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The effect marina design and recreational boating has on the spread of Non Indigenous Species

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1. Introduction

1.1 An overview

The spread of Non Indigenous Species (NIS) is a global problem and has major implications for the pattern of local biodiversity and ultimately the functioning of ecosystems. Invasive NIS are recognised as a major threat to biodiversity in article 8 on the Convention of Biological Diversity (CBD). Once NIS are introduced controlling them is expensive, time-consuming and can have negative effects on native biota. It recent years, the marine environment has seen an increase in recreational boating, international shipping and aquaculture but to date only the latter two have received international attention. In 2008 legislation changed to prohibit the use of Tributyltin (TBT) in anti fouling paints on all vessels entering or registered to the United Kingdom. This is because the accumulation of TBT has been found to have serious effects on marine biota; changing the sex of dog whelks and showing deformations in oysters (Savarese 2005). However TBT is the most effective pesticide available to deter sessile biota from attaching to the hull of a vessel. There are other alternatives, such as booster biocides (European Commission 2006) but development of them has had significant effects on non target species and limited effects on target species, therefore at present hull fouling is set to become a serious issue.

The purpose of this report is to research the effect marina design and recreational boating can have on the secondary spread of species which have been introduced into the marine ecosystem. It will focus on the seasonal recruitment of species in two contrasting marinas, one open and one enclosed, in South West England. The settlement of colonising sessile invertebrates will be analysed using test panels as proxies for hull colonisation, over the reproductive season.

1.2 Background

In February 2008 I attended a science fair at the Marine Biological Association (MBA) and was introduced to John Bishop, who is a leading scientist on the Marine Alien's team. We discussed marine introductions and my second year project on *Crepidula fornicata* an invasive slipper limpet found in the Fal estuary. There was talk of a possible third year project to follow along side an MRes research project. A few weeks passed and then an email arrived, the project could go ahead. This research project began in March 2008 and the field work took place from April 2008 until August 2008. This project has had funding from the Esme Fairburn Foundation and The Green Blue.

1.3 The Green Blue

The Green Blue is an environmental awareness initiative by the British Marine Federation and the Royal Yachting Association. Their aim is to promote the sustainable use of coastal and inland waters by streamlining the operations and development of the boating industry in a holistic manner. It educates and informs the recreational boating community about environmental impacts and is working towards an environmentally self-regulating boating community (The Green Blue 2008). It has funded an array of research projects in keeping with their mission.

2. Literature review

This chapter provides a detailed review of the most current literature on the subject of Non-Indigenous Species (NIS) focusing on; the introduction of species, hull fouling, the importance of marina and marina design, the settlement of sessile invertebrates and the effect of recreational boating on the secondary spread of NIS.

2.1 Marine introductions via commercial shipping

The movement of species from one location to another via shipping has had major impacts on many marine ecosystems throughout the world. The movement of juveniles and larvae for the aquaculture and aquarium industry are contributing to the introduction of NIS (Maltlock 2004). Ballast water (figure 1) is also highlighted as a major vector for introductions; damaging marine and fresh-water environments worldwide, causing vast economic expenditure to industry and natural resources (Dunstan & Bax 2008; Bax et al. 2008; Ruiz *et al.* 1997).

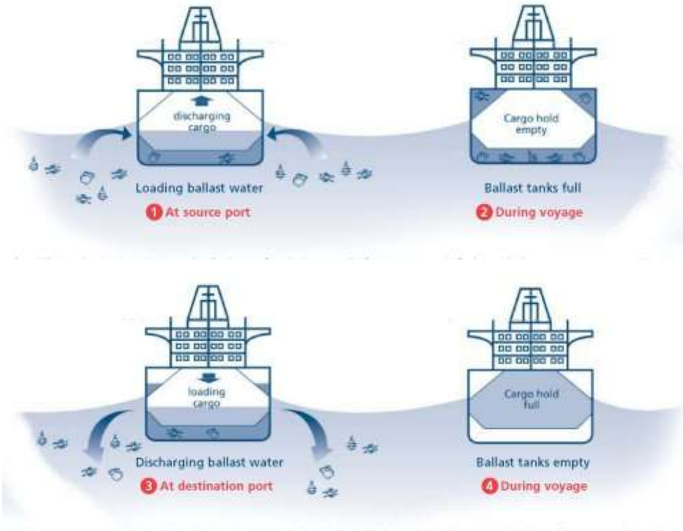

Figure 1. Ballast water is used by ships to provide balance and stability for un-laden ships. When cargo is unloaded ballast water is taken in, the ship returns to country of origin where new cargo is loaded and the ballast water is emptied. This water carries juvenile species and larvae thousands of miles around the globe for anthropogenic needs and changes the structure and community of ecosystems (IMO 2007).

The International Convention on the Control and Management of Ships' Ballast Water and Sediments introduced by the International Maritime Organization in 2004 awaits ratification and will require all international shipping to undertake high seas exchange. There is however no such convention for the problems associated with hull fouling (plate 1). Even though historical records indicated wooden boats were heavily incrusted with fouling organisms (Bax et al., 2003).

Plate 1. The hull of a ship is prone to fouling organisms. Paint and hull cleaning help decrease the spread of species; however it does not target all areas of a ship and some species are extremely persistent (Geocities 2006).

A study carried out in the North Sea by Gollasch (2000), analysed fauna transported by international ships to assess species introductions via hull fouling. A total of 257 species were analysed and 57% of the species found were non native to the North

Sea. Samples derived from hulls of ships contained 97% non native species compared to 38% from ballast water and 57% from sediment samples; indicating hull fouling is an important vector.

2.2 Hull fouling and anti-foul paints

A fouling organism is one that lives attached to submerged surfaces such as ships and pilings (Castro & Huber 2007). Effective anti-fouling paints incorporating organotins have noticeably reduced fouling biomass; however these paints have a limited period of effectiveness, which can be less than the ships' inter-docking period, dependant on sea temperature and abrasion (Minchin & Gollasch 2002). Vessels immersed over several years can allow fouling communities to develop and spread beyond their native distribution. Minchin and Gollasch (2002) propose that a short turnaround of vessels with attached mature sessile biota can lead to synchronised spawning and the production of sufficient larvae to form a founder population in a marina, according to season.

The different designs of floating structures provide varying conditions for fouling communities. These range from stationary platforms that may subsequently be moved (oil platforms), slow moving vessels (barges, yachts), to faster moving craft (hydrofoil ferries and naval vessels). Vessels with lower speeds appear to accommodate a greater biomass of fouling biota. Slow vessels may have a high residency and long immersion periods leading to extensive fouling communities (Southgate & Myers, 1985). Their relatively slow passage or inactivity enables fouling communities to settle over a longer time period. Many structures remain in harbour regions over long periods and their movements will allow transmissions of dense fouling communities with perhaps a greater probability of some becoming established (Minchin & Gollasch 2002).

The application of antifouling paint is a requirement under the International Maritime Organisation. Trybutyltin (TBT), a once common anti fouling paint, was banned in 1987 for recreational boats and in 2008 on all vessels, due to significant impacts on marine organisms. Since then the majority of paints have been copper based, in which the main biocide is cuprous oxide, this is toxic to marine life and thereby resists the build up of organic fouling (The Green Blue 2008), however not sufficiently. TBT is the still most successful pesticide to deter sessile assemblages colonising the hull of vessels. Another option has been the invention of biocide boosters, which are added to paint. However these have affected non target species and had little effect on fouling assemblages. The anti-foul products are controlled under the Biocidal Products Directive (98/9/EC).

2.3 The importance of marinas to the UK economy

There are 236 coastal marinas in the UK and Channel Islands providing 49,000 berths. This however is insufficient and many boat owners will travel large distances to use their vessels. The British Marine Federation (BMF, 2007) has identified the coastal marinas sector as an important contributor to employment, regeneration and tourism; equating up to £700 million and supporting over 22,000 jobs. With the rise of recreational boating; over 4 million adults participated in boating in 2007 and approximately 700,000 households own a boat, the size and type of marinas has also increased. The BMF now faces new challenges due to environmental restrictions which limit the growth and productivity of marinas especially with increased licensing costs for dredging. This study will benefit the BMF and other organisations such as the Green Blue with guidelines for the design of marinas and also scientific research that could help educate boat owners.

2.4 Marina design

Human modifications to the shoreline such as marinas modify wave propagation and tidal currents and can affect the recruitment of species in surrounding habitats (Connell & Glasby 1999; Glasby & Connell 2000; Floerl & Inglis 2003). Recreational vessels of >6m inhabiting marinas are generally at rest and can remain in port from an overnight stop to several months and up to a year, thus becoming part of the marina substrata (Floerl & Inglis 2003). Marinas can be categorised into two physical types, those with and without enclosures. Tidal exchange within enclosed marinas is restricted to either; a lock gate (plate 2) or a narrow channel, which determines the degree of flushing from outside waters; those lacking this protection are subject to increased tidal movements and currents (Blain 1992).

Plate 2. View from the inner marina at Port Pendennis towards the lock gate and outer marina, restricting the water flow.

Floerl and Inglis (2003) demonstrated how the physical characteristics of the location in which the boat is moored could have a large influence on both the size and frequency of recruitment which were greatly enhanced in partially enclosed marinas where available surfaces received large densities of settling larvae. Floerl and Inglis (2003), also contribute that the probability of a NIS engaging with an available vector is related to the abundance, availability and selectivity of the vector and the local supply of colonizing species. It is understood that larvae size and the frequency of repeat recruitments are critical determinants of the success of establishment by non-indigenous species (Carlton 1996; Ruiz et al. 2000); however Johnston and Keough (2000) comment how the use of toxic anti fouling compounds prevents the settlement of larvae. However as explained above this approach has limited success over a certain time period. In a study conducted by Floerl and Inglis (2003) measurements of hydrodynamics were also taken and showed that enclosed marinas retained water in the marina for up to 12 hours, reducing flushing time and water flow. Judge and Craig (1997) suggested that water flow plays a significant factor in species sustaining life processes and the recruitment of larvae to a substratum, indicating enclosed marinas may not facilitate increased colonisation. Studies in the laboratory, for example, have shown that both arborescent and encrusting sessile suspension feeders often exhibit flow-dependent feeding (Okamura 1984).

2.5 Colonisation of floating structures

Man-made urban structures such as pontoons provide an important habitat for a wide range of subtidal plants and animals, which would otherwise be attached to a nearby bedrock surface (Connell & Glasby, 1999; Arenas *et al.* 2007). However a great contrast can be seen in the composition and abundance of fouling communities inhabiting the two surfaces (Connell & Glasby, 1999; Connell, 2001). Pontoons benefit by being isolated from the seafloor, decreasing the chances of benthic predation, (Connell & Glasby 1999, Holloway & Connell, 2002). Many studies demonstrate the material of substrata (e.g. clay, sandstone, plastic, fibreglass) to determine the settlement of different assemblages. Anderson and Underwood (1994), illustrate how many species including barnacles and oysters recruit in larger numbers on concrete or plywood than on fibreglass or aluminium. A multivariate analysis showed assemblages on different substrata were significantly different after 1 or 2 months of submersion, but became more similar after longer periods. Thus is indicating that the nature of the substratum can affect both initial colonisation of particular species and the development of the assemblage over time. Holloway and Connell (2002) conclude that processes generating new habitats can cause assemblages to change in structure and composition and furthermore, urban structures may become habitats for introduced species.

2.6 Recreational boating as a vector for secondary spread

Recreational boating and the maritime leisure industry is a growing sector both economically and geographically (British Marine Federation 2007). In port there are opportunities for NIS to spread and settle onto smaller craft. Due to the prolonged time recreational vessels spend in port, it is possible for those vessels to accrue a fouling that corresponds to the locally established biota (Floerl & Inglis 2000). Another element to consider is that NIS can be spread with overland transport of hull-fouled boats on trailers (Minchin & Golasch 2002). As detailed earlier in section 1.6, there has been an increase in recreational boaters driving to different locations to launch their vessels.

Hull fouling from recreational boating has been associated with the spread of marine introductions with many studies concerned with the effects on inland water ways (Johnson et al. 2001). Others have looked at the effect recreational boating has had on the movement of vertebrates and algae (Floerl & Inglis, 2003) and research carried out by Mineur et al. (2008) concentrates on recreational boating as a vector for seaweed introductions. Floerl & Inglis (2003) suggests recreational boating activity has been implicated in the spread of a number of notable invasive species, including the Japanese kelp *Undaria pinnatifida* and the marine black-striped mussel *Mytilopsis sallei* (Thresher 1999).

To assess boat owner involvement, Mineur et al. (2008) conducted questionnaires to collate data on both the awareness boat owners about introduced species and also to judge how journey patterns and the management of their vessels influence hull fouling and ultimately the spread of invasive species. A hull survey was also carried out to produce a dataset of macro algae found on vessels which were in use. The research carried out in this study will illustrate the part recreational boating could play in the transfer of introduced species as well as the seasonal and longer term settlement which occur in marinas where these vessels are kept.

