The role of post-settlement processes in determining fouling assemblages on artificial reefs

By

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“Without basing a reef’s construction upon proper scientific principles it becomes at best a temporary high relief area of questionable value, or at worst an ocean junk pile whose major value has been as a promotional gimmick publicizing a special interest group” Turner et al. 1969

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SUMMARY

Eco-engineering of marine habitats is becoming more relevant as anthropogenic additions to hard substrate through ocean sprawl accelerate. Knowledge is needed on the material and structure useful for artificial hard substrate habitats, and on methods for effective monitoring. This project compared assemblages on artificial and natural reefs and then investigated post-settlement processes that could have contributed to the differences between the reefs. This study also compared and contrasted new methods for comparing and monitoring reefs. The outcomes from this project will help to inform coastal managers and other researchers as well as provide the foundation for practical interventions to increase biodiversity.

Fouling communities on established artificial reefs were examined and compared to determine substrate specific differences after decades of succession. Whereas most comparative studies only use a single artificial and natural reef, this project compared seven different aged reefs made from three different artificial materials and one natural substrate type. The community analyses derived from two methods, image analysis and physical sampling, were also compared. Reefs had dissimilar fouling communities even if they were the same underlying substrate type. The only purpose built reef, made from tyre modules was one of the least diverse sites and was probably affected by regular disturbances caused by its elasticity in the materials used.

Sampling fouling communities using image analysis showed greater similarity across substrates and reef types, because of lower level taxa identifications than obtained

from scrapings. Image analysis can underestimate assemblage diversity and miss cryptic invasive species, but was the most accurate method to efficiently sample colonial macroinvertebrates. The relationship between biotic complexity (macro algae and macroinvertebrate cover) and non-colonial invertebrates was also examined in this study using functional habitat groups. This study highlighted how the choice of methodology in visual surveys can influence the assessment of fouling communities.

Four common artificial substrates (concrete, rubber, steel, and wood) were deployed at four different sites along the coast of the Gulf of St. Vincent in South Australia every season and retrieved after three months. There were no seasonal differences in the recruitment onto the plates, but some site and substrate specific differences were recorded. This chapter indicated that there was continuous and wide spread larval recruitment in this southern temperate gulf and substrate specific recruitment trends over the sampled period.

Competition for space on hard substrates is well documented, but large macroinvertebrates and macroalgae also provide complex 3-D structures that could positively affect recruitment and perhaps functional diversity. As artificial reefs usually have communities that are different to those found on natural counterparts, similarity on a function level was examined instead. Functional diversity was explored using Biological Trait Analysis (BTA). To determine if sessile macroinvertebrates facilitate recruitment and increase species and functional diversity, three structurally complex macroinvertebrates were translocated onto settlement plates and colonisation compared after six months. The diversity of taxa was highest on the settlement plates that had the most complex shaped facilitator, a bryozoan. Functional diversity was not significantly different between any of the treatments, however, more mobile species had colonised plates with facilitators of greater structural complexity. This experiment emphasised the importance of biogenic habitat heterogeneity and positive relationships on artificial reefs. The results demonstrate that abiotic and biotic substrate differences affect the development of fouling assemblages on artificial reefs and that species and functional diversity can be increased by the presence of structurally complex organisms.

The overall findings of this study suggest that managing any hard substrate reef is a complex task. The idea of managing or creating ecosystems that have been lost to habitat degradation, may not be realistic or feasible. Approaching the task from the viewpoint of achieving minimal harm to the overall system and some functional similarity may be the best approach for marine resource managers.

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DECLARATION

I certify that this thesis does not incorporate without acknowledgment any material previously submitted for a degree or diploma at any university; and that to the best of my knowledge and belief, it does not contain any material previously published or written by another person except where due reference is made in the text.

Signed.

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CHAPTER 1. GENERAL INTRODUCTION

1.1 BACKGROUND

This study investigated how post-settlement processes structure fouling assemblages on marine hard substrates, substrate and regional differences in biodiversity and functions, and how commonly used assessment methods can affect the evaluation of fouling assemblages. Hard substrates are an increasingly common habitat type in the marine environment (Connell & Glasby 1999, Bishop et al. 2017), the term not only refers to rocky reefs and other natural rock but also many substrates that are artificial and possibly provide different habitat for marine fouling communities. The rate of coastal urbanisation has meant that in many coastal regions there are more artificial than natural substrates to colonise (Bulleri & Chapman 2010, Airoldi et al. 2015, Dafforn et al. 2015A, Martins et al. 2016). Artificial reefs and artificial substrates are often differentiated in the literature, although the term “artificial substrates” includes artificial reefs by the definition of their material components. The definition of an artificial reef used by Svane and Petersen (2001) to encompass any submerged structure that is susceptible to fouling, is more inclusive and relevant to studies comparing assemblages on multiple reefs. The number of artificial reefs that are available for the colonisation of marine organisms are increasing around the world as coastal urbanisation continues (Airoldi et al. 2015, Dafforn et al. 2015A, Martins et al. 2016). Examples of structures not usually considered as artificial reefs include wharves, shipwrecks, energy production structures and breakwaters (Butler 1986 Glasby 2000, Perkol-Finkel & Benayahu 2006, Coates et al. 2014, Ido & Perkol-Finkel 2015). Artificial reefs are useful to test ecological theory, because 1) they can provide identical structures from which replicate observations can be made and 2) their uniformity can help minimise any unquantified and unknown physical differences that occur in natural habitats, which may cause variations in assemblages (Glasby & Connell 1999). The dynamic nature of marine fouling communities on hard substrates is attributed to a mix of larval availability, temporal and spatial variations of abundances, structural complexity and disturbance.

1.2 PRE- AND POST- SETTLEMENT PROCESSES AND FOULING ASSEMBLAGES

There are four fundamental ecological processes that can change the abundances of individuals in populations; births, deaths, immigration and emigration. The first two are localised processes, whereas the last two can operate at much larger scales, linking populations through the long-distance movement of larval or mobile benthic stages (Cobb et al. 1997). In the marine environment, where many species are broadcast spawners, fertilised eggs and larvae are immediately subject to transport by currents, leading to a high connectivity between populations (Todd 1998, Le Corre et al. 2015). Species with planktotrophic larvae have a wider dispersal capability than those with lecithotrophic larvae or direct development (Todd 1998, Cahill et al. 2017). The distribution of populations of marine invertebrates is determined by the dispersal abilities of larvae and the extent of passive transport by the surrounding medium on a relatively large scale, and then by the availability of suitable settlement sites on a relatively small scale (Minchinton & Scheibling 1991). For macroalgae with microscopic propagules, dispersal is mostly over shorter distances (Bobadilla & Santelices 2005), but dispersing large numbers of algal thalli can reduce the risk of local extinction by spreading propagules over large spatial areas (Worm et al. 2001).

Supply-side ecology looks at variations and mortalities of larvae whilst in the plankton and how it affects benthic communities (Lewin 1986, Underwood & Fairweather 1989). Planktonic larvae are exposed to different environmental factors such as hydrology, predation, or settlement cues (Pawlik 1992). Variable seasonal differences in the plankton can also influence settlement as most species have peaks in larvae at a particular time of year, and whilst the time of year might be predicted, the size of the peak cannot (Keough 1983).

Invertebrate larvae actively select an environment that is advantageous and the presence of a specific bacterial biofilm community can provoke settlement (Wieczorek & Todd 1998, Kroehl & Hadfield 2004, Zhao et al. 2013). If the specific settlement cues are absent, larvae can postpone settlement and remain as larvae for hours or even months (Pechenik 1990). Physical factors such as surface contouring, complexity and heterogeneity of substrate, low shear microsites and the behaviour of the larvae can also be critical as to whether the larvae settles (Rodriguez et al. 1993).

For soft sediments, post-settlement processes are often the most significant cause for variations in a community and pre-settlement processes are not always the dominant determinant of spatial and temporal patterns (Olafsson et al. 1994). This study examines post-settlement processes that can drive variations in hard substrate assemblages (Wieczorek & Todd 1998). After the larvae settle upon hard substrates, they are usually subject to high rates of mortality (Hunt & Scheibling 1997). The causes of early post-settlement mortality include: delays in metamorphosing into adult forms, biological disturbances that include accidental ingestion by grazers, physical disturbance, physiological stress, predation and competition (Hunt &

Scheibling 1997). The effects of these ecological processes on the recruiting community can vary between sites subject, for example, to localised relationships between larval supply and predation intensity (Sams & Keough 2007; Vieira et al. 2012; Osman 2015). Temporal and spatial variations in abundances of early pioneer assemblages have been observed after initial settlement or recruitment has taken place (Hunt & Scheibling 1997).

The patterns in settlement of marine larvae are frequently inferred from recruitment (Rodriguez et al. 1993), which can be misleading, subject to the variations in post-settlement mortality (Keough & Downes 1982, Connell 1985). Settlement and recruitment of benthic marine invertebrates is a complex ecological process, determined by the interaction of biotic and abiotic factors which operate at different spatial and temporal scales (Rodriguez et al. 1993). Settlement is defined as the point where an individual first takes up permanent residence on the substratum (Keough & Downes 1982). According to Rodriguez et al. (1993), for benthic invertebrates with pelagic larvae, the term “settlement” should be used to describe the passage from a pelagic way of life to a benthic way of life. The use of the term “passage” encompasses the arrival onto the substrate and then the metamorphic changes undergone to settle onto the substrate. Recruitment then follows on from settlement, and implies a lapse of time from this metamorphosis. Settlement can involve an active choice by the larvae such as habitat selection, but recruitment involves no active choices (Keough & Downes 1982).

After marine invertebrates survive early post-settlement mortality events and then recruit into the fouling community, threats from competition as well as predation still remain. These mechanisms and processes drive the succession and shifting assemblages in marine fouling communities and are a source of much discussion in the literature (Mook 1981, Nydam & Stachowicz 2007, Claar et al. 2011). Biotic processes and their often unpredictable nature led Connell and Slatyer (1977) to propose three different models of community succession: 1) the facilitation model, in which later successional species benefit from earlier settlement of pioneers: 2) the tolerance model, where later species were successful whether the pioneers were there or not: and 3) the inhibition model, where earlier species inhibited later species from settling. The inhibition of large sessile invertebrates can have strong effects on community structure (Sams & Keough 2012). Marine fouling organisms can inhibit the settlement of other larvae through a variety of physical means, for example some bryozoans have spines and elaborate skeletons to inhibit settlement and encroachment (Pawlik 1992). For communities that follow the inhibition model, disturbance of a patch can create free space for settling larvae to gain a foothold in the community and so compete with the established species. For assemblages on artificial reefs, the facilitation model of succession (Connell & Slatyer 1977) can apply, as sessile species can create biogenic habitats for interstitial fauna (Mineur et al. 2012). Promoting the settlement of key facilitators, may help artificial reefs become more analogous to natural benchmark sites as well as stabilise and increase resilience in their communities (Coombes et al. 2015, Dyson & Yocom 2015).

1.3 DISTURBANCES IN FOULING ASSEMBLAGES

The ecological term ‘disturbance’ can be defined as any event that kills or displaces individuals from a community (Sugden et al. 2007). The creation of a new patch of substrate and the subsequent role in providing new space for settlement and recruitment depend on larval supply and processes such as post settlement mortality. The continued creation of new space by disturbance and the processes that follow are known as “patch dynamics” (Pickett &Thompson 1978). Patch dynamics are a reflection of nature’s unpredictability and the role of natural disturbances in maintaining diversity.

The term “patch” is used as it does not imply a specific area, but a spatial relationship between a space and its surroundings, so patch dynamics emphasises patch change (White & Pickett 1985). The larger the disturbed patch, the more recovery depends on the arrival of larval settlement from surrounding habitats, whilst smaller patches can be readily colonised by the surrounding undisturbed patches of the fouling community (Thrush & Whitlatch 2001). The dispersal characteristics of propagules and larvae are also important in determining the recruitment patterns (Dudgeon & Petraitis 2001). Small-scale disturbances can allow both pioneer and later successional species to be present in communities at the same time (Dial & Roughgarden 1998).

The concept of natural small-scale disturbances maintaining high diversity is known as the Intermediate Disturbance Hypothesis (IDH) and was first proposed for terrestrial plant communities and coral reefs (Connell 1978). The IDH was preceded by the concept that localised communities remain in equilibrium, where the abundances of species undergo constant or cyclical oscillations over generations (Sousa 1979). The IDH takes into account natural disturbances and patch dynamics in fouling communities. For example, grazing of algae in fouling communities leads to perennial and annual species being found together (Worm et al. 2001). In an intertidal boulder habitat where physical disturbances (movement of boulders) associated with wave action were common, red algae persisted in the fouling community despite always being succeeded by larger brown algae and barnacles (Sousa 1979). These findings suggested that the community was not reaching equilibrium as disturbances maintained higher diversity in the fouling community (Sousa 1979). Bailey-Brock (1989) observed that, in the disturbed grazing scars from feeding reef fishes, new bryozoan colonies were forming. These interactions reflect the IDH, where the grazing relationship between algae and the reef fishes can create space available for the settlement of new bryozoan colonies. The effectiveness of intermediate disturbance in maintaining high diversity in a localised fouling community is governed by the larval supply and pre-settlement processes. If there is high settlement of a superior competitor onto a patch, it can exclude subordinate settlers (Dial & Roughgarden 1998). The complex and hard-to-predict development of fouling assemblages are further complicated by the increasing numbers of anthropogenic-materials that have been submerged in the environment (Ido & Perkol-Finkel 2015). These can be subject to additional severe anthropogenic disturbances such as cleaning, movement and the introduction of exotic larvae (Glasby et al. 2007). Disturbances are important events for artificial substrates and something that must be taken into account by coastal managers.

1.4 FOULING ASSEMBLAGES ON ARTIFICIAL SUBSTRATES

The habitat created by submerged artificial substrates provide for complex ecological relationships between the inhabitants, newly-arrived settlers, visitors and other natural and artificial habitats in the region. From the earliest stages of a fouling assemblage, artificial substrates can have an effect on the settlement and then successful recruitment of fouling assemblages. Different biofilms on artificial substrates can facilitate the settlement of different groups of early pioneer invertebrate and algae assemblages (Kriwy & Uthicke 2011). The assemblages of invertebrates are sometimes less diverse (Reimers & Branden 1994, Martins et al. 2016) or more diverse (Perkol-Finkel et al. 2006, Zintzen et al. 2006) than those found on natural hard substrates. Comparisons with communities on natural reefs are hard, as often the adjacent artificial reef is accidental like a shipwreck (Perkol Finkel et al. 2006) or unintentional like a wind turbine pylon (Andersson et al.2009) leading to structural differences in the base substrate. Microhabitat complexity can further affect the diversity of fouling communities and their similarity with communities on adjacent natural hard substrate (Perkol-Finkel & Benayahu 2005, Martins et al. 2016; Loke & Todd 2016; Lavender et al. 2017; Liversage et al. 2017). Understanding how structure and ecological processes drive the development of fouling communities is important for evaluating whether the increased use of artificial structures for habitat restoration can substitute for natural habitat (Dafforn et al. 2015B; Strain et al. 2018).

Anderson and Underwood (1994) looked at recruitment of an intertidal estuarine fouling assemblage onto four different substrates (concrete, plywood, fibreglass and aluminium). The settling larvae displayed a preference for particular substrates; concrete and plywood panels were colonised by more species than the fibreglass and aluminium. In tropical seas, recruitment has also been found to differ across substrata (Perkol-Finkel & Benayahu 2004). The various structures and surfaces of many artificial substrates can promote the settlement of some fouling species. Concrete and wood are potentially more porous and/or rougher than fibreglass and aluminium substrates and have potentially greater surface area and/or greater microhabitat diversity for colonisation (Anderson & Underwood 1994, Coombes et al. 2015). The chemical properties of concrete can attract different epibenthic fauna than those colonising underwater steel structures (Andersson et al. 2009) and lead to long-term dissimilarities of communities compared to natural reefs (Perkol-Finkel et al. 2006). Wooden structures are also susceptible to marine borers such as *Teredo navalis*, which can establish populations on artificial structures (Borges 2014). Structural complexity and surface area, are also important on larger scales and can lead to fouling community being significantly different from surrounding natural substrates even over longer time scales. In the Red Sea, a shipwreck sunk over a century ago remained distinct from the coral reef it was lodged in, attributed to the structural differences between the two substrates (Perkol-Finkel et al. 2006).

The structure and orientation of artificial substrates can modify environmental conditions such as light exposure or shading. Assemblages on vertical surfaces were significantly different from those found on the horizontal surfaces of pontoons in Sydney Harbour, Australia (Connell 1999). Surface orientation together with substrate composition of concrete panels was found to affect the composition of the fouling community (Glasby 1999). The level of light availability in the water column will also affect the fouling assemblage, in particular the stratification of macro algae. For example, the available light determined the boundaries between the algae and bryozoan communities on the Montrose Alpha oil platform, as below 10 m algae rapidly gave way to hydroids and bryozoans on all pylons and other parts of the submerged platform structure (Forteath et al. 1982). Annual or seasonal algal growth that is associated with seasonal water turbidity in coastal seas further affect the fouling assemblage on artificial substrates (Bailey-Brock 1989).

1.5 STUDYING ASSEMBLAGES ON ARTIFICIAL REEFS

Newly-submerged artificial reefs provide opportunities to study the ecological effects of new artificial substrate. Artificial reef deployments are both large scale-disturbances and can be set up as manipulative long term experiments to study fouling communities. Experimental reefs with replicate modules are rare in the literature, because of the cost, planning and materials involved. However, with the proliferation of concrete modular reefs used for fisheries enhancement, they are becoming more common (Bohnsack et al. 1989, Wilding & Sayer 2009, Krohling et al. 2006, Hunter & Sayer 2002). There is even an emerging trend for sinking large surplus vessels for dive tourism purposes with little or no consideration given to the habitat the ship will provide. Naval vessels are built to a different standard than merchant ships and the skulled vessels allow studies on the assemblage that will settle upon it and exist for years to come (See Appendix 2).

Many community or assemblage studies are non-manipulative and observational in nature or manipulative experiments (disturbance or removal studies) (Chang et al. 2017). New methods and analysis techniques many help overcome these limitations. The number of methods that can be used by researchers to sample fouling assemblages is increasing (Dobretsov et al. 2014). These advancements like satellite imagery (Call et al. 2003), sidescan sonar (Arney et al. 2017) and image analysis software (Kohler & Gill 2006) have brought about changes in the way assemblages are sampled and analysed. New technologies may allow the investigation of macroscale questions that were previously impossible to address (Arney et al. 2017).

Functional group analysis can be used to examine how similar an artificial reef is to a natural reef, in terms of functions and is a relatively new way to perform an assessment. The Biological Traits Information Catalogue (BIOTIC) provides a list of 40 'traits', which can help to coalesce organisms into groups defined by multiple traits (i.e. feeding, larval stage, size etc) (MarLIN 2006). All of these tools are now relatively low cost and often open source, which will allow coastal management groups a greater variety of tools and methods to assess artificial reefs.

Whilst much work has been undertaken looking for increased fish stocks (Attraction vs. Production, Bohnsack 1989) for artificial over natural reefs, care needs to be taken to mitigate or limit any impacts that artificial reefs place upon the biota inhabiting the soft-bottom habitat they are nearly always deployed upon (Grossman et al. 1997). Soft-bottom habitats are influenced by natural hard substrates as well as artificial reefs, however the unique materials and structures found on the latter may influence adjacent benthic soft sediment communities differently (Barros et al. 2001; Wetzel et al. 2014, Heery et al 2017). Frazer and Lindberg (1994) looked at prey densities in soft-bottom benthos surrounding an artificial reef for three different predator phyla (octopus, stone crabs and fish). The densities of prey species significantly increased with distance from the reef modules (Frazer & Lindberg 1994) hinting at foraging occurring nearby to the reef. The artificial reef and its inhabitants can also alter the physical characteristics (grain size) of the surrounding soft-bottom substrate; sediments closer to reef modules have had coarser sediments than areas further away (Ambrose & Anderson 1990).

1.6 THE USE OF ARTIFICIAL REEFS IN SOUTH AUSTRALIA

South Australia first began to deploy purpose-built artificial reefs in the 1970s, after a visiting American academic recommended tyre reefs as a way to increase fish production as the habitats found in the Gulfs were similar to American waters (Branden et al. 1994). The reef program included tyre module reefs, one of which was used to study two years of seasonal variations in algal colonisation and growth (Reimers & Branden 1994). The catch rates and any increases in recreational fishing catches or effort were also studied (McGlennon & Branden 1994). There were also a large number of studies based on the fouling communities attached to pier pilings on some of the piers around the gulfs of South Australia. These studies have looked at: dispersal, settlement and recruitment to hard substrates (Kay & Keough 1981, Keough 1983, Butler 1986, Davis & Butler 1989); stability of the fouling community (Kay & Butler 1983, Butler & Connolly 1996); patch dynamics (Kay & Keough 1981, Butler 1991); and predation relationships (Keough & Butler 1979). Butler and Connolly (1996) studied the assemblages on a concrete pier over a decade, and found that the fouling community continued to change in terms of different species and abundances. There is renewed interest in the use of artificial reefs in Australia and South Australia (where this study took place) both for fisheries enhancement and ecosystem restoration (shellfish reefs) outcomes (McAffee & Connell 2017). The established epifaunal communities on existing artificial reefs, including the wharves and piers that are encompassed by Svane and Petersens’ (2001) definition are important comparisons sites or perhaps potential lessons to be learned from for new reefs. Especially when the rich diversity within the jetty epifaunal pylon communities is so well recorded (Kay & Butler 1983, Butler & Connolly 1996). This thesis addresses a need for further knowledge to inform the design of future artificial reefs in southern Australia. As well as the suitability of methods to evaluate the subsequent assemblages that recruit upon them.

1.7 STUDY OBJECTIVES AND THESIS STRUCTURE

The main aim of this thesis was to investigate the relevance of post-settlement processes in the creation of different fouling assemblages on artificial reefs, looking at commonly used materials. The study focused on mobile and sessile species of fouling assemblages on the reefs, but did not include vertebrate species such as fish. Therefore the author has chosen to not use the word ‘community’ as the study is excluding reef fish and larger mobile invertebrates.

The communities on any new purpose built artificial reefs in southern Australia will most probably be benchmarked against natural reefs by future researchers. However the similarity with other long established regional structures such as wharves and jetties, shipwrecks, with their documented diverse epifaunal communities (Butler 1986) should also be taken into account as these could be providing much of the larval supply for any new purpose built reefs. The epifaunal communities on the established jetty pylons are even more important when further coastal urbanisation rates are considered (Dafforn et al 2015A).

The study looked at processes that may determine some of the dissimilarity in fouling assemblages on artificial reefs constructed from the same or different artificial substrates, as well as the usefulness of different sampling methodologies like trait analysis for comparisons. This study used natural and manipulative field experiments to investigate the following;

1. Evaluate the common methods of assessment and the level of taxonomic resolution needed to assess fouling assemblages
2. Determine the composition of fouling assemblages on some of the artificial reefs and natural reefs within the study region as well as identify dominant species and functional groups in the assemblage.
3. Investigate the role of seasonal larval availability vs. active substrate selection for settling fauna and flora, inferred from recruitment.
4. Test whether facilitation and keystone species could increase functional diversity of artificial reefs as well as explore the usefulness of trait analysis for comparisons of assemblages

This thesis has been presented in the format of four data chapters (Chapters 2-5) for later publication, presenting the experimental studies and Chapter 6 is a synthesis of the results from all data chapters There is also an additional short discussion chapter in Appendix 2 on the renewed trend of sinking ex-Naval Vessels for use as artificial reefs, which could lead to long term effects for surrounding habitats because of the materials and construction involved. The study outcomes will help to inform new artificial reef developments.

CHAPTER 2. METHOD MATTERS IN THE DETECTION OF DIFFERENCES IN FOULING ASSEMBLAGES

2.1 INTRODUCTION

An increasing amount of artificial substrate has become available for colonisation by fouling assemblage in coastal areas (Glasby & Connell 1999, Dafforn et al. 2015A, Firth et al. 2016). Submerged anthropogenic substrates, called artificial reefs hereafter following Svane and Petersen (2001), are found in most urbanised marine environments (Andersson et al. 2009). They are often constructed from materials not found in natural reefs, for example; concrete, steel, wood and rubber (Butler 1991, Reimers & Branden 1994, Butler & Connolly 1999, Glasby & Connell 1999, Zintzen et al. 2008). The substrate material is usually related to the purpose of the reef, which can include: habitat restoration (Pickering et al. 1999); fisheries enhancement (Bohnsack 1989, Jensen 2002); resource extraction; or transport (Forteath et al. 1982; Butler & Connolly 1996). Different artificial substrates may be occupied by different fouling assemblages (Diamant et al. 1986, Perkol-Finkel et al. 2006), but comparisons between artificial and natural reef assemblages can be confounded by different sampling methods used (Relini et al. 2007).

Artificial reef studies often follow a descriptive approach for a single artificial reef or substrate (Forteath et al. 1982, Osenberg et al. 2002); however, comparative studies are important tools in determining assemblage differences. Assemblages on artificial reef substrates have been compared with adjacent natural substrates (Diamant et al. 1986; Ambrose & Swarbrick 1989; Perkol-

Finkel et al. 2006, Firth et al. 2016B), but single studies examining fouling assemblages on multiple substrates that are artificial and natural are rare and difficult because artificial reefs are often much smaller, younger and more isolated than natural reefs (Carr & Hixon 1997). Comparative analyses and reviews encounter the additional problem of different methods used in studies. Relini et al. (2007) analysed 30 years of studies on different artificial substrates, based on approximately 100 papers, yet the confounding variety of survey methods and sampling times made their meta-analysis tenuous. Increasingly more methods are available and accessible, because of technological advancements and cost reductions in purchasing or hiring new sampling tools such as high resolution sidescan sonars (Arney et al. 2017).

Comparison of sampling methods on reefs have largely focused on coral reefs and fish assemblages and mainly compared sonar, photos and visual census methods (Leujak & Ormond 2007, Lam et al. 2006, Arney et al. 2017). For fouling assemblages, comparisons between photo-quadrats with digital image analysis and the collection of the contents of the quadrat (scraping) has not been done before on temperate reefs. Sampling by scraping and collecting the complete fouling assemblage from defined quadrat areas has been used in many artificial reef studies (eg. Zintzen et al. 2006, Walker et al. 2007) to collect detailed information on species assemblages for later laboratory analysis and/or permanent specimen records. The time-consuming and destructive nature of scraping samples, does not allow this method to be used for monitoring temporal changes within patches of fouling communities. Sampling is always a trade off on the resources available, replication need (Canning-Clode & Sudgen 2014), and also dependant on weather, maritime heritage restrictions, scientific diving regulations and logistics.

