Running head: Surveying non-native species in an active port

Design and implementation of two surveys targeted at describing fouling communities and identifying non-native species within active ports.

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ABSTRACT

Ports have long been considered 'high-risk' areas for the introduction of non-native species (NNS) and should therefore be a focus of NNS monitoring. The industrial nature of active ports can, however, provide various problems when attempting to carry out monitoring programmes. Current methodologies designed to identify NNS and to describe fouling communities have not been developed specifically for use in active ports and can encounter a number of issues when used in these environments. Here, two surveys were developed and trialled within an active port in South Wales, UK, designed to describe fouling communities, identify NNS and overcome some of the major limitations to conducting surveys within ports. Over a six-month period, fouling communities dominated by solitary ascidians developed in each survey. Seven NNS were identified, mostly species already recorded in the 1950s, including the Mediterranean crab *Brachynotus sexdentatus*, and the more recently introduced Japanese skeleton shrimp *Caprella mutica*. Each survey was evaluated independently with respect to key factors, including the ability to detect NNS and practical aspects of using these survey methods in an applied context. We conclude that whilst each survey can function independently, the use of both survey types in conjunction offers the most robust solution to identifying NNS and describing wider fouling communities within active ports. This research has implications for the future monitoring and management of NNS within UK ports.

KEY WORDS: Non-native species; ports; maritime trade; fouling communities; benthic macrofauna; ecological surveys

INTRODUCTION

Non-native species (NNS) have long been considered as one of the biggest threats to biodiversity, the stability of marine communities, and ecosystem functioning (Bax *et al.*, 2003; McGeoch *et al.*, 2010; Molnar *et al.*, 2008; Rohde *et al.*, 2017; Sala *et al.*, 2000). NNS can give rise to significant ecological and economic damages, however, a major concern is their high variation and unpredictability of impacts (Katsanevakis *et al.*, 2014; Lovell & Stone, 2005; Pimentel *et al.*, 2001). Coupled with this is the differing nature of impacts, for example both positive and negative impact at the ecosystem service level rather than the overall perceived impact of species (Katsanevakis *et al.*, 2014). In general, the preferred approach is to prevent the introduction and spread of NNS rather than to undertake expensive eradication or control measures post establishment (Pyšek & Richardson, 2010; Puth & Post, 2005; Rohde *et al.*, 2017). Implementing effective monitoring programmes to identify the arrival of NNS, serving as an early warning, is key in preventing establishment (Anderson, 2007; Rohde *et al.*, 2017).

Marine organisms have likely been transported and become established around the world for thousands of years (Aubet, 2001; Carlton and Hodder, 1995). However, globalisation has led to the rapid increase in species introductions observed over the last few decades (Streftaris *et* al., 2005; Floerl *et al.*, 2009; Hulme, 2009; Maceda-Veiga *et al.*, 2013; Sardain *et al*., 2019). Maritime trade has long been recognised as the primary invasion vector for marine NNS (Bailey, 2015; Ruiz *et al.*, 1997; Williams *et al.*, 2013; Katsanevakis *et al.*, 2013), either through ballast water or hull fouling, meaning that ports are considered to be more at risk of invasion by NNS than natural coastal habitats.

Despite the strong link between ports, maritime trade and NNS, there is relatively little published research aimed specifically at describing communities within ports (Bailey, 2015). This may be due in part to the limitations in terms of ease of access and safety when working within active ports, as well as the lack of need or desire for port owners to publish any findings from private surveys that may have been undertaken within their ports. By contrast, marinas are frequently studied worldwide as habitats for NNS (Canning-Clode *et al.*, 2013; Guerra-García *et al*., 2015; Foster *et al.*, 2016; Shenkar *et al*., 2018). Whilst marinas offer more accessibility and safety than ports, the habitats and factors influencing communities are often different even to the nearest port. Marinas are more commonly associated with the local spread of NNS through recreational boating (Martínez-Laiz *et al.*, 2019), rather than being the initial site of species introduction, which should be the focus when attempting to prevent invasions. Effective management of NNS is made much more difficult when there is a lack of survey data (Campbell, 2011; Dahlstrom *et al*., 2011; Azmi *et al.*, 2015), highlighting the importance of establishing long-term local and regional monitoring efforts. This should be focussed on the most likely sites for novel introductions which, in most cases, are ports that are linked to the global maritime trade network.

Various methodologies for the monitoring of fouling communities and associated NNS have been trialled and published over the last few decades (e.g. Cohen *et al.*, 2005; Arenas *et al.*, 2006; Floerl *et al.*, 2012). Rapid assessment surveys (RAS) are a favoured method and have been successfully applied in ports and marinas around the world (Cohen *et al.*, 2005; Bishop *et al.*, 2015; Mineur *et al.*, 2012). However, the industrial nature of ports can provide difficulties when attempting to safely conduct this type of survey. Traditionally, RAS have targeted existing submerged structures (e.g. pontoons, buoys, ropes and chains; Cohen *et al.*, 2005; Arenas *et al.*, 2006; Mineur *et al.*, 2012), where well-established fouling communities can be surveyed without the need to deploy some form of settlement material. This type of survey benefits from being a quicker and cheaper method than most alternatives, and it covers a range of different structures, materials and habitats. It is, however, difficult to record small and cryptic organisms which may be inhabiting structures as the rapid assessment does not use destructive sampling (Rohde *et al.*, 2017). Further, it is not feasible to compare colonisation quantitatively among sites due to the non-standardised area units. In larger and more active ports, RAS may not always be a viable option due to the lack of long-term submerged structures, safe access to suitable sites, and port health and safety regulations.

Settlement and colonisation experiments are another chosen method for surveying fouling communities. This method has been heavily used over the past few decades and has been adapted into various designs, using a range of materials and deployed in a range of environments (Floerl *et al.*, 2012; Bangor University, 2015; Cook *et* al., 2015). Generally, some form of plastic is used as a virgin settlement surface for larval settlement and development, with most survey designs applying a single plastic tile suspended in the water column and deployed for a period of several months.

The advantage of this survey type is the ability to record quantified data and the option to choose suitable sites for deployment, which is particularly beneficial for use within active ports. The need for an extended deployment period with settlement surveys, often at least three or four months, and the associated higher costs that follow, are the main reasons why RAS have increased in use over the last couple of decades.

Various studies have applied both RAS and settlement experiments, either in an effort to compare the accuracy of each method or to provide a more robust survey (Cook *et al*., 2015; Hurst, 2016; Marraffini *et al.*, 2017). Perhaps the most important finding when comparing the two methods within the same study is the accuracy of identifying NNS (Lehtiniemi *et al.*, 2015). Comparisons have shown that both settlement surveys and RAS are liable to miss certain NNS but are reliable at identifying the majority of NNS present (Cook *et al*., 2015; Marraffini *et al.*, 2017). Cook *et al*. (2015) reported that settlement surveys and RAS each missed two species which were found in the other survey type, suggesting that the most robust surveys would incorporate elements of each survey type.

Arguably, the most comprehensive guide for surveying within ports is the HELCOM/ OSPAR combined strategy targeted for use within the Baltic Sea (HELCOM, 2013). Whilst this strategy suggests a preference for the combined use of RAS and settlement surveys, it concedes that RAS may not be a viable option in all ports. Despite this, no alternative adapted survey type is offered, with it being suggested that a traditional form of settlement survey alone would be sufficient (HELCOM, 2013).

It follows that both survey types could be adapted for use within ports, to include beneficial traits of each whilst overcoming some of the limitations to working within active ports. These modified surveys could also provide some key information which currently neither RAS nor settlement experiments offer and yet which may prove valuable in informing targeted biosecurity plans (e.g. colonisation rate and community succession over the deployment period).

The aim of this research was to design a survey method tailored to describe the fouling community within an active port, focussing on identifying non-native species that may be present. The objectives were to:

a) quantify the succession of faunal colonisation,

b) compare colonisation success and fouling communities among different sites within the port,

c) identify differences in faunal colonisation between materials typically present in ports

Two survey methods were developed and tested in an active port, the Port of Swansea, Wales, UK. The relative success of each survey method was assessed with respect to understanding the fouling community and detecting NNS. The potential role of the surveyed port as a vector for NNS into the region was considered.