2.7 Aims and objectives

The GreenBlue specified an area of research which was required to further educate and enhance their mission. The aims and objectives were detailed in response to this .This research follows an MRes project which investigated the entire south coast of England, however it concentrated solely on open marinas, with varying types of enclosure. The aims and objectives of this study incorporated the results of the MRes project as a comparison with the open marina (Premier). The 'strongly enclosed' category demonstrated in the MRes project will be used as a comparison to the lock-gate marina (Port Pendennis) in this study.

The main aim of this study was to investigate the effect marina configuration has on hull fouling from which a set of guidelines could be produced, that would influence future use and design of marinas.

These aims were achieved by the following objectives. To quantify the settlement of sessile species, test plates were used, providing proxies for in situ hull colonisation. To establish which species are sustained, longer term plates were exposed showing medium term community development. These objectives were reinforced by the MRes results. The null hypothesis predicts there to be no difference in abundance or diversity within the contrasting marinas.

3. Site location

This chapter outlines the study area and gives mapped detail for reference purposes.

3.1 The Fal Estuary

The Fal Estuary is a ria system on the south coast of Cornwall (figure 2), which developed in response to Holocene sea-level rise (Bird 1998). Due to this natural phenomenon the Fal Estuary (figure 3, plate 3) is the third largest natural harbour in the world.

The estuary extends 18 km inland from its mouth to the northern tidal limit at Tresillian and has a total shoreline length of 127 km. In general terms it can be divided into two regions; the inner tidal tributaries and the outer tidal basin together termed the Carrick Roads. The estuary is macrotidal with a maximum spring tide of 5.3 m at Falmouth, but mesotidal at Truro with a spring tide of 3.5 m. According to chart data, the estuary banks reach a maximum depth of 4m and the dredged channel a maximum depth of 33m (Featherstone & Du Port 2007) creating an estuary with well mixed waters

Figure 2. Map of South West region showing Falmouth on the south coast (Mapquest 2007)

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Figure 3. Map of the Carrick Roads and Fal estuary detailing the shape and surrounding cities, towns and villages (World guides 2007)

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Plate 3. The Carrick Roads looking south towards the ocean (Cycleau 2005)

Under the EU Habitats Directive the Fal Estuary has been designated a Special Area of Conservation (SAC) (DEFRA 1998, Cornwall County Council 2008), protecting many threatened species (figure 4).

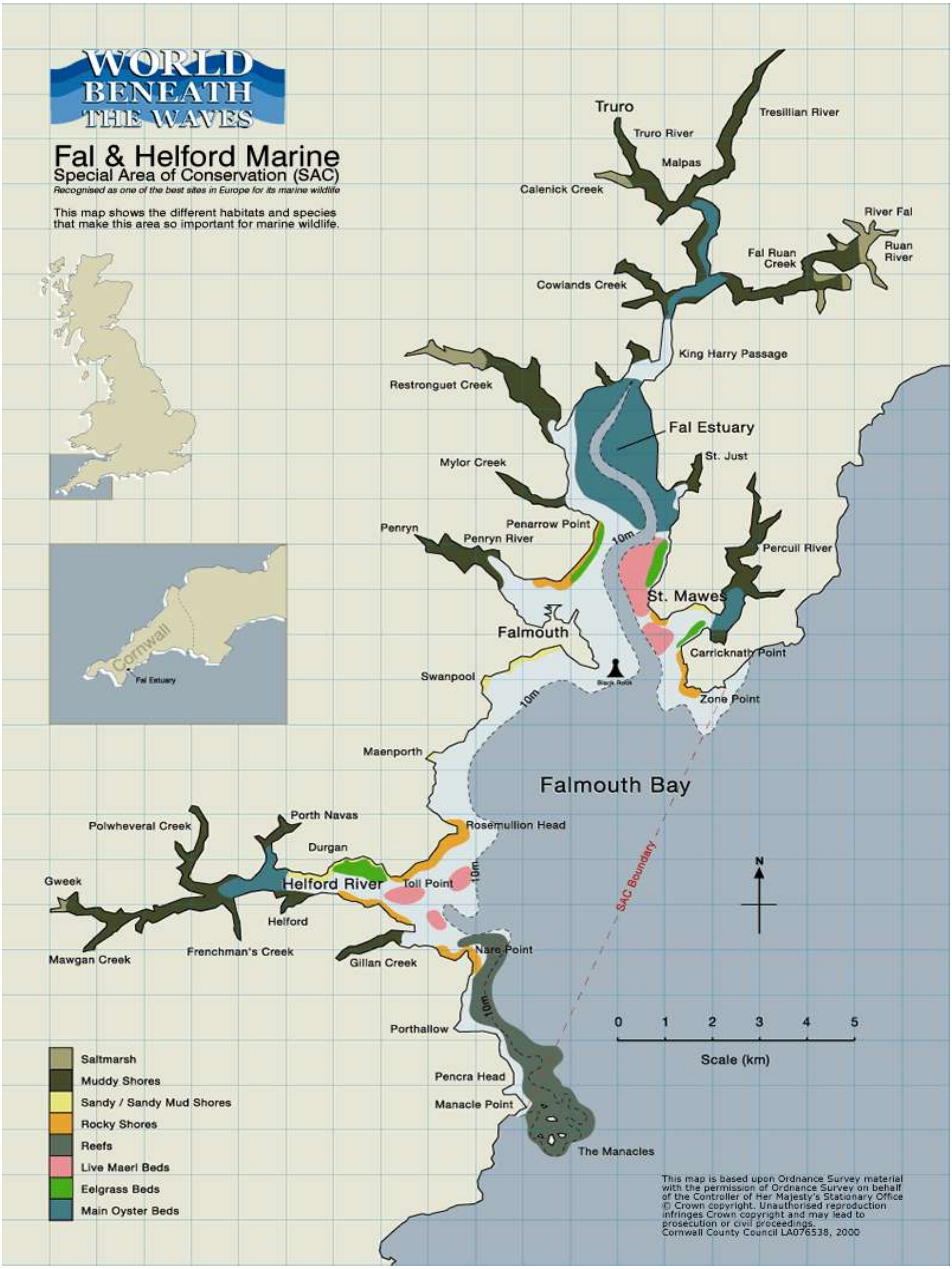

Figure 4. Map showing the species and habitats which are protected under the SAC designation in the Fal Estuary (World Beneath the waves, 2008)

3.2 Premier Marina

Premier Marina is positioned at the entrance to the Penryn River (appendix 1); it is an open marina (plate 4) and thus receives a steady water flow and well mixed waters from the Fal Estuary (Bird 1998). It does contain an inner marina but this is controlled by the tide. For the purpose of this experiment the site selected used only the outside berths (figure 5).The grid reference SW 798 338 defines the location of Premier marina.

Plate 4. Premier Marina, an aerial photograph showing the distinction of inner and outer berths (reeds online 2008).

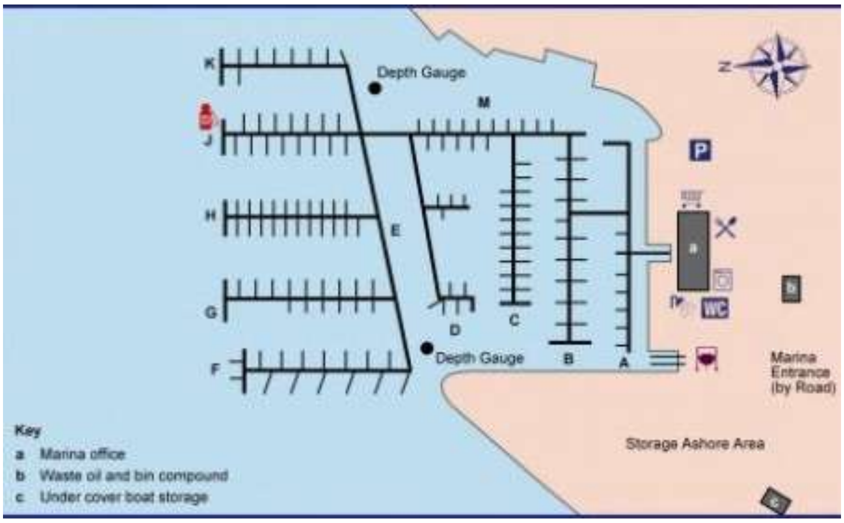

Figure 5. The layout of berths found at Premier Marina (reeds online 2008).

3.3 Port Pendennis Marina

Lying adjacent to the town centre Port Pendennis (appendix 1) is divided into an outer marina and an inner, locked gate (enclosed) marina (plate 5), only accessible three hours either side of high water. It creates a sheltered marine environment with limited mixed waters (Bird 1998). This refuge has become an arrival and departure point for many Trans-Atlantic and Mediterranean voyages due to its close proximity to the ocean. It also houses many large semi-residential yachts. For the purpose of this investigation the inner marina (figure 6) was the focus, enabling a comparison with Premier Marina. The grid reference SW 814 323 defines the location of Port Pendennis marina.

Plate 5. Photograph showing the area of the inner marina at Port Pendennis, illustrating an enclosed site containing mainly yachts (Port Pendennis 2007).

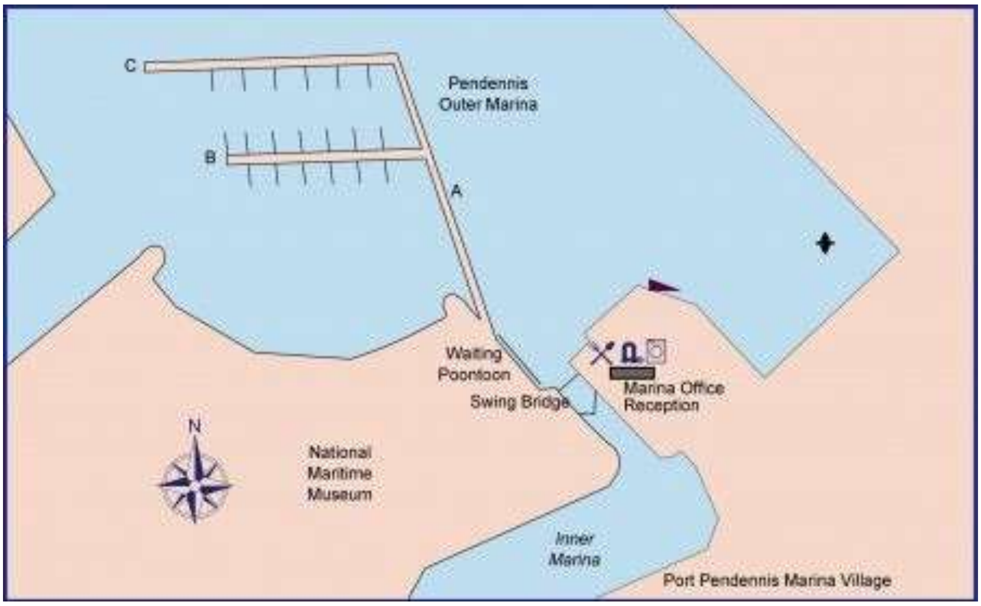

Figure 6. Map showing the sheltered inner marina at Port Pendennis, illustrating the lock gate, allowing only a small amount of mixing and water flow.

4. Materials and Methods

4.1 Time scale

To record and investigate settlement of sessile invertebrates the research had to be conducted in the reproductive season, when larvae and juveniles are most abundant. This research commenced in early April 2008, it was expected little colonisation would take place in the early months but a clear trend would be visible by covering each reproductive month from there on in. Preferably, the study should have continued well into September, however this experiment concluded at the end of August.

4.2 Equipment

A range of equipment was used to obtain the results. For the panels placed in the marinas; 15 x 15cm acrylic 3mm sheets were drilled with 0.5mm holes in the middle of opposite edges. Acrylic rope and grip ties cemented the structure together with a panel at the top and one hanging 180cm below. A lead fishing weight was used to hold the entire panel in place. Twenty litre containers were used to transport the short term panels from each marina and preserved using a 70% ethanol solution. Plastic clips were used as a non-corrosive protective barrier to separate the panels and avoid contact. A record was made of the order in which each panel was collected. The long term panels were collected in cool boxes and transported directly to the laboratory in sea water.

Analysis of the panels was undertaken in the laboratory using a Wild M8 dissection microscope and a 100 point grid measuring 15cm x 15cm. A supply of water was needed and the space was well-ventilated. For individual identification of species smaller dishes and a dissection kit were used.

4.3 Site selection

The marinas needed to display differing water flows; ideally one locked-gate marina and one open marina. In this study both marinas were in the same estuary. Two sites within each marina were chosen, representing an exposed location and a sheltered location. Within these areas a site A and B were selected and recorded on a map, for use at each deployment (figure 7 & 8).