Photo-quadrats are non-destructive, permitting multi-year assemblage comparisons and are relatively quick to collect in the field, but require time-consuming digital image analysis later in the lab. The percentage cover of organisms can be quantified using random points and assigning categories, previously using transparent overlays (Bailey-Brock 1989; Aseltine-Nielson et al. 1999) or projecting slides on to paper with a grid (Foster et al. 1991). The advent of high-quality underwater digital cameras and software packages such as Coral Point Count (Kohler & Gill 2006), have made percentage cover estimation from digital images a common method of sampling fouling assemblages (Macedo et al. 2006; Walker et al. 2007; Zintzen et al. 2008, Dumas et al. 2009). Photo-quadrats for percentage cover estimates are commonly used to analyse coral reefs, as they offer higher image resolution than video transects and usually cost less than other sampling methods (Dumas et al. 2009). Percent cover is a common measure of attached organisms, and digital analysis is one of the most precise methods (Meese & Tomich 1992). Digital analysis may require coarser taxa groupings of visually indistinct or cryptic species to determine percentage cover depending on image clarity (Zintzen et al. 2008).

Photos could overlook the structural complexity of fouling assemblages, which are typically multi-layered (Walker et al. 2007). Foster et al. (1991) found underestimates of percent cover and taxa when using photo-quadrats compared to *in-situ* arrays with randomised pointed rods where over story layers were moved aside, but higher precision of cover estimates in the photo-quadrats. Advancements in photogrammetry may improve future assessments of structurally complex habitats (Bryson et al. 2017).

Percentage cover can overestimate the abundances of some taxa. For example, Foster et al. (1991) found 1.6-2.3 times higher abundances for randomised points within a photo quadrat compared to in-situ counts within a grid on a rocky shore. Since 2006, the freely available Coral Point Count with Excel extensions (CPCe) software (Kohler & Gill 2006) has been used in over 200 peer-reviewed publications (Appendix 1 Supplementary Figure 2.1), mainly for sampling coral (tropical) reefs. CPCe can be easily adapted for temperate or sub-tropical reefs (Walker et al. 2007, Barrett et al. 2010) and it is likely that more temperate reef studies will use this and other similar software packages in the future.

Cost-effective sampling methods (such as image analysis and the collection of samples from quadrats) are critically important tools to detect non-indigenous species (NIS) and climate-related shifts in marine communities. Increased rates of commercial shipping and climate change have the potential to introduce new species and communities onto hard substrates around the world (Bax et al. 2003). Artificial reefs could act as important ‘beachheads’ or initial stepping stones for NIS by facilitating settlement of invasive species, and the continued deployment of artificial reefs that can be present in the environment for long time periods (Perkol-Finkel et al. 2006), is a concern for marine biodiversity (Sheehy and Vik 2010, Glasby et al. 2007).

I tested whether the choice of sampling methodologies affected the comparative assessment of hard substrate assemblages. The hypothesis was that assemblage differences between substrates can be detected if sampled with different methods. Two sampling methods, photo-quadrats with digital image analysis (hereafter called photo-analysis) and physical collection of the contents of the quadrat (hereafter called scraping), were compared to determine their efficacy in analysing and comparing fouling communities. This comparison was carried out on different materials of artificial reefs (Branden et al. 1994) and natural reefs to achieve a rigorous comparison unaffected by substrate-specific fouling assemblages. I also tested the community assessment at different taxonomic levels to account for the coarser taxonomic resolution in the photo-analysis.

2.2 MATERIALS & METHODS

2.2.1 STUDY SITES

The fouling assemblages studied were on five artificial reefs (ARs) and two natural reefs (NRs), located in the lower part of the Gulf of St. Vincent (GSV) and on the north coast of Kangaroo Island, South Australia (Figure 2.1). All sites were situated near Investigator Strait, characterised by strong current patterns (Bye 1976). The Gulf St Vincent (GSV) contains predominantly soft-sediment seafloor, with seagrass meadows, some natural hard substrate provided by a large bivalve (*Pinna bicolor*), coastal rocky reefs (Bryars & Rowling 2009) and limestone shelf reefs (Westphalen 2010). The rocky reefs are typically covered in brown macroalgae such as *Sargassum* spp. and *Cystophora* spp. (Westphalen 2011). The invertebrate fouling assemblage includes sponges, ascidians, bryozoans, hydroids, corals, bivalves, polychaetes and crustaceans (Butler 2008).

Sites were chosen based on substrate, access and logistics, which did not allow for a balanced design. Only wooden pilings could be replicated across regions, but examples of other reef types were mostly only accessible at single locations during this study period. The SS *Hougomont,* is a steel-hulled vessel sunk in the 1930s off the lower Yorke Peninsula. The SS Hougomont is in a remote location and had not been sampled before other than by maritime archaeologists. I accompanied a survey team during field work, which was the first time the fouling assemblage has been documented on the vessel. The sampling on this vessel was covered by a restrictive permit issued by the Commonwealth Government and the number of scrapings were limited to preserve the exterior of the heritage listed vessel. No parts of the hull were removed for this study.

In South Australia, jetties or piers are common, given the shallow waters in the gulfs, and the historical use of coastal shipping. The jetties at Edithburgh (EB), Rapid Bay (RB) and Kingscote (KJ) were selected as sites where wooden substrate has been submerged. The irregular maintenance and addition of new pylons of the jetties makes estimating their age impossible. The fouling communities on jetty pylons at Edithburgh and Rapid Bay have also been studied previously (Butler 1986). The pylons at all three sites were constructed of jarrah (*Eucalyptus marginata*), a hardwood that it is resistant to marine borers such as *Teredo navalis*. The Kingscote Tyre reef (TR) built in 1986, is an example of artificial reefs that have been designed as a fish-attracting device. The natural limestone reefs at Frenchmans Cap (FC) and Second Valley (SV) consisted of limestone shelves.

Water depths at the study sites varied from 9 m for the jetties and wreck, to 7 m for the natural reefs and tyre reef. These depths allowed divers to spend a few hours on site. Ambient substrate around the artificial reefs was mostly coarse sand with seagrass patches, although the tyre reef was surrounded by fine sediment and scallop beds. The sites sampled from natural reefs were located within an area of 40 m^2 of the rocky shelf, similar to the area available at the other sites.

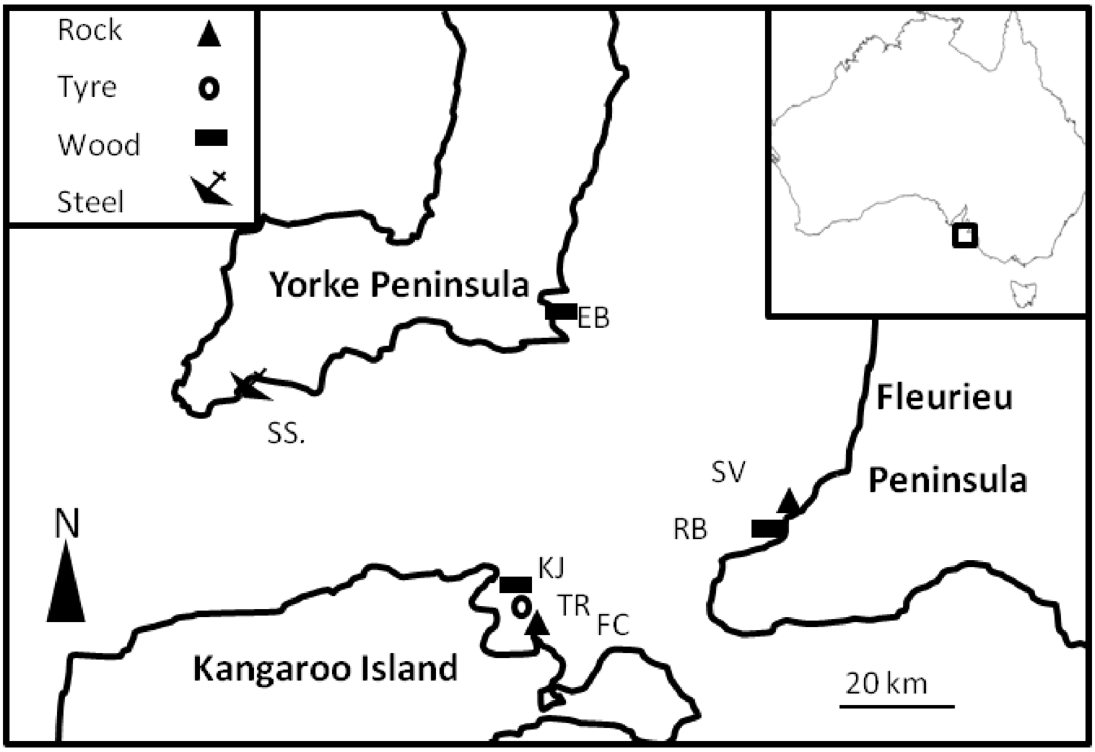


Figure 2.1. Map showing the location of the lower Gulf St Vincent in South Australia (insert is continental Australia), the sampling sites and their materials. SS: SS Hougomont, EB: Edithburgh Jetty, SV: Second Valley Reef, RB: Rapid Bay Jetty, KJ: Kingscote Jetty, FC: Frenchmans Cap Reef, TR: Kingscote Tyre Reef.

2.2.2 SAMPLING METHODOLOGY

All samples were collected by a small team of divers on SCUBA. Photographs of the quadrats were taken first (12mp Olympus 1080 µTough camera and Sea & Sea strobe) before the contents of the quadrats were scraped into large ziplock bags and preserved in 10% formalin/seawater solution for later analysis. The samples were taken from four aspects (N, E, S & W) if possible, which was only the case at the jetty sites, whereas only three aspects were sampled at the remaining sites. The prevailing lit side of the vertical surfaces was north. A large quadrat (0.5 x 0.5 m) was used to sample SS, but at the three other reef types (tyres, jetties and rock), a smaller quadrat (0.25 x 0.25 m) (Canning-Clode & Sudgen 2014) was used to account for the smaller size of the sampled surfaces (The jetty pylons were smaller than 0.5m in diameter). At the natural reefs and wreck, four quadrats were haphazardly placed over three 5 m long distances (n = 12). Because of the collapse of the centre of the vessel (*SS*) and the flat surface topography of the shelf reef at Frenchmans Cap, the locations of the quadrats were horizontal and vertical surfaces at these two sites. The tyre reef consisted of distorted pyramid shaped modules (approx. 1.5 x 1.5 m) and three haphazardly-selected modules in different parts of the reef were sampled on each side (n = 9) at a height of 0.5 to 1.5 m above the sea floor. At the jetty sites, three pylons were haphazardly chosen from different locations around the jetty. Samples from pylons were taken at heights of at least 1 m above the sea floor and 2 m below the low tide mark so that the sampled area was not exposed at low tide or subject to scour or burial. Each of the pylons was sampled on all four aspects (total n = 12).

Organisms from the scrapings were analysed in the laboratory and identified to the finest possible taxonomic level. The organisms were recorded using three different measures of distinct components: (i) mobile epifauna and some solitary sessile animals (eg. *Pyura* spp. ascidians) were counted as individuals per surface area; (ii) macroalgae were quantified by volume displacement in a large beaker; and (iii) colonial animals, such as ascidians (eg. *Botrylloides leachii* and *Didemnum* sp.), hard encrusting bryozoans were recorded as present or absent, because scraping can fragment the colony. The abundances (individual counts and volume displacement) for the scrapings were converted to individuals and mL per area of the smaller quadrat ($0.0625m^2$).

The digital photographs of each quadrat were analysed for percentage coverage to quantify the sessile invertebrates and algae, using Coral Point Count with Excel extensions V.6 (Kohler & Gill 2006) and a species list developed from the scrapings as per Dumas et al. (2009). One hundred random points were selected on the images because it provided the best coverage of the quadrat, including all of the taxa present in the pilot study, but still remaining relatively time efficient. Algal identifications on the photos were only made to genus level for the major divisions of Chlorophyta, Heterokontophyta and Rhodophyta.

2.2.3 STATISTICAL ANALYSIS

The two methods resulted in two different species lists and datasets with some overlap (See Table 2.2 and supplementary Table 2.1). The average species density at each site was compared for both methods using a single factor (method) permANOVA. The Kulczynski resemblance matrices for the species lists (based on presence/absence) obtained using the photo-analysis and scrapings were compared using second stage (2Stage) analysis (Clarke et al. 2006). The total number of species in each sample, for both methods was compared using a Euclidean resemblance matrix and a single factor (method) permANOVA. For the abundances, scrapings had to be separated into three community measures with their own resemblance matrices, based on the measures collected: colonial sessile invertebrates (presence/absence P/A), non-colonial invertebrates (# individuals) and macroalgal volume displacement (mL). Differences in abundances for both methods and measures were compared using single factor permANOVA analyses for substrate and site because of the unbalanced sampling design. The four community similarity resemblance matrices based on photo-analysis (percentage cover; Euclidean distance), scraping colonial invertebrates (P/A; Kulczynski coefficient), non-colonial invertebrates (abundance counts; Bray-Curtis similarity) and algal displacement (volume; Euclidean distance) were compared to each other at two taxonomic levels (family and finest taxonomic resolution identifiable) using 2Stage analysis with a Spearman rank correlation (Clarke et al. 2006). 2stage analysis is a rank correlation between two similarity matrices and gives values between zero (no relation) and 1 (highly similar) (Clarke et al. 2014). The two taxonomic levels were analysed separately because identifications in the photos were often limited to family level, whilst specimens from scrapings could be differentiated to species level in most cases. Principal Coordinate Ordination (PCO) plots at both taxonomic levels were used to visualise the similarity of fouling communities based on each method and measure. The outcome is further displayed in a 2Stage MDS plot. Community differences based on the method, measure as well as the taxonomic level used were further tested using four, single fixed-factor permANOVAs (factor = substrate, reef type, site and aspect). All statistical tests were performed in PRIMER v6 with permANOVA+ add-on.

2.3 RESULTS

The fouling communities on the natural and artificial reefs in Gulf St Vincent were rich in encrusting and mobile organisms. The two methods used (scrapings and photo-analysis) had very different taxonomic resolutions, with 197 taxa in total recorded by scrapings (Appendix 1), but only 39 taxa (Appendix 1) based on photo-analysis from the same quadrats.

2.3.1 COMPARISON OF DIVERSITY ASSESSMENT BETWEEN METHODS

Both the total number of species collected as well as the taxonomic composition of the fouling community depended on the method used. Based on scraping samples, 117 different plant and animal families were found, represented by 168 invertebrate and 29 plant taxa. The phylum with the largest number of families represented in the scraping samples was Mollusca (24 families), followed by Arthropoda (20 families). In the photo-analysis, 35 different families were recorded, represented by only 39 macroalgae and invertebrate taxa over all sites. The phylum containing the most families in the photo-analysis were the sponges (9 families), which also represented the largest number of taxa from photo-analysis (10 taxa), followed by the ascidians (5 taxa). Molluscs, arthropods, echinoderms and annelids were either not observed or under-represented in the photo-quadrats (Figure 2.2). Green algae were found with the most similar number of taxa using both methods (Figure 2.2).

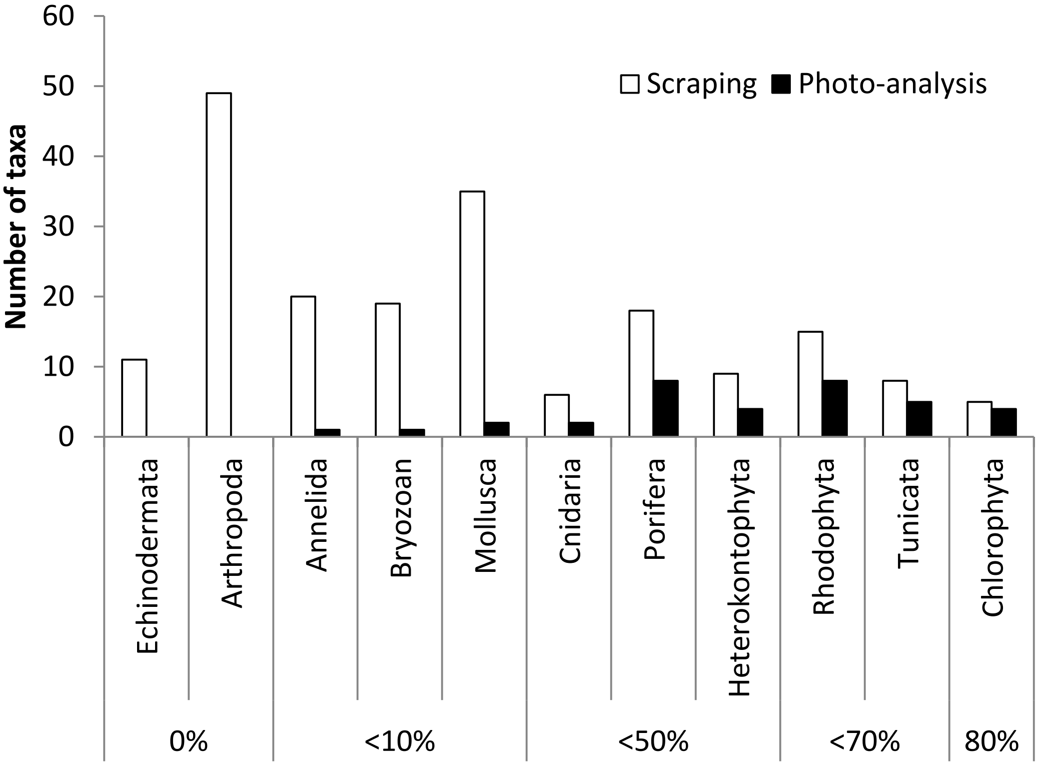


Figure 2.2. Number of taxa found for phyla for each sampling method across all sites. The percentage overlap between the total numbers of taxa detected with both methods also shown. Phyla are ordered from the least similar to the most similar number of taxa found.

The total number of taxa at each site varied between 9 (Kingscote Tyre Reef) and 21 (Frenchmans Cap) for photo-analysis, and between 29 (Frenchmans Cap) and 136 (*SS. Hougomont*) based on scraping samples (Figure 2.3). There was a significant difference in the number of taxa found in the samples from all of the reefs, depending on the sampling method (Pseudo-$F_{1,160}$ = 57.706, P = 0.001). The average taxa density per quadrat was lower based on the photo-analysis than the scrapings (Figure 2.3). These differences were more pronounced on artificial reefs than natural reefs (Figure 2.3). The steel and tyre reef sites had on average 10 more species per scraping sample than the percentage cover photo-analysis from the same sampled quadrat area.

Canopy-forming macroalgae were present with *Sargassum* spp. and *Cystophora* spp., as well as *Caulerpa longifolia* (Agardh 1823) at one of the sites (Kingscote Jetty). Encrusting algae included Rhodophyta (e.g. *Peysonnelia* sp., *Gracilaria* sp.) and brown turfing algae (e.g. *Padina* sp., *Lobophora* sp.). Sessile animals of the fouling communities present at most sites were *Chondrilla* sp. (Porifera), didemnid ascidians and *Pyura* sp. (Tunicata). Mollusca (Osteriidae & Malleidae) were only recorded on the natural reef at Frenchman’s Cap and the tyre reef (Figure 2.3). The scrapings of the fouling communities further revealed a species-rich mobile animal component, dominated by Crustacea (e.g. tanaids and crabs such as *Halicarcinus* sp.), gastropods and burrowing bivalves (Figure 2.4).

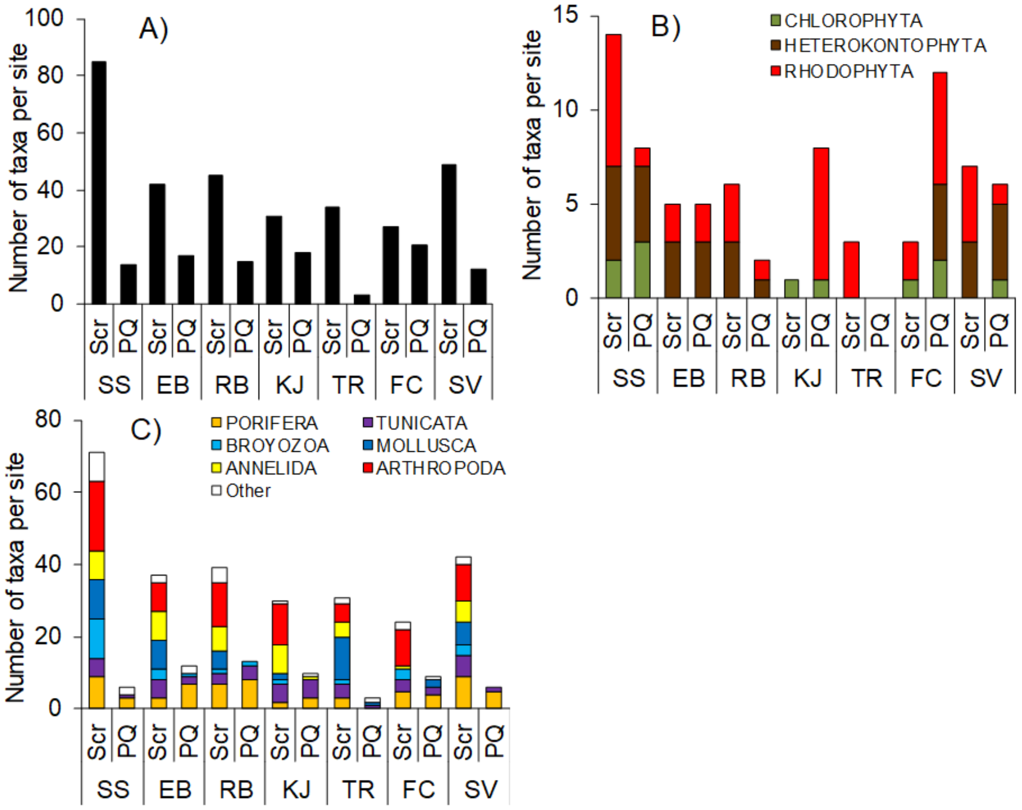


Figure 2.3. A comparison of the number of taxa per phylum found at each sampling site using the scraping (Scr) and photo analysis (PQ) method. A) total taxa count, B) taxa of macroalgae, and C) taxa for macroinvertebrates. Low numbers of Echinodermata, Cnidaria and Platyhelminthes have been combined into the category ‘Other’. Site abbreviations, SS: *SS. Hougomont*, EB: Edithburgh Jetty, RB: Rapid Bay Jetty, KJ: Kingscote Jetty, TR: Kingscote Tyre Reef, FC: Frenchman’s Cap, SV: Second Valley.

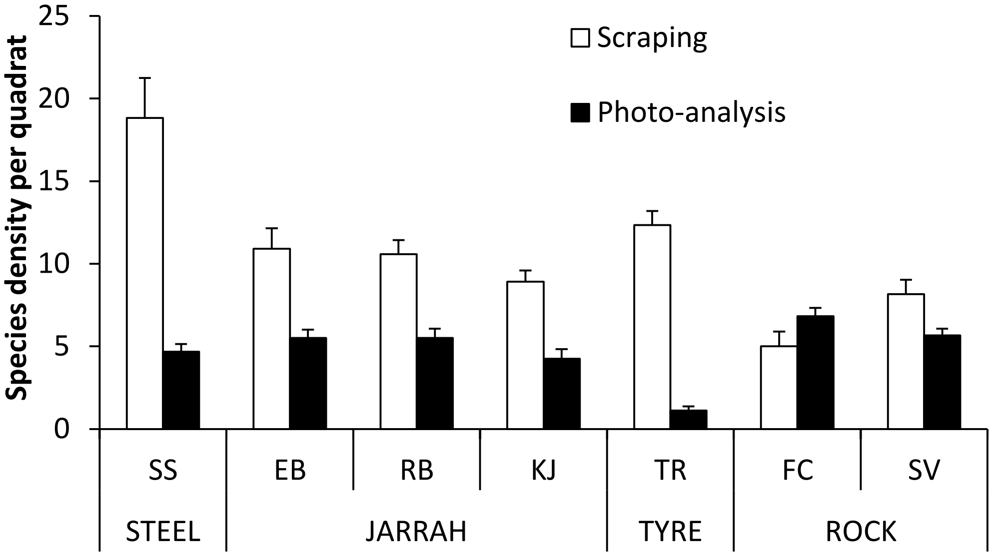


Figure 2.4. Average (+SE) the number of taxa per small quadrat area (0.0625 m^2) from both sampling methods for each site sample. Site abbreviations; SS: *SS. Hougomont*, EB: Edithburgh Jetty, RB: Rapid Bay Jetty, KJ: Kingscote Jetty, TR: Kingscote Tyre Reef, SV: Second Valley, FC: Frenchmans Cap.

The two methods used gave different resolutions for particular phyla. The scrapings yielded a higher number of families than the photo-analysis, especially within the Mollusca and Arthropoda. The photo-analysis missed many of the more cryptic fauna (arthropods, echinoderms, bryozoans and molluscs) that were the most species- and family-rich groups in the scraping samples. Three NIS were found in the scrapings: the tanaid *Tanais dulongii* on the steel reef (1 site), the colonial ascidian *Botrylloides leachii* on both rubber and wood (4 sites), and the solitary ascidian *Ciona intestinalis* on wood and steel (4 sites).

For macroalgae, scrapings provided for a finer taxonomic resolution for brown and green algae, whereas red algae occurred with a similar number of families in both the scrapings (9 families) and the photo-analysis (8 families). The species lists (presence/absence) resemblances obtained from the photo-analysis and the scraping methods were not similar on the finest taxonomic level (2Stage analysis, Range -2 to 2, Spearman correlation = 0.007), but more closely matched at the family level (Range: -2 to 2, Spearman correlation = 0.42).