MATERIALS AND METHODS

Study area

Research was conducted within the Port of Swansea, South Wales, UK. This port is an enclosed area consisting of three connected docks linked to the Bristol Channel via a lock. The oldest of these three docks, the Prince of Wales Dock, was constructed in the late 19th century with the other two docks, King's Dock and Queen's Dock, being constructed in the early 20th century. Historically the Port of Swansea has traded largely in copper, coal, tinplate and oil, of which only coal remains to be traded today following a decline in trade throughout the 20th century. Along with coal, the port now regularly trades in dry bulks, scrap metals, timber, and general cargo, as well as having an aquaculture production site designated within Queen's Dock for the culture of blue mussels (*Mytilus edulis*; Linnaeus, 1758). Around 600,000 tonnes of cargo are traded annually with an average of 81 ships per week transiting in an out of the lock, including pilotage and tug vessels (ABP, unpublished).

Temperature within the port ranged from ca. 11°C through November to a maximum of ca. 22°C during July, average temperature for the entire survey period was ca. 17.5°C. Mean salinity was recorded as 28.5, with no significant stratification. Whilst the Bristol Channel experiences a tidal range of up to 13m, water levels within the port are maintained at around 10 – 12 m through regular pumping directly from the Bristol Channel to replace water lost primarily through lock operation. These docks therefore offer a unique insight into an isolated subtidal habitat which is influenced by water from the Bristol Channel as well as any potential species introductions through maritime trade or aquaculture.

Site selection

A total of 3 sites were selected for the deployment of survey materials, called Zone A, B and C in this study (Figure 1). Due to the level of activity within the Port of Swansea, safe operation was a key factor in identifying suitable deployment sites. Sites were selected based on a) the availability of surface mounting points (e.g. mooring bollards, fences, shackles etc.), b) the proximity to active working berths or derelict infrastructure for safety reasons and to minimise the chance of removal of materials, c) the proximity to other sites to ensure a wide coverage across the docks. Each zone containing two deployment sites, one for each distinct survey type, located within close proximity of one another (< 10 m). Zone A did not meet this criterion due to the busy operational quay limiting safely accessible mounting points; mixed material survey materials for this zone were hence deployed at the nearest suitable location (Figure 1). Zones B and C do contain two deployment sites located within < 10 m of each other.

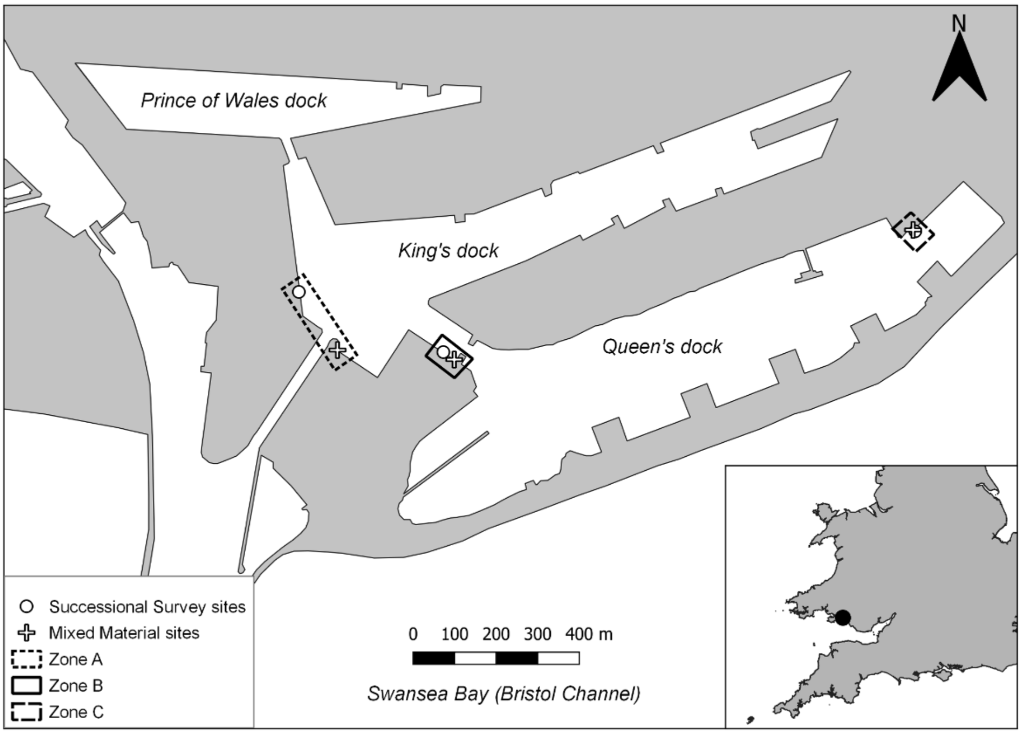

Fig. 1. Map outlining the position of survey sites within the Port of Swansea. Each zone contains a location for the deployment of both a ‘Successional Settlement Survey’ and a ‘Mixed Material Survey’. Zones are outlined showing paired sites.

Successional Settlement Survey (SSS)

Acrylic (PMMA) tiles (225cm^2, 15 cm x 15 cm, grey in colour) were used as the settlement material. 6 tiles per site were lightly sanded using 40 grit sandpaper and mounted, using cable ties, within an aluminium frame (Figure 2). Each frame was suspended in the water column using polypropylene rope, attached to a fixed surface mounting point (e.g. a mooring bollard; Figure 2). Frames were suspended initially to a depth of approximately 4 m, although the water level in the port can vary meaning that depth did not remain constant during deployment. Materials remained in deployment for 6 months from deployment in May 2018 until collection in November 2018.

This survey type was designed to provide quantified measures of certain ecological parameters, namely species abundance and percentage cover, as well as informing on the colonisation rate and whether there is a successional change in community assemblage over the deployment period. Mounting six tiles within one frame also overcame some of the logistical issues of working within an active port, most notably the lack of availability of safe working areas.

Fig. 2. Successional Settlement Survey materials prior to deployment.

Mixed Material Survey (MMS)

This survey type comprised various materials that are commonplace in most ports, each acting as a settlement surface for larval settlement and development. Materials included brick, soft wood (constructional timber, pressure treated), rope (natural fibre and polypropylene), steel, plastic (acrylic tiles as used in the Successional Settlement Survey, and PVC tubing), and a cotton fibre mop head (to represent more complex fibrous materials). Both forms of plastic included sanded and un-sanded variations to investigate any potential settlement preferences based on material roughness. Materials were connected in a set sequence along lengths of rope (Figure 3) and, as with the Successional Settlement Survey, suspended in the water from a fixed surface mounting point. Depth of deployment ranged from approximately 3 – 6 m, based on the length of the materials and fluctuations in water level. Materials were deployed for 8 months from May 2018 to early February 2019. This survey was designed to investigate whether there is any material preference for settlement of organisms, non-native or native, and whether community composition varied between materials.

Fig. 3. Mixed Material Survey materials prior to deployment.

Sampling

Materials for each survey type were deployed during May 2018 at three sites (Zones) within the Port of Swansea (Figure 1). Zones were visited monthly over a deployment period of 6 months and 8 months for the SSS and MMS, respectively. For the SSS, one acrylic tile was removed from the frame each month and taken for laboratory-based taxonomic identification of the species present. MMS materials remained untouched throughout deployment. Materials were collected after 8 months, following a detailed description of colonisation and identification of species *in situ*.

Laboratory analysis

Samples collected as part of the SSS underwent laboratory-based analysis. Acrylic tiles were destructively sampled, whereby organisms were systematically removed and identified. Analysis consisted of a visual taxonomic identification to the lowest possible taxon of macrofauna present on tiles and in scrape samples.

A combination of dissection and compound microscopes were used where necessary, and identification of species was achieved with the aid of various guides including '*Handbook Of The Marine Fauna Of North-West Europe*' (Hayward & Ryland, 2017), '*British Marine Amphipoda*' (Lincoln, 1979) and Linnean Society taxonomic materials. When necessary, organisms (e.g. amphipods and polychaetes) were fixed and preserved for short periods of time in 70% ethanol to aid identification.