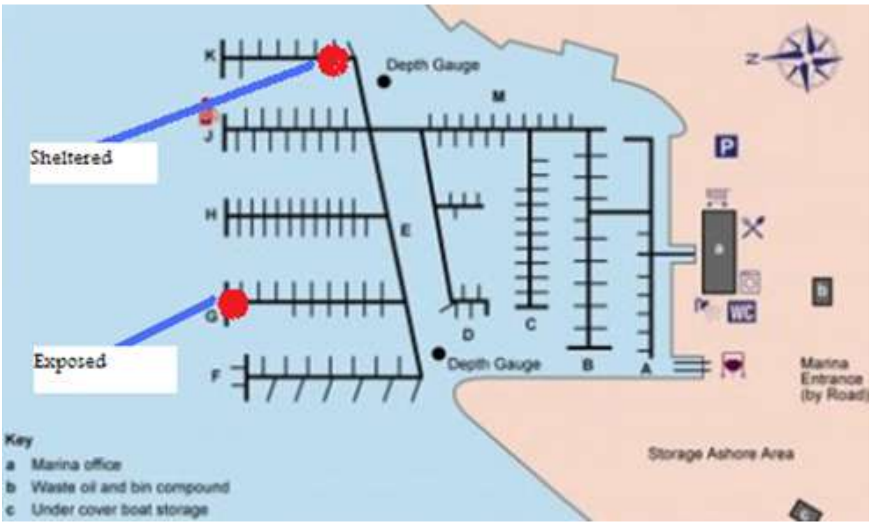

Figure 7. Map to show the exposed and the sheltered locations at Premier marina, the open marina. At each of these areas there will be a site A and site B.

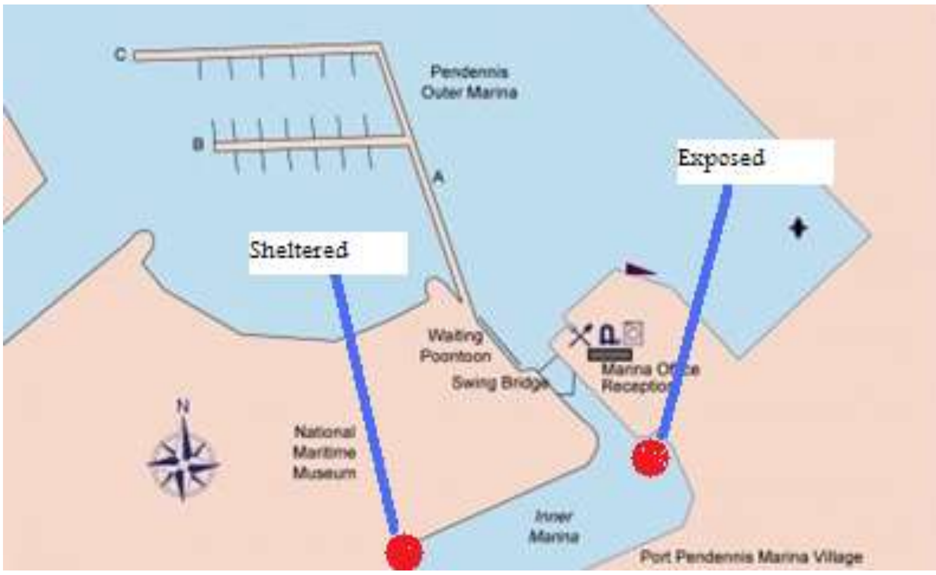

Figure 8. Map to show the exposed and the sheltered locations at Port Pendennis marina, the closed marina. At each of these areas there will be a site A and site B.

To significantly define water flow plaster of Paris clods were deployed at four of the deployments (plate 6). These were hung at the depth of the deepest panel in a neighbouring location. To calculate the water flow the clods were weighed before and after and then a percentage loss was calculated. The first reading was subtracted from the second reading to obtain an absolute loss, after which the calculation below was followed:

$$\frac{Absolute\ \ loss}{primary\ \ weig\,ht} \times 100 = \text{percentage loss}$$

Plate 6. A plaster of Paris clod, hung at four regular intervals to measure the water flow at each marina. The clods above represent the contrasting marinas; even in the absence of scales the difference in water flow is clearly identifiable.

4.4 Deployment

The short term and long term panels were identical, but within each site, the long term panel stayed in position for the duration of the experiment and the short term panel was changed on a two week rolling cycle. It was necessary to make the first deployment at Spring Low Water to ensure the depth was correct so there was no connection with the benthos or any other substrata. Further still each surface plate was hung 10 -20 cm below the surface with each site measurement identical for comparison.

Table 1. Sampling periods of each settlement panel deployed (D – Deployment of settlement panels; C – Collection of settlement panels; LT- Long Term Panels)

Set 1		**Set 2**		**Set 3**		**Set 4**		**Set 5**		**Set 6**		**LT**	
D	**C**	**D**	**C**	**D**	**C**	**D**	**C**	**D**	**C**	**D**	**C**	**D**	**C**
01/05	14/05	14/05	28/05	28/05	10/06	10/06	24/06	24/06	04/07	04/07	19/07	01/05	19/07

4.5 Data collection and storage

As outlined above each two week panel was stored in a plastic container separated by a non-corrosive barrier and preserved in 70% ethanol (plate 7). It was imperative that the order in which the panels were kept was recorded for future identification. The panels were kept in a cool dark place until needed. A refuse bag was used at each site for the weights and string which were generally covered in algae and needed to be cleaned for the following week.

Plate 7. The short term panels are collected in 20 litre plastic containers and preserved in 70% ethanol.

Plate 8. Collection of long term panels using cool boxes and sustaining the animals in sea water for transportation to the laboratory.

The long term panels were collected (plate 8) at the end of the study, to quantify longer term settlement. These were stored in cool boxes preserved in sea water and transferred directly to the laboratory. It was found best to separate and protect the panels within the cool boxes as squashing the animals inhibited the identification process.

4.6 Recording the data

A 1 cm border was left around the edge of each panel and so the sample accounted for only the species on the plate surface and not on the edges. Each panel was sampled, both long and short term.

4.6.1 Short term panels

A shallow transparent dish larger than 15 x 15cm was used combined with a grid on the base that showed an axis of numbers and letters marked in permanent pen. This was used as a guide and reference point. The panel was submerged in 70% ethanol and placed under a Wild M8 dissection microscope (plate 9). The lines of the grid were followed and as a species appeared it was recorded by tally on a sheet (appendix 2). When a panel was recorded it was necessary to make a note of the sequence it came in and close the container which limited the fumes.

Plate 9. The Wild M8 dissection microscope and laboratory space at the Marine Biological Association.

4.6.2 Long term panels

The long term panels showed developed animals and therefore required a large tray, for example a cat litter tray. Water or ethanol was used to submerge these; however some panels were so encrusted with animals they were left bare. The species were recorded by grid point analysis. A 100 point grid, 15 x 15cm, was placed over the panel (plate 10). The species directly beneath each grid cross section was recorded as a tally. The 100 grid point represents 100% therefore each record is 1%. When a species was present but not under a cross section, it was recorded as P and given 0.5% in the data table. It was useful to have a prepared sheet of species detailed from the literature (appendix 3).

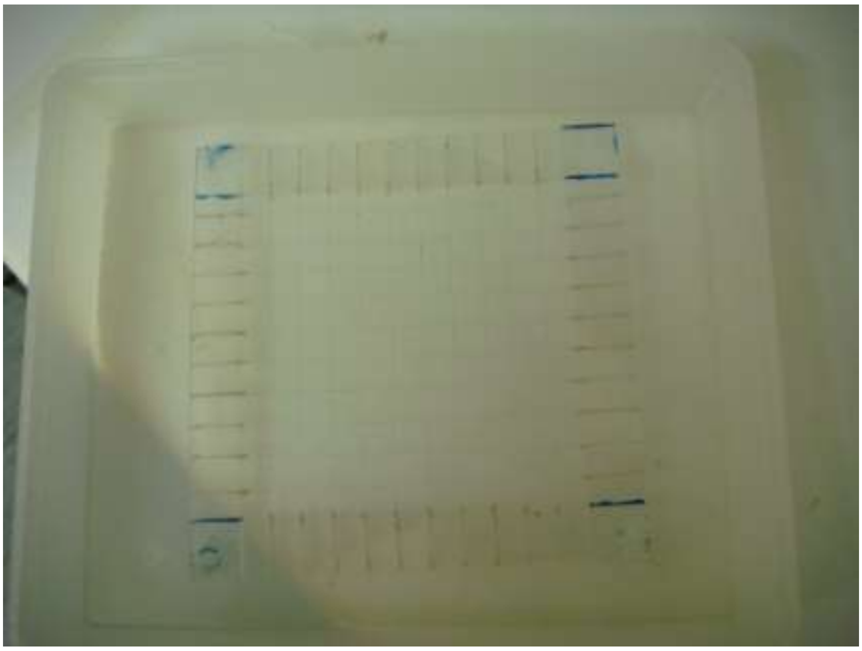

Plate 10. A 100 point grid is placed over the panels and the species directly underneath a cross section is recorded as 1%.

4.7 Data analysis

The data was analysed using univariate (ANOVA) and multivariate (PRIMER) methods. Multivariate methods were designed to analyse many species over multiple environmental variables. It was characterised by basing comparisons on two or more samples, on the extent to which samples share particular species, at comparable levels of abundance. This was therefore used for both long term and short term panels. Techniques used were a method of hierarchical agglomerative clustering (Clarke & Warwick 2001) and non-metric multi-dimensional scaling (usually shortened to MDS, Clarke & Warwick 2001).

Both methods; clustering and MDS start explicitly from a triangular matrix of similarity coefficients computed between every pair of samples. It attempted to reduce the complexity of data; showing not just species which dominated each data set but the occurrence of rare species as well. One way of doing this was to restrict attention to a single similarity coefficient, but allow a degree of transformation; Bray-Curtis similarity has been used for this study.

4.7.1 Clustering

Within this study clustering has been used for finding natural groupings where samples were more similar to each other than in other groups. The groups then cluster, fused with the highest mutual similarities then gradually lower in similarity level. The result of hierarchical clustering has been represented in a dendrogram.

4.7.2 MDS
MDS constructed a configuration of the samples in a specific number of dimensions, which aimed to satisfy all the conditions imposed by the similarity matrix. Dependent on the degree of similarity of a sample it was either placed close to or further away from each other. For the purpose of this study the MDS has been shown in two-dimensional form, though it can be effectively demonstrated in three-dimensions on electronic media.

To characterise degrees of significance ANOSIM has been used and SIMPER was performed to categorise which species allowed for the significance.

4.7.3 ANOVA
Univariate analysis of ANOVA has been completed in Minitab and used exclusively for short term panels. It aimed to assess; which marina had the greatest amount of larvae settlement, irrespective of taxa and give a significance value for this. It also distinguished NIS settlement between marinas. A two-way crossed variance test assessed whether the data was suitable for the significance test, by conforming to Bartlett's test where the P value had to be less than 0.5. The General Linear Model was used for significance analysis.

5. Results

The analysis was conducted using both PRIMER and ANOVA. Detailed below are the findings from these analyses.

5.1 Short term panels
In total, ten taxa were identified on the panels in addition to bare space (appendix 4), which was used as a taxon equivalent, but not as a frequency in multivariate analysis. Taxa were identified to the lowest possible taxonomic level; either species or genus. The exceptions were spirorbids, hydroids and some solitary ascidians. Among the taxa scored were solitary and colonial ascidians, encrusting and arborescent bryozoans, hydroids, barnacles and segmented worms. Across the two week panels, settlement was limited and except for hydroids most settlement was a matter of days old. This made identification complex.

As an overview of the periods of sampling, figure 9 graphically illustrates the individual sets, showing which sets recruited the most settlers over a short term time scale.

There was an even distribution of highest settlement values; sets 1, 2 and 5 showed Premier marina to have the highest amount of settlement, and sets 3, 4 and 6 showed Port Pendennis as having the greatest settlement. Set 1 (Fig. 9) deployed between 1st April to the 14th April and set 4 (Fig. 9) deployed from 10th June to the 24th June were characterised by the showing greatest difference in the number of settlers. Set 1 was characterised as the highest settlement for Premier, while set 4 for Port Pendennis was observed to have the highest settlement. On set 6, deployed from 4th August to the 19th August a small variation was seen in the number of settlers between the marinas. The most pronounced settlement was recorded in Set 4 and Set 6 (Fig. 9), deployed from 10^{h} June to 24th June and 4th August to 19th August, respectively.