2.3.2 COMPARISON OF ABUNDANCE ASSESSMENTS BETWEEN METHODS

The abundances in the scrapings were quantified using two different measures, individuals (ind. per m^2) for the fauna (Figure 2.5A) and volume displacement (mL per m^2) for macroalgae (Figure 2.5B). The tyre reef had extremely high densities of molluscs with 300 individuals per m^2 on average (Figure 2.5A), which were not detected by the photo-analysis (Figure 2.5C). These high densities were attributed to the small (<10 mm) bivalve *Musculus nanus,* which occurred amongst aggregations of the tunicate *Pyura* sp. The number of macroinvertebrates for scrapings varied significantly between substrate types (permANOVA Pseudo-$F_{3,80}$ = 4.9511, P = 0.0001) and sites (Pseudo-$F_{6,80}$ = 4.2776, P = 0.0001).

The most abundant macroalgal species was *Caulerpa longifolia* (Chlorophyta) (at 1286 mL per m^2), yet this species was only found on the wooden jetty at KJ. The most abundant Rhodophyta was *Peysonnelia* sp. with an average of 188 mL per m^2 at FC (Figure 2.5B). Heterokontophyta were most prominent at the steel wreck (SS) (188 mL per m^2). The volume of macroalgae also varied significantly between substrates (Pseudo-$\mathrm{F}_{3,80}$ = 7.1264, P = 0.0001) and sites (Pseudo-$F_{6,80}$ = 9.0891, P = 0.0001).

The abundances for photo-analysis were all quantified using a single measure, percentage cover. The percentage cover values per m^2 for the higher taxa groupings varied across the four tested substrate types. The single steel (SS) and the two rocky reefs (FC, SV) had a high percentage cover (>40 %) of brown algae (Figure 2.5C), mainly *Sargassum* and *Cystophora* species. The photo-analysis also quantified bare patches (category ‘empty’) in which no taxon categories were observed.

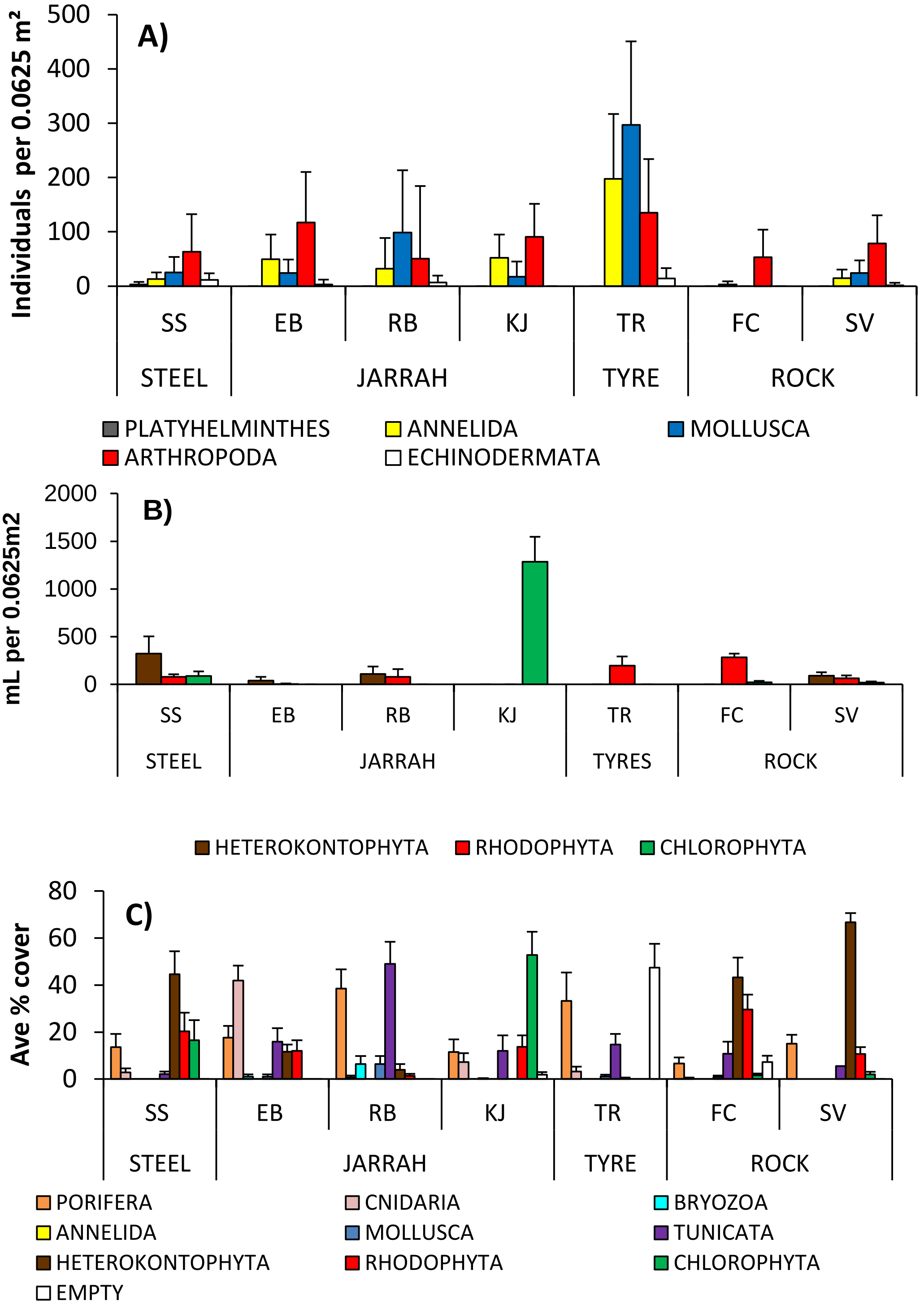


Figure 2.5. Mean (+SE) abundances per 0.0625 m^2 for three units of measurement for sampled sites: A) Non-colonial and macro-invertebrates abundances; B) algal displacement; and C) percent cover per 0.0625 m^2 of modular macroinvertebrates and macroalgae.

Abundances based on the photo-analysis also varied within substrate types. The three wooden sites had very different percentage cover values. KJ was dominated by Chlorophyta (*Caulerpa longifolia*, average coverage of 54.1%), and some replicate quadrats were completely covered by it (Figure 2.5C). The percentage cover of macroinvertebrates and macroalgae varied only significantly between substrates (Pseudo-$F_{3,6}$ = 8.2708, P = 0.0326).

The photo-analysis was not able to quantify the majority of macroinvertebrates including some abundant cryptic sessile invertebrates, such as *M. nanus* that were detected in the scrapings. Yet, the photo-analysis was able to quantify the abundances of sponges, corals and colonial tunicates, whilst scrapings could only quantify solitary tunicates such as *Pyura* sp. because encrusting species were severely damaged during the collecting process.

2.3.3 COMPARISON OF FOULING ASSEMBLAGES BETWEEN METHODS AND TAXONOMIC LEVELS

As the two methods obtained different taxonomic compositions and abundances, they also depicted different distinctions or similarities between fouling assemblages. Statistically, the permANOVA results for both methods and measures found that fouling assemblages were significantly different between reef types, sites and substrate, and analyses on both taxonomic levels gave the same results (Table 2.1). Significant differences by aspect were only found in the photo-analysis at both taxonomic levels. The assemblage assessments that were yielded by scrapings showed no significant effect of aspect, even for the macroalgal assemblages (Table 2.1).

Table 2.1. Single-factor permANOVA (Euclidean distance) results for fouling assemblages based on photo-analysis (PQ) and scraping (Scr) methods, the scrapings have been separated into different measures: (Non-colonial) macroinvertebrates (individuals per 0.0625 m^2), colonial macroinvertebrates (p/a) and macroalgae (displacement volume per m^2). Four factors (site, substrate, aspect and reef type) were tested (* P≤0.05, ** P≤0.01,*** P≤0.001, ns non-significant).

Method	**Assemblages**	**Taxonomic level**	**Site**	**Substrate**	**Aspect**	**Reef Type**
PQ	Photo-analysis	Family	***	***	*	***
Scr	Macro-invertebrates		***	***	n.s.	***
Scr	Colonial		***	***	n.s.	***
Scr	Macroalgae		***	***	n.s.	***
PQ	Photo-analysis	finest	***	***	*	***
Scr	Macro-invertebrates		***	***	n.s.	***
Scr	Colonial		***	***	n.s.	**
Scr	Macroalgae		***	***	n.s.	***

How well methods and measures captured differences in fouling assemblages was analysed for coarser (phylum or family) and finer (genus or species) taxonomic levels. In both cases, the PCO plots based on photo-analysis gave different groupings of assemblages from the various study sites than PCO plots based on scraping samples (Figures 2.6 and 2.8). Furthermore, the PCO plots based on the three different enumeration measures for scrapings (algal displacement, mobile invertebrates and colonial invertebrates) showed considerable differences in assemblages subject to the taxonomic level used. This was not the case for PCO plots based on photo-analysis, where the resemblance matrices for the two taxonomic levels were closely matched (2Stage comparison Spearman Rho = 0.99), showing little was left out of the assemblage assessments by identifying samples below family level.

Based on photo-analysis, fouling assemblages differed between the majority of artificial and natural reef substrates, with samples from the tyre reef depicted as a tight cluster (Figures 2.6D and 2.8D) because of the high percentage of ‘empty’ space that was not captured in the scraping method. Based on scrapings, PCO plots of algal displacement showed a group of natural reef samples that was quite distinct from other natural and artificial reef samples (Figure 2.6C and 2.8C), which did not emerge in PCO plots of mobile invertebrates (Figure 2.6A and 2.8A) nor presence/absence of colonial invertebrates from scrapings (Figure 2.6B and 2.8B). Assemblages of colonial invertebrates and mobile invertebrates were thus far less distinct between the various sites, as samples from natural reefs were more scattered on the PCO plots and similar to the artificial substrates, regardless of the taxonomic level used (Figure 2.6 and 2.8, A & B). Yet for mobile invertebrates, the similarity of some wooden samples had decreased with the finer taxonomic level used (Figure 2.6A and 2.8A). Macroalgae showed more defined assemblages for the different substrates, however, the macroalgae in some steel samples were more similar to samples from other substrate types (Rock and Wood) at the family level (Figure 2.6C and 2.8C).

Community analyses based on the two methods and measures used were compared using 2Stage analyses (Table 2.2) and an nMDS plot (Figure 2.7). The resemblance matrices of algal displacement and photo-analysis samples were the most closely-related ones, at the family level (Spearman Rho = 0.29) and even more so at a finer taxonomic level (Spearman Rho = 0.38), as both showed a distinct separation between natural and artificial assemblages (Figure 2.6C and D, Figure 2.8C and D, values in Table 2.2). The resemblance matrices from other measures of scraping samples showed little relatedness (low Spearman Rho values, Table 2.2) and especially the mobile invertebrates were very different from the resemblance matrix from photo-analysis samples (see Spearman Rho values Table 2.2). The increase in taxonomic distinction did increase the 2Stage relatedness for some community measures (mobile invertebrate and colonial invertebrates, Spearman Rho = 0.03 to 0.12, Figure 2.7), but further decreased it for resemblance matrices of mobile invertebrates and the photo-analysis (Spearman Rho = -0.39 to -0.48) as well as between colonial invertebrates and photo-analysis (Spearman Rho = -0.03 to -0.17) (Figure 2.6 and 2.7, Table 2.2).

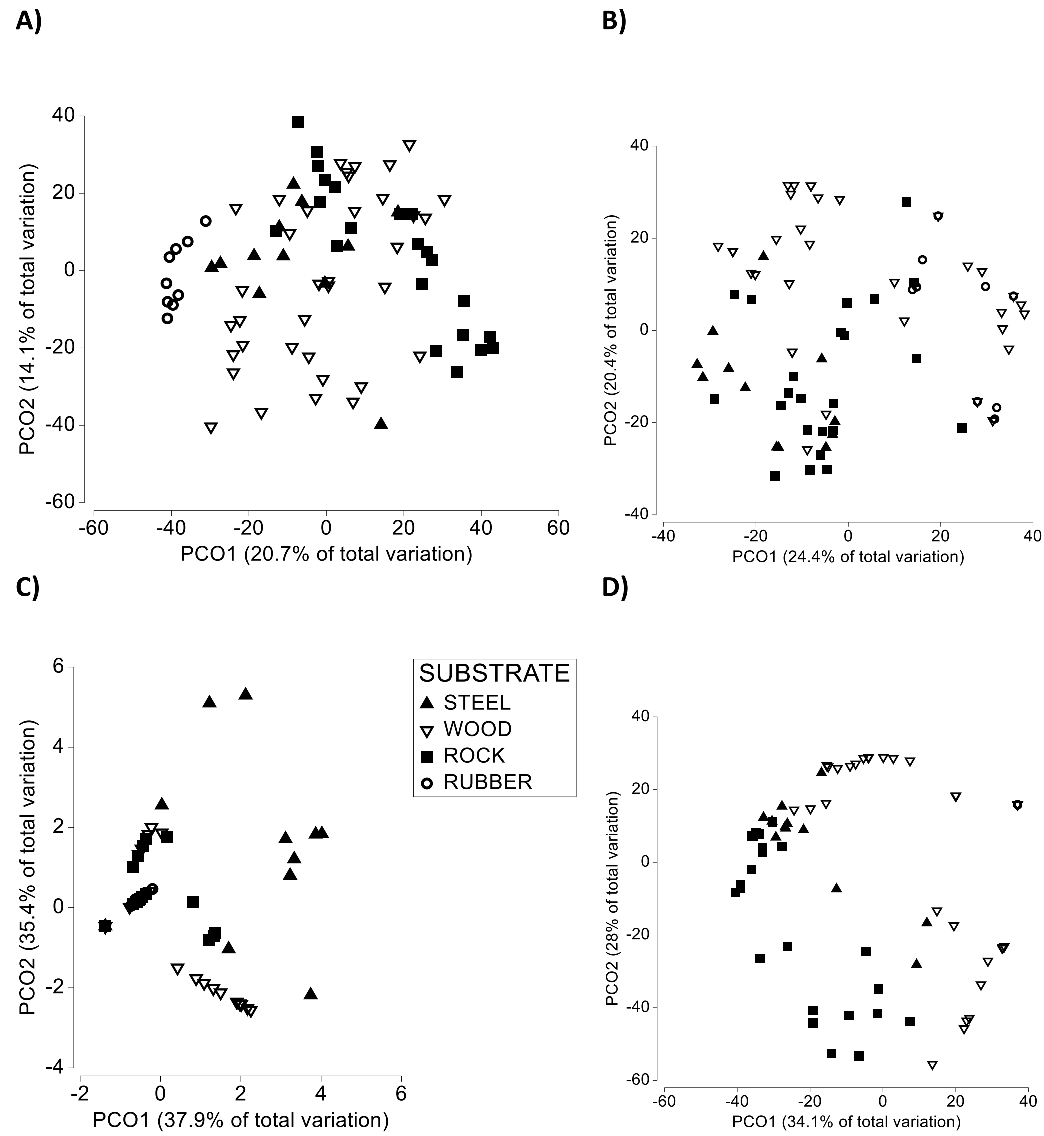


Figure 2.6. 2Stage comparison of higher level community resemblance matrices using PCO plots for the one percent cover and three scraping community measures: A; scrapings mobile invertebrates (Bray-Curtis), B; colonial invertebrates P/A (Kulczynski), C; macroalgae (Phyla) displacement volume mL (Bray-Curtis) and D; percentage cover (Bray-Curtis). **2Stage** resemblance values in Table 2.2

Table 2.2 2Stage comparison values of higher and lower-level assemblage resemblance matrices from the PCO plots (Figure 2.6), showing relatedness using Spearman (-1 to 1) correlation for the one percent cover and three scraping community measures. The higher the correlation the more similar the resemblances are. nMDS plot Figure 2.7.

	Resem scrapings Family	**Resem algae mL lowest**	**Resem algae mL phyla**	**Resem colonial Family**	**Resem colonial Lowest**	**Resem percent cover Family**	**Resem percent cover Lowest**
Resem scrapings Family							
Resem algae mL lowest	0.14						
Resem algae mL Phyla	0.11	0.91					
Resem colonial Family	0.16	0.26	0.23				
Resem colonial Lowest	0.17	0.27	0.23	0.91			
Resem percent cover Family	0.17	0.19	0.19	0.16	0.15		
Resem percent cover Lowest	0.10	0.13	0.14	0.08	0.07	0.73	
Resem scrapings Lowest	0.81	0.19	0.16	0.20	0.21	0.11	0.05

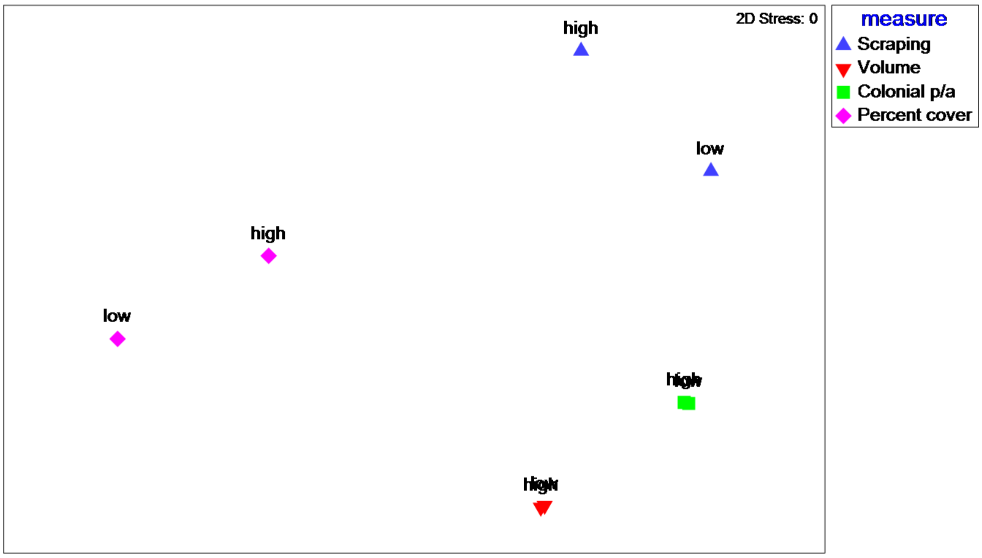


Figure 2.7 nMDS plot based on 2Stage rank correlations of resemblances (Table 2.2) for assemblages from the different measures (scraping, volume, colonial p/a and percent cover), and using different taxonomic resolutions (high and low taxonomic level identifications).

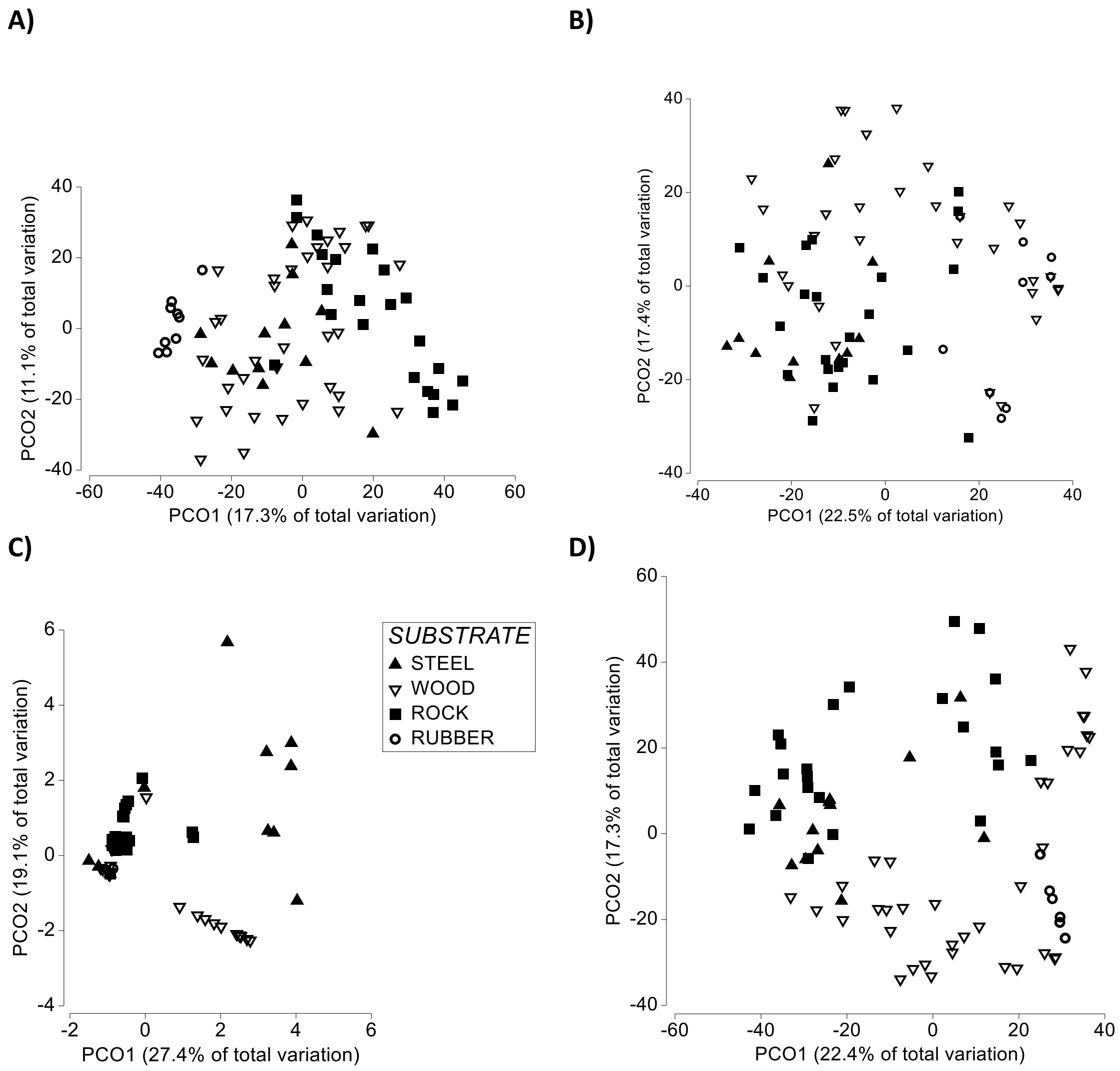


Figure 2.8. 2Stage comparison of the lowest taxa-level community resemblance matrices for the one percent cover and three scraping community measures: A; scrapings mobile invertebrates (Bray-Curtis), B; Colonial invertebrates P/A (Kulczynski), C; algal displacement volume mL (Bray-Curtis) and D; percentage cover (Bray-Curtis). 2Stage resemblance values in Table 2.2.

2.4. DISCUSSION

The two methods compared during this study yielded different insights into fouling communities, as each was better at sampling particular groups of organisms. Overall both methods showed that fouling communities on artificial substrates are distinct from those found on natural reefs. Distinctions between communities on different substrates have been extensively reported in the literature (Perkol-Finkel & Benayahu 2006, Glasby et al. 2007). This study was carried out at a single point of time, and did not aim to detect changes in fouling communities over time.

2.4.1 COMPARISONS OF THE TWO SAMPLING METHODS

Significantly more taxa were detected in samples from scrapings than those in photo-analysis. The main discrepancy between the methods was because of the low representation of molluscs, crustaceans, echinoderms and annelids in the photo-analysis. These phyla accounted for most of the diversity in the scraping method but, with their smaller sizes and interstitial habitats, could not be detected in the photo-analysis. This is an acknowledged limitation of sampling with photo-quadrats and point count analysis (Bohnsack 1979, Maggorrian & Service 1998). Water visibility can also limit detection and may lead to the exclusion of photos from some sites and potentially unbalance sampling designs (Zintzen et al. 2006). Specimens from the scraping samples can be identified in the laboratory with a greater taxonomic certainty, as individuals are physically present rather than being part of an image on a screen. For an intertidal rocky habitat, where water visibility is not usually relevant, photo-quadrats and *in-situ* observations of specimens showed a 90% similarity of species list between the methods (Macedo et al. 2006). In the subtidal, time limitations for divers can limit the number of useable replicate images for suitable photo-analysis (Zintzen et al. 2008), but more replicate images usually can still be collected by photography within the same timeframes than by undertaking scraping of the substrate.

The taxa lists from each method were more strongly correlated at the Family level than at the finest taxa level, as expected. Both sampling methods showed higher richness on artificial reefs than on the natural reefs; however, the photo-analysis showed a greater species overlap between natural and artificial reefs. Similarities were noted between assemblages on the reefs sampled in Kangaroo Island based on photo-analysis but not when using the scraping method. Similarity patterns based on the photo-analysis could possibly be obscuring the contributions that artificial reefs make in maintaining regional fouling assemblage diversity, which was shown in the more species-rich scraping samples. Fouling assemblages on artificial reefs are usually very different to those on natural reefs, often caused by apparent substrate preferences of larvae (Andersson et al. 2009; Glasby et al. 2007) and structural differences between artificial and natural reefs (Perkol-Finkel et al. 2006). This comparison shows that method matters for depicting the magnitude of these differences.

The photo-analysis data missed many of the non-sessile and cryptic macroinvertebrates that, when collected in the scrapings, formed some of the most abundant taxa. An example of this was the burrowing bivalve *M. nanus,* only present in communities on artificial reefs in association with aggregations of the ascidian, *Pyura* sp. The tyre reef had the most abundant fouling community in the scraping samples, driven by large abundances of molluscs, arthropods and cnidarians. This contrasted sharply with the percentage cover abundances for the same site, where the most abundant category was ‘empty space’. Tyre reefs have the ability to flex during storms thus losing many hard encrusting species (Collins et al. 2002), and so, following storms, both methods would underestimate the habitat importance of tyre reefs, but photo-analyses are especially susceptible with a reliance on larger encrusting macrophytes and invertebrates.

Three non-indigenous species (NIS) were detected, two tunicates and one cryptic tanaid from the northern hemisphere. The tunicates, *Botrylloides leachii* and *Ciona* sp., are commonly found on coastal artificial reefs in the GSV region (pers. obs.). Only the conspicuous colonial *B. leachii* was detected with both methods, while the tanaid and *Ciona* sp. were only detected in the scraping samples. Overlooking the presence of NIS amongst other small cryptic species highlights serious concerns about the use of photo-analysis for detecting non-indigenous species especially in their early arrival stages, except large and conspicuous invaders such as the Pacific sea star *(Asterias amurensis)* (Hewitt 2002). As well as underestimating or not detecting groups of individuals, photo-analysis can also overestimate abundances of some species if random points happen to be tightly clustered on a particular colonial organism within the sample area (Bohnsack 1979; Macedo et al. 2006). This has the potential to depict greater similarity between sites than if other abundance measures such as individual counts are considered. The correct selection of the numbers of random points in the overlay can help to overcome some of these errors (Zintzen et al. 2006), although longer periods of time may be required to analyse images. This selection is best done using a pilot study, as CPCe (Kohler & Gill 2006) can allow up to 500 points and the suitable number of points will depend on patchiness within the community (per. obs.). A pilot study is always needed to collect voucher specimens to develop the species list (Walker et al. 2007). Using multiple people can also minimise errors by sorting and allocating categories on an image to cross check results.