Data analysis

PERCENTAGE COVER ANALYSIS

Percentage cover of PMMA tiles (SSS and MMS) and selected other materials forming the MMS (brick, PVC pipe, steel and wood) was calculated from photos, using ImageJ software. Images were set to a known scale and covered areas were measured, with percentage calculated using a known total surface area of materials. Three materials (mop head, polypropylene rope, sisal rope) were omitted from the percentage cover analysis due to the inaccuracies in being able to measure coverage. For the SSS only, percentage cover was recorded for both the front (facing into the water column) and rear (facing into the port wall) orientation of settlement tiles. All statistical analyses in this section were performed within RStudio v.1.2.1335 (R Core Team, 2017).

Percentage data for both survey types was converted to proportion (range 0 – 1) before any statistical analysis. Data from the SSS were found to be non-normally distributed based on Shapiro-Wilk normality tests ($p < 0.05$) for both proportion and arcsine transformed proportion data. A beta regression was used to statistically analyse the effect of 'Month' and 'Orientation' on the observed percentage cover. Post-hoc Mann-Whitney U tests were used to analyse the pairwise differences between month groups.

As with the SSS, proportion data recorded within the MMS were found to be non-normally distributed based on Shapiro-Wilk tests of both proportion and arcsine transformed data ($p < 0.05$). A Kruskal-Wallis test was used here to analyse the effect of Material on the observed percentage cover. Dunn's Tests were performed as post-hoc pairwise analyses between material groups.

WHOLE COMMUNITY ANALYSIS

Primer 6 v.6.1.13 with PERMANOVA v.1.0.3 software (Anderson *et al*., 2008; PERMANOVA+ for PRIMER software) was used to analyse whole community abundance data between samples collected within the SSS and within the MMS. Four species identified could only be recorded by measure of area covered (cm^2), rather than abundance counts, and these remained within the analysis. Data were first transformed using a Log(x+1) transformation. This transformation was selected in order to downweigh a small number of highly abundant species, thus increasing the importance of species diversity within the analyses, as well as to accommodate for the combined use of abundance and coverage data within the same analyses.

The Bray Curtis similarity index was used to create a similarity matrix, from which non-metric multidimensional scaling (nMDS) and PERMANOVA analyses were performed. PERMANOVAs were designed with two factors (Zone and Month for the SSS, and Zone and Material for the MMS) and one response variable (values in the similarity matrix). No interaction term between factors was included.

The model used the permutation of residuals under a reduced model with type III (partial) sum of squares and 9999 permutations. Pairwise PERMANOVAs were used ad hoc to analyse the differences between certain factor groups (Anderson *et* al., 2008). Factor groups were Zone: A, B, C; Material: Brick, Mop, PMMA (sanded), PMMA (unsanded), Polypropylene rope, PVC (sanded), PVC (unsanded), Sisal rope, Steel, Wood.

CROSS SURVEY ANALYSIS

Species richness data (as total number of species recorded per sample, irrespective of surface area) was used in cross-survey analyses. These analyses were conducted to evaluate the effectiveness of each survey type at describing the whole fouling community, as well as identifying NNS. A Fisher-Pitman permutation test (Berry *et al.*, 2002) was conducted within R v. 3.6.2 (R Core Team, 2017), whereby the effect of the factor Survey Type (2 levels: SSS, MMS) on the response variable species richness was analysed.

RESULTS

Successional Settlement Survey (SSS)

A total of 40 different taxa across 9 phyla were identified as part of the SSS (Supplementary Table S1). Of these, 7 may be classified as non-native species (NNS) within the UK. Arthropoda was the most represented phylum with 13 different species, whilst only one species each of Echinodermata, Platyhelminthes and Porifera were identified. In terms of total abundance and coverage Chordata was the most common phylum, of which all but one of the species were within the class Ascidiacea. A total of 1264 individuals of *Ciona intestinalis* (Linnaeus, 1767) were recorded over the 6-month survey period, making this the most abundant species.

The similarity in communities among zones and months were visualised by nMDS (Figure 4). Both factors, Zone and Month, significantly affected the structure of the faunal communities; ‘Zone’ (PERMANOVA, pseudo-F = 3.19, p = 0.0029) and ‘Month’ (PERMANOVA, pseudo-F = 5.05, p = 0.0003). Pairwise tests among Zones showed a significant difference in community assemblage between Zone C and Zone A (PERMANOVA, t = 1.411, p = 0.0299).

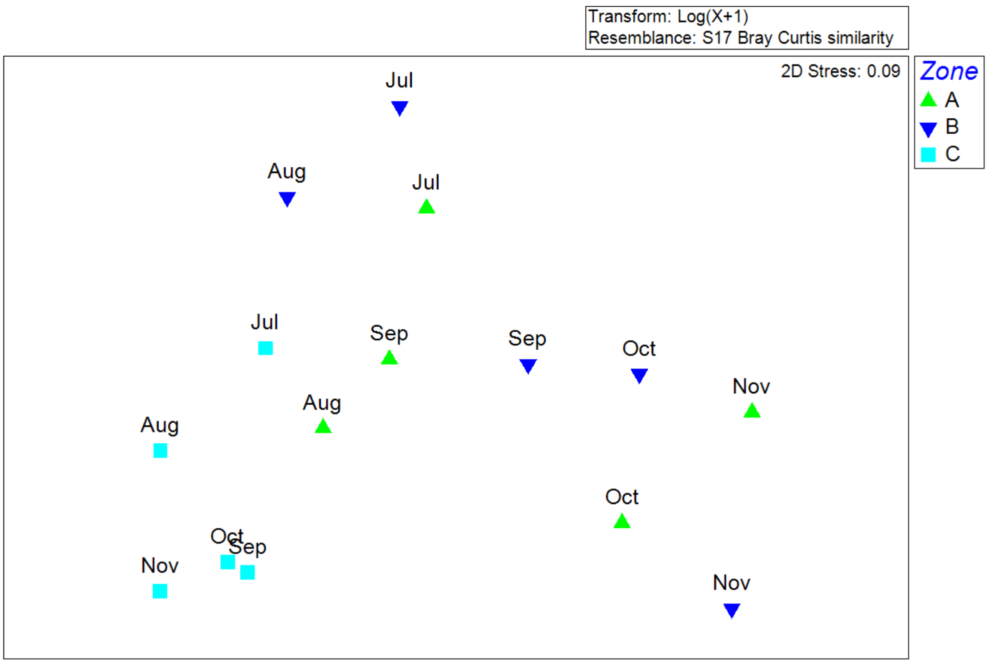

Fig. 4. nMDS plot of colonising species communities collected within the Successional Settlement Survey. Plot based on a resemblance matrix created using a Bray Curtis similarity indices of Log(X+1) transformed abundance data. Samples labelled by factor ‘Month’; symbols represent location factor ‘Zone’; June samples were removed from plot since communities were so species-poor that they could not be plotted in a meaningful way in relation to subsequent months.

The total number of species recorded per sample increased consistently to a maximum mean of 17.33 species per sample in October before falling to 14 species per sample in November (Figure 5). *Ascidiella scabra* was amongst the first organisms to begin colonisation in July and rapidly increased in abundance to a peak mean of 82.67 individuals per sample in September before falling through October and November. The abundance of amphipods declined at a similar time to *A. scabra*. A similar downward trend from September through to November can be seen in the total number of non-native species (NNS) recorded per sample, from a maximum mean of 3.67 species per sample in September to 2.33 species per sample in November. Conversely, the coverage of colonial ascidians and the abundance of *Aurelia aurita* polyps began to increase from September through to a maximum recorded mean coverage in November of 6.33 cm^2 per sample for colonial ascidians and mean abundance of 50 individuals per sample for *A. aurita* polyps.