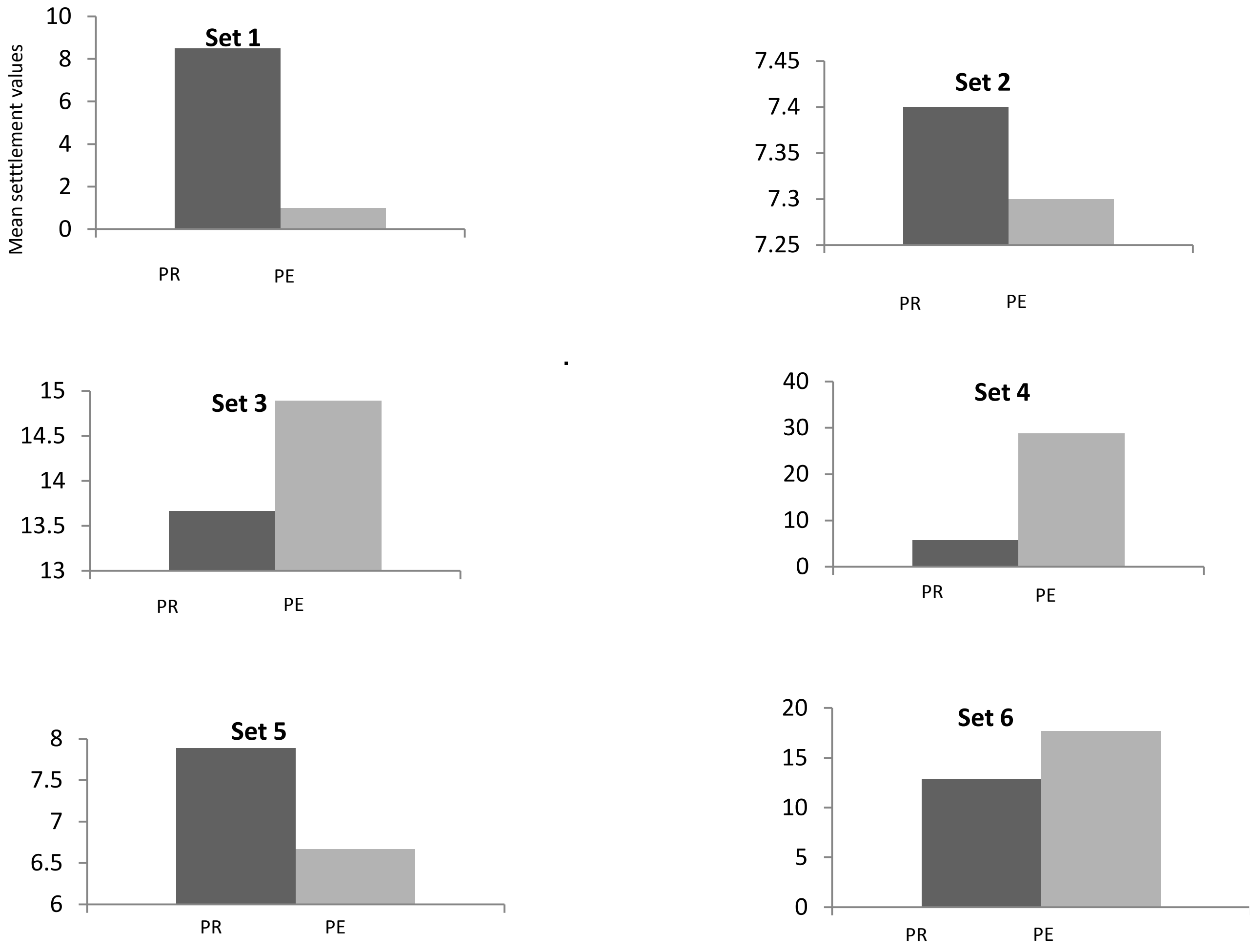

Figure 9. Total frequency values of settlement in marina, over six sets of short exposure to sea water from April 2008 to August 2008. PR – Premier marina, an open marina; PE – Port Pendennis, a locked gate marina.

5.1.1 Univariate analysis of total taxa

Initially, the total settlement of larvae that colonised contrasting marinas was compared by univariate analysis of AVONA. A main effects plot (figure 10) characterises the mean and range of each interaction by total settlement.

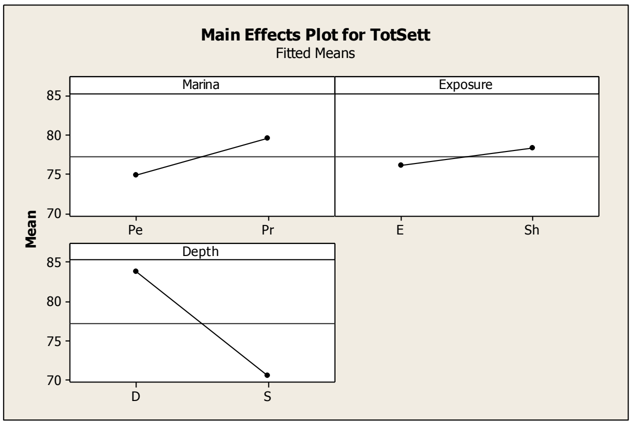

Figure 10. Main effects plot characterising the mean and range of each interaction

The main effects plot shows the greatest range to be observed in depth and the least in marina and exposure. Figure 10 demonstrates panels placed at lower depths have a greater amount of settlement from those nearer the surface.

It should be noted that ANOVA gives a clear understanding of which marina has shown the greatest propagule pressure but the analysis is limited as taxa are not represented. For example, all Barnacles could be found in Premier whereas the predominant ascidians could be present in Port Pendennis but ANOVA would just clump all taxa together. To rectify this, Barlett's test was conducted (figure 11) observing P=0.585, after which the General Linear Model (table 2) analyses the total settlement of each marina, compared with each interaction.

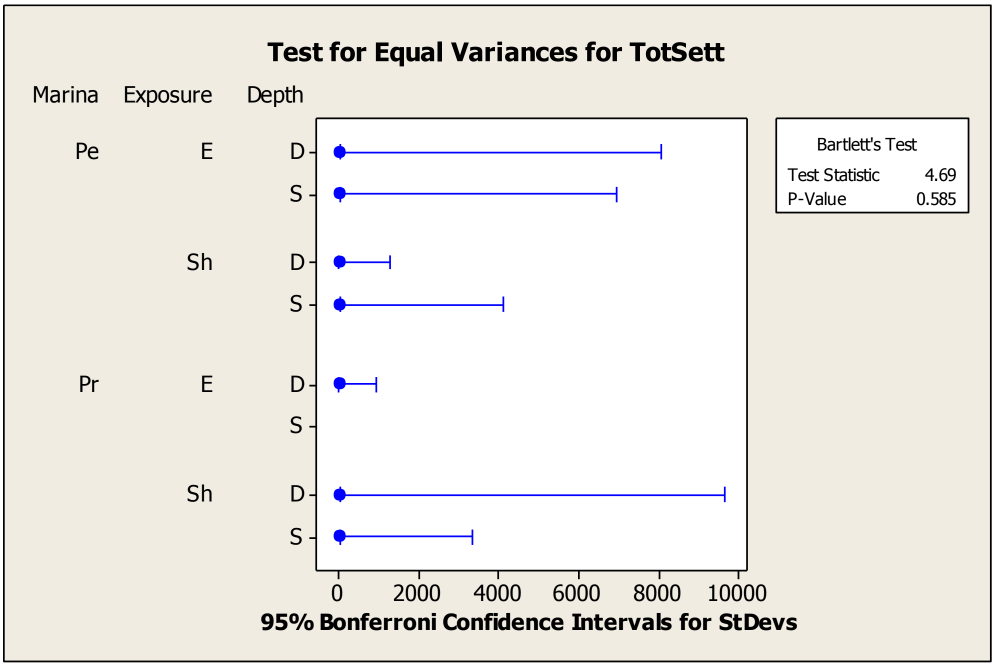

Figure 11. Test for equal variances, for total settlement, observing Bartlett's test as P=0.585

The analysis in table 2 characterises all interactions as showing no significant difference between marinas. The data was then refined to concentrate on the main interactions of marina, exposure and depth, it was then re-tested (table 3).

Table 2. General Linear Model for total settlement over all interactions

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Marina	1	85.6	85.6	85.6	0.14	0.714
Exposure	1	18.1	18.1	18.1	0.03	0.866
Depth	1	689.1	689.1	689.1	1.16	0.312
Marina*Exposure	1	1278.1	1278.1	1278.1	2.16	0.180
Marina*Depth	1	45.6	45.6	45.6	0.08	0.789
Exposure*Depth	1	150.1	150.1	150.1	0.25	0.628
Marina*Exposure*Depth	1	126.6	126.6	126.6	0.21	0.656
Error	8	4737.5	4737.5	592.2		
Total	15	7130.4				

After adjustment, there was a slight increase in significance, but not a significant difference between the total settlements of taxa between varying interactions (table 3).

Table 3. General Linear Model for total settlement over main interactions

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Marina	1	85.6	85.6	85.6	0.16	0.694
Exposure	1	18.1	18.1	18.1	0.03	0.856
Depth	1	689.1	689.1	689.1	1.30	0.276
Error	12	6337.7	6337.7	528.1		
Total	15	7130.4				

To illustrate the amount of settlement within each marina, the raw data can be expressed as a Boxplot (figure 12). It shows the interquartile range from the data plots, demonstrating the mean settlement for each marina. The greatest settlement is seen in Port Pendennis at the exposed, deep site and in Premier at the surface, deep site, which agrees with the main effects plot (figure 10). These trends cannot be analysed any further, as already stated, there is no significant difference observed.

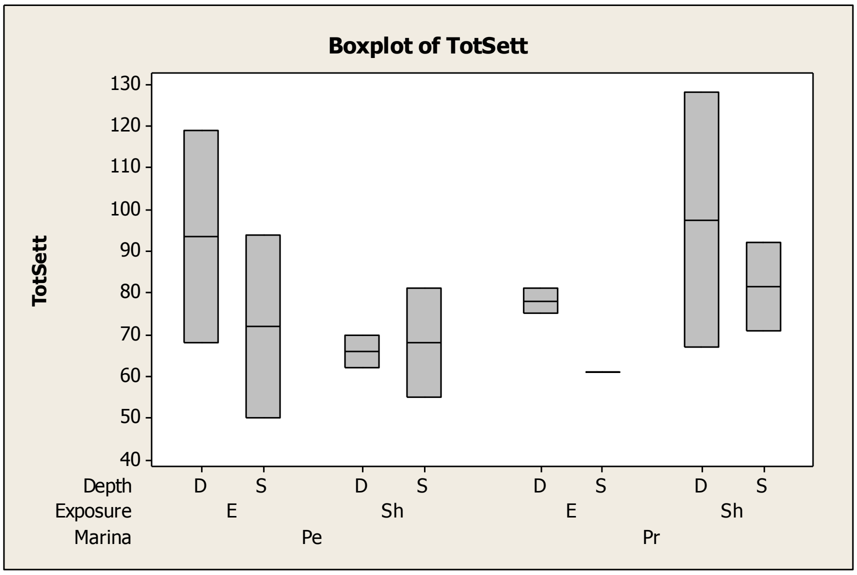

Figure 12. Boxplot to show the interquartile range and mean for total settlement of all taxa between the contrasting marinas of Port Pendennis and Premier.

Equally, ANOVA of total individual taxa present on each panel showed no significant differences (table 4).

Table 4 .Analysis of Variance for Total Taxa, using Adjusted SS for Tests

Source	**DF**	**Seq SS**	**Adj SS**	**Adj MS**	**F**	**P**
Marina	1	7.563	7.563	7.563	4.97	0.046
Exposure	1	0.562	0.562	0.562	0.37	0.554
Depth	1	3.063	3.063	3.063	2.01	0.181
Error	12	18.250	18.250	1.521		
Total	15	29.438				

5.1.2 Univariate analysis of Non-Indigenous species

An ANOVA was again carried out to analyse the settlement of Non-native species (NIS) established in each marina (appendix 5). Erect Bryozoan was categorised as NIS because the only erect Bryozoans found on long term panels were *Bugula neritina* and *Tricellaria inopinata.* Initially the equal variance test showed a limited P-value of 0.132 and therefore the data was re-tested by square-root, illustrating an improved P=0.418 (figure 13).

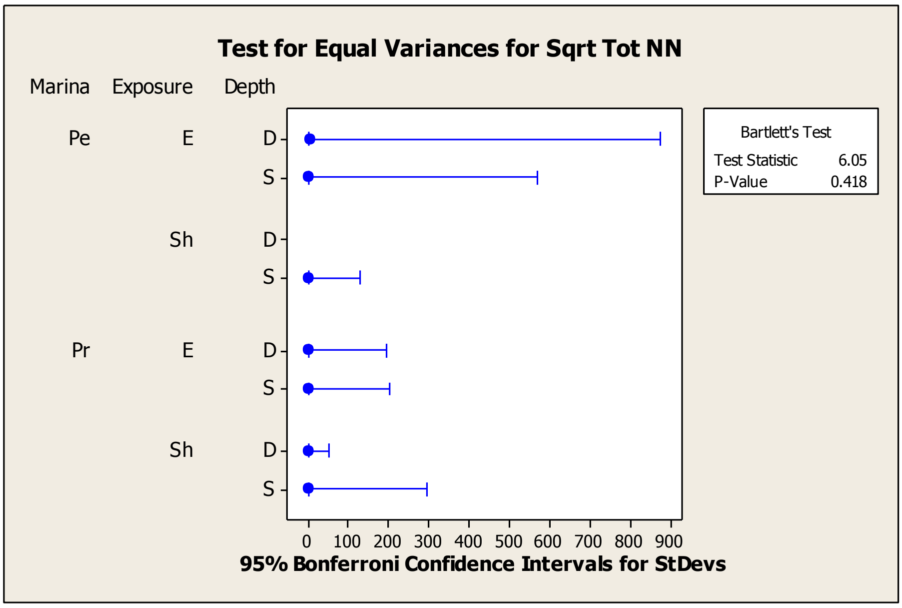

Figure 13. Test for equal variances by square root for NIS across contrasting marinas.