The greater taxonomic resolution in the scraping method (even though both Family and finest taxa levels found substrate to be significant) gave different similarities between samples and substrates than the photo-analysis. Whilst family level showed larger-scale patterns in fouling communities, finest taxonomic levels are more useful in assessing and evaluating how similar all, or parts, of an artificial reef are to a natural benchmark reef. Aggregating macrofauna data into higher taxa categories will not always show the responses of a community to change (Somerfield & Clarke 1995). The two different species lists and different community similarities between substrates for each method, does show that comparing separate studies that have utilised different sampling methods may distort the similarities between fouling communities on artificial and natural reefs.

2.4.2 RECOMMENDATIONS ON SAMPLING METHODS

The two collection methods yielded different assessments of fouling communities and each is suitable for sampling different groups of taxa in fouling communities. The lack of disturbance to the fouling community is the most obvious benefit for using photo-analysis, as it does allow monitoring over time without disrupting space occupancy. A problem is the lower taxonomic resolution of the photo-analysis, which inhibits the detection of NIS and less-visible shifts within the interstitial macroinvertebrates that are the main contributors to diversity in these temperate reef samples. Finer taxon-level identifications are important for investigating natural history, biodiversity, indicator species and small-scale processes (Waite et al. 2004), but the reduction of taxonomic resolution can also lead to a reduction in the number of variables (taxa) and zeros in the sample data (Vanderklift et al. 1996).

Improvements in camera quality may overcome some of the shortcomings in the future. Stereo cameras and other forms of digital analysis have been able to measure larger species underwater (Harvey & Shortis 1995). Further developments in 3D camera technology may mean that future researchers will be able to better quantify the more cryptic species. The use of video cameras to zoom in on cryptic invertebrates in the quadrat could overcome limitations of photo-analysis with random point software (Leujak & Ormond 2007) as well as allowing macro-photographs of prominent taxa.

The importance of fine-scale taxonomic resolution to a study needs to be carefully considered but will also depend on the available resources and especially the study aims and objectives (Waite et al. 2004, Ellingsen et al. 2005). Hence, photo-analysis alone may currently be unsuitable for detailed diversity assessments of temperate reefs as it can over-exaggerate community similarities between natural, artificial and other artificial reefs in regions. In conjunction with other methods such as environmental DNA (eDNA, Bohnman et al. 2014), photo-analysis can help to estimate the cover of fouling communities on artificial substrates (Zintzen et al. 2006), as well as detecting macro-scale shifts in community over time on monitored patches, thus making it an important monitoring tool. Visual estimation by a specialist observer is more accurate (Canning-Clode & Sudgen 2014), however advances in image recognition software may improve accuracy coupled with digital photogrammetry. This will remove the need for a specialist to view every picture and only view anomalies, allowing for increased sample sizes and better assessments. Increased use of digital images to collect data will be a common method used in future reef monitoring programs, but current technology is not yet suitable for detecting changes in community structure except in highly visible species. For this study, scrapings were used to look at interstitial fauna and photographs with percent cover to look at epifauna, to minimise destruction and obtain a comprehensive assessment of fouling assemblages.

Plate 1. Images of macroinvertebrates from fouling assemblages in the Gulf of St. Vincent.

CHAPTER 3. A COMPARISON OF FOULING ASSEMBLAGES ON NATURAL AND ARTIFICIAL REEFS IN THE GULF OF ST VINCENT.

3.1 INTRODUCTION

Submerged artificial substrates are found along urbanised coastlines around the world (Andersson et al. 2009, Firth et al. 2016A, Strain et al. 2018), constructed from materials that are different to the natural hard substrates (Glasby & Connell 1999). The definition of artificial reef in this study encompasses any anthropogenic structure that is susceptible to fouling (Svane & Petersen 2001), which recognises overlooked and relatively common features that might not be considered as reefs. Artificial reefs are submerged for a variety of reasons including; habitat restoration (Pickering et al. 1999), fisheries enhancement (Bohnsack 1989, Jensen 2002), resource extraction (Forteath et al. 1982), harbour infrastructure (Butler & Connolly 1996, Johnson et al. 2011) or sunken vessels (Perkol-Finkel et al. 2006).

Previous ideas around habitat limitation and the production of new fish populations (Bohnsack 1989), have led to the creation of artificial reefs constructed from many different materials of opportunity. Specialist fisheries enhancement reefs can have similar fish assemblages to natural reefs (Ambrose & Swarbrick 1989), but many commercially targeted fish species do not benefit from artificial reefs as was previously thought (Bohnsack 1989). Artificial reefs can support abundant invertebrate and fish assemblages, but when compared to natural reefs can differ structurally and functionally (Burt et al. 2009). The assemblages are usually very different to those found on natural reefs, when two reefs are compared (Aseltine-Nielson 1999, Perkol-Finkel et al. 2009, Hiscock et al. 2010). The assemblages of macroalgae and invertebrates on the reef can remain distinct from natural hard substrate, despite decades of colonisation and succession, because of the differences in structure and materials (Perkol-Finkel et al. 2006).

Artificial reefs usually have large areas of vertical surfaces that provide a range of conditions within a small spatial scale that are not found on natural reefs (Perkol-Finkel et al. 2006). The structural and physical properties of artificial substrates can lead to them becoming important ‘beachheads’ for non-indigenous species (Glasby et al. 2007), particularly floating pontoons (Dafforn et al. 2009) in enclosed environments such as harbours (Floerl & Inglis 2005). The continued establishment of certain kinds of artificial reefs could be detrimental for marine biodiversity, by hosting different assemblages of macroalgae and invertebrates (Glasby et al. 2007, Firth et al. 2016B). In some marine regions, artificial reefs are the only subtidal substrate available for settlement of sessile organisms, making them important ‘hard substrata islands’ in predominately soft bottom regions (Zintzen et al. 2006, Heery et al. 2017). Two shipwrecks in the North Sea were estimated to support fouling communities of 150-280 species, as the hard substrate provided attachment sites and habitats for a range of species not typically found in the surrounding soft sediments (Zintzen et al. 2006). New hard substrate reefs can also negatively affect the surrounding soft sediment communities, either through increased foraging from the reef species (fish and invertebrates) or changes in current regimes and sedimentary environment (Davis et al. 1982, Barros et al. 2001, Langlois et al. 2005, Heery et al. 2017).

The benefits of artificial reefs for the invertebrate and macroalgae assemblages are little known, because of a lack of comparisons and a focus on fish communities (Svane & Petersen 2001). A comparative approach to fouling assemblages is necessary, to determine the level of similarity between artificial and natural reefs. Comparing a single artificial reef type to a natural benchmark site, does not take into account the variety of hard substrates found in coastal areas, when adjacent reefs can be constructed from different artificial materials (Firth et al. 2016B) and can have dissimilar fouling assemblages of flora and fauna. There are few comparative studies on the assemblages of multiple artificial and natural reefs (Carr & Hixon 1997), and many only evaluated early recruitment to small patches of substrate (Hiscock et al. 2010). The assessment of artificial reefs will usually depend on the objectives of the reef, e.g. fisheries enhancement (Carr & Hixon 1997). The variety of artificial reefs that have been submerged for reasons other than habitat restoration (Firth et al. 2016A, 2016B Bishop et al. 2017) and fisheries enhancement will often be overlooked, the need for comparative studies is only just emerging.

Despite a long history of artificial reef construction in Australia (Branden et al. 1994), only five studies on artificial reefs, compared to North America (94) and Europe (72), were included in the review on artificial reefs by Baine (2001). There is a large number of old existing reefs created during government sponsored reef building programs from the 1960s until the mid-1980s all around Australia, listed comprehensively by Branden et al. (1994). The South Australian State Government first deployed purpose built artificial reefs constructed from tyres in the 1960s, after a visiting academic recommended artificial reefs as a way to increase fish production as there was a lack of hard substrates (Branden et al. 1994). The Gulf of St Vincent (GSV) is predominately soft bottom with seagrass meadows with some hard substrate provided by a large bivalve mollusc (*Pinna bicolor*), coastal granite boulder reefs (Bryars & Thiele 2009) as well as some limestone shelf reefs. The region has also had a long maritime history that provided numerous other non-purpose built artificial reefs (shipwrecks, piers and refuse), as well as the dedicated reefs for fisheries enhancement. There is also an anecdotally large and unquantified illicit recreational fishing reef program involving small scale 'personal' reef construction by fishers, often occurring over generations using surplus vessels and even large farm machinery scuttled at night. The fouling assemblages on GSV artificial reefs were characterised by having decade long succession (Butler & Connolly 1996) and variable seasonal patterns of recruitment onto new or disturbed substrate patches (Reimers & Branden 1994). This study aimed to describe and compare flora and fauna in fouling assemblages on three different established (10+ years) artificial substrates (wood, rubber and steel), to those found on natural rocky reefs in the Gulf of St Vincent. The main hypotheses were: (i) fouling assemblages on all artificial substrates will be distinct from those on natural reefs; (ii) fouling assemblages on different artificial substrate types will be dissimilar.

3.2 MATERIALS AND METHODS

3.2.1 STUDY SITES

The fouling communities at seven reef sites were sampled from June to early September 2011. The sites were located in the lower part of the Gulf St. Vincent, Investigator Strait and on the North Coast of Kangaroo Island, South Australia (Figure 3.1). There are strong currents in the Investigator Strait and the general movement of water across the Gulf is in an eastward direction (Bye 1976). The reefs were selected to cover a range of natural and artificial substrates as well as for logistics and access. They were all located in the Gulf St. Vincent bioregion and corresponded with bio-units based on seascape and ecological similarities for marine planning (Day et al. 2008). Where possible, subject to artificial reef availability, comparisons were made across the bio-units by treating them as a factor 'region' in the design. The sites Second Valley Reef (natural) and Rapid Bay Jetty off the Fleurieu Peninsula were located in the Yankalilla Bay and Light River bio-unit. The *SS Hougomont* and Edithburgh Jetty off the Yorke Peninsula lay within the Orontes bio-unit and on the northern coast of Kangaroo Island, Frenchmans Cap Reef (natural), Kingscote Tyre Reef and Jetty were part of the Nepean bio-unit.

The Second Valley reef has previously been surveyed in 2010 and was classed as healthy (Westphalen 2011). The fouling communities on the jetties at Edithburgh and Rapid Bay have been studied previously by Butler (1986), whereas the other sites had not been surveyed before. Four of the reefs (*SS Hougomont*, Kingscote Jetty, Frenchmans Cap and Kingscote Tyre Reef) were sampled on a joint expedition with the Department of Environment and Natural Resources, Maritime Heritage Branch and the Kangaroo Island Natural Resources Management Board (KINRMB). Sampling of the protected shipwreck was undertaken using permits granted by the Commonwealth Government and the State Government under supervision from a Maritime Archaeologist.

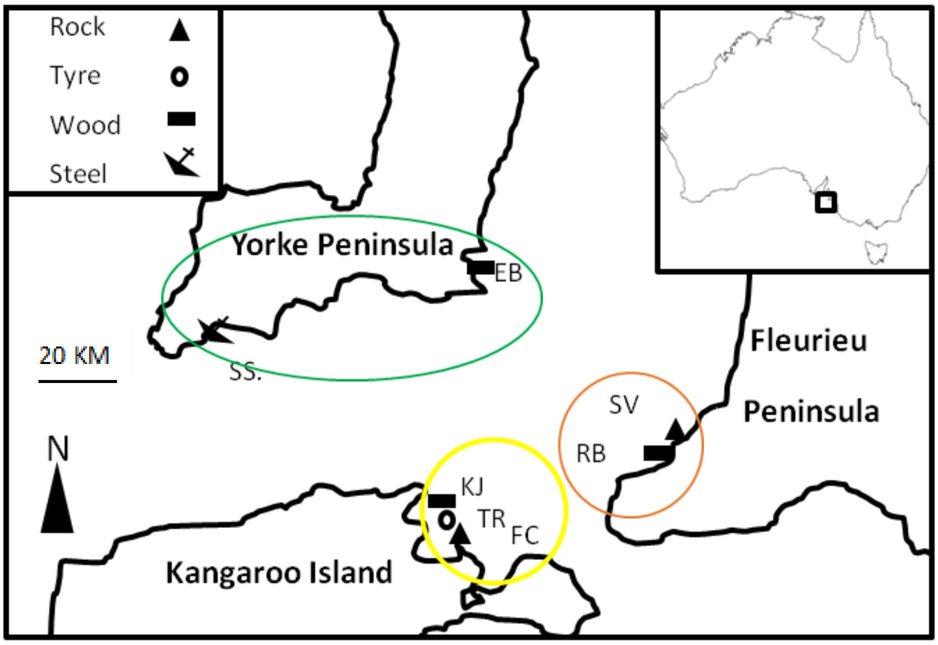


Figure 3.1. Map of the sampling sites in the Gulf of St. Vincent, Investigator Strait and north coast of Kangaroo Island. Site codes; SV (Rock): Second Valley, FC (Rock): Frenchmans Cap, RB (Wood): Rapid Bay, KJ (Wood): Kingscote Jetty, EB (Wood): Edithburgh Jetty, SS (Steel): *SS. Hougomont*, TR (Rubber): Kingscote Tyre Reef). Bio-unit site groupings are depicted red (Yankalilla Bay and Light River), yellow (Nepean) and green (Orontes).

3.2.2 SAMPLING METHODS AND ANALYSIS

The vertical surfaces at each site were sampled using 5 m transects and quadrats, with the contents being photographed, then removed for later identification and counts in the laboratory. The aspect was recorded for each sample (North, South, East, West), to test for light availability or shading. The prevailing lit side of the vertical surfaces was north. This was tested and found to be non-significant for abundances of macroinvertebrate, (permANOVA) $p > 0.05$) but further analysed for percent cover data of three wooden jetty sites. At each site 12 sample quadrats were taken from the surfaces of the reefs; the tyre reef was only 9 replicates because of the site and logistics. The *SS Hougomont* was sampled with 0.5 m x 0.5 m quadrats similar to Dobretsov et al. (2014), however all subsequent sites were sampled with 0.25 m x 0.25 m quadrats because of the size of the pylons at the other jetty sites (less than 0.3 m across). The abundances of the non-colonial invertebrates from the wreck were divided by four to standardise to the same area as the smaller quadrat (0.0625 m^2) and not extrapolated to individuals per m^2.

The non-colonial and mobile invertebrates (hereafter called non-colonial invertebrates) were identified and counted from the individuals in the physical samples of the quadrats. The use of two methods to sample different parts of the community was necessary, as each one cannot sample all of the community accurately. The species list was determined from the preserved contents of the quadrats (Walker et al. 2007). The abundances of large encrusting invertebrates, hereafter called colonial invertebrates and macroalgae were determined from the images taken *in-situ*, using random point percent cover estimations using a software package called Coral Point Count with Excel extensions (CPCe, Kohler

& Gill 2006). Categories were assigned to the 100 random points overlayed onto the image, using a species list developed in CPCe using the data obtained from the physical sampling of the quadrats.

3.2.3 DATA ANALYSIS

The data analysis was conducted using Primer/PERMANOVA+ Version 7. A univariate permANOVA was carried out on the Euclidean resemblance matrix for the total numbers of species across the factor ‘substrate’, followed up by pairwise testing. Similarly, the total abundances of non-colonial invertebrates (mobile, interstitial and attached individuals) were tested for substrate differences following 4th root transformation using single factor permANOVAs. The abundances of macroalgae and colonial invertebrates were respectively tested using separate univariate permANOVA analyses.

Regional comparisons could only be made for the three wooden jetties between the three regions (Nepean, Orontes and Yankallila & Light Bay), whereas no further artificial reef sites were logistically available across regions for other substrates. The effects of shading on the percentage cover of macroalgae and colonial encrusting invertebrates was examined at the three jetty sites (Edithburgh, Kingscote and Rapid Bay) (aspect factor levels; North, East, West, South). A two factor (Region and Aspect) permANOVA test was used to test for significant differences in the percentage cover assemblages of macroalgae and macroinvertebrates. A SIMPER analysis was performed to determine within group similarities (North, East, South, West).

A Bray-Curtis resemblance matrix for each component (abundances and percent cover data sets) was represented in a non-parametric MDS plot for both assemblages using bootstrapping, whereby 95% bootstrap regions are fitted around bootstrap averages, giving an indication of confidence intervals (Clarke et al. 2014). Variability was further assessed with tests of homogeneity of dispersion (PERMDISP) and test results included in the tables. Two multivariate single factor permANOVAs were used to determine if sampled assemblages could be best discriminated by substrate (4: steel, rock, rubber, wood). Community patterns were illustrated using shade plots combining a cluster analysis on species or functional groups using Whittaker's Index of Association, and constrained by a cluster analysis with Type 1 SIMPROF tests (Somerfield & Clarke 2013, Clarke et al. 2014).

The relationship between percentage cover and the non-colonial invertebrates (individual counts from scrapings) were explored using functional grouping of epibiota as predictor variables in a Distance Based Linear Model (DistLM). These functional groups were representative of the structural characteristics of the macroalgae and colonial macroinvertebrates found in the percentage cover analyses. The relationships between the structural functional groups and the non-colonial macroinvertebrate assemblages from DISTLM were graphed with distance-based redundancy analysis (dbRDA) plots, with significance tested using marginal tests.

3.3 RESULTS

3.3.1 SAMPLED FLORA AND FAUNA

A diverse range of invertebrates and macroalgae colonised the sampled reefs, 168 different invertebrate and 29 different macroalgal taxa were recorded in the quadrats (Table 3.1, Appendix 1 Table 2.2). Arthropods were the largest group of taxa found, followed by molluscs (Table 3.1). Rhodophyta accounted for most of the algae (Table 1), as there was the large number of red algae taxa recorded on the *SS. Hougomont*. Three introduced species were found; the tanaid *Tanais dulongii,* the tunicates *Botrylloides leachi* and *Ciona intestinalis. Tanais dulongii* were found only on the steel samples (1 site), *Botrylloides* sp. were found on both tyres and wood (4 sites overall) and *Ciona* were found on the rock, wood and steel (6 sites overall).

The artificial and natural reefs shared only 35 % of the macroalgae and 29 % of the invertebrate taxa (Figure 2), with the majority of taxa (62 % of macroalgae and 64 % of invertebrates) found only on artificial substrates. Only one macroalgae (*Sonderpelta* sp.) and 11 macro-invertebrates were unique to the natural reefs. Twelve taxa were unique to the tyre reef, including several species of scavenging gastropods and the sponge-dwelling mytilid *Trichomya hirsuta*. The fouling assemblage on the steel wreck had 63 unique taxa, including seven species of feather stars (Comasteridae) and brittle stars (Ophiuroidea). Some taxa were shared with the natural reef sites: for example, a pycnogonid that preys on bryozoans, a large encrusting bryozoan *Cheilostomata* sp. and a large sponge, *Darwinella* sp. that also occurs on wooden jetty pylons at other sites around the Gulf of St Vincent (pers. obs.). The epifaunal sponge *Leucosolenia* sp., commonly found attached to large macroalgae, was recorded on the wooden pylons and the natural rocky reefs.

Mobile taxa made up the majority of taxa found in the preserved quadrat samples, there were 53 arthropod taxa but only 4 were sessile (Cirripedia). The largest group of sessile fauna were bryozoans, because of the numbers to taxa from this phylum found on the *SS. Hougomont.* The natural reefs did not have the highest diversity in terms of actual species numbers (Table 3.1). The site with the highest number of taxa was the steel shipwreck (*SS Hougomont*), whilst the site with the least number of taxa was the natural rocky reef at Kangaroo Island, Frenchmans Cap, with only 29 taxa. The other rocky reef site at Second Valley had twice as many taxa present as Frenchmans Cap. Arthropoda was the largest representative phylum on the wreck of the *SS Hougomont*, with 37 taxa found, this was also the largest phylum at all other sites with the exception of the Kingscote tyre reef (Table 3.1), where Mollusca contributed most of the taxa.

Differences in total taxa numbers were found between substrates (species number of steel > wood > rock > tyres) and also within same substrate groups (rock: Second Valley > Frenchmans Cap; wood: Edithburgh > Rapid Bay > Kingscote Jetty) (Table 3.1). The number of taxa on the reefs was significantly different between substrates (Table 3.2). Pairwise comparisons revealed that the diversity (number of taxa) on all of the artificial substrates was significantly different ($P<0.01$) to the natural rocky reefs (Table 3.2). However, the diversity on the tyre reef was not significantly different to the wooden and steel artificial reef sites ($P>0.01$, Table 3.2).

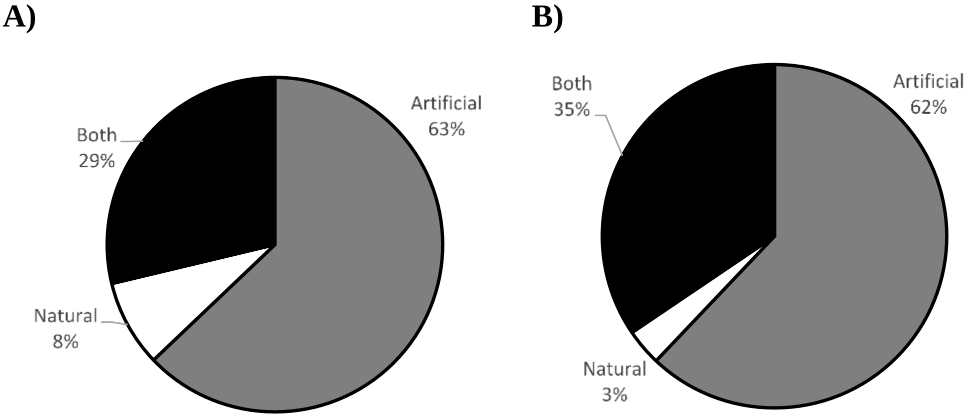


Figure 3.2 Percentage of the total number of taxa **A.** for macroinvertebrates found only on artificial reefs, natural reefs or on both types of substrates and **B**. for macroalgae found on only of artificial reefs, natural reefs or on both types of substrates.

Table 3.1. Total number of taxa (richness) for invertebrates and macroalgae found in the preserved scrapings at each for the 7 sites and their substrate materials, over all sampled quadrats (*SS. Hougomont* n=12, Kingscote Tyre reef n=9, Rapid Bay, Edithburgh and Kingscote Jetties n=12, Second Valley and Frenchmans Cap n=12). Total number of taxa per phyla is also depicted.

	TOTAL TAXA	***SS. HOUGOMONT***	**KINGSCOTE TYRE REEF**	**RAPID BAY JETTY**	**EDITHBURGH JETTY**	**KINGSCOTE JETTY**	**SECOND VALLEY**	**FRENCHMANS CAP**
Material	ALL	Steel	Tyres	Wood	Wood	Wood	Rock	Rock
PORIFERA	**18**	10	3	7	3	2	9	5
CNIDARIA	**6**	5	1	3	0	1	1	2
PLATYHELMINTHES	**2**	2	0	0	0	0	0	0
ANNELIDA	**20**	13	4	7	11	12	9	1
MOLLUSCA	**31**	17	12	6	6	2	7	0
ARTHROPODA	**53**	37	8	19	20	17	17	11
ECTOPROCTA	**19**	16	2	2	4	1	3	3
ECHINODERMATA	**11**	8	2	2	2	0	1	0
TUNICATA	**8**	4	4	3	5	5	5	3
CHLOROPHYTA	**5**	5	0	0	0	1	1	1
RHODOPHYTA	**15**	12	3	3	1	0	3	3
HETEROKONTOPHYTA	**9**	7	0	3	4	0	5	0
Species Richness	**197**	136	39	55	56	41	61	29

Table 3.2. Table of results for multivariate permANOVA analyses of total number of taxa, pairwise comparisons of the total number of taxa, total abundances of non-colonial invertebrate individuals, and univariate permANOVAs of the colonial and macroalgal assemblages and the non-colonial invertebrate assemblages. The residual was 74 for all non-pairwise tests. Bold indicates that permDISP tests for the factor were also significantly different, indicating variability among levels within the factor.

	df	**Pseudo-*F***	**t**	**P**$_{(perm)}$ **or P**
Total number of taxa				
Substrate	3	10.87		**0.0001**
Pairwise (substrate)				
Steel, Wood	46		5.111	<**0.001**
Steel, Rock	34		5.524	<0.001
Steel, Tyres	19		2.145	**n.s**.
Wood, Rock	58		3.110	**<0.01**
Wood, Tyres	43		2.034	n.s.
Rock, Tyres	31		3.415	<**0.01**
Total abundances of macroalgae				
Substrate	3	71.74		**<0.001**
Total abundances of colonial invertebrates				
Substrate	3	15.098		**<0.001**
Total abundances of non-colonial invertebrates				
Substrate	3	6.211		<0.001
Colonial invertebrates and macroalgae assemblages				
Substrate	3	4.710		**<0.001**
Non colonial invertebrates assemblages				
Substrate	3	7.846		<**0.0001**

3.3.2 ABUNDANCES OF COLONIAL INVERTEBRATES AND MACROALGAE

The percentage cover values for the higher taxa groupings varied across the four reef substrate types. The single steel wreck and the two rocky reefs had a high percentage cover of brown algae (Figure 3.3), compared to the wood and tyre reefs. At Second Valley, brown algae covered two thirds of the average quadrat. Frenchman's Cap had less brown algae coverage with 34.5%, but had an average foliose red algae coverage of 31.5%. The abundances of colonial invertebrates and macroalgae were very different on the three wooden jetties, both within the group and compared to the other three reef substrates. The green algae, *Caulerpa longifolia* covered many of the pylons at the Kingscote Jetty (Figure 3.3) and some quadrats had 100% coverage. The other two jetties did not have any green algae present and only a few large brown and red species were recorded (see Appendix 1). The only other reef substrate where green algae appeared was on the rocky reefs, but with low percentage cover values and *C. longifolia* was not present.