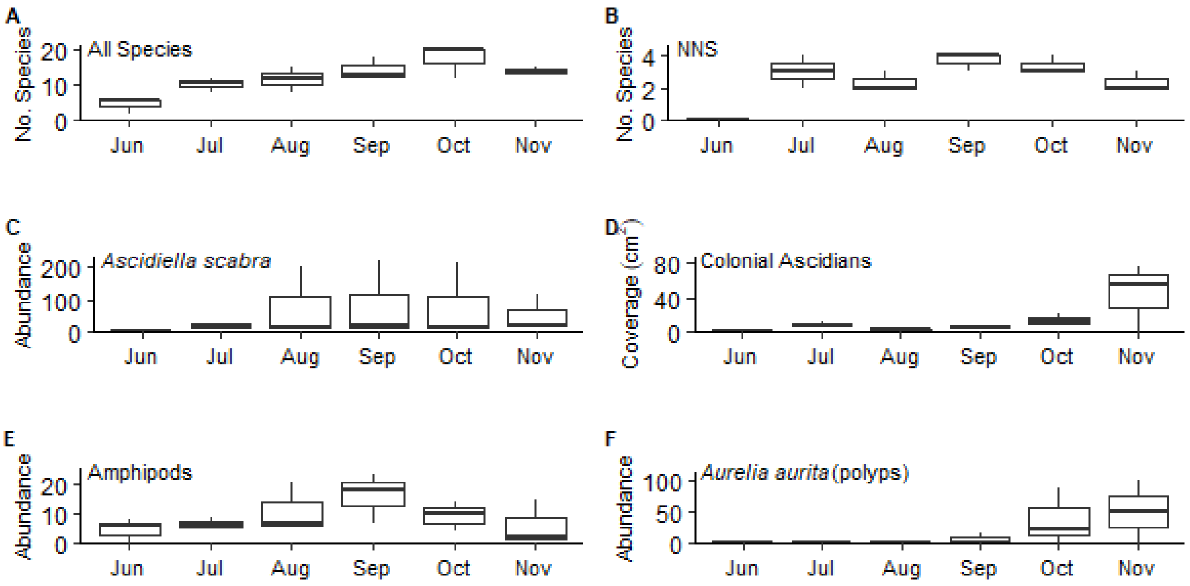

Fig. 5. Colonisation of settlement tiles in Swansea Port (450cm^2, n=3). A: total number of species recorded. B: number of non-native species (NNS). C: abundance count of *Ascidiella scabra*. D: surface area coverage (cm^2) of colonial ascidians. E: abundance count of amphipods. F: abundance count of *Aurelia aurita* polyps.

Percentage cover was recorded for both the front (facing away from the port wall) and the rear (facing towards the port wall) of each tile each month (Figure 6). Two months after deployment colonisation reached over 90% and 70% coverage for the front and rear of tiles, respectively. Coverage of the front of tiles remained over 90% for the reminder of the survey period. The rear orientation of tiles took until September to reach ca. 90% coverage, with the maximum coverage of 97% being achieved in October. There is no evidence here that the factors ‘Month’ (Beta regression, $z = 1.399$, $p = 0.162$) and ‘Orientation’ (Beta regression, $z = -0.579$, $p = 0.563$), nor the interaction of these factors (Beta regression, $z = 0.212$, $p = 0.832$) significantly affect percentage cover.

Pairwise analyses between months showed that percentage cover increased significantly from June to September (Mann-Whitney U test, $p = 0.0449$), June to October (Mann-Whitney U test, $p = 0.0081$), and June to November (Mann-Whitney U test, $p = 0.0043$); percentage cover was not significantly any other month groups (Mann-Whitney U tests, $p > 0.05$).

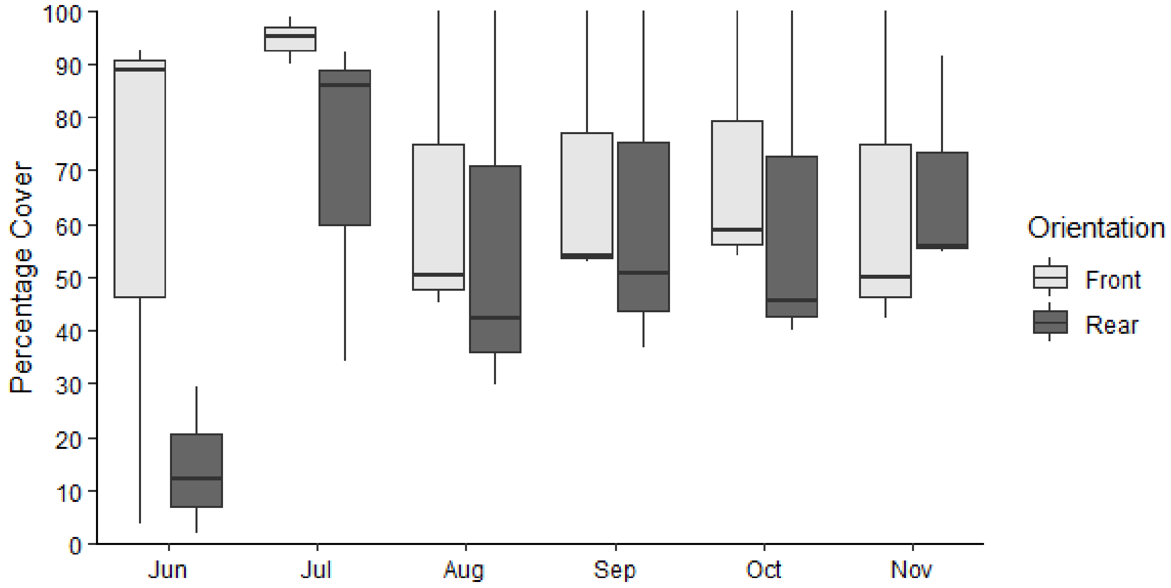

Fig. 6. Percentage cover of PMMA tiles recorded each month within the Successional Settlement Survey ($225cm^2$, n = 3). ‘Front’ refers to the orientation of tiles facing away from port walls, ‘Rear’ refers to the orientation of tiles facing the port walls.

Mixed Material Survey (MMS)

15 species were recorded within the MMS surveys (Supplementary Table S2). As with the SSS, Arthropoda was the most represented phylum with 7 species. Species with the highest total abundances were within the phylum Chordata, the most abundant species here being the Ascidiacea *Ascidiella scabra* and *Ciona intestinalis* with a total recorded abundance of 264 and 337, respectively, across all materials and all zones (Supplementary Table S2). Several species were recorded from only one material: *Spirobranchus triqueter* and *Palaemon serratus* (Pennant, 1777) from sanded PMMA tiles, *Carcinus maenas* (Linnaeus, 1758) and *Macropodia rostrata* (Linnaeus, 1761) from mop heads and *Bugula neritina* (Linnaeus, 1758) from unsanded PMMA tiles.

Community analysis indicated that both material type (PERMANOVA, pseudo-F = 2.57, $p = 0.0011$) and zone (PERMANOVA, pseudo-F = 3.44, $p = 0.0008$) significantly influenced the colonising fauna (Figure 7). However, there was no apparent grouping of material types, except for sisal rope due to the presence of only one organism, *Ciona intestinalis*, on the material. Pairwise comparison of all materials did not identify significant differences between isolated materials (PERMANOVA, $p > 0.05$; due to a small sample size of three within each material group the number of permutations completed was below the required level to consider the analysis reliable).

Samples from Zone C were clustered together and pairwise analysis showed a significant difference between the communities within zones A and C (PERMANOVA, t = 1.411, p = 0.033). All other pairwise comparisons were not significant.