ANOVA presented the NIS data through the General Linear Model to give P-values. The data was analysed over all interactions but did not give any significant values (table 5).

Table 5. Analysis of Variance for Square root Total settlement of NIS, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Marina	1	4.009	4.009	4.009	1.25	0.296
Exposure	1	4.015	4.015	4.015	1.25	0.295
Depth	1	0.223	0.223	0.223	0.07	0.798
Marina*Exposure	1	4.674	4.674	4.674	1.46	0.262
Marina*Depth	1	0.418	0.418	0.418	0.13	0.727
Exposure*Depth	1	0.038	0.038	0.038	0.01	0.916
Marina*Exposure*Depth	1	2.492	2.492	2.492	0.78	0.403
Error	8	25.623	25.623	3.203		
Total	15	41.493				

The data was re-tested concentrating solely on the simple interactions; marina, exposure and depth (table 6), but again no significant values were observed.

Table 6. Analysis of Variance for Square root NIS, using Adjusted SS for Tests.

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Marina	1	4.009	4.009	4.009	1.45	0.252
Exposure	1	4.015	4.015	4.015	1.45	0.252
Depth	1	0.223	0.223	0.223	0.08	0.781
Error	12	33.245	33.245	2.770		
Total	15	41.493				

The boxplot (figure 14) expresses which NIS were present in the greatest abundance, regardless of taxa within contrasting marinas. It can be observed the lock gate marina of Port Pendennis (Pe), illustrated the panel position of 'exposed and deep' to contain the highest abundance of settling NIS. This trend although apparent cannot be expressed further due to a lack of significance (table 6).

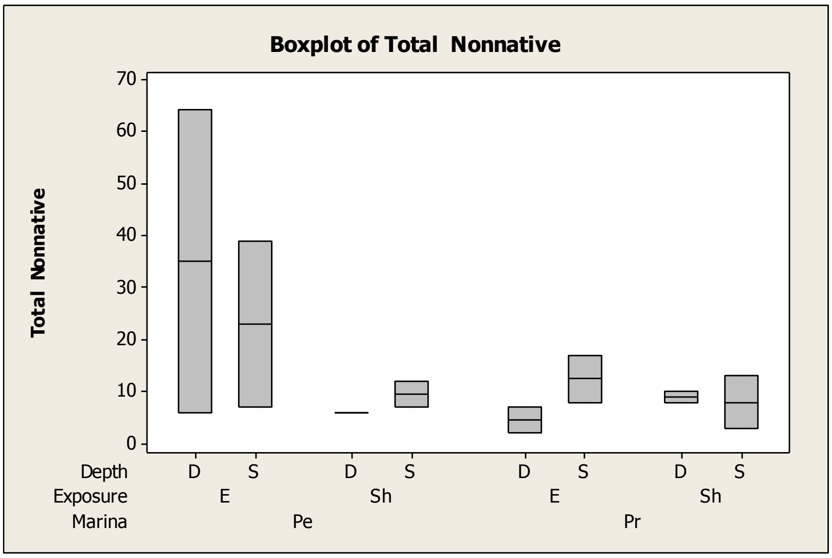

Figure 14. Boxplot of total settlement of NIS colonised in contrasting marinas.

5.1.3 Multivariate analysis of all taxa

There was no significant difference between marinas when comparing univariate analyses of total settlement of taxa across short term panels, but further multivariate analysis were applied to single out species across variable factors.

The factors assessed were marina, exposure and depth. ANOSIM analysis indicated significant comparisons between these factors (table 7).

Table 7. P values obtained for two-way crossed comparisons for assemblages exposed to sea water over short durations, at different marinas and positions within those marinas.

Factor	P value *(significant)	Global R value
Marina vs Marina	0.006*	0.583
Exposed vs Sheltered	0.007*	0.316
Deep vs Shallow	0.84	-0.109

Considering the high dissimilarity (P=0.84) between deep and shallow positions within the two marinas (table 7) this data was removed from further multivariate analysis.

CLUSTER analysis indicated the factors of marina and exposure varied dissimilarity. Figure 15 shows the results of hierarchical clustering using group-average data. The raw data was expressed as species per panel for each sample period from April to August with Bray-Curtis similarities calculated on √-transformed abundances. The dendrogram in figure 15 provides a sequence of fairly convincing groups, between the corresponding marinas (P=0.006). The Pendennis 'exposed and deep' site was part of the Pendennis grouping but showed the least similarity to the other sites within this group. Premier marina is observed as showing a similarity with two identical sites (exposed, sheltered) from Pendennis.

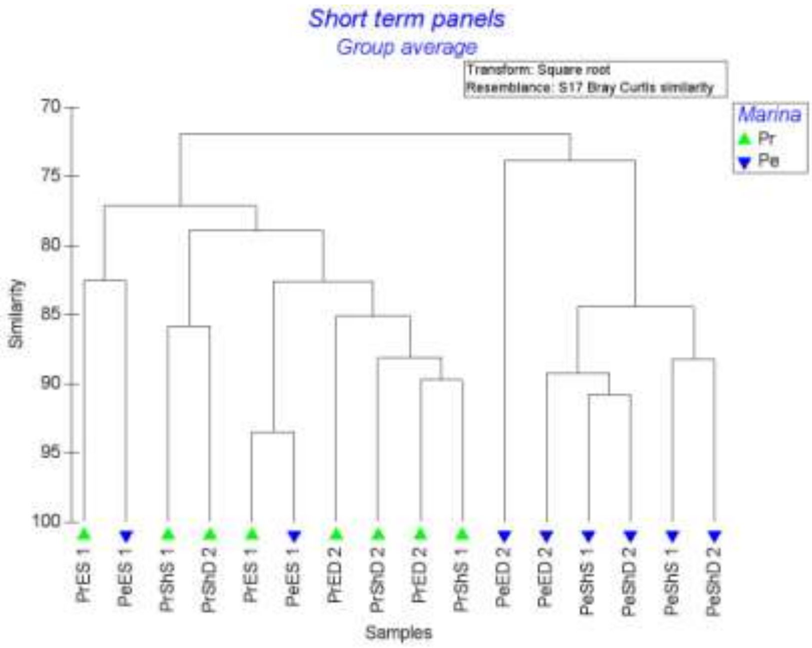

Figure 15. Premier and Port Pendennis Marina. Dendrogram of hierarchical clustering of 16 sites, using group average linking of Bray Curtis similarities calculated on √-transformed abundance data. The different sites within marinas are as follows; PR – Premier, PE – Pendennis, Sh – Sheltered, E – Exposed, S – surface, D – Deep.

To further illustrate the observations made from the dendrogram (figure 15), a non-metric MDS plot can be used (figure 16).

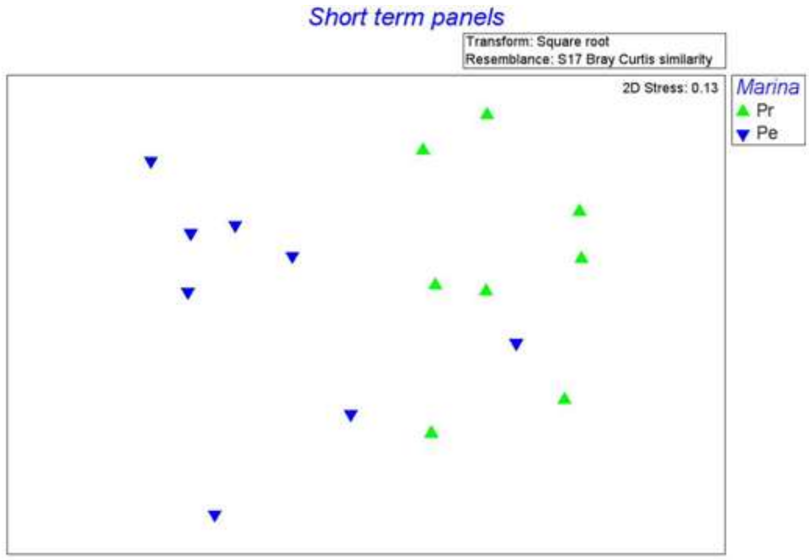

Figure 16. Similarity of marinas. MDS ordination of the 16 samples based on √-transformed abundances and Bray-Curtis similarity *(stress = 0.13)*

Figure 16 displays a 2-dimentional MDS ordination of 16 samples, based on √-transformed abundances and a Bray-Curtis similarity matrix, its aims to show the similarity of species relationship within contrasting marinas, Port Pendennis is grouped in blue and Premier is shown in green and it characterises two fairly distinct grouping.

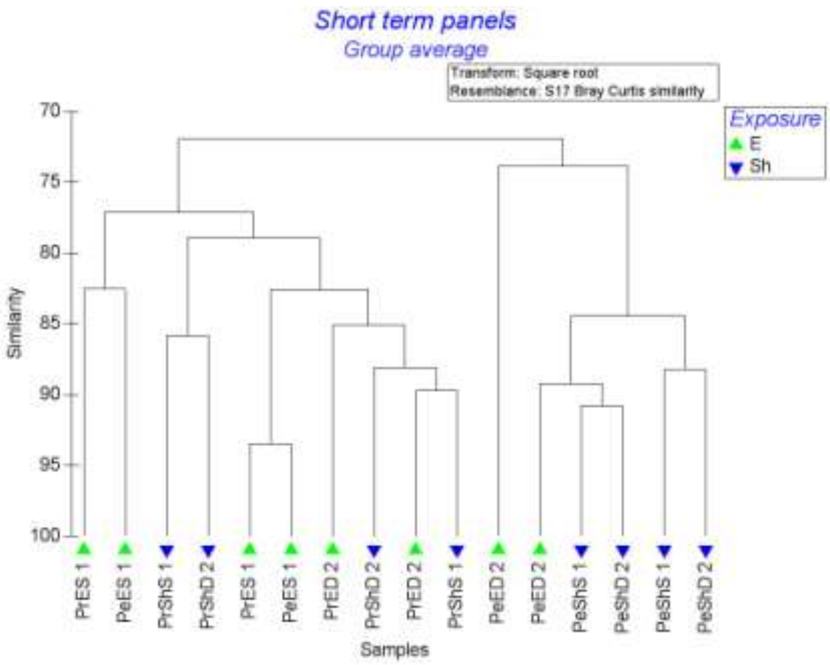

Figure 17. Premier and Port Pendennis Marina showing similarity of exposure. Dendrogram of hierarchical clustering of 16 sites, using group average linking of Bray Curtis similarities calculated on √-transformed abundance data. The different sites within marinas are as follows; PR – Premier, PE – Pendennis, Sh – Sheltered, E – Exposed, S – surface, D – Deep.

The same configuration was applied to exposure of marina, using CLUSTER analysis. The raw data was expressed as species per panel for each sample period from April to August with Bray-Curtis similarities calculated on √-transformed abundances. The dendrogram provides a sequence of fairly dissimilar groupings (figure 17), between the correlation of exposed and sheltered, within each marina.

The divide of similarity is apparent between exposed and sheltered sites at 73%. From the clustering there is a distinction between Premier and Pendennis, with exposed sites having a higher similarity to species found within Premier and sheltered spots have, in some cases a similarity to those of Pendennis marina.