Edithburgh, another of the wooden sites had a large percentage cover of the hard encrusting coral *Culicia* sp., followed by large encrusting tunicates and porifera (Figure 3). On the pylons at Rapid Bay, tunicates had the greatest surface coverage (Figure 3), especially the encrusting colonial tunicates (Didemnidae and *Botrylloides* sp.). Low flat encrusting sponges (Porifera) such as *Dendrilla* sp. and *Euryspongia* sp. formed the next largest groups at this site, similar to Edithburgh. The tyre reef had a high percentage of 'empty' (category unassigned) space in the quadrats. This is most probably covered in biofilms but lacked identifiable macroalgae or macroinvertebrates that could be determined from the photos. The only other site with an occurrence of ‘empty’ space, was the rocky reef at Frenchmans Cap (Figure 3.3). The next highest coverage at the tyre reef were sponges (average 29.3%) and tunicates (average 16%), comparable to the jetty sites. The total abundances of macroalgae ($P<0.001$) and colonial invertebrates ($P<0.001$) varied significantly with the substrate of the reef (Table 3.2).

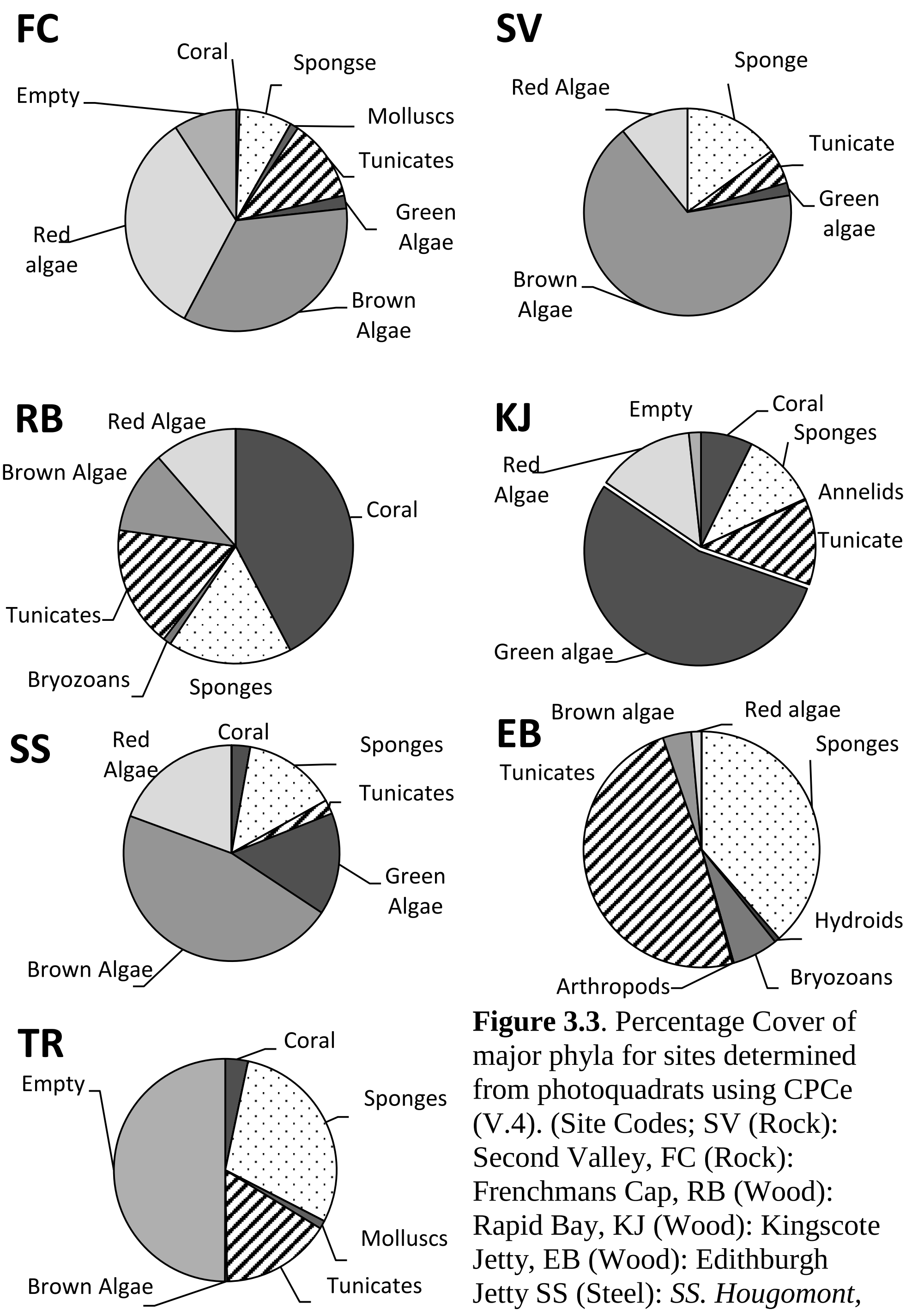


Figure 3.3. Percentage Cover of major phyla for sites determined from photoquadrats using CPCe (V.4). (Site Codes; SV (Rock): Second Valley, FC (Rock): Frenchmans Cap, RB (Wood): Rapid Bay, KJ (Wood): Kingscote Jetty, EB (Wood): Edithburgh Jetty SS (Steel): *SS. Hougomont*, TR (Rubber): Kingscote Tyre Reef).

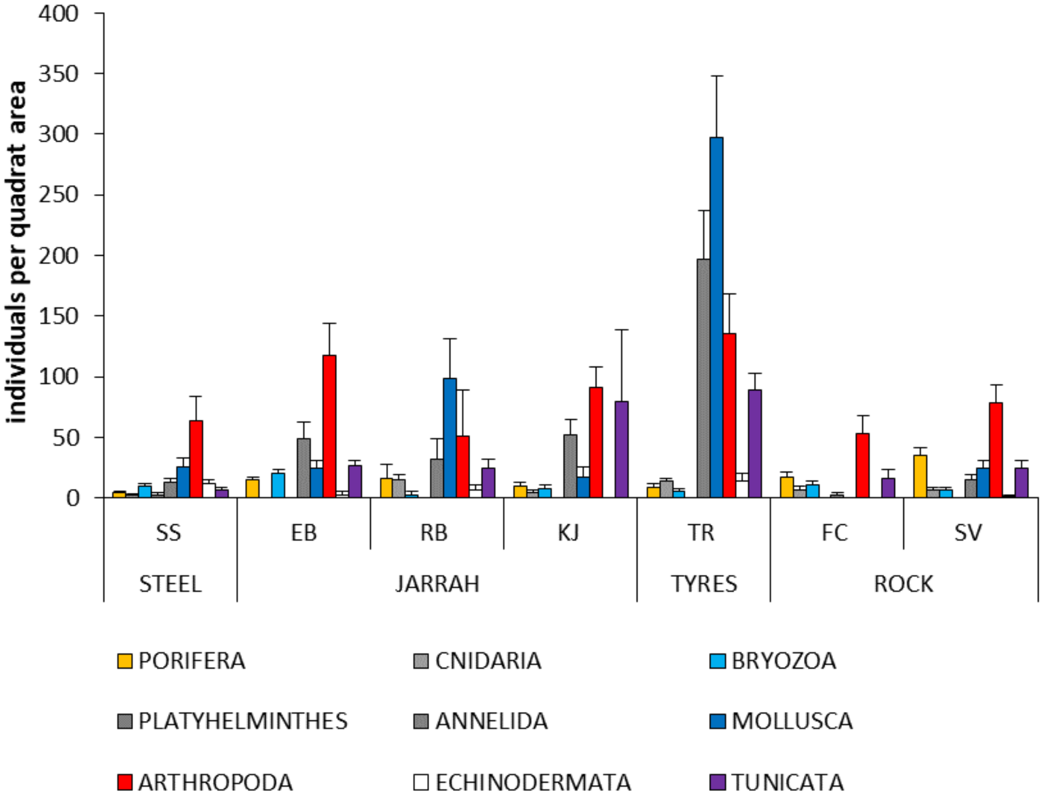


Figure 3.4. Abundances (average number of individuals per 0.0625 m^2 with SE) of non-colonial macroinvertebrates at each site collected from sampled quadrats. Site Codes; SS: *SS. Hougomont*, (Steel), EB: Edithburgh Jetty, RB: Rapid Bay, KJ: Kingscote Jetty (Jarrah), TR: Kingscote Tyre Reef (Rubber), FC: Frenchmans Cap (Rock), SV: Second Valley (Rock).

3.3.3 ABUNDANCES OF NON-COLONIAL INVERTEBRATES

The most abundant non-colonial invertebrates, were the bivalve *Musculus nanus* (142 ind. per sample), the ascidian *Pyura* sp. (131 ind.) and a tanaid (115 ind.). The tyre reef had extremely high densities of molluscs with 300 individuals per quadrat on average (Figure 3.4). This was caused by the previously mentioned high numbers of the small (<10 mm) bivalve *Musculus nanus*, which occurred amongst aggregations of the abundant tunicate *Pyura* sp., *M. nanus* was also on the other artificial reefs in smaller numbers, but no individuals were recorded from the rocky reefs. A sipunculan worm *Phascolosoma* sp., was also recorded in large numbers in the tyre reef samples (137 ind. per quadrat) whilst it occurred in lower numbers (<15 ind. per quadrat) on the other reefs. *Phascolosoma* sp. was also found amongst aggregations of *Pyura* sp. The most abundant malacostracan was a small crab, *Halicarcinus ovatus* (Hymenosomatidae) that was recorded at all sites, except the Edithburgh Jetty. It was the most abundant macroinvertebrate on the pylons at the Kingscote Jetty.

3.3.4 REGIONAL AND SMALL SCALE DIFFERENCES IN FOULING ASSEMBLAGES ON WOODEN JETTIES

Regional differences in fouling assemblages were detected for the three jetties which had different abundances of macroinvertebrate and algal cover from each other (Figure 3.3, Table 3.3). The assemblage from the jetty at Kingscote was distinct from those at Edithburgh and Rapid Bay (Figure 3.5). This is primarily because of the high percentage cover of the green alga, *Caulerpa sp.* as mentioned above. This species contributed to 83.70% (SIMPER) of the within sample similarity at this site. The other sites had great abundances of macroinvertebrates (sponges and stony corals) on the sampled pylons than algae. The only other major contributor to within group similarity was a brown algae at Edithburgh (SIMPER): 12.42% of the within group similarity). There was no significant difference in the macroalgae or macroinvertebrate assemblages by aspects (North, South, East and West) (Figure 3.5, Table 3.3). Within group similarity was <20% (SIMPER) for all four directions (North, East, South and West). On the north facing side of the jetties, where light is most prevalent on exposed surfaces in the southern hemisphere, the biggest contributors to within group similarity were two sponges (*Euryspongia* spp. 16.79% and *Callyspongia* spp. 16.44%).

Table 3.3. Two factor multivariate permANOVA analysis of the Colonial macro-invertebrate and macroalgae assemblages for the factors Region and Aspect on the wooden jetty pylons at Edithburgh, Rapid Bay and Kingscote.

Source	**Df**	**Pseudo-F**	**P(perm)**
Region	2	12.397	**0.0001**
Aspect	3	1.1452	n.s.
Region x Aspect	6	1.1199	n.s.
Res	24		

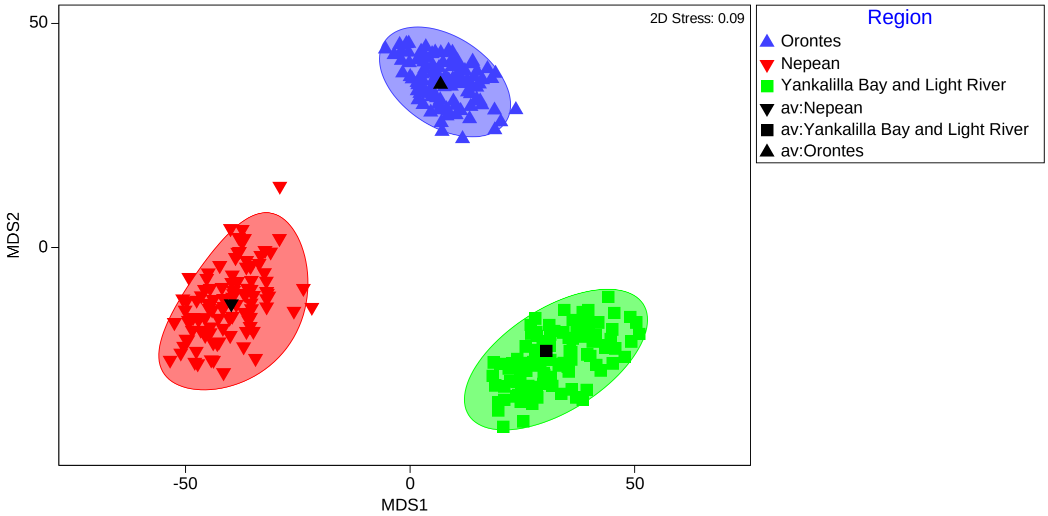


Figure 3.5. nMDS bootstrap plot of fouling assemblages (measured by percent cover) as well as the group averages on the three jetty sites differentiated by the factor Region (Orontes: Edithburgh, Nepean: Kingscote, Yankallila Bay: Rapid Bay).

3.3.5 SIMILARITY OF ASSEMBLAGES BETWEEN SUBSTRATES

The fouling assemblages were analysed in two parts reflecting the different measures of abundances, the non-colonial invertebrates derived from individual abundances and the colonial invertebrates and macroalgae from the photo-quadrats. For both measures, fouling assemblages differed significantly between substrates, and also had significant dispersion (Table 3.2), with a higher variability for non-colonial than colonial invertebrates and macroalgae (Figure 3.6). The non-colonial invertebrate assemblages on the two natural reefs were similar to each other based on both measures (Figure 3.6). Colonial invertebrate and macroalgal assemblages on the *SS Hougomont*, were more similar to those found on the natural reefs (Figure 3.6B). This was driven by comparable macroalgae assemblages at each site (Figure 3.7B). The colonial and macroalgal assemblages on the three wooden jetties were not as similar (Figure 3.6B) as the assemblages of non-colonial invertebrates at the same sites (Figure 3.6A). The colonial invertebrate and macroalgal assemblage and the non-colonial invertebrate assemblage on the tyre reef were very dissimilar to all the other substrates.

Shadeplots illustrate how the species composition differs between the artificial and natural reefs (Figure 3.7). All of the most common taxa of non-colonial macroinvertebrates were found in abundance on the steel ship wreck, and subsets of these taxa were found on the wooden jetties, making these two types of artificial reef more similar to each other (Figure 3.7A). The tyre reef had only high abundances of several non-colonial macroinvertebrates , (e.g. *Phascolosoma* indet., *Pyura sp.* and tanaids), however it had very high abundances of bivalves (*Musculus nanus, Hiatella australis,* and *Ostrea angasis*), which were also found on other artificial reefs, but not on natural reefs (Figure 3.7A). The tyre reef was also distinct based on photo-quadrats, as colonial macroinvertebrates and macroalgae were almost absent (Figure 3.7B). The cover of macroalgae and colonial macroinvertebrates was very different between the natural reefs and ship wreck and the wooden jetties (Figure 3.7B). The epibiota on the natural reefs and steel ship wreck were more similar, but each of the three wooden jetties had a more unique epibiotic flora and fauna, with *Caulerpa* sp. being abundant at Kingscote and the jewel coral *Culicia* sp. at Edithburgh (Figure 3.7B).

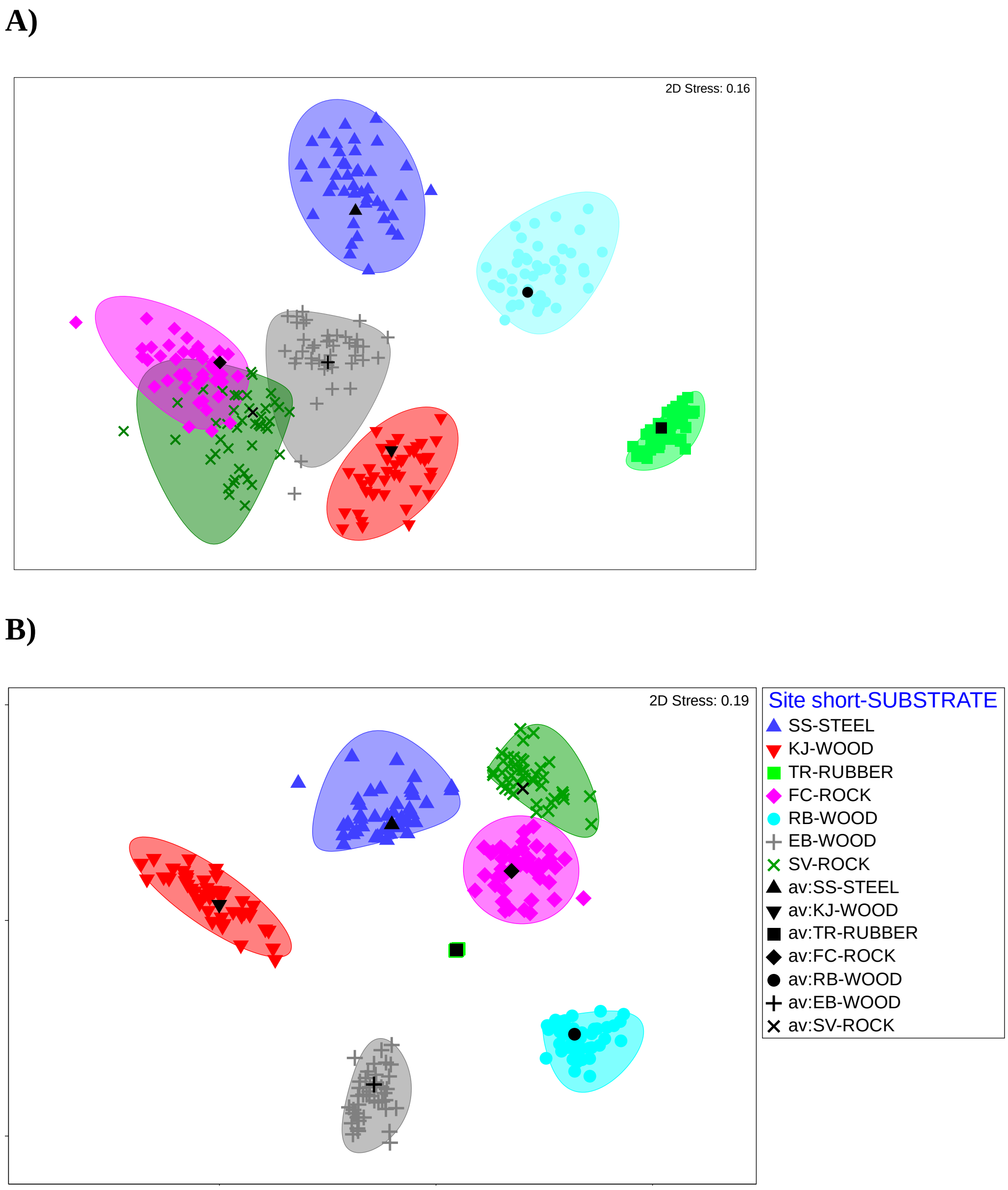


Figure 3.6. Non-parametric MDS plots for **A)** Non-colonial invertebrate assemblages (determined from the individual abundances), and **B)** Colonial invertebrate and macroalgal assemblages (determined from the percent cover) on the 7 sampled reefs. Within site variation is shown by the fitted 95% bootstrap envelopes around the bootstrap average for each site and substrate. Site codes, FC; Frenchmans Cap, SV; Second Valley, EB; Edithburgh Jetty, RB; Rapid Bay, KJ; Kingscote Jetty, SS; SS Hougomont, TR; Kingscote Tyre Reef.

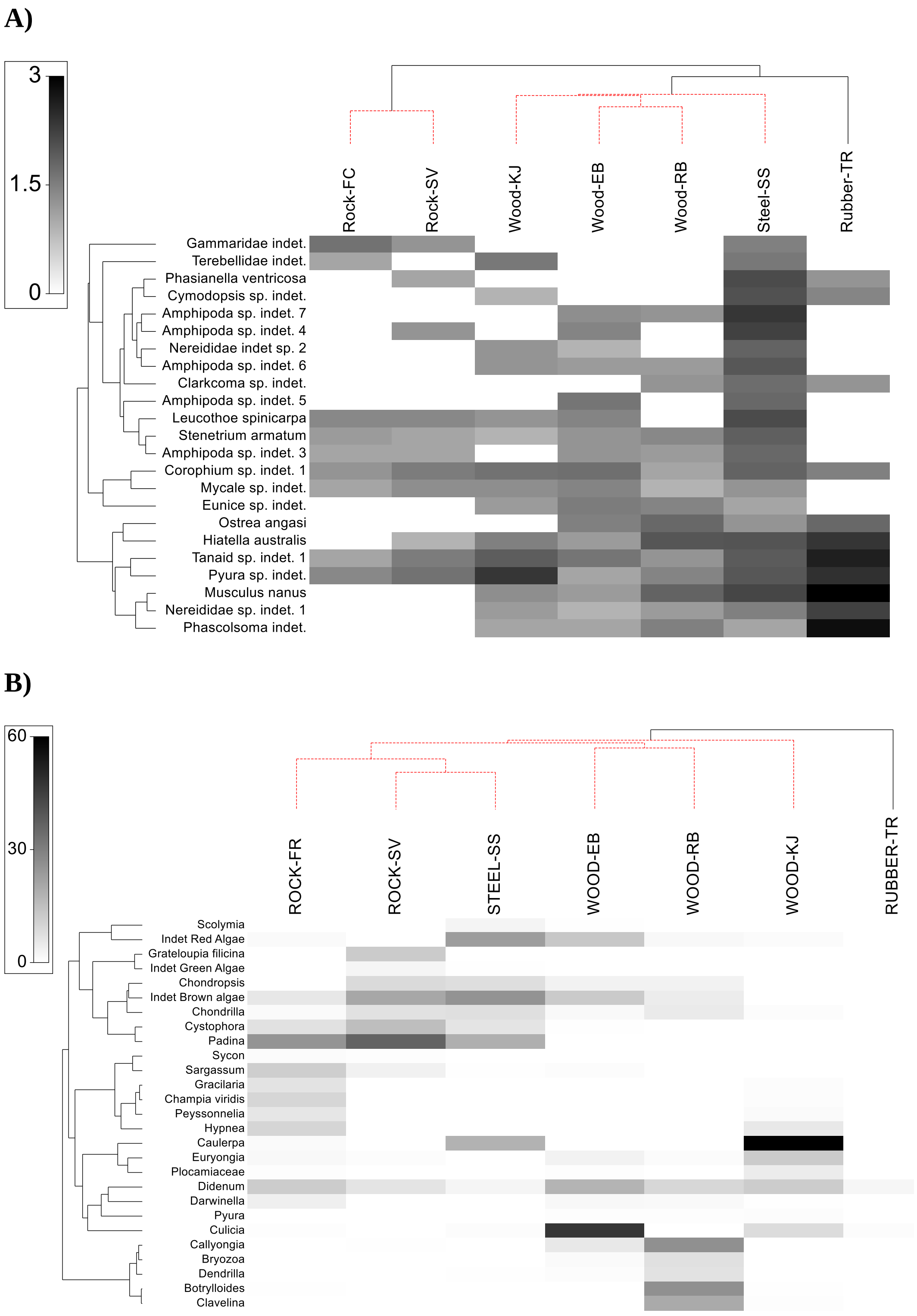


Figure 3.7. Shade plots of fouling assemblages across substrates and sites for **A)** non-colonial macroinvertebrates and **B)** colonial macroinvertebrate and macroalgae assemblages over all 7 sites. The depth of the shading corresponds with abundance. Rare species were excluded from this analysis. Species and sites are ordered by separate cluster analysis, and the site cluster is constraint by SIMPROF test outcome with black lines showing significantly different clusters, and red lines denote clusters that are not significantly different.

3.3.6 RELATIONSHIP BETWEEN EPIBIOTIC STRUCTURAL GROUPS AND NON COLONIAL MACRO-INVERTEBRATES.

To explore whether the assemblages of mobile and interstitial non-colonial invertebrates were affected by the epibiota, structural habitat differences provided by colonial invertebrates and macroalgae across substrates and sites were analysed in a shade plot (Figure 3.8) and further used as predictor variables in a DISTLM analysis (Fig. 3.9). To define habitat forming groups, eight categories were chosen to reflect functional groups that can provide common structure that may promote macroinvertebrate assemblages (amphipods, bivalves, annelids, echinoderms) that were sampled by scraping (Figure 3.8). The epibiota on the three artificial substrates and the natural reefs could be differentiated by the habitat provided (Fig. 3.8). The rocky reefs at Second Valley (SV) and Frenchmans Cap (FC) provided more heterogeneous habitat structure than the artificial sites. This appeared to be driven by the percent cover variations in brown algae, mainly Chaetophyta, filamentous red algae and *Malleus* sp. (Bivalvia) (Figure 3.7). The epibiotic structure of the artificial reefs was more determined by small jewel corals (Figure 3.6), such as *Culicia* sp (Cnidaria) as well as local variations in green algae, which was particularly prevalent on the Kingscote Jetty. With the exception of the tyre reef (almost no epibiotic structure present), the groups Medium Foliose (small brown and red algae) and Soft Encrusting (colonial ascidians e.g. didemnids) were found at all of the sites (Figure 3.8). The steel wreck had similar structural habitat composition as the natural reefs at FC and SV (Figure 8). The large foliose group (brown macroalgae) were missing from the three Jetties, but they did have more hard encrusting taxa such as corals and bryozoans (Figure 3.7B). The assemblage of non-colonial mobile invertebrate fauna of the natural and artificial reefs was determined by the habitat structure provided by the epibiota (Figure 3.9). Marginal testing showed that this group and soft encrusting (*Botrylloides* sp.) did significantly affect non-colonial and interstitial macroinvertebrate assemblages (Table 3.5).