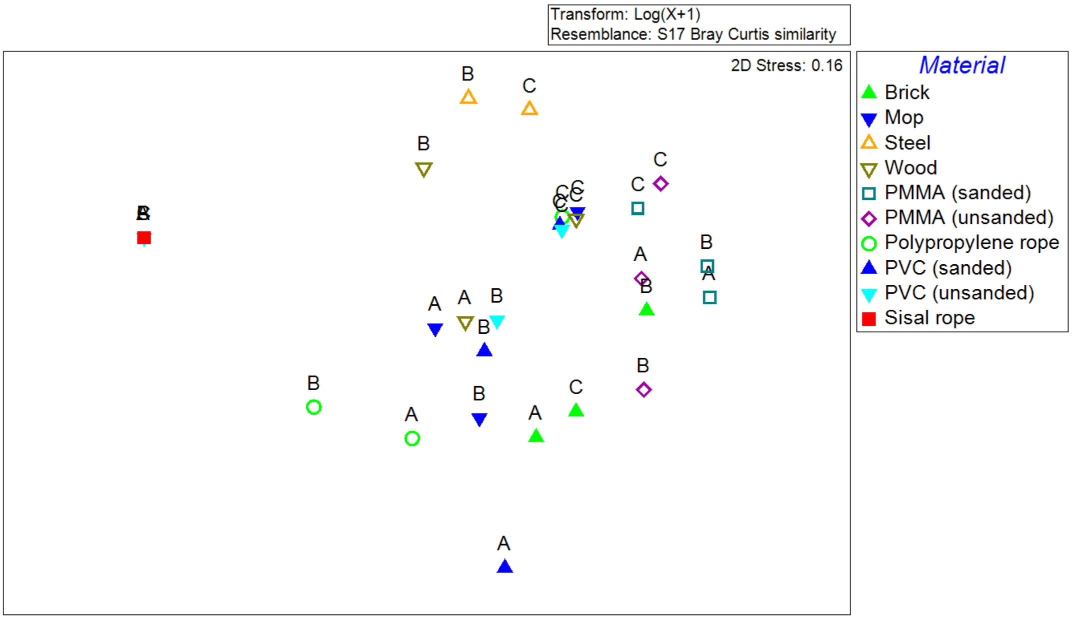

Fig. 7. nMDS plot of samples collected within the MMS. Raw data transformed using a Log(X+1) transformation. Plot based on a resemblance matrix created using a Bray Curtis similarity index. Samples labelled by factor ‘Zone’; symbols represent factor ‘Material’.

Seven of the ten materials present within the MMS had the total colonised area measured and converted to percentage cover (Figure 8). Each form of PMMA tile (sanded and unsanded) along with wood were among the most heavily colonised materials. Sanded PMMA and wood had the highest median coverage of 34% and 36%, respectively, after eight months of deployment. Steel was consistently recorded with the smallest amount of colonisation with a median of 4%. Material type was found to have a significant effect on the observed percentage cover (Kruskal-Wallis, $p = 0.031$). Significant differences in percentage cover were identified between: sanded PMMA and sanded PVC; sanded PMMA and steel; unsanded PMMA and steel; wood and steel (Dunn’s Test, $p < \alpha/2$ where $\alpha = 0.05$). No significant differences in percentage cover were recorded between all other material pairs (Dunn’s Test, $p > \alpha/2$ where $\alpha = 0.05$).

The total abundance of organisms recorded from each material was standardised by surface area to abundance counts per 500 cm^2 (Figure 9). PMMA tiles had the greatest abundance of organisms per 500 cm^2 with median values of 130 (sanded PMMA) and 56.7 (unsanded PMMA). The mop head saw the lowest abundance per 500 cm^2 with 1.4. However, the copious strands of the mop head had a far greater surface area than any other material (6960 cm^2); on average 29.5 ± 25.0 sd organisms were recorded per mophead. Material type significantly influenced the abundance of organisms per 500 cm^2 surface area (Kruskal-Wallis, $p = 0.01$). Pairwise tests revealed significant differences between the following material types: sanded PMMA and mop head, sanded PMMA and polypropylene rope, sanded PMMA and sisal rope, sanded PMMA and steel, unsanded PMMA and mop head, unsanded PMMA and sisal rope, unsanded PMMA and steel (Dunn’s Test, $p < \alpha/2$ where $\alpha = 0.05$). No significant differences in the abundance per 500 cm^2 were recorded between any other material pairs (Dunn’s Test, $p > \alpha/2$ where $\alpha = 0.05$).

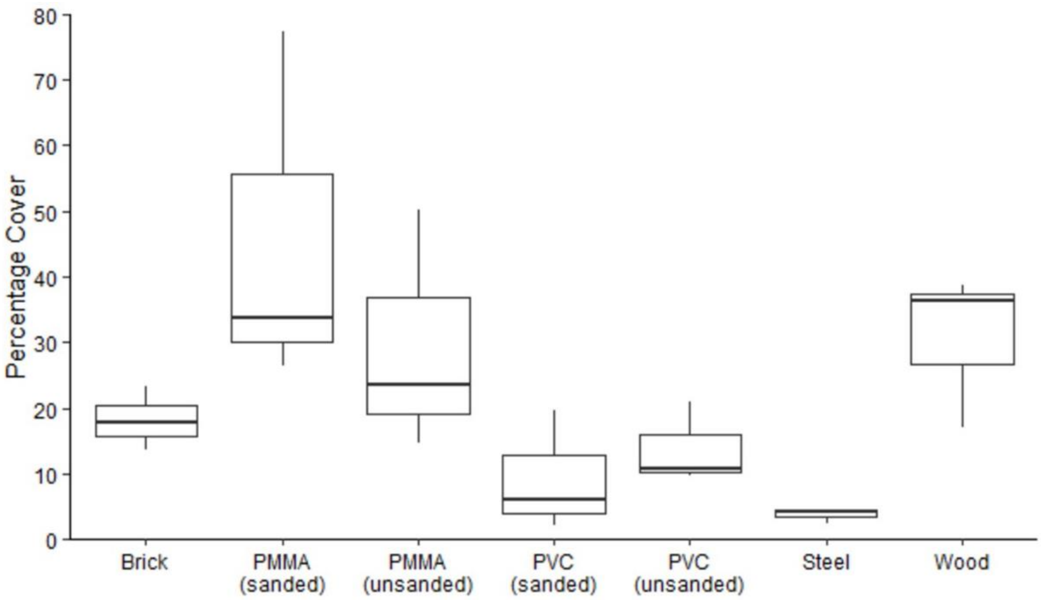

Fig. 8. Percentage cover of organisms present on selected materials from within the MMS after eight months. n = 3 for each material.

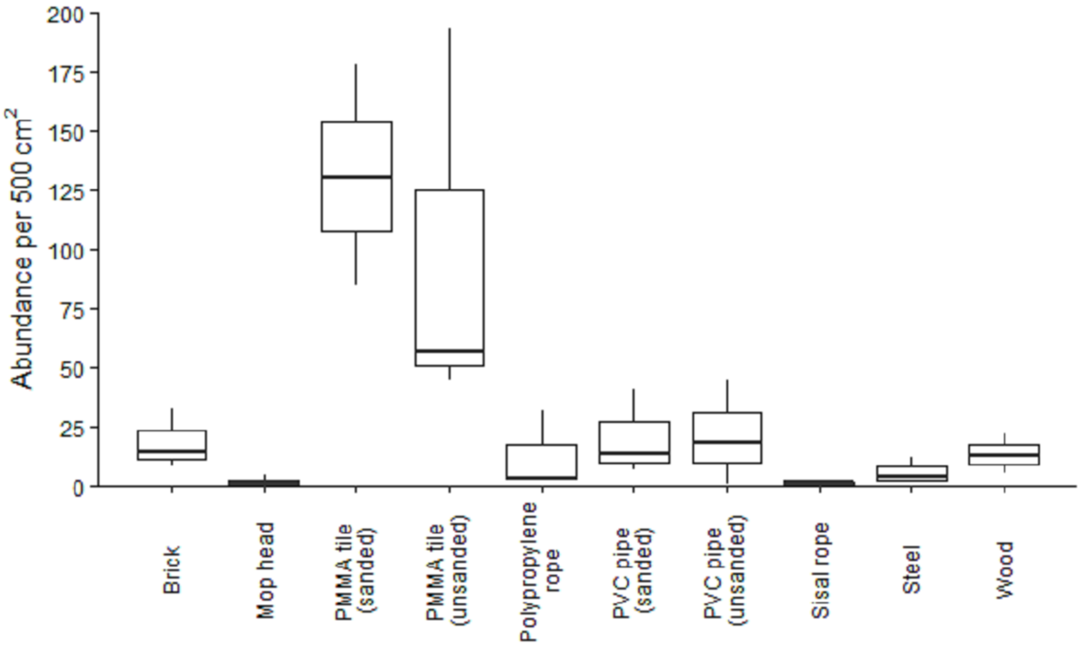

Fig. 9. Total abundance of organisms per 500 cm^2 recorded on each material used within the MMS. Abundance counts standardised using the surface area of each material to allow for direct comparison. n = 3.