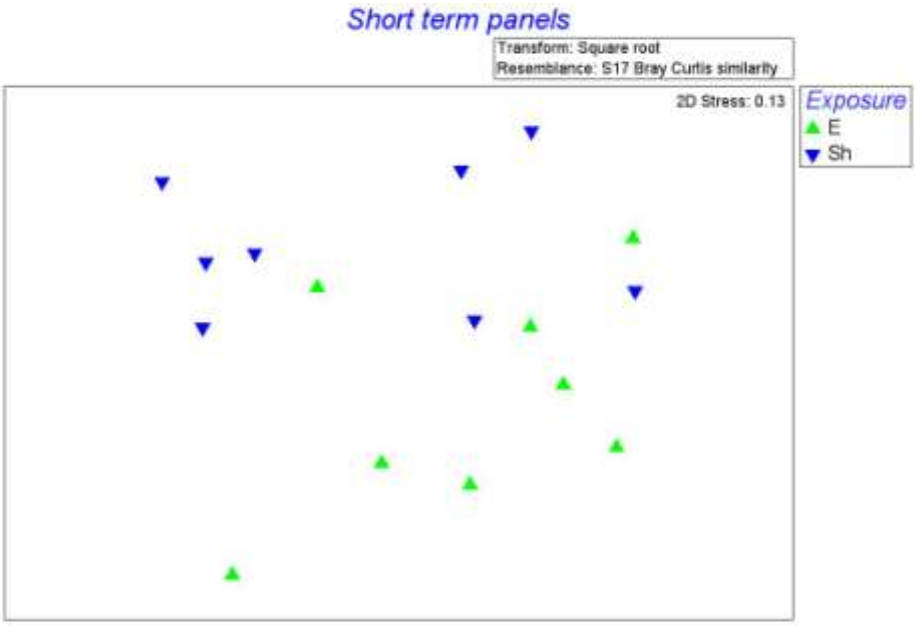

Figure 18. Similarity between exposure groups. MDS ordination of the 16 samples based on √-transformed abundances and Bray-Curtis similarity *(stress = 0.13)*

The 2-dimentional MDS ordination shows the separation of species composition from exposed areas to sheltered areas. This further represents the cluster dendrogram, and confirms the similarity of species by distinctive groupings, which are significant within each exposed and sheltered sample area (P=0.007).

Both marina configuration (open and lock-gate) and exposure (exposed and sheltered) showed significant differences (table 7). SIMPER was preformed to identify the main taxa responsible for the differences observed (table 8). Assemblages present in open marinas were characterised by the presence of and starting with the highest abundances; *Hydroid sp., Diplosoma, Botryllus* and *erect Bryozoan.*

Assemblages found in the lock-gate marina were characterised by the presence of and starting with the highest abundances; *Diplosoma, Hydroid sp., Elminius, Botryllus, Solidary Ascidian* and *Ciona.*

Table 8. SIMPER two way analysis using Bray-Curtis similarity, showing the average dissimilarity abundances of taxa within the contrasting marinas.

	Premier	Pendennis				
Species	Av.Abund	Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%
Hydroid sp.	5.29	3.28	5.06	1.94	20.05	20.05
Ciona intestnialis	0.00	1.99	4.88	1.51	19.32	39.37
Elminius modestus	1.82	3.35	4.42	0.98	17.51	56.88
Asicidian Solidary	2.71	2.21	2.64	1.05	10.47	67.35
Diplosoma sp.	4.87	5.23	2.17	1.44	8.60	75.96
Botryllus schlosseri	2.61	2.24	2.14	1.32	8.46	84.41
Erect Bryozoan	1.87	1.58	1.69	1.51	6.70	91.11

The average abundances for taxa within the open marina (Premier) shows *Hydroid sp* to have the highest dissimilarity of 5.06% and the enclosed marina is characterised by *Diplosoma* (5.23%)*.* The least dissimilarity is observed as erect Bryozoans at 1.69% (table 8).

5.2 Long term panels

As would be expected the long term panels showed far greater settlement (appendix 6). ANOSIM showed a significant difference in the settlement structure between marinas and between depths but there was no significant value in exposure of panel (table 9).

Table 9. P values obtained for two-way crossed comparisons for assemblages exposed to sea water over a longer duration at different marinas and positions within those marinas.

Factor	**P value** * **significant**	**Global R value**
Marina vs marina	0.001*	0.807
Deep vs shallow	0.027*	0.333
Exposed vs Sheltered	0.265	0.094

The settlement onto panels in differing exposures in either marina was not significant (P=0.265) and therefore further analysis was not undertaken on this factor.

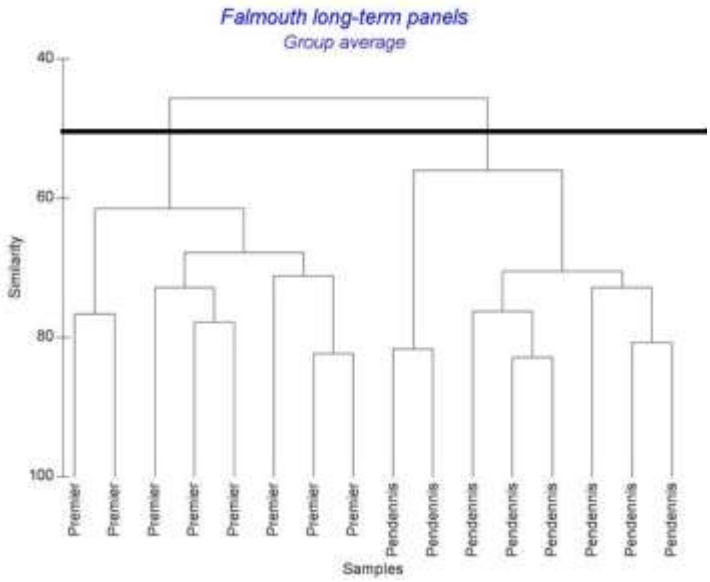

Figure 19. Premier and Port Pendennis Marina showing dissimilarity of settlement. Dendrogram of hierarchical clustering of 16 sites, using group average linking of Bray Curtis similarities calculated on √-transformed abundance data.

To further illustrate the divide of species which settled in contrasting marinas, a CLUSTER dendrogram is constructed (figure 19) taken from a similarity matrix (appendix 7). The dissimilarity of species, indicated by the black line, is observed at 50%. There structure of marinas is clearly significant, representing different community development over longer exposure.

A significant difference was also characterised in depth of panel (P=0.027). A 2-dimensional MDS ordination displays these groupings (figure 20). The grouping of deep vs shallow was equally categorised into marina type. Thus the colonisation was representative across marina type and depth of panel within both marinas. To observe the clustering of dissimilarity (figure 31) the MDS ordination distinguishes an obvious grouping of Port Pendennis to Premier marina. The green circle signifies the separation of similarity at 50% (figure 20).

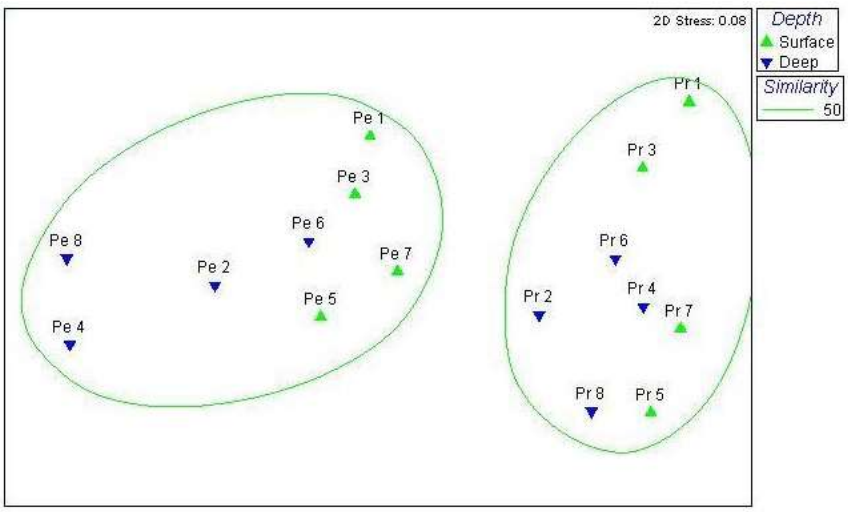

Figure 20. Similarity between marina groups and depth of panel. MDS ordination of the 16 samples based on √-transformed abundances and Bray-Curtis similarity *(stress = 0.08)*

As previously observed, there was a significant difference of settlement within marinas and depth over longer exposure of panels to sea water, therefore SIMPER could be used to observe the species which categorise these trends.

The settlement within Premier marina was characterised as, starting with the highest abundance; blank space, *Diplosoma sp., Ascidiella aspersa, Elminius modestus, Tricellaria inopinata.* In the enclosed marina (Port Pendennis), the following species represent the settlement found, commencing with the highest abundance; *Ciona intestinalis, Ascidiella aspersa, Diplosoma sp.* and black space. Table 10 represents the average abundances as a percentage in contrasting marinas. Over all species Ciona *intestinalis,* a solitary ascidian is observed to be the most abundant settler (8.89%) and is found in Port Pendennis, the second highest (3.92%) is *Eliminius modestus* a non-native species and was found predominately in Premier marina.

Table 10. SIMPER two way analysis using Bray-Curtis similarity, showing the average dissimilarity abundances of taxa within the marinas of Port Pendennis and Premier.

	Premier	**Pendennis**				
Species	Av.Abund	Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%
Ciona intestinalis	2.18	8.89	9.04	2.27	16.98	16.98
Elminius modestus	3.92	0.09	5.11	2.08	9.60	26.58
Blank space	6.96	3.36	4.93	1.60	9.27	35.86
Obelia longissima	3.23	0.00	4.16	1.58	7.82	43.68
Diplosoma sp.	5.87	5.01	3.65	1.18	6.85	50.53
Tricellaria inopinata	3.26	0.48	3.63	1.86	6.82	57.35
Bugula neritina	2.70	0.73	3.48	1.01	6.54	63.89

SIMPER was again used to analyse the dissimilarity of species which gave a significant difference of P=0.027 between contrasting depths table 11.

Table 11. SIMPER two way analysis using Bray-Curtis similarity, showing the average dissimilarity abundances of taxa over deep and shallow exposures.

	Shallow	Deep				
Species	Av.Abund	Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%
Diplosoma sp.	6.65	4.24	4.90	0.99	13.50	13.50
Ciona intestinalis	4.21	6.86	4.41	1.02	12.17	25.67
Ascidiella aspersa	5.49	7.17	3.17	1.51	8.74	34.41
Blank space	6.01	4.31	3.02	1.10	8.32	42.73
Bugula neritina	1.65	1.78	2.88	1.28	7.93	50.66
Botryllus schlosseri	2.81	1.58	2.80	1.25	7.72	58.39
Tricellaria inopinata	2.51	1.23	1.84	1.19	5.06	63.45

In general across all shallow panels, the colonial ascidian *Diplosoma sp.* was found in the highest abundance (6.65%), whereas the highest abundance for deep panels were characterised by *Ciona intestinalis* a solitary ascidian, observed at 6.68%.

5.3 Water flow measurements

To measure water flow at the contrasting marinas of Port Pendennis and Premier, plaster of Paris clods were deployed at four intervals over the 14 week sampling period. Table 12 indicates a clear distinction of water flow in contrasting marinas, with the enclosed marina of Port Pendennis demonstrating a decreased water flow across each period, to that of the open marina (Premier).

Table 12. Clod measurements from contrasting marinas, recording water flow by calculating percentage loss.

Date	Clod no.	Marina position	Before	After	Percentage loss (%)
01/05 – 14/05	2	Premier	635.09	434.72	**31.55**
01/05 – 14/05	3	Pendennis	662.38	299.7	**54.75**
28/05 – 10/06	7	Premier	620.32	459.2	**25.97**
28/05 – 10/06	15	Pendennis	610.06	252.51	**58.6**
10/06 – 24/06	6	Premier	670.5	475.77	**29.04**
10/06 – 24/06	13	Pendennis	649.34	329.79	**49.21**
04/07 – 19/07	5	Premier	678.23	482.55	**28.85**
04/07 – 19/07	10	Pendennis	681.98	381.45	**44.07**

6. Discussion

6.1 Settlement

Quite surprisingly, settlement values recorded during this experiment (for the short term panels) were much lower that expected in either marina. Throughout the sampling period (figure 9), no trends were expressed (table 2 &3), and at the peak the reproductive summer periods of set 5 and 6 no higher than average recruits settled. Neither of the marinas sampled presented more than 150 recruits in all six replicate panels at the same sites over the total sampling time frame. This is clearly a much lower value in overall recruitment than those obtained in previous studies (Floerl & Inglis 2003). It is also apparent from the short term sampling that none of the factors tested; marina, exposure nor depth showed higher levels of total taxa settled (table 4). This is viewed as abnormal behaviour for settling larvae, especially within open and enclosed marinas (Arena et al 2006, Floerl & Inglis 2003). These results may possibly be due to frequent events of heavy rain that occurred throughout the season: an unusually variable, extreme and unpredictable weather pattern. Such weather patterns have been found to conspire with other physical and environmental processes linked to the release of propagules by fouling organisms. For example, Connell (2001) found that heavy rain and gales significantly affected estuarine environments and contributed to reduced settlement of epibiota.