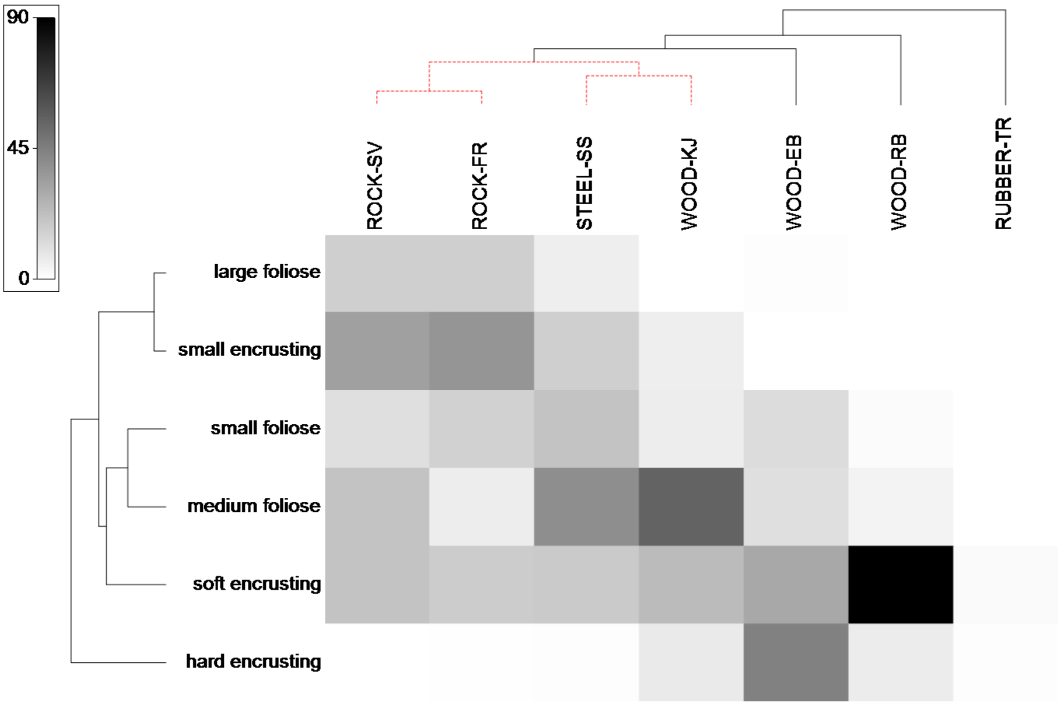


Figure 3.8. Shade plots of average percent cover assemblages of functional habitat forming groups of macroalgae and macroinvertebrate cover over all 7 sites. The depth of the shading corresponds with abundance. Rare species were excluded from this analysis. Species and sites are ordered by separate cluster analysis, and the site cluster is constrained by SIMPROF test outcome with black lines denoting significantly different clusters, and red lines cluster which are not significantly different.

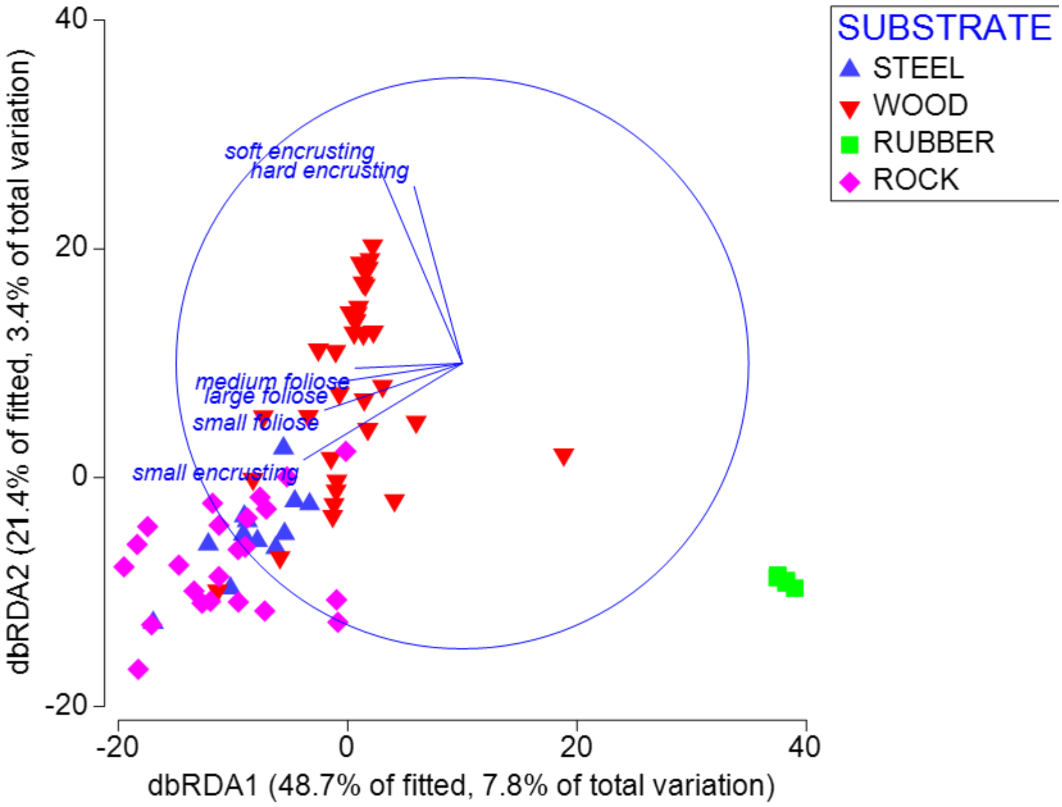


Figure 3.9 Distance based Linear Model Redundance Analysis (dbRDA) (DistLM) plots of non-colonial macroinvertebate fauna on artificial and natural reefs constrained by functional habitat forming groups of the epibiota as predictor variables, displayed in the vector overlay. Sites; Rock; Frenchmans Cap, Second Valley, Wood; Edithburgh Jetty, Rapid Bay, Kingscote Jetty, Steel; SS Hougomont, (Marginal test results are in **Table 3.5**).

Table 3.5 Marginal tests for the Distance based Linear Models (DistLM) (**Figure 3.9**) for functional groups of macroinvertebrates and algae from the percent cover data. Significant results in bold.

Group	**Pseudo-*F***	**P**
Hard Encrusting	2.2111	**0.0051**
Soft Encrusting	2.2477	**0.0037**
Soft Tubes	1.0268	n.s.
Rotund	1.3372	n.s
Medium Foliose	1.1052	n.s.
Large Foliose	1.1115	n.s.
Small Encrusting	1.4962	n.s.
Small Foliose	1.2903	n.s.

3.4 DISCUSSION

The hard substrates provided by the different artificial reefs were supporting more diverse assemblages than was what found on the natural reefs. Artificial reefs provide opportunities for colonisation by fouling species in environments that had a little or no natural hard substrate available such as the Gulf St Vincent. Many artificial reefs are typically deployed into areas of low productivity (for some reef/commercial fishes) as well as onto soft sediments (Svane & Petersen 2001, Heery et al. 2017). Most artificial reefs are usually not equivalent to natural hard substrates in materials and structure, resulting in assemblages that are different to the surrounding reefs (Perkol-Finkel & Benayahu 2004). While the deployment of large artificial reefs such as the tyre reef, *SS Hougomont* and jetties can be considered an anthropogenic disturbance to the soft sediment habitats they are deployed upon (Walker et al. 2007, Heery et al. 2017), this study has shown that they can increase biodiversity by providing habitat not otherwise present.

Artificial reefs can support diverse communities, but usually it is not analogous to what would be found on natural hard substrates (Connell & Glasby 1999, Burt et al. 2009). Some of the artificial reefs sampled in this study were more diverse than the two comparative natural reefs. Several non-indigenous species were detected in the sampling, but these showed no preference for any particular substrate, natural or artificial. Despite this, the majority of the species found on the artificial reefs were considered endemic (Edgar 2008). Even with the lack of large numbers of invasive species, the assemblages on artificial reefs were different to those on natural reefs. The assemblage on the wreck of the *SS Hougomont* was the most similar to those found on the two natural reefs, the

Kingscote tyre reef was the least similar and the jetties were somewhere in between the other two artificial reef types. The fouling assemblage on the steel wreck overlapped with those found on the natural reefs and the other artificial substrates, both taxonomically and when functional groups were assigned to macroalgae and colonial macro-invertebrate epibiota.

The position of the jetty pylon side to light (aspect) did not significantly affect the fouling assemblages, however, they were distinct by region. This finding contradicts previous findings where light availability has been found to structure reef assemblages for both macroalgae and invertebrates (Forteath et al. 1982, Firth et al. 2016B). The reasons for this might be in the relatively shallow waters (<15 m) where these reefs are found, light availability might not vary or the structure above not shade the pylon significantly.

The regional differences of fouling assemblages on the wooden jetties could be explained by proximity to other hard or soft substrates as source of larvae, competition, resources and predation pressures (Osman 2015, Ponti et al. 2015, Langlois et al. 2005, Barros et al. 2001), that caused differences within substrate groups or between them on small spatial scales (Osman 2015). Region-specific supply side ecological processes (Underwood & Fairweather 1989) that can affect recruitment may have had an effect on the early colonisation or subsequent colonisation of the reef following disturbance.

3.4.1 DISCUSSION OF ASSEMBLAGES ON SUBSTRATE TYPES

The two natural rocky reefs surveyed had both the second highest and the lowest species richness values respectively. The two reefs were also covered with *Cystophora* sp. and *Sargassum* sp. brown macroalgae, typical of many of the healthy reefs in the region (Westphalen 2011). The natural reefs had some encrusting invertebrates but most were low relief sponges (*Dendrilla* sp.) and tunicates (Didemnidae).

The steel wreck, the *SS Hougomont*, where the mid-section had collapsed in on itself formed a structure analogous to the rocky reefs in the region (vertical relief < horizontal relief) and possibly explains the similar macroalgal assemblages. The wreck supported a lot of taxa covering the surface that had a significant effect on the assemblages of mobile macro invertebrates. Typically the stern and bow sections had large areas of vertical relief and this contributed to some of the differences to the other sites for the non-colonial invertebrate assemblages. These assemblage differences caused by structure have been observed between surfaces on the recent shipwreck reef (Walker et al. 2007) and between another wreck and adjoining coral reef (Perkol-Finkel et al. 2006). Steel is a now a popular material that is being used to construct purpose built fishing reefs (Perkol-Finkel & Benayahu 2007) and reefs for tourism (Walker et al. 2007, Hiscock et al. 2010). Purpose built steel reefs are skeletal geometric designs with the emphasis on fish production (Sonu & Grove 1985), the reef layouts are complex structures for the fouling communities. Artificial reefs formed from steel vessels are very different structures, having large patches of vertical and horizontal surfaces available for colonisation. If the vessel is intact, the external surfaces of the vessel can be covered in large numbers of filter feeding invertebrates (Zintzen et al. 2008). The *SS. Hougomont* most probably had a very different fouling assemblage initially when there was more vertical relief. The changing nature of the reef surfaces over the last eighty years because of degradation by corrosion, has made the wreck more similar to the natural reefs in this study. The reef still supported a more diverse range of taxa than the natural reefs and any of the artificial reefs.

The tyre reef was the only reef sampled that was installed with the objective of increasing fish production (Branden et al. 1994), however the fouling assemblage on the reef was very different to the natural reefs as well as the other artificial substrates. There were also large areas of the reef that were not visibly covered in encrusting colonial invertebrates or macroalgae despite the reef being almost three decades old. Tyre reefs can flex during storm events and this can disturb or cause damage to encrusting flora and fauna, leading to the creation of bare patches on what are mature reefs (Collins et al. 2002). The findings of this study suggest that fouling assemblages on this tyre reef are subject to regular physical disturbances.

The assemblage on a concrete reef in the Gulf St Vincent was still undergoing significant change decades after installation (Butler & Connolly 1996), despite not having to contend with a flexible substrate base like a tyre reef. The early recruitment of invertebrates to substrates made up of rubber was found to be significantly lower than other substrates such as concrete, steel and coral (Fitzhardinge & Bailey-Brock 1989). Low recruitment, coupled with regular disturbance may mean that tyre reefs are more susceptible to settlement by non-indigenous species; however, the regular disturbance may also mean that these reefs are less vulnerable to hard encrusting invasive species. Tyre reefs have an additional risk to the marine environment in the leeching of heavy metals and organic compounds over time (Collins et al. 2002). Tyres were used in the creation of the majority of the artificial reefs in the large scale reefing program in South Australia (Branden et al. 1994), but tyres as reef material has lost popularity, following the disintegration of a reef and the loose tyres destroying an adjacent coral reef (Collins et al. 2002)

The jetties, whilst not purpose built reefs, are important hard substrate habitats with unique fouling assemblages. These jetties were located in regional ports that are now mostly used for recreational purposes (fishing and diving). Previous work by Butler et al. (1991), found that the fouling assemblages on the pylons at Edithburgh were very different over short distances to those on natural hard substrates. The variable recruitment, over one year, onto five jetties (including Edithburgh and Rapid Bay) sampled in this study has been demonstrated previously (Butler 1986), thus early recruitment after disturbances at these sites may create very different initial assemblages. Whilst the three sampled jetties had similar non-colonial invertebrate assemblages, the high percentages of macroalgal cover (*Caulerpa* sp.) on the jetty at Kingscote caused the reef's macroalgae and colonial invertebrate assemblage to be less similar to the other two reefs. The greatest rates of algal growth in the southern hemisphere typically occur in the spring and early summer months (Reimers & Branden 1994). Algal growth is important in subtropical waters and may determine the sequence of benthic recruitment to newly immersed structures (Bailey-Brock 1989, Fitzhardinge &

Bailey-Brock 1989). Algae can create new biotic structure for settlement/refuge or stratify the fouling community and inhibit the settlement of other species. In the North Sea the algal communities on the submerged sections of an oil platform covered the first 10 m of the submerged pylon with hydroids and bryozoans appearing in higher abundances below (Forteath et al. 1982). Kingscote Jetty was covered in *Caulerpa* sp. on the outer pylons, with a sponge dominated community on the seafloor visible to the sampling dive team under the middle of the structure. The outer pylons of the other two jetty sites were covered with filter feeders (sponges, corals, bryozoans, polychaetes, tunicates). These sites had less numbers of macro invertebrates compared to those with more macroalgal cover.

Algae usually compete with corals for space (or light) and interactions between the two are frequently interpreted simply in terms of algal superiority, often because of reduced herbivory or increased nutrient availability (McCook et al. 2001). This suggests that whilst shading and light availability can structure fouling communities (Forteath et al. 1982, Glasby 1999), at Edithburgh and Rapid Bay, another driver is allowing filter feeders to outcompete algae for space on the outer, least shaded pylons.

3.4.2 RECOMMENDATIONS FOR COMPARISONS

The comparisons of different artificial reefs to the natural reefs in this study, showed that similarity in fouling communities between the artificial reefs to the natural reefs varied with substrate and also region. Some artificial substrates are better than others in developing fouling assemblages similar to natural hard substrates (Connell & Glasby 1999). The fractured nature of reef development in the Gulf St Vincent (Branden et al. 1994), coupled with the presence of non-purpose built reefs has provided many different substrates that have created diverse fouling assemblages. A broad definition of artificial reefs (Svane & Petersen 2001) is important for the multi-substrate comparative studies advocated by Carr & Hixon (1997). The broad definition of artificial reefs includes structures such as piers, shipwrecks and breakwaters that contribute much to the patchwork of hard substrates in regions. The inclusion of these structures in this comparative study has shown that non-purpose built reefs are often more similar, more diverse and more stable than the purpose built reef, using a material that was officially sanctioned (Branden et al. 1994).

The patchworks of substrates and regional variations in assemblages present a considerable management challenge in coastal seas as future environmental changes may have different effects depending how resilient a reef is. Artificial reefs themselves can and usually are found to have different assemblages of encrusting and mobile invertebrates as well as macroalgae to natural reefs (Perkol-Finkel et al. 2008). In areas with little natural hard substrate such as the Gulf St Vincent, artificial reefs are increasing the diversity of fouling assemblages and thus probably influencing the supply of larvae and propagules within the region. The choice of substrate is therefore critical to ensure the stability and limit the dissimilarity of the assemblage, and its subsequent larval production to the natural reefs. This is true not only for the development of purpose built reefs but for any submerged artificial structure.

CHAPTER 4. SEASONAL RECRUITMENT VS. SUBSTRATE PREFERENCES OF RECRUITING LARVAE AT FOUR SITES IN THE GULF ST VINCENT.

4.1 INTRODUCTION

As urbanisation increases along coastlines (Bulleri & Chapman 2010, Dafforn et al 2015A, Firth et al. 2016A), it is important to understand the dynamics of fouling communities on artificial substrates (Glasby 2000). The use of materials for coastal structures such as piers (Butler 1986) and wind turbines (Andersson et al. 2009) have created fragmented patchworks of artificial and natural reefs (Branden et al. 1996, Perkol-Finkel et al. 2006). A wide variety of materials has been used for habitat restoration and to build reefs for fisheries enhancement, for example tyres (Bailey-Brock 1989), steel (Perkol-Finkel & Benayahu 2004), polypropylene pipes (Edelist & Spanier 2009) and concrete (Seaman 2007. The use of purpose built artificial reefs to address specific objectives (tourism, habitat restoration, fish stock increases) has often had limited or inconclusive success (Baine 2001). Further complicating the management or application of artificial reefs, is the presence of “unintentional” or “overlooked” artificial reefs (Firth et al. 2016A). Any artificial submerged substrate is susceptible to fouling, and thus could be considered an artificial reef (Svane & Petersen 2006). This definition of an artificial reef is more encompassing, taking into account the variety of structures found in coastal seas such as wharves, wind farms, oil rigs and sunken vessels.

The fouling assemblages on hard substrates in temperate seas, can consist of a diverse group of algae, sponges, cnidarians, bryozoans, molluscs and tunicates (Butler 1998, Zintzen et al. 2008). The life histories of most of the species recruiting into these assemblages, include a microscopic, free-living planktonic stage (Pechenik 1999). The diversity of the larvae and algal propagules in the plankton is seasonal (Bailey-Brock 1989, Andersson et al. 2009) and depends on the life histories of adult populations. The settlement of larvae and successful recruitment into fouling assemblages can determine populations (Rodriguez et al. 1993, Sams & Keough 2012). Variable seasonal differences in the plankton community can also influence settlement, as most species have peaks in recruitment at a particular time of year, and whilst the time of year can be predicted, the size of the peak cannot (Keough 1983). The supply of larvae and their subsequent successful recruitment is important for the makeup of fouling communities (Underwood & Fairweather 1989). Distances from source populations and larval mortality in the plankton may mean that some recruitment in remote areas may be poor, whilst other areas can have large abundances of the same species settling. It is then unlikely that the reefs in different regions or within a region will contain the same assemblage of species (Underwood & Fairweather 1989). At any stage larvae are vulnerable to natural mortality through predation, and competition.

The act of settlement will also structure fouling assemblages, as larvae can actively select their settlement sites from different available substrate patches. Site selection is important, as it can account for some of the variability in the recruitment survival rates (Blythe & Pineda 2009). The settlement of some larvae might depend on specific types of substrates or the presences of facilitators who provide additional structure, such as bivalves (Farinas-Franco et al. 2013). Early differences between the assemblages on different artificial reefs (pylons) suggest that larvae may prefer to settle on one particular substrate or that survival rates were higher on one type (Andersson et al. 2009). The surface structure of reef materials can influence settlement and later abundances even with millimetre variations (Coombes et al. 2015).

The addition of anthropogenic hard substrates means that larvae now have a wider variety of reefs to select from, their substrate choice can affect the subsequent succession at that site. The fouling assemblages on artificial reefs can remain distinct from those on natural ones over long periods of time (Perkol-Finkel et al. 2006). The assemblages on artificial reefs can also have a higher diversity or greater abundances of species than those on natural substrates (Connell & Glasby 1999). Higher abundances of hydroids have been found on several isolated artificial reefs that were surrounded by predominately soft sediments regions (Zintzen et al. 2008). The reefs, in addition to providing habitat that would not otherwise be available to sessile filter feeders and predators, can increase predation on larvae in the plankton moving over the soft sediments, potentially changing the local larval supply.

As artificial reef substrate comparisons are often approached from habitat restoration or conservation objectives, the underlying question in many studies is how similar one or two artificial reefs are to a natural reef benchmarking site (e.g. Perkol-Finkel et al. 2006, Hunter & Sayer 2009). Comparative studies assess the reefs success in reaching its objectives using a natural benchmark site or sites, however many substrates were deployed without any measurable conservation objectives to be measured by. The continued proliferation of artificial reefs in coastal (Firth et al 2016A, 2016B) and even deep seas where hard substrates are limited, means that another question can be asked, how similar are the fouling communities on these artificial substrates within regions? In the tropics and northern hemisphere, recruitment has been compared on multiple artificial reef substrates (Fitzhardinge & Bailey-Brock 1989, Perkol-Finkel 2006, Hunter & Sayer 2009). Assemblages of early recruits have been found to be distinct across multiple artificial reefs (Fitzhardinge & Bailey-Brock 1989, Connell & Glasby 1999), however seasonal fluctuations in propagule and larvae recruitment can also lead to the formation of different fouling assemblages on artificial reefs (Bailey-Brock 1989, Underwood & Anderson 1994).

Given the differences in seasonal conditions, there are limits to how far understanding ecological patterns and processes from warmer water studies can be extrapolated and applied to cold temperate communities (Andersson et al. 2009).

The objective of the present study was to compare the seasonal recruitment onto commonly found artificial substrates at different temperate sites. The aim was to test whether it is seasonal larval trends or substrate preferences that will determine the recruitment and subsequent development of an early fouling assemblage onto an artificial reef. The experiment will test the hypothesis, that there will be seasonal variation in recruitment across substrate types.

4.2. METHODS

4.2.1 SITES AND EXPERIMENTAL PROCEDURE

The experiment was conducted at four sites (Edithburgh, Wool Bay, Rapid Bay and Second Valley) in the Gulf of St Vincent (GSV), South Australia (Figure 4.1). Two sites, Rapid Bay and Second Valley were on the eastern side of the GSV on the Fleurieu Peninsula. Rapid Bay has two jetties adjacent to one another, a long older jetty and a newer one made from concrete and steel. This experiment took place on the newer jetty, as it enabled a surface team to retrieve samples from the dive team below. The remaining two sites, Edithburgh and Wool Bay were on the western side of the GSV, on the Yorke Peninsula. Each of the peninsulas was considered to be a region in the experimental design with two sites nested in each.

All of the experimental sites had piers onto which large rectangular steel frames (2 m x 1 m) were affixed. The piers are constructed of steel and hard wood *(Eucalyptus marginata*) pylons and are maintained but not treated with any antifouling paint. The frames were placed with the approval of local councils or Government Departments. Settlement plates were attached with zip-ties to the rigid plastic grid of each frame.

The settlement plates were submerged and mounted each season by a small team of divers. The depth at which the experimental frames were situated was 6 m at Edithburgh, 3-5 m Second Valley, Rapid Bay and Wool Bay. The shallow depths allowed divers to spend longer working on the frames during collection. The frames were securely attached to the north side of the four piers. The settlement plates were 100 x 100 mm squares, as used by Underwood & Anderson (1994) and Simpson et al. (2017). The plates were constructed from four materials that have been used in artificial reefs in the region. Steel and wood (*Eucalyptus marginata*) were directly replicated, whilst rubber with a shallow (5 mm deep) grip pattern and a fibre cement building sheet (hardiflex) simulated the surfaces of well used tyres and concrete reefs respectively. The hardiflex sheeting has a rough side and smooth side, for experimental purposes the rough side was deployed on the outside of the frame.

For each substrate, five plates were deployed every season at each site (Table 4.1), with 80 plates collected each season. The plates were deployed by placing them haphazardly onto a grid at each time interval. Over the experimental timeframe, two out of 16 settlement frames were lost to storms at Second Valley (spring) and Rapid Bay (autumn). These were subsequently replaced by new frames with the next seasons replicates (Table 4.1). For collection after three months deployments, each plate was covered with a sampling bag and then removed from the frame, to minimise the loss of any organisms

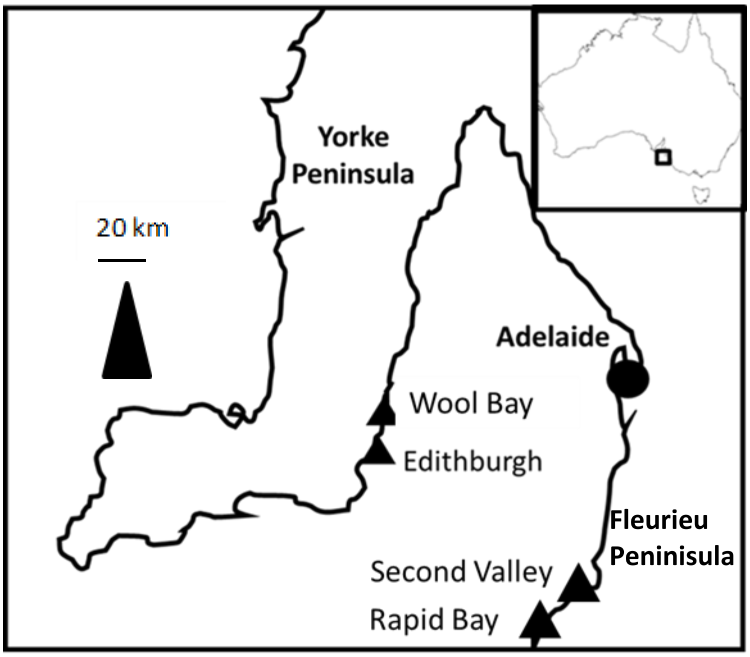


Figure 4.1 Map of the lower Gulf of St. Vincent, South Australia showing the locations of the four experimental sites, Wool Bay, Edithburgh, (nested in region: Yorke Peninsula) Second Valley and Rapid Bay (nested in region Fleurieu Peninsula).

Table 4.1 Table of experimental sets (5 replicates of each 4 substrates at each site) retrieved or lost over the 12 months of experimental timeframe.