Non-native species (NNS)

A total of 7 NNS were recorded during this research. All 7 species were identified from within the SSS, with only 5 of the 7 identified within the MMS (Table 1). *Caprella mutica* (Schurin, 1935) and *Monocorophium acherusicum* (Costa, 1853) were the two species found exclusively within the SSS. *Bugulina stolonifera* had the greatest average abundance of all NNS in the SSS as well as MMS, and a second bryozoan, *Bugula neritina*, had the lowest average abundance.

Table 1. Mean abundance of NNS per 500 cm^2 (± standard error) of each NNS recorded within the two survey types: Successional Settlement Survey (SSS) and Mixed Material Survey (MMS).

NNS	**Survey Type**	
	SSS	**MMS**
Austrominius modestus	13.7 ± 6.9	72.3 ± 49.9
Brachynotus sexdentatus	0.3 ± 0.3	0.7 ± 0.3
Bugula neritina	1.0 ± 1.0	0.7 ± 0.7
Bugulina stolonifera	115.3 ± 20.1	74 ± 50.6
Caprella mutica	18.0 ± 10.0	-
Monocorophium acherusicum	5.3 ± 2.4	-
Styela clava	3.0 ± 3.0	8.3 ± 5.8

Cross-Survey Analysis

Species richness data (as the total number of species recorded in samples) were analysed across the two survey types to identify the effectiveness of each survey type at describing fouling communities as well as identifying NNS. Considering all species, the SSS attracted a larger number of species compared with the MMS (Figure 10). This difference was found to be statistically significant (Fisher-Pitman permutation test, $Z = -2.0207$, $p = 0.0433$) and therefore demonstrates that survey type was a significant factor in determining the number of species recorded. Survey type was found to have no significant effect on the number of NNS recorded (Fisher-Pitman permutation test, $Z = -0.488$, $p = 0.6256$).

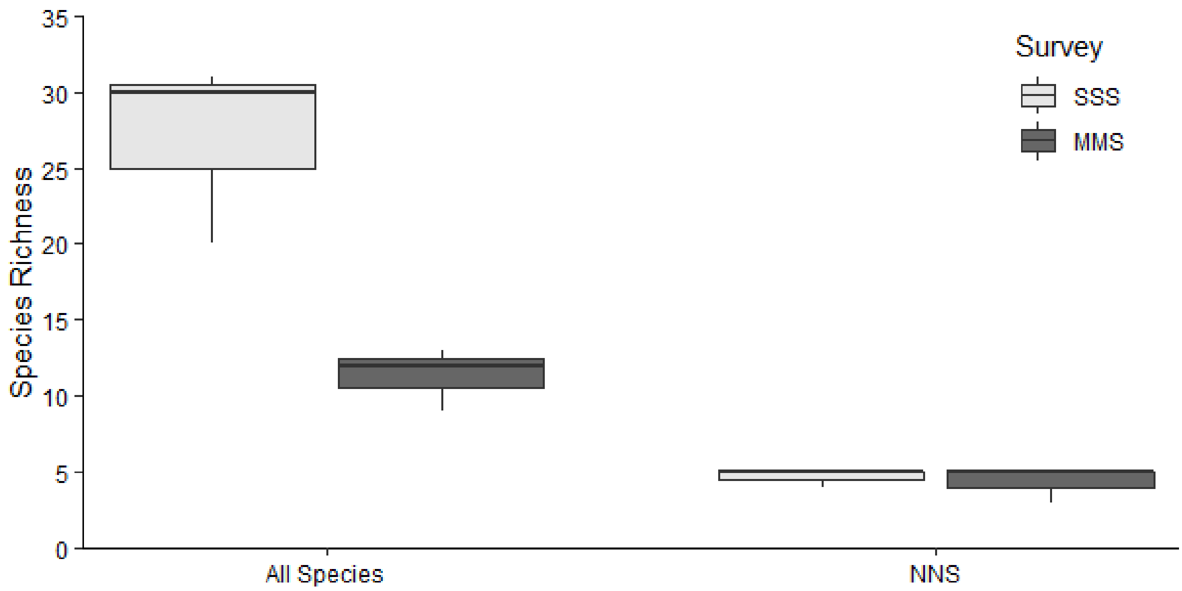

Fig. 10. Average species richness recorded with the two survey types ‘successional settlement survey’ (SSS) and ‘mixed material survey’ (MMS). Species per 2700 cm^2 for SSS (sum of 6 tiles per zone), 13533 cm^2 for MMS (sum of all materials per zone); n=3.

DISCUSSION

Fouling communities

Selection of materials by sessile benthic organisms is more complex than that of mobile organisms, relying on a wide range of factors including orientation, position, material, light, pollution, recruitment, competition, predation and biofilms, amongst others (Sutherland and Karlson, 1977; Osman, 1977; Harris and Irons, 1982; Keough and Downes, 1982; Glasby, 1999, 2000; Glasby and Connell, 2001; Dobretsov *et al*., 2005; Blockley and Chapman, 2006; Qian *et al.*, 2007; Tyrrell and Byers, 2007; Nydam and Stachowicz, 2007; Crooks *et al*., 2011). Applying two different survey types greatly improved our knowledge of the fouling community present within an industrial port environment. The rapid rate of colonisation of settlement tiles within the successional settlement survey (SSS) was striking. Colonisation to over 90% coverage took only two months for the sides of tiles facing the water column. Colonisation during this time period (May through to July) was expected as this coincides with the annual phase of benthic larval settlement, although the process can be highly variable (Keough, 1983; Ronowicz *et al.*, 2014). Colonisation rates were comparable to those observed in a similar study conducted in marinas in North Wales (Bangor University, 2015), even though the study focussed on individual species and did not record overall coverage.

Of the 38 species identified in total, 18 fell into the functional group of filter feeders, including the three most abundant species: *Ciona intestinalis, Ascidiella scabra* and *Bugulina stolonifera*. In natural ecosystems filter feeders can play a key role in structuring phytoplankton communities and in nutrient cycling, and therefore controlling primary production to an extent (Stein *et al.*, 1995; Sánchez *et al.*, 2016). Given that the Port of Swansea is an enclosed system, it is likely that filter feeders, particularly the abundant solitary ascidians, play a major role in determining clearance rates and forming the observed communities. Filter feeders have also been identified as keystone species in other systems (Persson *et al.*, 2007), the effect of which may be further enhanced through the production of faecal pellets which may support a range of different organisms such as the detritivores identified within this study (Ostroumov, 2005). It was observed that ascidian species would readily settle directly onto the tests of *A. scabra*, with up to five different ascidian species being recorded on one individual. *A. scabra* has a cartilaginous test making it rigid with a rough texture, creating a viable surface for larval settlement. By contrast *C. intestinalis*, the only other frequently abundant and large solitary ascidian identified, has a more flexible and softer test, and consequently no organisms were observed to settle directly onto this species. It follows therefore that *A. scabra* may be a key species in increasing biodiversity within certain fouling communities.

By removing one settlement tile per month as part of the SSS it was possible to investigate community succession during the first six months of deployment. It provided greater survey power by recording species that were not present on settlement materials after six months but were present for intermediate stages within the six month period, thus providing a more complete insight into fouling communities rather than single ‘snapshots’ in time as is often the case when using settlement panels (Bangor University, 2015; Cook *et al*., 2015; Hurst, 2016). Community structure was found to change over the course of the survey period, which we suggest here may be influenced by the presence of *A. scabra*. Dense aggregations of *A. scabra* appeared to support a number of additional taxa, such as various amphipod species, through the initial four months of colonisation. This is made more apparent given the fall in the total number of species recorded per sample occurring concurrently with the fall in abundance of *A. scabra*.

Succession in this study saw the increase in coverage of colonial ascidians following the decline in *A. scabra* abundance. It is thought that the more two-dimensional habitat created by the colonial ascidians, compared to that created by dense *A. scabra* aggregations, provides less space, shelter and access to food, which are important factors in habitat selection by cryptic organisms (Aikins & Kikuchi, 2001). This resulted in the transition from a more species-rich fouling community, when *A. scabra* was present in high abundance, to a more species-poor community as colonial ascidians increased in dominance.