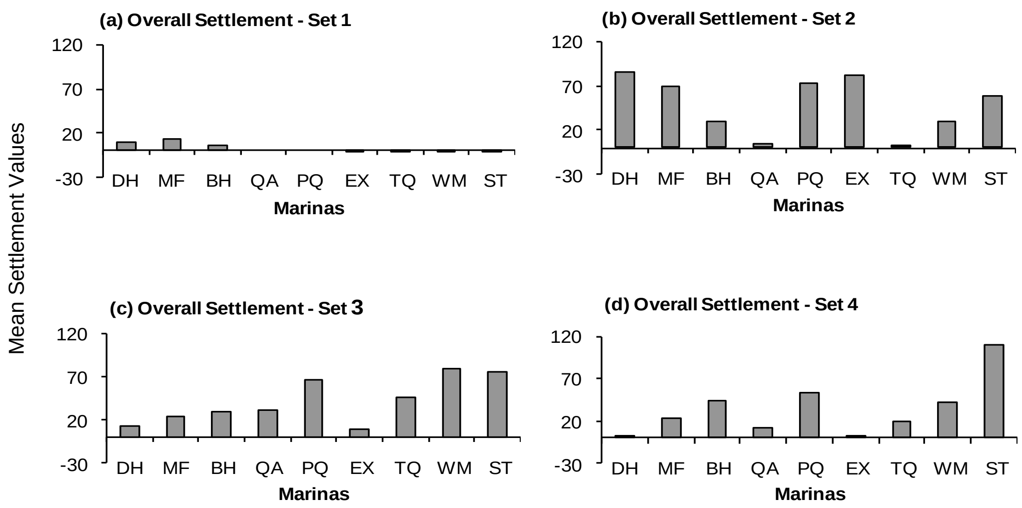

Figure. 21. Total frequency values of settlement in marinas, over the four sets of short exposure to sea water, deployed from March 2008 to July 2008. Marinas are organised according to increase in degree of enclosure (DH – Darthaven marina; MF – Mayflower marina; BH – Brixham marina; QA – Queen Ann's Battery marina; PQ – Poole Quay marina; EX – Exmouth marina; TQ – Torquay marina; WM – Weymouth marina; ST – Salterns Marina, Santos 2008).

A similar situation occurred during a sampling of the entire south coast of England; where limited recruitment was observed onto panels at the same time of year (figure 21). Despite this, some sets showed significant differences between enclosures of marinas.

Nevertheless, these results did show distinct patterns in the settlement of fouling organisms onto available surfaces in the open and the enclosed marina, over two different time periods. In both short term and long term sets, sessile assemblages found in Port Pendennis marina were characterised by the predominance of similar taxa. In contrast, Premier marina (open marina) appeared to support very differing assemblages over the two time scales.

The enclosed marina of Port Pendennis was typified by the presence of *Ciona intestinalis* and *Dipolsoma schlosseri* during both long and short term time frames. The open marina of Premier showed little representation of settlement from short term to long term, the predominant assemblages present on short term panels were found to be *Hydriod sp.,* solitary ascidian and *Botryllus schlosseri,* and the long term were characterised by *Elminius modestus, Obelia longissim* and*, Diplisoma schlosseri.* Both *Tricellaria inopinata* and *Bugula neritina* were present on both the long and short term panels.

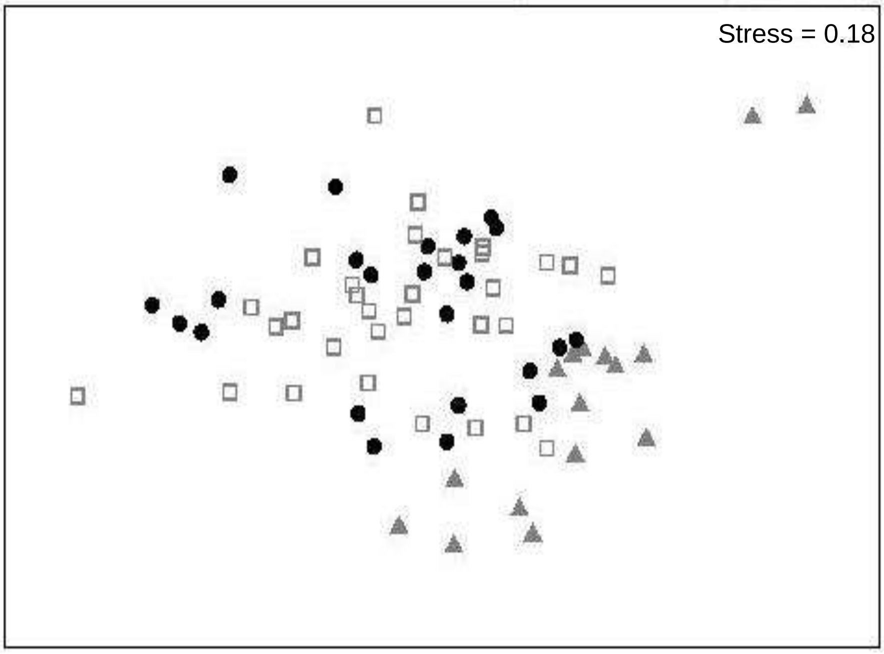

Figure. 22. MDS ordination of all long term panels (open squares – Strongly Enclosed marinas; shaded circles – Weakly Enclosed marinas; shaded triangles – Open marinas).

Probably the most pronounced difference between assemblages across marinas, in both long and short term panels, was the fact solitary and colonial ascidians accounted for more than 60% of the most conspicuous taxa in the enclosed marina, whereas in the open marina, ascidians formed less than 30% of taxa recorded. These trends were found to be fairly consistent with the entire south coast of England, where the results showed 50% of the most conspicuous taxa derived from colonial ascidians in the strongly enclosed marinas (Santos 2008).

From the MDS plot (figure 22) of the entire south coast of England, distinct groupings can be observed for each marina. This is substantiated by degrees of significance (table 13) which indicate open marinas to be significantly different from weakly enclosed and strongly enclosed marinas. The level of enclosure of each marina was determined by the creation of an enclosure index; by dividing the combined extent of openings (allowing water exchange with the adjacent sea) by total perimeter of the marina. The index values are as follows; smaller than 0.1 defined Strongly Enclosed marinas, between 0.1 and 0.3 defined Weakly Enclosed marinas and higher than 0.3 defined Open marinas (Santos 2008).

Table 13. R-values obtained with ANOSIM routine for pairwise comparisons of assemblages in marinas with different degrees of enclosure (O – Open marinas; SE – Strongly Enclosed marinas; WE – Weakly Enclosed marinas; * $p < 0.001$, ^{ns} – not significant).

Pairwise Tests	
Global R	0.246*
O vs SE	0.487*
O vs WE	0.364*
SE vs WE	0.014^{ns}

The observations in table 13 are replicated in this study where ANOSIM shows a significant difference between contrasting marinas in the same estuary (table 14).

Table 14. P values obtained for two-way crossed comparisons for assemblages exposed to sea water over a longer duration at different marinas and positions within those marinas.

Factor	P value * significant	Global R value
Marina vs marina	0.001*	0.807
Deep vs shallow	0.027*	0.333
Exposed vs Sheltered	0.265	0.094

6.2 Implication of settlement

The results for enclosed marinas in both this study and the MRes study of the south coast indicate an increased settlement of colonial and solitary ascidians. Marinas that are fully enclosed by a lock-gate have a limited tidal exchange (McMahon 1989) and may experience greater environmental stress, than areas through which water can flow. Lock gate marinas have been shown to reduce water circulation inside the marina. However, the density of recruitment by sessile marine invertebrates is typically a function of larvae flux. Experiments using concentrations of invertebrate larvae have shown that the density of recruits is generally greatest in conditions of rapid water flow (Judge & Craig 1997). In this study however, current flows were measured as minimal within the enclosed marina (table 12) and therefore one possible explanation for elevated ascidian settlement could be that they result from exceptionally large concentrations of competent propagules retained within the confines of the marina basin. This has been observed in a study undertaken by Floerl & Inglis (2003).

Equally, ascidian species produce lecithotrophic larvae whose short larval lifetimes (minutes to days) can reduce the distance that larva are transported, which facilitate recruitment back into parent populations and contribute to the dense populations in enclosed marinas (Osman & Whitlatch 2004). These taxa are characterised by rapid growth and they successfully compete for space, often dominating the fouling assemblages. Some studies have shown 50 to >70% of ascidians settlers survive the first twenty-four hours (Hunt & Scheibling 1997) increasing their ability to colonise. Stachowicz et al. (1999) have recorded their invasion to be made easier in low-diversity communities in where there are fewer competitors for any newly opened space. This also is proposed by Webb & Keough (2000) who comment that an increase of solitary and colonial ascidians within an enclosed marina maybe due to decreased competition. This trend echoes that found in this study, where a possible decrease in diversity of taxa has led to an increased abundance of ascidians (table 15) within the enclosed marina of Port Pendennis.

Table 15. SIMPER two way analysis using Bray-Curtis similarity, showing the average dissimilarity abundances of taxa within the marinas of Port Pendennis and Premier over long term exposure to sea water. Note the apparent diversity of taxa established over long term exposure within the open marina (Premier), compared to the limited establishment of mainly Ascidian species in the lock-gate marina (Pendennis).

	Premier	**Pendennis**				
Species	Av.Abund	Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%
Ciona intestinalis	2.18	8.89	9.04	2.27	16.98	16.98
Elminius modestus	3.92	0.09	5.11	2.08	9.60	26.58
Blank space	6.96	3.36	4.93	1.60	9.27	35.86
Obelia longissima	3.23	0.00	4.16	1.58	7.82	43.68
Diplosoma sp.	5.87	5.01	3.65	1.18	6.85	50.53
Tricellaria inopinata	3.26	0.48	3.63	1.86	6.82	57.35
Bugula neritina	2.70	0.73	3.48	1.01	6.54	63.89

The lack of ascidians present in open marinas has also been determined by an increase of predation. Predators have been found to consume newly settled recruits and juvenile life-stages in areas with rapid water flow (Osman & Whitlatch 2004), limiting their establishment.

There was limited diversity and abundance of taxa observed on short term panels in both the open and lock-gate marina, compared to the long term panels which demonstrated increased diversity and abundance, especially in the open marina. The literature suggests when larvae alight on a surface they move rapidly over it, in more or less a straight line. Where favourable stimuli are not encountered (or unfavourable stimuli received) they will swim away and explore another surface, the impression is given that the larvae is, in some way testing the suitability of substrata (Harris 1990). One reason given for unsuitable substrata is that most larvae seem to settle more readily on surfaces that have a bacterial film (Harris 1990). This is perhaps one reason for limited settlement to short term panels as inadequate bacterial film established in two weeks, whereas the long term panels provided a favourable surface.

Early post-settlement mortality could also be a cause of a lack of recruitment onto short term panels. Recruitment has been defined in many different ways, but Booth and Brosnan (1995) suggest that survival through high mortality in the first few days to weeks after settlement may be a biologically meaningful definition. These first few days after settlement are a critical period for many sessile invertebrates. Hunt and Scheibling (1997) found that the mortality of some sessile taxa was up to six times higher than normal during the first few days. The lecithotropic stage of larvae, present in ascidian development, experience a depletion of stored nutrients, due to a delay of metamorphosis which then results in decreased size and unsuccessful recruitment. This could account for the large abundance of ascidians on long term panels compared to the limited recruits found on short term panels. Accidental ingestion or 'bulldozing' from grazers on epibiota has been documented as a contributory factor in post-settlement mortality. However, this vulnerability to disturbance by grazers is seen to decrease with size (Harris 1990), hence affecting the young juveniles. Predation by macro fauna has been shown to affect early settlement by sessile invertebrates. Exclusion of predators by cages altered the abundance (bryozoans) spatial distribution (ascidians) and size distribution (bryozoans and hydroids) of recruits of different taxa but had little effects on spirorbids. Interestingly, predation on colonial species such as ascidians, affected juvenile rather than adult colonies. The removal of all zooids (to simulate predation) killed all juveniles in less than twenty days but did not cause mortality in adults which quickly regenerated (Osman & Whitlatch 2004). In previous studies (Hunt and Scheibling 1997) algae have been observed as a component in the overgrowth of sessile invertebrates which has caused significant mortalities among recently settled recruits. In this study, although not recorded, algae formed a large percentage of fauna on the short term panels, but this was recorded as blank space. Hence, this could have significantly contributed to the over-growth of any newly settled species. This concludes the possibilities for a reduction in the abundance and diversity of species on short term panels.

On long term panels the diversity of taxa was observed to be significantly different on surface panels compared to deep panels (P=0.027). On the contrary, the short term panels showed no significant values and possible explanations for this have been explained above. The species *Diplosoma listeranium* was found in abundance on surface panels and predominantly in the lock-gate marina. Some studies (Hunt & Scheibling 1997) have indicated bright light as a cause of early mortality in various species of ascidians, however, *Diplosoma listeranium* has been found to be an exception to this and survives well in well lit sites (table 11).

Competition is always a factor in conditions of limited nutrient supply and/or availability of suitable habitat (Harris 1990). This phenomenon could account for the presence of certain taxa, e.g. *Hydriod sp.* and *Elminius modestus* on short term panels whilst being absent from the long term panels.

