stonolifera	-	-	0	-	-	1
Total species detected	9	8	9	6	7	8

Overall, the identity of the non-native species recorded in the study marinas via both techniques was very similar; both techniques effectively recorded *Corella eumyota*, *Didemnum vexillum*, *Asterocarpa humilis*, *Ficopomatus enigmaticus*, *Bulgula neretina*, *Tricellaria inopinata*, *Schizoporella japonica*, *Austrominius modestus* and the mobile species *Caprella mutica* (Table 16). However, some differences did occur between techniques with some species; *Styela clava* and *Undaria pinatiffida* were only detected within the RAS approach and *Borylloides violaceous* and *Austrominius modestus* were only recorded when monitoring with settlement panels.

8.1.3 Discussion of RAS vs Settlement Panels

In terms of detecting non-native species, both techniques recorded a similar suite of non-native species indicating that they may be equally useful in terms of monitoring non-native species. Apart from the discovery of *U. pinnatifida*, both techniques recorded non-native species already recognised as being established or present within the locations they were recorded, suggesting that neither technique would be particularly more effective than the other at detecting the new arrival of a non-native species. Comparisons made here did indicate that RAS was more effective than settlement panels in detecting the presence of *S. clava* and *U. pinnatifida*; RAS participants easily observed mature specimens of both species attached to the pontoons of the marinas and their distinctive morphological characteristics enabled them to be easily identified. However, the absence of *Styela clava* on settlement panels does not indicate that this technique is ineffective. This absence may be due to the late breeding season of *S. clava* where peak larval release and recruitment occurs mainly in the autumn (Parker *et al.* 1999), which may have begun after the settlement panels were retrieved for analysis. In terms of developing an Inshore Monitoring Network, this example demonstrates how the seasonality of a non-native species should be a consideration when considering the development of monitoring techniques. Clearly, the detection of a priority-threat non-native species may require a more continuous or strategic based monitoring strategy based on life-history patterns.

The detection of non-native algae using settlement panels may be particularly problematic, as algal specimens were not identified within the scope of this study as they were not developed enough to be distinguishable from native species with similar appearance or characteristics. Similarly, within the RASs conducted by the MBA, little focus was given to non-native algal species, but this was mainly because separate algal-based rapid assessment surveys were commissioned by NRW to address this. These algal surveys were similar in nature to the one detailed within this report and were also conducted by Francis Bunker (section 6). Such action emphasises that the importance of relevant experts within specific fields is already recognised, particularly when conducting any kind of RAS survey. As with the algal study presented here, a substantial quantity of specimens were collected during the algal surveys, necessitating a subsequent period of laboratory identification afterwards highlighting that there are also difficulties when trying to identify algal species in the field.

There may specific limitations associated with settlement panels; as the panels monitor settlement they need a supply of larvae to detect a species, an early stage arrival of non-native would probably release very few larvae/propagules and it is unlikely these would be detected by a small number of settlement panels suspended in the water column within a marina. Similarly, a non-native species would not be detected upon a panel unless it was releasing propagules. Evidently, there are benefits from using RAS as they may alert us to the presence of species that may not occupy or be easily identifiable on a settlement panel, they may also be effective as an early alert mechanism as the targeted surfaces within a RAS are the first sites that are usually invaded. However, the accuracy of these types of studies relies very heavily on the availability of experts with relevant skills to conduct the survey. Neither monitoring approach is very effective at monitoring mobile non-native species; this may be an area where techniques need to be developed to get information on the distribution of non-native mobile species e.g. amphipods.

The use of settlement panels for monitoring is not without merit; species that preferentially settle on horizontal surfaces or do not live close to the water surface may detected more effectively via settlement panels, and such surfaces would be very difficult to access and survey within a RAS meaning a non-native could be overlooked. There are also other benefits to consider, the settlement panel approach is cheap in terms of materials and does not rely on the availability of a number of taxonomic experts. In

addition, the precise analysis of the panels allows for collection of abundance data about native and non-native communities, and may even allow for further study about the invasion potential of a species but this approach is costly in terms of time.

Clearly, each method has its own benefits and limitations; in order to detect as many non-natives as possible within the limitations of these techniques, the use of both techniques for monitoring sites would be a useful approach within an Inshore Monitoring Network. For example, if the results from RAS and settlement panels were combined for Holyhead marina a total of eleven non-native species would have been detected rather the nine species detected using each method alone. This combined approach may be limited by financial and logistical constraints, but with the threat of non-native species ever-present such actions should be considered in order to detect new arrivals as effectively as possible.

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Appendix A: Presentation given at the conference in Belgium on marine invasive non-native species.

BENELUX TALK.pdf

Appendix B: An inventory of seaweeds collected from Holyhead Marina - 30th September 2014 by Francis Bunker (Non-natives species are highlighted).

Scientific name	Qualifier	Holyhead	Holyhead	Holyhead	Holyhead	Holyhead
		Visitor Exposed	Vistor Sheltered	Vistor exposed (side near entrance)	Resident inside middle (both sides)	Resident inside middle second position
		14/09/2014	14/09/2014	14/09/2014	14/09/2014	14/09/2014
Antithamnionella ternifolia		R			R	
Bryopsis hypnoides		R				
Bryopsis plumosa			O	R		O
Callophyllis laciniata		R	R			
Ceramium	uncertain of species		O			
Ceramium virgatum		R	C	F	F	O
Chondrus crispus		O	C	O		R
Cladophora	uncertain of species	R				
Colpomenia peregrina					R	O
Corallina officinalis		F		R		
Corallinaceae	around edge of pontoons	F		F		
Cryptopleura ramosa		R		R		
Desmarestia aculeata		F				
Ectocarpales		O			O	R
Ectocarpus siliculosus		R				
Ellisolandia elongata		O		R		
Fucus	grazed	R				
Fucus vesiculosus			O		O	
Gelidium				R		
Hypoglossum hypoglossoides				R		
Laminaria digitata				O		
Laminaria hyperborea		C		F		
Lomentaria clavellosa			R	R		R
Lomentaria orcadensis				R		
Mastocarpus stellatus			R			
Neosiphonia harveyi			R	R	O	O
Nitophyllum punctatum				R	O	O
Palmaria palmata		O		O		
Phycodrys rubens		P		R		

Polysiphonia brodiei				R		
Polysiphonia elongata		C	P	R	R	R
Polysiphonia fucoides		R		R		
Polysiphonia stricta				O		F
Porphyra				R		
Pylaiella littoralis			C	F		
Rhodophyllis divaricata				R	R	R
Saccharina latissima		R	A	A	A	A
Ulva	flat	O			F	O
Ulva clathrata			F			
Ulva compressa	flat and tubular forms	C		O		
Ulva flexuosa					F	
Ulva linza			F			
Ulva rigida				F		
Undaria pinnatifida			O	R		