Knowledge of how densely different materials are colonised and which species colonise each material can contribute to informing port management plans. This may be, for example, to adopt strategies to increase port biodiversity by focussing more on the types of materials that are present within the port. Community composition, total colonised area and the abundance of organisms per 500 cm^2 were all found to be significantly influenced by material type. PMMA in particular was consistently found to support a greater abundance and total colonised area than most other materials, which may have wider implications for the distribution of organisms associated with marine litter (Miralles *et al.*, 2018). Fibrous materials, such as rope and mop heads, were amongst the least colonised materials. This is thought to be based largely on larval habitat selection preferences, where more solid and secure substrata such as plastic, wood and brick is favoured (Osman, 1977). Steel too would ordinarily be considered a viable substrate for larval settlement. However, accelerated low water corrosion (ALCW; Marty *et al.*, 2014; Smith *et al.*, 2019) was found to impede the colonisation of steel, likely through chemical interaction with larvae or by creating a physical barrier for larval settlement (Smith *et al.*, 2019). Contrary to observations made in the MMS, both of the sessile species recorded from only one material, *Bugula neritina* and *Spirobranchus triqueter,* have been reported to colonise a wide range of materials in previous studies (Li *et al.*, 2016; Gündoğdu *et al*., 2017), and indeed in the SSS survey within this study. Whilst it is difficult to directly compare observations made here to previous colonisation studies, particularly ones from different environments, it is probable that a number of the factors listed above are resulting in the reduced colonisation of some materials by certain organisms. Given the high abundance of *Ascidiella scabra, Ciona intestinalis* and *Bugulina stolonifera* recorded in this survey across most materials, it seems likely that competition for space and food would be the primary factor in limiting colonisation by various other species. Competition is likely enforced by a lack of larval recruitment, particularly with *B. neritina* as this species was recorded only five times across both survey types, which suggests there may be low larval recruitment for this species within the port. This information could be applied by port operators to promote certain communities within ports, or to increase the efficiency of port activities and processes by using specific material types.

Non-native species (NNS)

A total of 7 non-native species (NNS) were identified and recorded as part of this study, with only one species, *Brachynotus sexdentatus*, not being considered 'established' within the UK (NBN Atlas, accessed online 4th December 2019). The date of first record for these species range from as long ago as 1875 for *Bugulina stolonifera* (Ryland, 1960) to as recent as 2000 for *Caprella mutica* (Willis *et al.*, 2004). Each of the established NNS can be found at various locations around the UK, having spread beyond the site of first introduction (Ryland, 1960; Eno *et al*., 1997; Bracewell *et al.*, 2012).

More locally to Swansea, all but *C. mutica* have been reported from the South Wales coastline with *B. stolonifera*, *Bugula neritina* and *Brachynotus sexdentatus* (Risso, 1827) having been recorded from within the Port of Swansea in the late 1950s (Naylor, 1957). *B. sexdentatus* has in fact only ever been recorded in the UK from within the Port of Swansea and, along with *B. neritina*, was thought to have been naturally eradicated from the port following the closure of the Tir John power station in the 1970s (Eno *et al*., 1997; Arenas *et al.*, 2006). Water within the port had been artificially heated whilst the power station was in operation, through the discharge of heated effluent, creating a suitable habitat for the warmer water natives *B. sexdentatus* and *B. neritina* (Keough and Chernoff, 1987; Cuesta *et al*., 2000). Arenas *et al*. (2006) reported the presence of *B. neritina* from various locations around the UK, in contradiction to Eno *et al*. (1997), although no surveys were conducted within the Port of Swansea. It is likely that each of these species remained within the port through a successfully reproducing population rather than being reintroduced. It would appear though that the abundance of each species within the port has reduced since the last comprehensive survey was completed in the 1950s (Naylor, 1957). This may well be due to the cooling of the dock water which may have shifted the competitive edge back to some of the native species or indeed NNS, such as *B. stolonifera*, which have been recorded in high abundance within this study.

It is difficult to accurately comment on how, or even when, these NNS may have been first introduced to the port due to the lack of baseline data. Given the port activities it seems plausible that shipping is the likely pathway for all non-native introductions here. Ports are widely regarded as potential vectors for NNS, where they may be first introduced to a region within a port before spreading more locally along a natural coastline (Bailey, 2015). Regarding the NNS recorded in this study, only *B. sexdentatus* and *C. mutica* have not been previously recorded from elsewhere within the South Wales region. Therefore, these two species are at risk of being dispersed from the port into the natural environment of the Bristol Channel. Whilst it is unknown exactly when *C. mutica* was first introduced to the Port of Swansea, it can, however, be assumed that if the habitats and environmental conditions in the Bristol Channel were suitable for *C. mutica* then it would by now have spread out of the port. *C. mutica* is a common NNS worldwide and is regularly reported from within ports and marinas (Ashton *et al.*, 2007a). These reports along with experimental studies indicate that *C. mutica* can survive in temperatures ranging from -1.8 °C to 25 °C (Schevchenko *et al*., 2004) but would likely not survive prolonged exposure to salinities below 18 (Ashton *et al.*, 2007b). Temperature ranges within in the Bristol Channel fall comfortably within the tolerance of *C. mutica*, however the tidal nature of this region can result in salinities of 17 on low tides (Henderson *et al.*, 2012). Whilst the lower range of salinities here are short lived, it could potentially be the reason that *C. mutica* has not been recorded from the Bristol Channel. Competition from native caprellid amphipods (Shucksmith *et al.*, 2009) such as *C. linearis* (Linnaeus, 1767), which is present in South Wales (NBN Atlas, accessed online 6th December 2019), or the possibility that *C. mutica* is in fact present in the Bristol Channel but has either not been observed in surveys or the data have not been reported in the public domain, may also be reasons for the perceived absence of *C. mutica* in the Bristol Channel.

A lot less is known about *B. sexdentatus*. Whilst temperatures within the Port of Swansea can range from approximately 5 °C to 25°C (unpublished), it is unlikely that *B. sexdentatus* would be able to tolerate the harsh conditions of large tidal range and temperature and salinity fluctuations that define habitats within the Bristol Channel, given that it is native to Southern Europe. The remaining five NNS have been previously recorded from the South Wales region (NBN Atlas, accessed online 6th December 2019) therefore the current risk of non-native invasion beyond the port is minimal for these species.

Port-to-port and port-to-marina transport of NNS remains a risk through either ballast water or hull fouling and should therefore be considered in the port biosecurity risk management protocols. It is, unfortunately, impossible to say whether the NNS appeared first within the Port of Swansea or elsewhere within the Bristol Channel.

Effectiveness of survey methods

When discussing the effectiveness of each survey type designed for this study, the success of overcoming specific challenges of surveying within active ports must be considered, as well as the effectiveness of identifying species (both native and non-native), the quality of data provided and some of the more logistical aspects such as cost and level of required expertise.

Previous methodologies for the study of fouling communities in ports describe the use of rapid assessment surveys (RAS; Cohen *et al.*, 2005; Arenas *et al.*, 2006; Rohde *et al.*, 2017), settlement surveys (Floerl *et al.*, 2012; Ronowicz *et al.*, 2014; Tyrrell & Byers, 2007) or a combination of the two (HELCOM, 2013; Cook *et al.*, 2015; Hurst, 2016). The SSS described in this study is a development of more conventional settlement surveys whilst the MMS is, in effect, an adaption of a RAS, whereby materials are assembled and deployed rather than using materials that already exist in the port. This aims to overcome the problem of a lack of existing materials that can be safely accessed, which is the primary difficulty of using RAS within ports. The materials within the MMS would need to be in deployment for a number of years to be directly comparable to RAS, which may be considered for longer-term monitoring. Both survey types successfully overcame the primary limitations to conducting fouling community surveys within ports. All survey materials deployed in May 2018 remained undisturbed and were successfully retrieved in the winter of 2018. No materials interfered with port operations and researchers were able to comfortably work within the port health and safety regulations. This success was due in a large part to extensive field site visits and discussion with port authorities during the planning phase to limit the risk of interference in port activities and potential removal of materials.