	Season			
Sites	**Spring**	**Summer**	**Autumn**	**Winter**
Rapid Bay	Retrieved	Retrieved	Lost	Retrieved
Second Valley	Lost	Retrieved	Retrieved	Retrieved
Edithburgh	Retrieved	Retrieved	Retrieved	Retrieved
Wool Bay	Retrieved	Retrieved	Retrieved	Retrieved

4.2.2 SAMPLE ANALYSIS

Digital images were taken of the settlement plates using a camera (12 mp Olympus µtough) mounted onto a tripod, in the laboratory. The diversity and the numbers of taxa were calculated using data obtained from sorting the plates in the laboratory. The abundances of mobile and sessile non-colonial invertebrates were counted in the laboratory using a dissecting microscope and represented using individuals per 100 cm^2 (density). Colonial invertebrates (tunicates, sponges, bryozoans etc.) and some non-colonial invertebrates (e.g. barnacles, tube worms and large solitary ascidians) were measured as percent cover, using an overlay of 100 random points and assigning categories. Coral Point Count with Excel extensions (CPCe V.4.1) (Kohler & Gill 2006) was used to determine the percent cover from the digital images of the settlement plates. The category list for the CPCe was developed from the species list from the sorted plates over all 4 seasons. Family level and indeterminate taxa categories were included in this species list for multiple taxa that might not be able to be distinguished from each other in the pictures (e.g. *Sargassum* sp.). A bare or open space category was used in the software package, which leaves out the unclassified/empty random points as only abundances of organisms were measured.

4.2.3 STATISTICAL ANALYSIS

The total number of taxa per phyla was calculated using the diversity data from physically examining the settlement plates, the number of taxa were split into sessile and mobile taxa and calculated for sites, substrates and seasons. The total number of taxa per replicate was tested with a univariate four factor permANOVA analysis using a Euclidean distance resemblance matrix for substrate, season and site (nested in region). The community multivariate analyses were split into two parts; the fouling assemblages of colonial invertebrates and macroalgae (percent cover) and non-colonial invertebrates (number of individuals). The average dissimilarities (%) of the recruiting assemblages on each substrate and season were compared using a SIMPER analysis and the within group similarities (%) were also shown. The community analyses were tested using Bray-Curtis resemblance matrix and a four factor permANOVA (substrate, season, sites (nested in region)) for both measured assemblages. Further multivariate hypothesis testing was carried out using a Canonical Analysis of Principle coordinates (CAP) routine, to test how well groups could be assigned, using each of the four tested factors. The results were compared in a table for each measured assemblage as per Smith et al. (2013).

4.3. RESULTS

4.3.1 DIVERSITY OF ASSEMBLAGES

Across all four substrates, sites and seasons, 67 invertebrate taxa and 12 macroalgal taxa were recorded on the settlement plates. The fouling assemblages recruiting onto the substrates was comprised of cnidarians (*Obelia* sp. and *Canularia* sp.), sponges (*Leucosolenia* sp.), bryzoans (encrusting Cheilostomata) annelids (sabellids, serpurlids, spirorbids), molluscs (bivalves and gastropods), arthropods (isopods, amphipods, tanaids, pycnogonids, decapods) and tunicates (didemnids). Arthropoda was the most diverse phylum (8- 20 taxa per replicate) across all seasons, substrates and sites (Figure 4.2A, B, C, D. Encrusting bryozoans and molluscs were the next richest phyla (Figure 4.2A, B, C, D). Macroalgae were represented by brown, red and green divisions, and the number of macroalgal taxa peaked in spring and decreased over summer, autumn and then increased again in winter for all of the substrate (Figure 4.2). The filamentous brown algae *Hincksia* sp., was found on all substrates for all seasons and sites, while red algae were only found on the Yorke Peninsula sites (Figure 4.4C).

The total number of recruiting taxa over all of the substrates from all of the sites, peaked in spring, and decreased over summer and autumn (Spring 70 taxa >Summer 58 taxa >Autumn 54 taxa <Winter 66) (Figure 4.3B). The four substrates had similar numbers of taxa on the plates per season (Figure 4.3A). However, there were different seasonal peaks in diversity between the four substrates (Figure 4.3B). The number of recruiting taxa showed a small increase during winter for three substrates types, with the steel substrate being the only exception (Figure 4.2A). The steel plates appeared to lose a lot of the fouling through corrosion on the plate, sloughing off the surface (Figure 4.4). The overall number of taxa found at sites also varied and the two Yorke Peninsula sites had the most taxa over all seasons (Figure 4.3C), although two sets of seasonal data were missing from the other region. Site (nested in region) interacting with both substrate and season (Table 4.2) was significant for the total number of taxa found on the plates.

Table 4.2. Univariate permANOVA results for four factor (Substrate, Season, Region, Site (nested in Region)) analysis of total number of taxa.

Factor	**df**	**Pseudo-*F***	**P(perm)**
Season	3	2.7077	n.s.
Substrate	3	1.046	n.s.
Site (Region)	2	31.5	**0.001**
Region	1	0.040	n.s.
Season x Site(region)	2	14.692	**0.0001**
Substrate x Site(region)	9	4.3122	**0.0001**
Season x Substrate x Region	9	0.60981	0.7624
Season x SubstratexSite(region)	6	7.7329	0.0001
	Res 223		

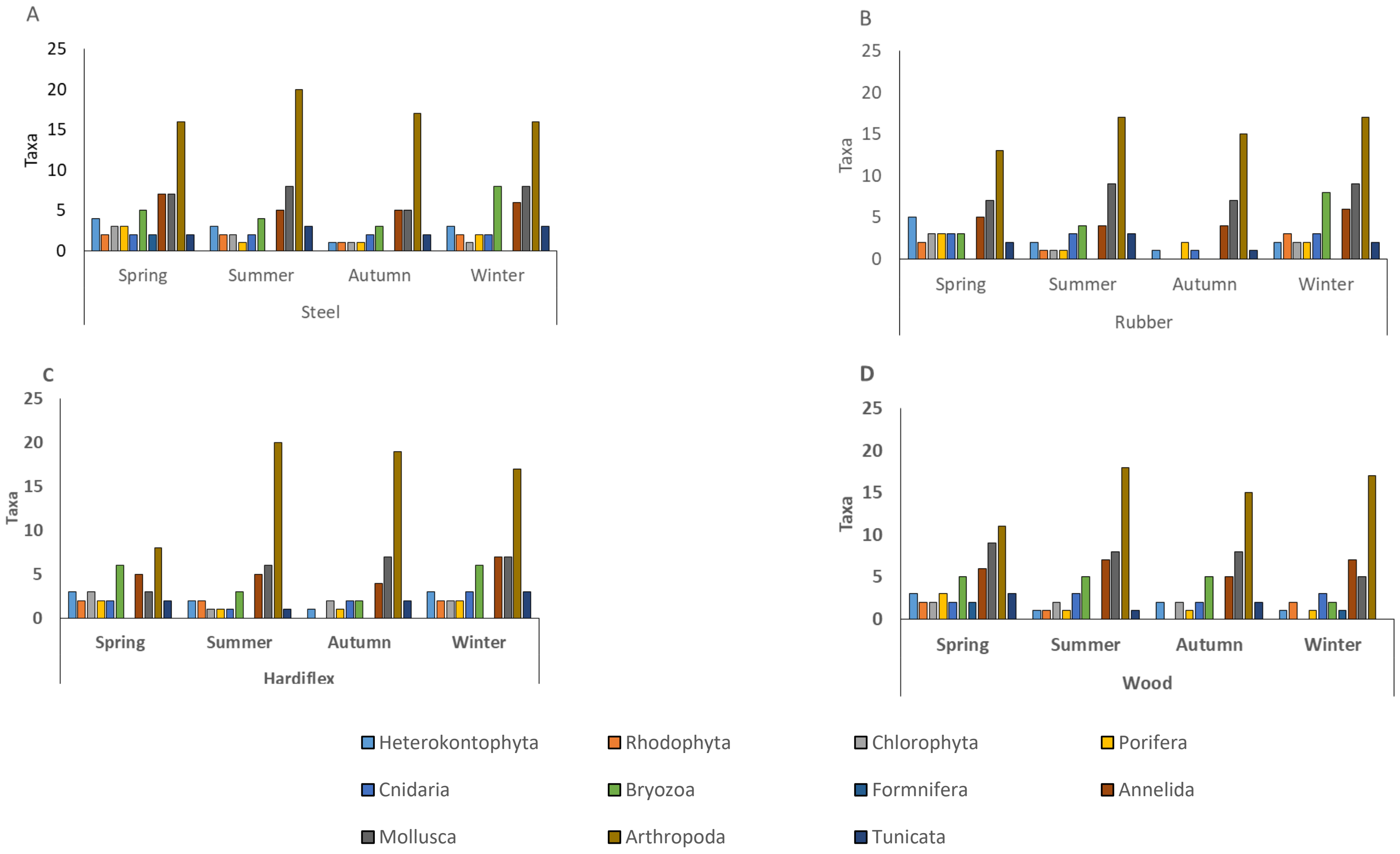


Figure 4.2. Total number of taxa (invertebrates and macro algae) recruiting onto each substrate (A: Steel, B: Rubber, C: Hardiflex, D: Wood) across all sampled sites in each season. The four seasons are defined as Spring (September,-October-November), Summer (December-January-February), Autumn (March-April-May), Winter (June-July-August).

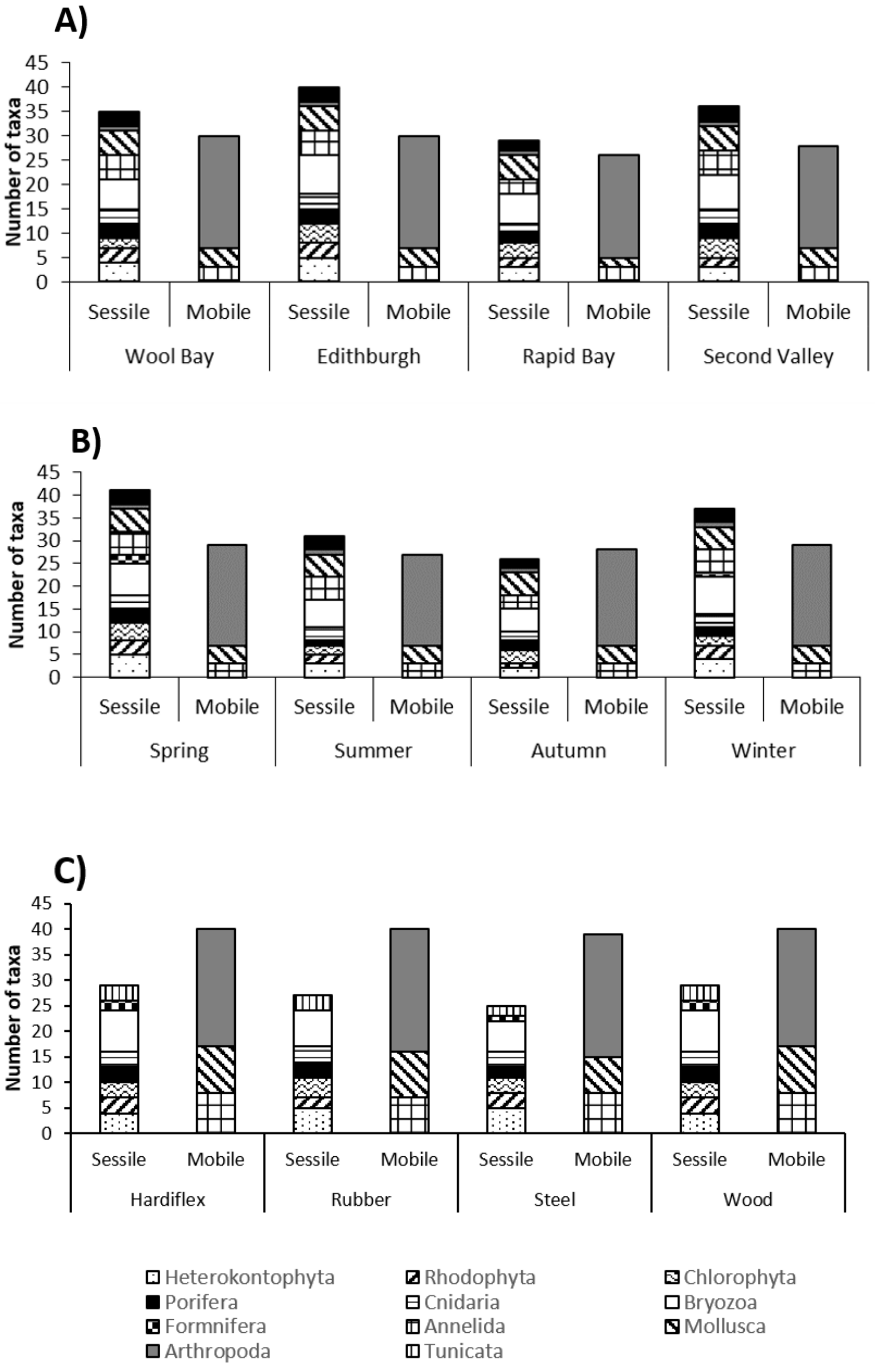
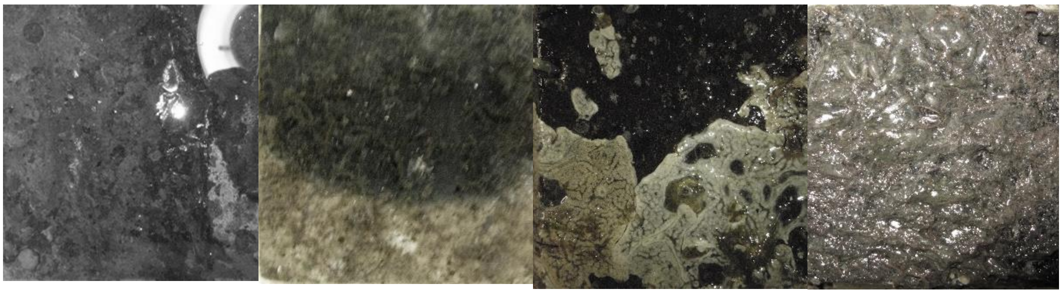


Figure 4.3 A: Total number of sessile or mobile taxa for each phylum found on each site. **B:** Total number of sessile or mobile taxa for each phylum found in each season for all pooled four sites. **C:** Total number of sessile or mobile taxa for each phylum found at each substrate pooled over four seasons and four substrates.



Steel Hardiflex Rubber Wood

Figure 4.4. Examples of the fouling assemblages on the settlement plates at the Edithburgh Jetty during the Austral Summer 2013.The steel plate has some filamentous brown algae. The hardiflex plate is mostly covered with encrusting bryozoans. The rubber plate is covered with Didemnid ascidians while the wood plate is covered with green algae.

4.3.2 MULTIVARIATE ANALYSES

4.3.2.1 COLONIAL INVERTEBRATES AND MACROALGAE

The macroalgae and colonial invertebrate assemblages on steel over all sites and seasons were the most similar (Table 4.3A, 66.8%). The assemblages on the steel and hardiflex plates were the most dissimilar of all the substrates (Table 4.3A). SIMPER analysis found that the coverage of brown algae (filamentous) contributed most to the similarity between groups for steel and wood substrates (contributing to 88.2% of steel, 60.8% of wood within group similarities) and less so for rubber and hardiflex (contributing to 46.9% of rubber and 24% of hardiflex, within group similarities). The fouling communities in Spring and Autumn were the most similar (Table 4.3B), while the communities in Winter were only 38.1% similar (Table 4.3B).

There were significant interactions between site (nested in Region) and season as well as between site (nested in Region) and substrate (Table 4.4). The interactions with region influenced the other results. The regions were significant determinants for the fouling assemblages found in each season and on each substrate. Significant differences were found between sampling sites (nested in Region).

Table 4.3: SIMPER analysis for colonial invertebrates and macroalgae recruiting **A:** onto substrates (pooled over all four sites and seasons), and **B**: seasonally (pooled over all four sites and substrates). Underlined numerals show average similarity within substrate groups, whilst non-underlined numerals show average dissimilarity between substrate groups.

A	**Wood**	**Hardiflex**	**Rubber**	**Steel**
Wood	48.89%			
Hardiflex	63.20%	36.99%		
Rubber	51.59%	59.03%	51.64%	
Steel	53.43%	74.40%	59.03%	66.88%
B	**Spring**	**Summer**	**Autumn**	**Winter**
Spring	47.20%			
Summer	56.59%	42.63%		
Autumn	53.24%	58.09%	47.18%	
Winter	58.34%	59.11%	59.11%	38.08%

Table 4.4: Four factor multivariate PERMANOVA for macroalgae and colonial invertebrates (percent cover): Region, Site (Nested in region), Substrate, Season.

Factor	**df**	**Pseudo-F**	**P**
Region	1	2.72	n.s
Site (Region)	2	2.507	**0.001**
Substrate	3	10.30	n.s.
Season	3	1.42	n.s.
Region x Season x Substrate	9	3.6311	**0.001**
Site (Region) x Season x Substrate	12	2.4982	**0.001**
	Res. 224		

4.3.2.2 NON-COLONIAL MACROINVERTEBRATE ASSEMBLAGES

The spring assemblages of non-colonial macroinvertebrates were the most variable (least similar) across all of the sample sites and substrates (Table 4.5A). Settlement by mobile invertebrates in spring was also very different to the other seasons (78-81 % dissimilarity). The assemblages of non-colonial macro invertebrates found on the steel were the most unique (>70 % dissimilarity) but assemblages varied (24.4 %) (Table 4.5B). Season and substrate were not significant factors on their own for non-colonial macro-invertebrates, but Region and its interactions with the other factors (Substrate, Season), were the most significant factors for assemblages of non-colonial invertebrates (Table 4.6).

Table 4.5. SIMPER analysis for non-colonial invertebrates recruiting **A**: seasonally (pooled over all sites and substrates), and **B**: onto substrates (pooled over all four sites and seasons). Underlined numerals show average similarity within substrate groups, whilst non-underlined numerals show average dissimilarity between substrate groups.

A	**Spring**	**Summer**	**Autumn**	**Winter**
Spring	11.6%			
Summer	81.4%	45.3%		
Autumn	81.7%	60.9%	36.6%	
Winter	78.8%	57.9%	58.9%	46.2%

B	**Wood**	**Hardiflex**	**Rubber**	**Steel**
Wood	28.25%			
Hardiflex	67.90%	33.44%		
Rubber	69.08%	65.96%	32.98%	
Steel	74.23%	72.07%	70.68%	24.38%

Table 4.6: Four factor PERMANOVA for non-colonial assemblages recruiting on to settlement plates, with Region, Site (Nested in region), Substrate, Season.

Factor	**df**	**Pseudo-F**	**Perm**
Region	1	3.5347	**0.0001**
Substrate	3	4.7668	**0.0001**
Season	3	20.699	**0.0001**
Site (Region)	2	11.965	**0.0001**
Region x Season x Substrate	9	4.066	**0.001**
Site (nested Region) x Season x Substrate**	12	3.771	**0.001**
	Res 224		

4.3.3 TESTING HYPOTHESES USING CAP FOR BOTH ASSEMBLAGES

Canonical analysis of principle coordinates (CAP) was used to test hypotheses of how well samples can be assigned into groups determined by each factor (Table 4.8A and 4.8B). Substrate and season CAPs were represented in two dimensional space (Figures 4.7 and 4.8), but did not show any obvious groupings based on the two chosen factors for either non-colonial invertebrates or colonial invertebrates and macroalgae. Samples were not clustered in seasonal groups in the Season CAP (Figure 4.8A and 4.8B). Samples from spring were dispersed amongst the other seasons and the samples were only 5 % correctly explained by that factor. Substrate type was a better discriminator of the colonial and macro-algal assemblages than the season (35 % correct vs. 57 % correct) (Figure 4.7A and 4.8B). The non-colonial invertebrate assemblages in the substrate CAP (Figure 4.7B) were less dispersed and were clustered into groups that reflected substrate groupings. Samples that were found on steel were tightly grouped in the CAP (Figure 4.7B) (90 % correct), while wood (42.9 % correct), rubber (34.3 % correct) and to a lesser extent hardiflex (61.4 % correct), were more interspersed (Figure 4.7B). The non-colonial invertebrate samples when tested over four different discriminate hypotheses (Substrate, Season, Region and Site) using CAP analysis, were best determined by region which was only 43% misclassified (Table 4.8A). Region was also a factor that explained the most groups for the colonial and macroalgae groups (Table 4.8B).

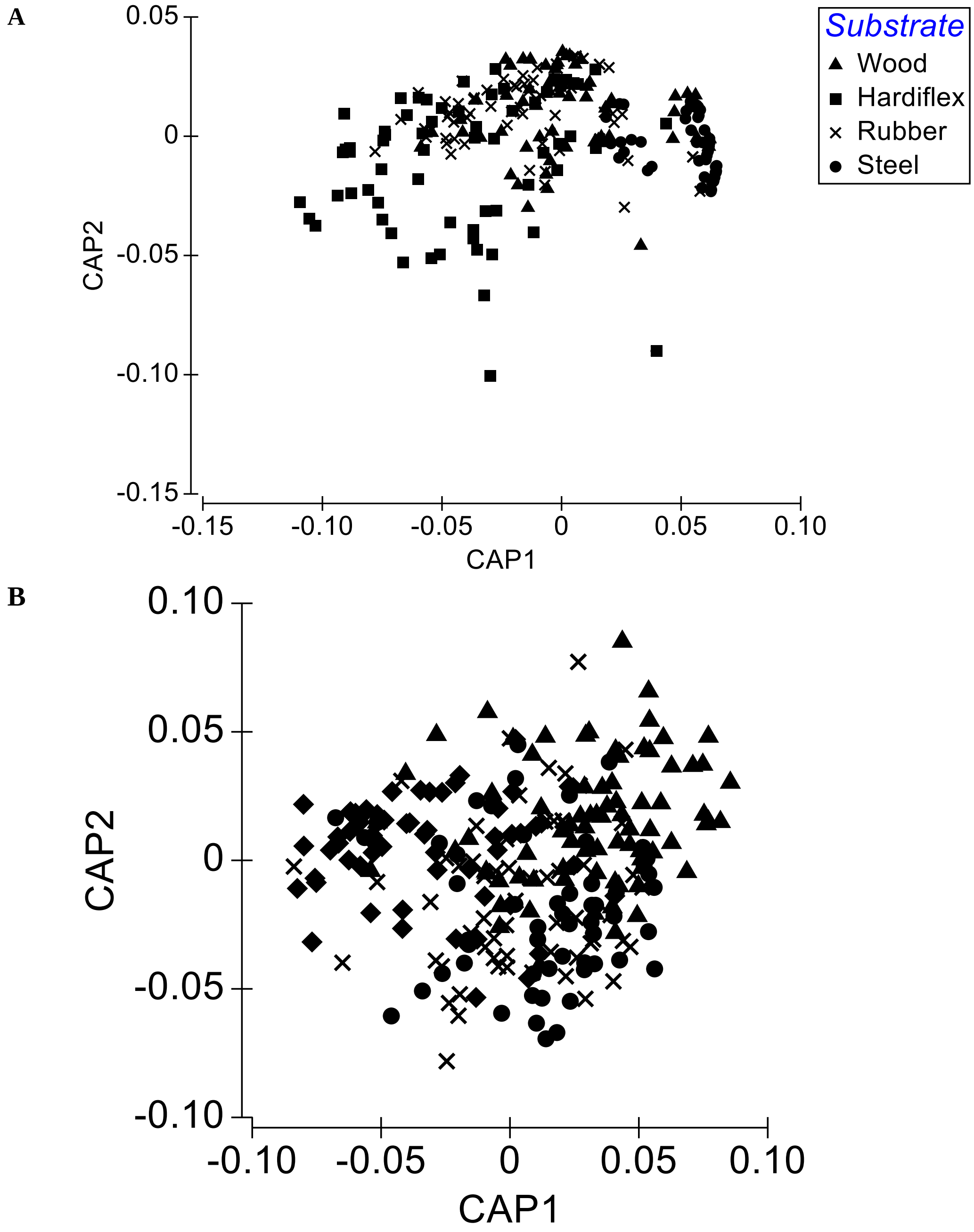


Figure 4.7 A. CAP analysis testing the hypothesis that substrate can predict groups for colonial invertebrates and macroalgal assemblages. CAP diagnostics values appear in Table 4.7. **B.** CAP analysis testing the hypothesis that substrate can predict groups for non-colonial invertebrates. CAP diagnostics values appear in Table 4.7.

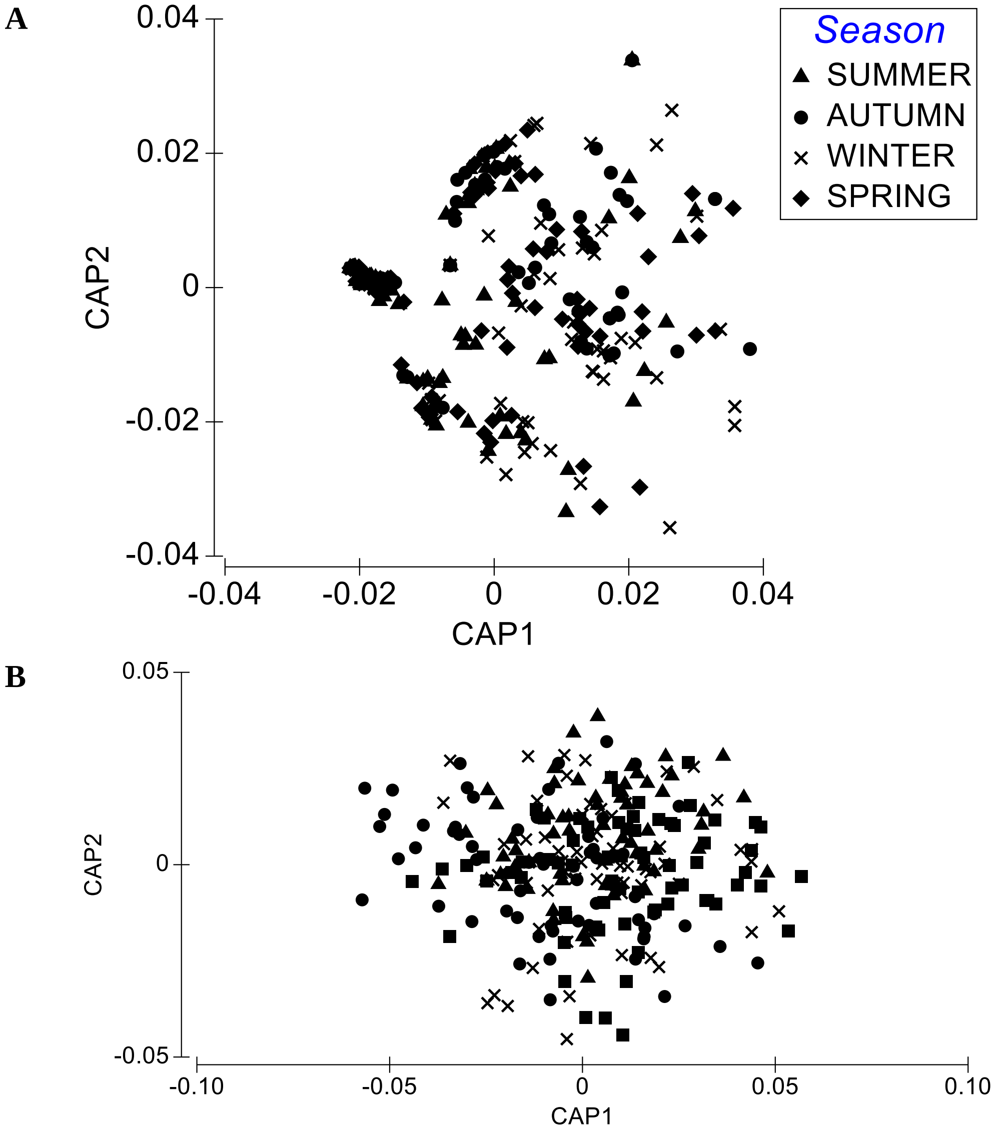


Figure 4.8 A: CAP analysis testing the hypothesis that season can predict groups for colonial invertebrates and macroalgae. CAP diagnostics appear in Table 4.8. **Figure 4.8 B:** CAP analysis testing the hypothesis that season can predict groups for non-colonial invertebrates. CAP diagnostics appear in Table 4.8.