6.3 The presence of non-indigenous species

Over the entire sampling period a total of six NIS were recorded, they were: *Eliminius modestus, Bugula neritina, Tricellaria inopinata, Botrylloides violaceus, Styela clava* and *Corella eumyota.* However when a statistical analysis was carried out comparing the various factors: marina type, depth of panel and exposure of panel; no significant value was attained (table 16). This was clearly not the case in other comparable studies (Arena et al. 2006, Floerl & Inglis 2003). The sampling period was spread over five months, a fairly small time frame, with testing taking place in a pair of contrasting marinas (one open, one lock-gate), therefore the results are by no means definitive and could be substantiated further to enhance trends found in the settlement of NIS.

Table 16. Analysis of Variance for total NIS by square root, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Marina	1	4.009	4.009	4.009	1.45	0.252
Exposure	1	4.015	4.015	4.015	1.45	0.252
Depth	1	0.223	0.223	0.223	0.08	0.781
Error	12	33.245	33.245	2.770		
Total	15	41.493				

Although a total of six taxa of invasive species were found, in some cases as little as five occurrences were found over the total sampling period. This was true for the taxa *Bugula neritina, Tricellaria inopinata, Styela clava* and *Corella eumyota.* Any occurrence with numbers this small will inevitably be statistically insignificant (Clarke & Warwick 2001).

The introduced barnacle *Elminius modestus* was recorded as the highest settler in the lock-gate marina on the short term panels and in the open marina on long term panels. As a species, it has been found to buck other trends observed when taxa colonises marinas (Arena et al. 2006, Floerl & Inglis 2003). *Elminius modestus* has been observed to prefer open marinas, and this was the case in this study on the long term panels. Floerl and Inglis (2003) have suggested the reason for this to be either its preference for higher flow environments or to the pre-emption of limited space, leading to competition from other taxa in enclosed marinas. Potentially, overcrowding by colonial and solitary ascidians on the long term panels in the lock-gate marina lead to it becoming out competed.

Interestingly, global climate change has been seen to have major implications on ecosystems resulting in increase in numbers and changes in NIS. Occhipinti-Ambrogi (2007) noted that the recruitment pattern of *Botrylloides violaceus* coincided with a period of low recruitment of other native ascidians. The competition for open space on the substrate was heavily influenced by the timing of recruitment and this in turn was found to be temperature dependant. Changing seasonal patterns may begin to favour the settlement of NIS at a particular time of year and thus have long lasting consequences in preventing the recruitment of native species later. Although this cannot be determined in this study it should be noted in the open marina *Botrylloides violaceus* was present in a higher abundance than ascidians and this could be due to increased temperatures.

6.4 Settlement of epibiota onto manmade structures

Marinas, by their nature change the configuration of the environment in which they are built, introducing artificial habitats into relatively small area. Heterogeneity of a habitat can play an important role in determining the structure of epibiotic assemblages (Bulleri & Chapman 2004). Marinas are three-dimensional environments providing a variety of intertidal habitats differing in material, orientation, shading and wave-exposure. It has been determined by various studies that the nature of substrata will have profound effects on epibiota (Arena et al. 2006, Booth & Brosnan 1995, Floerl & Inglis 2003, Connell 2000, Connell & Glasby 1999, Glasby & Connell 2001). There have also been consistent differences found between assemblages on vertical surfaces compared with those on horizontal undersides. Thus it could be considered artificial structures like pontoons and vessels introduce two new habitats into marinas and therefore into waterways. Bulleri & Chapman (2004), among others have demonstrated that these differences are due to varying conditions such as light, predation, larvae behaviour and in many studies (Bulleri & Chapman 2004; Osman & Whitlatch 2004; Glasby & Connell 1999) a distinct difference in water-flow or wave action. Water flow and wave action have also been linked to abundances of assemblages found in different types of enclosure and attached to different substrata (Glasby & Connell 1999; Floerl & Inglis 2003). As discussed previously, this study has confirmed that certain taxa, in this case the ascidian, have reacted well to the substrata and changing conditions present within an enclosed marina and become an abundant species.

6.5 Guidelines for marina design

Each artificial habitat which changes the composition of the natural environment, such as an enclosed marina, cannot act as a replacement for a natural habitat; as it provides a novel environment which can lead to different types of intertidal assemblages (Bulleri & Chapman 2004). The combination of different types of artificial habitat in a relatively small area may facilitate the colonisation of populations of organisms able to take advantage of the new physical and biotic conditions with unpredictable effects on the native biota and the colonisation of floating manmade structures, such as vessels (Connell & Glasby 1999; Glasby & Connell 2001).

Although water flow was not measured, both the findings from this research and the study covering the entire south coast of England concur with the investigation carried out by Floerl and Inglis (2003), which demonstrated a distinct and consistent difference in the rate of recruitment of fouling organisms to available surfaces in enclosed boat harbours compared with those in open areas. Other factors to consider are the local tide amplitude, the volume of the marina basin relative to the size of the entrance and the amount of freshwater input into the marina system, each of these can affect the residence time of water within an enclosure and therefore significantly affect larvae retention and survival. Unnatural fresh water influx such as the heavy rainfall which occurred during the sampling period of this study can result in the recruitment of a smaller number of taxa. This was also demonstrated in the study undertaken by Floerl & Inglis (2003).

As this research has not identified all of the attributes necessary for marina design, the results from this study should be considered in line with set of parameters which demonstrate all of the necessary functions of a new marina. However, when designing a marina it would be advisable to develop a plan which includes the following:-

- The marina should use materials that recruit similar assemblages to those in a nearby natural environment, refer to (Bulleri & Chapman 2004; Connell & Glasby 1999; Glasby & Connell 2001) for detailed research.
- It is also apparent from this study, the MRes project covering the south coast of England and other studies (Floerl & Inglis 2003), that the degree of enclosure should be considered, with an emphasis in the design on allowing for a steady water flow.
- As demonstrated in this study increased assemblages will develop at a depth of 2m compared to the surface, therefore channels or varying depths should be avoided.

6.6 Implications for recreational boating

Artificial structures such as boats and pontoons create a certain amount of shading over the marine environment. Glasby (1999) looked at the potential effects of shading on epibiotic assemblages, which provided clear evidence that shading can affect colonisation by many taxa. Light reductions in long-established assemblages' leads to a significant increase in ascidians and bryozoans (Glasby 1999). This has implications for the adequate use of antifouling paints; ideally, the hull of a vessel should be washed down and repainted each year, though invariably vessels are left in much longer and develop a heavy encrusting of fouling assemblages (Chambers et al. 2006). Vessels and pontoons also differ from natural, hard substrata as they have the ability to float. Floating substrata can create a clear difference in assemblages to fixed substrata (Glasby 1999). This is linked to the differing physical characteristics of the substrata. Floating substrata are, at some point, always exposed to the water surface, and hence subjected to greater intensities of light, which has been demonstrated to have significant effects on larvae dispersal and recruits (Glasby 1999).

The findings demonstrated in this work have implications for the spread of non-indigenous fouling organisms. Recreational boating has been noted for the spread of number of invasive species including the Japanese kelp (*Undaria pinnatifida*) and the marine black-stripped mussel (*Mytilopsis sallei*). The probability of a NIS engaging with an available vector in a source location is related to the abundance, availability and selectivity of the vector and also the local supply of colonising propagules (Mineur & Johnston 2008, Floerl & Inglis 2003, Ruiz et al. 1997). This study reinforces other studies suggesting that the physical characteristics of the location in which a vessel is moored could have an influence on both the size and frequency of recruitment events. Floerl & Inglis (2003) and Mineur & Johnston (2008) have demonstrated a similarity in composition and abundance of fouling assemblages which recruit to both Perspex panels covered in aged copper-based anti fouling paints and the hull of vessels in the surrounding marina. Therefore boats moored in an enclosed marina which have a limited amount of antifouling paints will leave the marina with a considerably larger population and variety of fouling organisms than comparable vessels leaving an open well flushed marina. On average the amount of individual recruitments will be greater in an enclosed marina, although in this study it was dependant on the taxa, therefore a large number of vessels can be exposed to the same pulses of recruitment at any one time, thus leaving the same marina carrying very similar assemblages. These two features of propagule supply (recruitment size and frequency of repeat recruitments), appear to be critical in determining the secondary spread of non-indigenous species (Carlton 1992; Ruiz et al. 1997; Floerl 2002, Floerl & Inglis 2003). The results from this study and the MRes research suggest an enclosed marina basin amplifies these events.

It has been acknowledged that enclosed marinas can increase the secondary spread of NIS, but it should also be noted that enclosed marinas could replicate the same effect with native species. Falmouth is a busy port, for both European and trans-Atlantic voyages, which might potentially increase the possibility of introducing what is native in England to other countries.

6.7 Conclusions

The marina industry has a witnessed a rising commitment to boating in the UK and Channel Islands, with over 700,000 households owning a vessel. This has lead to an increase in the size and type of marina, with implications for the natural environment in which they are built (Arena et al. 2008; Bulleri & Chapman 2004; Floerl & Inglis 2003; Connell & Glasby 1999; Glasby & Connell 2001; Webb & Keough 2000). Enclosed marinas are popular with vessels remaining in port for a longer period due to their sheltered nature from waves and water movement. This study has been concerned with the ability for an enclosed water basin to have major implications on the spread of non-indigenous species, which have been highlighted in other studies by Floerl & Inglis (2003) who linked a lack of water flow to increased recruitment within an enclosed area. In this investigation the short term panels gave limited recruits and after univariate analysis of ANOVA was conducted all factors tested; marine type, depth of panel and exposure of panel were absent of a significant value. However after multivariate analysis on the short term panels, ANOSIM gave significant values for marina type (P=0.006) and exposure of panel (P=0.007), showing distinct taxa significant to each factor. The open marina (Premier) and the enclosed marina (Pendennis) were characterised by similar assemblages. However, there was a lack of *Ciona intestinalis* within Premier but showing higher abundances of Hydroid, Bryozoan and Barnacles (60% of total taxa). This concurred with exposed and sheltered areas throughout both marinas which demonstrated colonial and solitary ascidians present in much greater numbers (55% of total taxa) within sheltered areas and a higher abundance of Hydroid in exposed areas. On panels exposed to seawater over a longer exposure the enclosed lock-gate marina developed similar assemblages to those on the short term panels, on the contrary the open marina supported a different diversity of taxa over a longer exposure.

As the colonial and solitary ascidians, consisting of *Diplosoma sp., Botryllus schlosseri* and *Ciona intestinalis*, dominated both sheltered areas and the enclosed marina, contributing as the most conspicuous taxa, this trend was discussed. It was found enclosed water basins have generally a limited diversity of taxa which ascidians profit on, enabling their successful recruitment as an abundant species. Even though ascidians were the most prominent taxa, the short term panels showed little settlement of this taxon and others, compared to those of the long term panels. A possible result could be due to early post-settlement mortality which included; predation, limited nutrients from delayed metamorphosis, ingestion from grazers and a dominance of algae over growth.

So it is clear from this study cemented by that of the MRes project, which covered marinas across the south-coast of England that an open marina develops diverse assemblages in proportion to one another whereas the enclosed marina allows certain taxa to thrive. Floerl and Inglis (2003) among others, indicate the size of recruitment and the frequency of this event happening are heightened in an enclosed marina which can significantly effects the secondary spread of non-indigenous species.

It is therefore offered to the marina industry, organisations such as The Green Blue and the recreational boating community to demonstrate these findings when developing, designing or lobbying for a new marina.

6.8 Evaluation

This study did not reach its full potential of recruitment to short term panels due to varying possibilities; external elements of unnatural weather patterns are thought to be significant. This potential was also not realised in the MRes study which demonstrated a lack of recruitment to short term panels. Therefore clearer trends, in particular for NIS, may be established if the same study was retested. Climate change has been demonstrated to increase NIS, therefore extending the study over winter would be of interest.

Algae was identified to be a possible cause for early post-settlement mortality, therefore further research should identify and record all taxa present on panels, compared to this study which solely recorded sessile invertebrates. Anti fouling of the hull of vessels is a requirement by the International Maritime Organisation and although the paint has in general aged when a vessel is docked in a marina, due to inadequate replenishment of anti fouling paint, a vessel has a degree of protection applied to it. In this study the panels were sanded to aid colonisation, not as a deterrent and therefore it is suggested for other research to use panels with an application of aged copper based anti fouling paint, as a comparison to the sanded variety.

The MRes project was conducted over nine marinas with three degrees of enclosure, thus two marinas per level. In this study a pair of contrasting marinas was tested and therefore this study would be improved by including more marinas, comparative of the same nature (open and lock-gate). The quantitative data from this study could be further enhanced with the introduction of a control site, it would aim to replicate the natural environment and as a constant for both types of marina.

This area of research still needs a great deal of work to be under taken to fully understand the consequences of enclosed water bodies, especially enclosed marinas as vectors for the secondary spread of non-indigenous species.

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