As a direct comparison between survey types, the SSS was more effective than the MMS at identifying species, with an average of 27 species present per sample in the SSS compared to only 11 in the MMS. Both survey types did, however, record a similar number of NNS per sample, although two NNS (*Caprella mutica* and *Monocorophium acherusicum*) were recorded only from within the SSS. This indicates two key points: firstly, the MMS appears to attract a greater proportion of NNS per sample than the SSS; and secondly that the SSS is more successful at describing whole fouling communities. It could be argued then that there is no need to deploy the MMS, as the SSS offers more in terms of data on fouling communities and can identify more NNS. The MMS does though offer important insight for stakeholders in terms of biosecurity planning. Clearly, a combination of the SSS and MMS provides the most useful data with consideration not only of what species are inhabiting the Port of Swansea, but also which materials may be of interest for future management.

Since the materials used in each survey were deployed as accurately as possible to a set depth and with the slight fluctuation in the port water level (between 10 – 12m) over the course of deployment, depth effects may have influenced the observed fouling communities both between survey types and within the MMS, where materials extended over a couple of metres in sequence.

Depth is known to be a factor in determining the formation of biofilms and settlement of some organisms (Hurlbut, 1991; Head *et al*., 2004; Kazmi *et al*., 2020) and has been found to be a significant factor in determining the community composition in a previous study (Lezzi and Giangrande, 2018). Accommodating multiple SSS frames in sequence at set depths, similar to that done by Lezzi and Giangrande (2018), would enable depth as a factor to be investigated without the need for additional deployment sites. However, weight should be considered if doing this in future and retrieval of survey materials may not be feasible without mechanical assistance during months of peak colonisation.

The quantity of data collected in this study varied between survey types primarily due to the way in which the data was collected. Species identified from the SSS were all recorded in the laboratory once a month for six months, whilst the MMS utilised field-based identification only once following the deployment period of eight months. This is believed to be the primary reason for the significant difference between the number of species identified within the SSS and MMS, as there were six times as many samples collected and laboratory based analysis allows for the identification of more cryptic and smaller organisms that may be missed during field identification. A consideration for future applications would be to adapt the MMS survey procedure to include monthly field examinations, thus incorporating the element of succession and seasonality, which would likely increase the total number of recorded species and reduce the disparity between the MMS and SSS. Having three of each survey type deployed within the port provided minimal replication, particularly when considering the level of replication required for powerful statistical analyses. This is also an important factor when considering the probability of detection of rare species. Many studies that use settlement panels to detect NNS do not make reference to the probability of detection when determining appropriate sample sizes (e.g. Canning-Clode *et al*., 2013; Bangor University, 2015; Hurst, 2016; Marraffini *et al*., 2017), however it is an important factor in determining the confidence that all NNS would be identified if present within a system and therefore the reliability of the survey method (Floerl *et al*., 2012; Ma, 2020). Within ports it would be difficult to significantly increase the sample as the availability of sites to deploy materials that satisfy both the research aims and port authorities are generally very limited (HELCOM, 2013). Due to the practicalities of working within active ports there must be leeway for a degree of compromise between increasing the number of replicates and operating safely within a potentially dangerous environment. We suggest therefore that sample size should be increased, with a view to increasing the probability of detecting NNS, when it is possible to achieve this safely. It follows that the importance of this research tends more towards the descriptive aspect of identifying species and the applied focus of informing future port monitoring and biosecurity management strategies, in line with current legislation (Environment (Wales) Act, 2016; Regulation (EU) No 1143/2014). Whilst both surveys yielded quantified measures of species richness and abundance, these were more easily calculated from the SSS due to the standardised size of PMMA tiles compared to the varied surface areas of materials used within the MMS. A consideration for port authorities applying these methods in the future would be to reflect on what sort of data would be valuable. If it is simply a case of listing which organisms are inhabiting the port, then quantified data would be less of a priority.

There are several practical aspects that need to be considered, which weigh for and against each survey type: cost, time, level of expertise required, and equipment required. For each of these factors the differences between each survey types comes down to the use of laboratory-based sample analysis. Each survey type cannot be separated in terms of the time required to deploy and retrieve materials; however, the more in-depth laboratory analysis takes considerably longer than field-based analysis.

Laboratory analysis also requires a higher level of expertise and equipment than would be required to do more basic visual analysis in the field. These factors contribute to the overall cost that would be incurred for port authorities to implement the survey methods, with the accessibility of experienced taxonomists along with the necessary equipment and resources being an important consideration for future applications.

It should also be noted that settlement surveys are just one way in which to monitor NNS and describe the wider fouling community. The use of molecular techniques such as metabarcoding and the isolation of environmental DNA (eDNA) from water samples is becoming increasingly popular in the field of invasion biology, where the applications can include screening for target NNS and tracing the origin of NNS as well as more broadly identifying organisms present in a specific environment (Rius *et al*., 2015). Collecting water samples for the extraction of eDNA can be successfully and safely conducted within port environments, and has been proven effective at identifying some of the NNS identified within this study, suggesting this a viable option for NNS screening (Borrell *et al*., 2017; Holman *et al*., 2019). The use of eDNA and metabarcoding for the quantification of abundances, as well as being able to identify all organisms to species level, is perhaps currently limiting the use of this method. Alternatively, underwater video and the use of remotely operated vehicles (ROVs) can be an effective tool in identifying visually distinctive species and can cover large areas habitat (e.g. Cánovas-Molina *et al*., 2016, Meyer *et al*., 2020). In the past, the use of ROVs has generally been constrained by cost to broad scale and meso-scale surveys (Bo *et al*., 2014; Cánovas-Molina *et al*., 2016), however there is now an increasing use of mini-ROVs for smaller scale surveying which would be possible within ports (Buscher *et al*., 2020). Whilst video methods are likely to miss detail when it comes to identifying cryptic species, identifying organisms to species level, and providing a detailed insight into community structure, the speed with which data can be collected means this method could prove effective in screening for visually distinctive NNS within ports. On balance, data obtained from the settlement surveys outlined here are currently the most effective compromise between cost, effort and level of detail, that can provide useful insights for managers into community structure and community development, which may be overlooked by alternative survey methods.

Conclusion

Deployment of the two survey designs outlined in this study was successful in terms of both overcoming the major constraints of conducting field surveys within active ports as well as providing a comprehensive description of the fouling communities present within the Port of Swansea. This success hinged on developing a strong working relationship with Associated British Ports, the operator of the Port of Swansea, which enabled for effective planning and implementation of surveys; a point which would likely be essential for conducting further surveys within active ports. A total of 38 species were recorded to species level, including 7 non-native species (NNS). Communities were found to be dominated by filter feeders, with large abundances of the solitary ascidians *Ciona intestinalis* and *Ascidiella scabra* as well as the non-native bryozoan *Bugulina stolonifera*. It is thought that some of these filter feeders, particularly *A. scabra*, serve as keystone species providing settlement surfaces and thereby supporting further colonisation by additional taxa. Community succession was evident over the course of the survey period which we suggest here may be driven by the succession of *A. scabra* by colonial ascidians. Colonial ascidians increase in colonisation as the abundance of *A. scabra* falls, leading to a change in habitat type and therefore community structure.

Material type was found to play a significant role in determining community composition, and the knowledge gained on which materials support greater species richness or higher proportions of NNS has considerable implications for port management and could prove essential in developing biosecurity and biodiversity plans. There is little concern over the NNS recorded from within the port, given that the only NNS not to have been previously recorded in South Wales are unlikely to survive in the environmental conditions of the highly tidal Bristol Channel. Transport of NNS from port-to-port through ballast water or hull fouling is the only real concern regarding further dispersal of NNS, highlighting the importance of the port to continue adhering to biosecurity guidelines.

A combination of both survey types is the clear approach in terms of providing a detailed analysis of fouling communities as well as offering practical insights to stakeholders regarding port management. It is recommended that the practical applications of survey implementation, particularly in terms of the available expertise and resources, prior to the deployment of survey materials, need to be carefully considered.

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