Table 4.8. CAP analysis results for **A**: non-colonial macroinvertebrates and **B** Colonial invertebrates and macroalgae. Summary of CAP analysis results) for each community component (Factors: Substrate, Season, Site and Region).

A: Non-colonial invertebrates				
Hypothesis	**Substrate**	**Season**	**Region**	**Site**
variable # groups compared	280	280	280	280
PC axes chosen m	16	10	16	16
Proportion of variation explained	98%	79%	97%	98%
Significance of trace certificate	0.001	0.001	0.001	0.001
Significance of delta statistic	0.001	0.001	0.001	0.001
Total % of samples misclassified	57%	44%	43%	57%
# samples correctly classified out of total	120	158	160	120
B: Colonial invertebrates and macro-algae				
Hypothesis	**Substrate**	**Season**	**Region**	**Site**
variable # groups compared	280	280	280	280
PC axes chosen m	3	3	3	5
Proportion of variation explained	92%	93%	93%	93%
Significance of trace certificate	0.001	0.0002	0.553	0.444
Significance of delta statistic	0.001	0.006	0.553	0.517
Total % of samples misclassified	68%	64%	50%	74%
# samples correctly classified out of total	88	100	141	72

4.4. DISCUSSION

Substrate specific recruitment was found for some colonial invertebrates and macroalgae, but substrate had little effect on the more mobile non-colonial invertebrate recruitment. Substrate choice did affect the coverage of some colonial invertebrates and may determine if some parts of the fouling assemblages can persist. The fouling assemblages on the four substrates had similar species richness and most of the taxa were found across all of the substrates.

The lack of substrate specific recruitment was unexpected, as larvae are known to select settlement sites (Farinas-Franc et al. 2013) and even postpone metamorphic life stages until a site is found (Blythe & Pineda 2009). There were substrate specific differences (multivariate) between the assemblages of non-colonial invertebrates and macro-algae. The presence on every substrate may be explained by limited free patches coupled with strong regional supply (Osman 2015) and that larvae can give up in their search for preferred substrate (Blythe & Pineda 2009).

The steel and rubber panels had the lowest percentage cover of macro-algae and non-colonial invertebrates, which could be attributed to their surface properties. Steel can have high percentage cover of encrusting corals in the tropics, but corrosion can mean that the substrates can wear away quickly over relatively short time scales causing the loss of any biotic cover (Fitzhardinge & Bailey-Brock 1989). Many steel reefs are constructed using surplus vessels, whose anti-fouling hull coatings can continue to structure the fouling community years after scuttling (Hiscock et al. 2010, See Appendix 2). The surfaces on tyre reefs can also structure the fouling assemblages by flexing during storm and lose much of the encrusting fauna (Collins et al. 2002). Tyres were a common reefing material in the 1970s (Branden et al. 1994), as they were low cost and required little effort to sink them. Recruitment diversity and abundance onto a tyre reef in Hawaii was significantly less than on four other substrates sampled (Fitzhardinge & Bailey-Brock 1989), as larvae were either avoiding the tyres or the substrate's flexing during storms caused the loss of encrusting flora and fauna (Collins 2002). The panels in this study could flex and bend unlike the other substrates panels. Other studies have found that there were no significant differences between the fouling communities on artificial reefs constructed from tyres and concrete (Collins et al. 2002).

The rigid or inflexible surfaces of the other two tested substrates, hardiflex and wood allowed for the development of encrusting invertebrates and macroalgae. These two substrates had diverse assemblages of encrusting bryozoans at the end of each season. Hardwoods have been used in the marine environment for thousands of years (piers, wharves and fishtraps). Fixed and set aggregate substrates, represented in this study by hardiflex panels, are usually concrete and are becoming more common in the marine environment (Baine 2001). Concrete is resistant to marine borers and used to support large modern maritime infrastructure features such as oil rigs, breakwaters, wind turbines and piers. The structural characteristics of concrete can shape the communities found on its surface with concrete sections of a wind turbine differing to those on the adjacent steel sections after 12 months (Andersson et al. 2009).

The site where the experimental plates were mounted (nested in region) was the most important factor for the recruitment of many taxa. Variations in regional assemblages are common in single substrate settlement plates in southern Australia (Simpson et al. 2017, Foster et al. 2014, Smale 2012; 2013) across both large and small spatial scales. The adjacent habitat at each site was important for the recruiting macroinvertebrates, suggesting immigration from nearby substrates is a key process in early fouling communities and probably determines many post settlement interactions for settling larvae, such as predation and herbivory. Some of the differences in the fouling assemblages between sites in this study, were most probably caused by the established fouling communities on adjacent hard substrates (Wendt et al. 1989). The surrounding pylons at two of the sites (Rapid Bay and Edithburgh) were sampled as part of Chapters 2 and 3 with both having different fouling assemblages. The presence of adjacent assemblages or source populations may have influenced the recruitment of larvae through competition or foraging pressure, which has explained differences between sites in a previous study (Fitzhardinge & Bailey-Brock 1989). Larval availability and distribution in the water column can lead to variability on both small and large spatial scales (Rodriguez et al. 1993).

The recruiting colonial invertebrates (tunicates, bryozoans, cnidarians, spirorbids etc.) and macroalgae were expected to display clear seasonal peaks (Fitzhardinge & Bailey-Brock 1989). Underwood & Anderson (1994) found different seasonal assemblages over four artificial substrates, with macroalgal growth peaking in summer/spring. The coverage of macroalgae was thus expected to peak in spring and summer, as recruiting assemblages can be dominated by either macro-algae propagules or colonial invertebrates depending on the season (Fitzhardinge & Bailey-Brock1989, Reimers & Branden 1994). In the Northern Hemisphere, colonial species settled less in the cooler months (Mook et al. 1980). Yet, there were no detectable overall seasonal peaks for macroalgae diversity or assemblages found in this experiment.

Whilst variation in fouling communities on artificial substrates can occur on relatively small spatial scales (Glasby 2000, Sams & Keough 2012, Smale 2013), in this study strong differences were not evident in the early recruitment assemblages after 3 months and post-settlement processes may be playing a stronger role. Recruitment of the same species onto the plates might have been different if they had been deployed for multiple seasons, as recruits of some sessile species such as bivalves can be attracted to established adults (Hunt & Scheibling 1997). The early recruitment patterns in this study have shown that substrate and season are not as important as the deployment site for early recruitment to new artificial substrates. This applies to substrates that are deployed in close proximity to established fouling communities.

The development of macro-algae and the colonial invertebrates is important as it can provide additional biotic structure, which can facilitate the settlement of non-colonial invertebrate larvae (Osman & Whitlatch 1995). Transplanted clusters of the bivalve, *Modiolus modiolus* have significantly increased species richness, diversity and individual abundances on artificial reefs that received the clusters (Farinas-Franco et al. 2013). Interstitial amphipods, which would benefit from biotic structures, were the most diverse group of non-colonial invertebrates found on all of the experimental settlement plates and usually settle in biotic structures and differences in cover can contribute to the differences between substrates (Aikins & Kikuchi 2001). However, the recruiting colonial invertebrates and algae were not yet large enough to provide habitat thus, the majority of non-colonial invertebrate individuals recruiting onto the plates were probably adults or juveniles immigrating or foraging from the adjacent substrates.

Understanding whether long term developmental differences persist between assemblages on artificial substrates will require longer experimental time frames lasting decades (Butler & Connolly 1996, Perkol-Finkel & Benayahu 2005, Farinas-Franco et al. 2013). Post-settlement processes, facilitation and the structural differences between artificial substrates may play a greater role in explaining differences between fouling assemblages in close proximity to each other than early substrate specific recruitment. The role of facilitation on artificial reefs is further explored in the next experiment (Chapter 5).

CHAPTER 5. BIOGENIC STRUCTURES FACILITATE RECRUITMENT ON ARTIFICIAL REEFS.

5.1. INTRODUCTION

As the advance of ocean sprawl (Firth et al. 2016A, 2016B) coincides with coastal habitat degradation and biodiversity loss (McCauley et al. 2015), a growing demand for ecological engineering of physical infrastructure has emerged (Moschella et al. 2005, Firth et al. 2014, Strain et al. 2018). Eco-engineering solutions can include structural mimics or habitat-forming taxa (Dafforn et al. 2015, Ferrario et al. 2016, Strain et al. 2016). Ecosystem engineers can increase diversity on complex hard substrates or specialised reefs like eco-concrete blocks, creating rapid increases in diversity (Ido & Perkol-Finkel 2015). Facilitation may thus help to increase resilience of heavily urbanised coastal habitats (Dyson & Yocom 2015). An understanding of positive interactions within communities is particularly important for managing structures that could be stressful for native fouling assemblages or marine regions (Halpern et al. 2007), such as coastal artificial reefs that are deployed upon soft sediment habitats.

Positive interactions such as facilitation occur when at least one participant is benefiting and no harm is caused to either (Stachowicz 2001, Halpern et al. 2007). Positive interactions are important for community development in soft sediments, where facilitation, not inhibition, is the dominant process that determines succession (Gallagher et al. 1983). In tropical coral reefs, the diversity of small invertebrates and fishes is related to the complexity in the shape of scleractinian corals (Idjadi & Edmunds 2006). For hard substrate communities, increases in the habitat complexity, which can be created by physical ecosystem engineers, will result in greater diversity and/or abundances of associated fouling assemblages (Cruz-Suzeiro et al. 2010). Many marine organisms, such as amphipods are known to preferentially recruit onto surfaces where a specific type of colonial or sessile invertebrates and/or algae is present (Baskett & Salomon 2010).

Colonisation and succession are influenced by the establishment of large sessile invertebrates through facilitation, inhibition and tolerance (Connell & Slatyer 1977). The early stages of assemblages on reefs are highly competitive and most early interactions are inhibitory (Dean & Hurd 1980). Some habitat forming organisms can have positive effects by facilitating or at least tolerating, the recruitment of other organisms (Halpern et al. 2007). Increases in the recruitment numbers of small, mobile and cryptic macroinvertebrates can arise from positive relationships with large space providing sessile organisms such as algae (Schroeter et al. 2015, Cruz-Rivera & Hay 2001).

Facilitation is also an important component of invasion success, and the identification of foundation species that are preferred by non-indigenous species (NIS) can inform biosecurity management (Altieri et al. 2010). Positive native-invader interactions may help to explain invasion success in areas where resilience would be expected because of high natural diversity (Stachowicz & Brynes 2006). The facilitation of invasive species by natives is expected to get worse as climate change progresses (Altieri et al. 2010). Increasing resilience of fouling communities through careful marine management should be a consideration in the planning stages of an artificial structure.

The foundation species that facilitate the recruitment of other organisms can be also termed, physical ecosystem engineers (Jones et al. 1994). Ecosystem engineers can change physical structures, complexity and heterogeneity of the environment having a marked influence on the associated communities (Jones et al. 1994, 1997). Ecosystem engineers or foundation species are primary structuring agents in both marine and terrestrial systems (Yakovis et al. 2008). For example, the removal or addition of barnacles changes the species composition of recruiting fauna (Bros 1987). Numbers of barnacles are positively correlated with the number of species and the abundances of mobile individuals within the same patch (Bros 1987, Yakovis et al. 2005). Other physical ecosystem engineers like blue mussels, can have negative effects on recruitment (Durr & Wahl 2004). Biogenic reefs especially those created from shell or tube building molluscs or annelids are important for coastal protection and ecosystem function (Firth et al. 2015).

Interactions between habitat forming foundation species and their interstitial residents are not always unidirectional, as smaller organisms can reciprocate with positive effects for their hosts, creating mutualisms that can underpin ecosystem functions (Hay et al. 2004). Over large spatial scales, positive interactions can lead to higher interstitial diversity by increasing both the number and variety of habitats available. However the impacts of positive interactions on localised diversity has only been studied in a few cases for fouling communities (Stachowicz 2001).

Variations in community assemblages can lead to a series of alternative states of a fouling community on a reef type with different facilitating species present. Alternative states of communities occur when interacting species exist in different abundances and are dominated by different sets of taxa depending on the environmental conditions (Petraitis & Dudgeon 2004). Facilitation of recruits could be a possible feedback loop, which can maintain alternative states in communities (Petraitis & Lantham 1999). The alternative states of communities on artificial reefs may also mean that functional groups of organisms can vary across reefs, depending on the foundation or facilitating species present.

Invertebrates and macroalgae on artificial substrates are known to differ in abundance and also diversity to communities found on both natural and other artificial materials (Perkol-Finkel & Benayahu 2004, Firth et al. 2016b). The community differences begin in early stages of the assemblages (Fitzhardinge & Bailey-Brock 1989) and significant dissimilarities remain decades after submersion (Perkol-Finkel et al. 2006, Chapter 2). The seasonal nature of the recruitment of many invertebrates and macroalgae (Keough et al. 1983) and long succession times (Butler & Connolly 1999), may mean that communities found on the same artificial reef substrate type may vary, depending on the time of submersion and age. The different assemblages of invertebrates and algae mean that different functional groups may be present on artificial reefs compared to those that are found on the adjacent natural hard substrates (Munari 2013), changing ecosystem functions over small or large spatial scales.

Most of the work on functional groups on artificial reefs has focused on the role of functional groups of fishes (eg. Brickhill et al. 2005, Burt et al. 2009). So far, only one study has looked at different functional groups of benthic invertebrates found on an artificial reef, at a single stretch of concrete breakwater (Munari 2013). Functional groups of organisms can be formed by assigning each taxon traits, such as life history, feeding habits, size and life style, which is known as Biological Trait Analysis (BTA) (Bremner et al. 2006). Using functional groups may be better to measure the responses to disturbance on alternative state communities on artificial reefs than traditional taxa based analyses (Mouillot et al. 2013).

The role of positive interaction through the facilitation of recruitment by habitat-forming taxa, and resulting taxonomic and functional composition of assemblages on artificial reefs were investigated using an experimental approach. Facilitating species and structures were transplanted onto artificial substrate to investigate the role of foundation species on the recruitment of invertebrates. Species providing biogenic structures were selected from fouling communities found on the structures at the experimental site (mussels, bryozoans and corals) and tested as physical facilitators. The hypotheses tested were that plates with more structurally complex facilitators would be colonised by a more diverse community, and that all facilitator plates would contain equivalent functional groups. Findings from this experiment will inform the eco-engineering design of artificial reefs to enhance biodiversity.

5.2 METHODS AND MATERIALS

5.2.1 STUDY SITES

This study was carried out on a small pier at Edithburgh in the Gulf of St Vincent, South Australia. The Gulf has few natural hard substrates available for recruitment, but a patchwork of hard substrates is provided by different artificial reefs scattered throughout the gulf. The Edithburgh pier (Figure 5.1) is surrounded by soft sediment and seagrass meadows, with some natural hard substrates provided by the eroding cliffs on the shore and patches of the large bivalve *Pinna bicolor* in adjacent sediments.

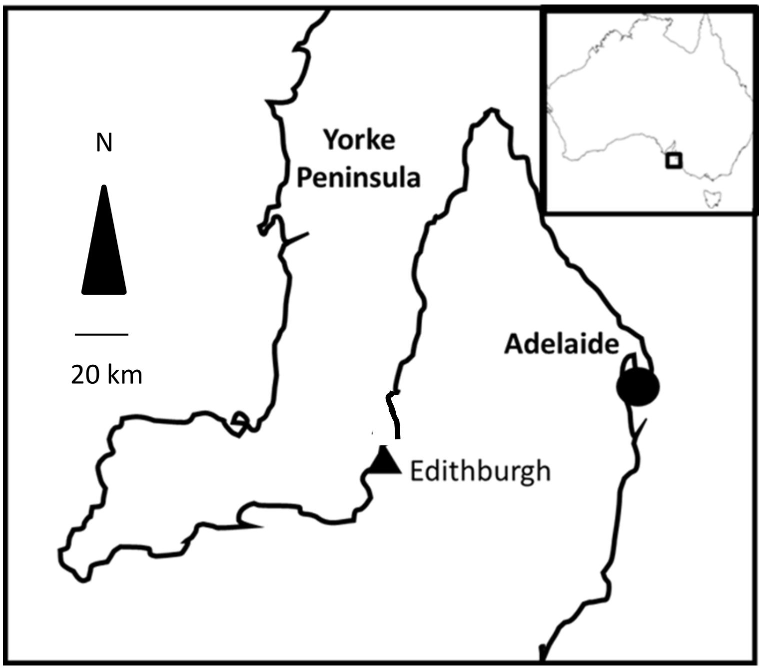


Figure 5.1: Map of the Gulf St Vincent, South Australia. The experimental site at Edithburgh is depicted on the map. The location of the city of Adelaide is also shown across the Gulf from the Yorke Peninsula.

5.2.2 EXPERIMENTAL PROCEDURE

The experiment was conducted on settlement plates (200 mm x 200 mm) constructed from hardiflex fibre sheeting, which has a surface resembling the micro topography of concrete. Five replicate plates were used for each experimental treatment, which comprised three facilitator species and three control treatments (total plates used was 30). Live specimens of the selected facilitators, mussels (*Mytilus* sp.), bryozoans (Order Cheilostomata, *Celleporaria* sp.) and hard/stony corals (*Culicia* sp.) were gathered from the sites by divers and placed in separate aerated containers, before being attached to separate settlement plates each using an inert and commonly used aquarium epoxy (Selleys Knead-it Aqua). The facilitators covered approximately 50% of the surface area of the plate (Figure 5.2). The controls were a bare plate control, procedural control (Epoxy) and abiotic structural control (rocks). To test for general structural effects, the treatments also corresponded to the structure provided based on estimated volume, shape and height, with no relief by the bare control, low relief by the epoxy control, medium relief by the rock and mussel treatments, and high relief by the bryozoan and coral treatments.

The plates were attached to a large steel frame and mounted underwater on the exterior of a northward facing pylon. Replicate plates for the facilitator species and the three controls bare (control), rock (abiotic structure) and epoxy (procedural) were randomly placed on the steel frame using a grid system. The plates were placed 5 cm apart from each other. The experiment was left for six months over the Austral-summer 2012/2013, to allow for recruitment and post-recruitment processes to take place on the plates. Divers checked the frame and attachment of the plates every two months. The plates were removed from the frame in June 2013 and frozen until later analysis in the laboratory. The invertebrates and algae were identified using dissecting microscopes. Non-colonial macro-invertebrates were counted as individuals whilst the algae and colonial invertebrates were recorded as present/absent in the species list.

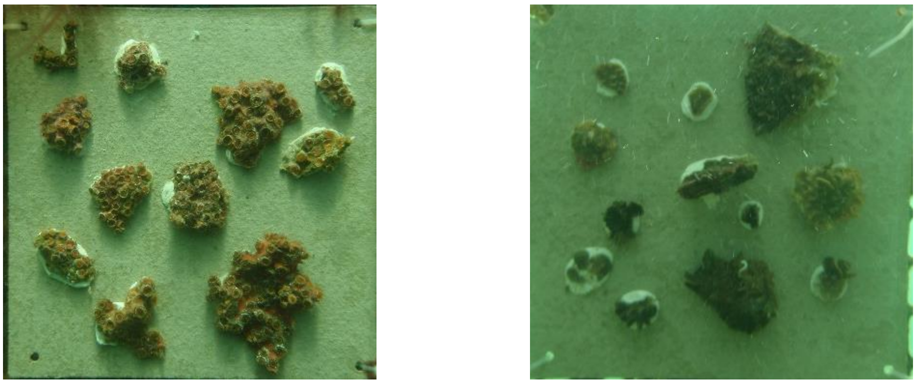


Figure 5.2. Examples of the experimental plates for the coral (*Culicia* sp., left) and bryozoan (right) treatments.

5.2.3 FUNCTIONAL CLASSIFICATION

Biological traits were selected to determine functional groups present in the faunal assemblages on the settlement plates. The traits (Table 5.1) reflected the feeding mode and environmental position of the organisms found on the plates. The environmental position trait was especially important for grouping some of the burrowing taxa such as bivalves that were not sessile or attached (Appendix 1 Supplementary Table 2.3). Infauna-top refers to those taxa found on the exterior of the plate, the infauna-middle group comprises of those that were found within or amongst the facilitators. The modes were combined to form nine functional groups, and taxa were assigned to them following Bremner et al. (2006). The traits were assigned based on literature searches for the organism and laboratory examinations and observations. The European MarLIN database (MarLIN 2006) was also used to inform on the traits. The underlying treatment or facilitating taxa (bryozoan, corals, mussels), were not included in the functional group analysis to avoid skewing the results and in most cases their abundances needed to be measured in percent cover and thus were not comparable.

Table 5.1. Functional group classification using combinations of traits (feeding and environmental position) to differentiate macroinvertebrates on fouling communities (full list appears in **Appendix 1. Supplementary table 2.3**).

Trait	Modes
Feeding mode	Filter Predator Scavenger Herbivore
Environmental Position	Infauna_top Bentho-pelagic Epibenthic Infauna middle

5.2.4 DATA ANALYSIS

The statistical data analysis for this study was undertaken in PRIMER/PERMANOVA+ V.7. The overall diversity of the assemblages was measured using the number of taxa including the colonial invertebrate and macroalgae that were recorded as present/absent in the laboratory. The total number of species and also number of functional groups per plate were tested for significant differences by treatment type (facilitators) using a single factor univariate permANOVA analysis. The numbers of macroinvertebrates that could be recorded as individuals (annelids, molluscs, arthropods) were used to calculate the diversity of non-colonial macroinvertebrates (Shannon-Wiener index H’) for each treatment type. The functional diversity for each treatment was also calculated using the Shannon-Wiener index. The abundances of individuals were compared across treatment types using a single factor permANOVA. Differences in species numbers, functional groups and their abundances were respectively tested for structural relief.

Functional assemblages were illustrated using a shade plot with a cluster analysis on groups using Whittaker's Index of Association, and constrained by a cluster analysis with Type 1 SIMPROF tests (Somerfield & Clarke 2013, Clarke et al. 2014). Community differences were tested with multivariate permANOVA (factor treatment) and further assessed with SIMPER analyses, looking at within group (Treatment) similarity percentages for both taxa and functional group assemblages. The major contributing taxa to those similarities (>10%) were presented in a table. Canonical Analysis of Principle coordinates plots (CAP) were used to test and visualise in two dimensions whether functional group assemblages could be best discriminated by either the structure (high, medium, low, no relief) or treatment type (facilitator species or controls). 2nd Stage tests were used to test how similar the resemblance matrices for the assemblages were for the treatments and relief factors.

5.3. RESULTS

5.3.1 TAXONOMIC DIVERSITY OF INVERTEBRATES AND ALGAE

Five algae and seventy-four different invertebrate taxa recruited onto the settlement plates over the experimental time frame. The mussel treatment plates had the least diverse assemblage which consisted mainly of tube building annelids and the experimental epoxy control had the second lowest number of taxa. The high relief bryozoan plates had the largest numbers of taxa (Table 5.2), and arthropods were the largest contributor to the total number of taxa. This phylum represented most taxa in all of the assemblages found on the different structural treatment. Amphipods were the most diverse group of arthropods found on the plates. The structure that was added to the plate influenced the total numbers of all taxa that recruited into the assemblage (Pseudo-$F_{5,32}$=3.1861, P=0.0206).

The bare control plates did not have the lowest number of taxa, but were colonised by a largest number of bryozoan taxa (Table 5.2), which were all flat encrusting and low relief species from the order Cheilostomata. All of the structural treatments were colonised by the brown algae *Hincksia* sp. (Heterokontophyta), a low relief turfing algae. No green algae were detected on any of the provided structure types. The diversity (Shannon-Wiener index) of non-encrusting macro-invertebrate taxa, which were measured as individuals (annelids, molluscs and arthropods) was greatest on the plates with rocks (Mussels < *Culicia* sp. < Bare < Epoxy < Bryozoa <Rock). The assemblage on the mussel treatment was the least diverse with the lowest number of taxa and H' index value (Table 5.2). The colonial tunicates, *Clavelina* sp. *Botrylloides leachii*, observed on the surrounding pylons, and recorded in chapters 2 and 3, and were also found on the plates in this chapter.

5.3.2 FUNCTIONAL GROUP DIVERSITY

The combined biological traits applied to the taxa found formed nine different functional groups of invertebrates (Table 5.1) out of 16 possible combinations. The epoxy control had the lowest number of functional groups (Table 5.2) whilst the rock treatment had all of the defined 9 functional groups within the assemblages. Both the epoxy control and mussel treatments had a low number of taxa, but although two more functional groups were present on the mussel treatment, they had the lowest functional diversity of all the treatments (Table 5.2). The total number of functional groups found in across treatments was not significantly different ($P > 0.05$).

Table 5.2. Number of taxa from phyla found on the experimental plates after six months exposure. Diversity (Shannon-Wiener diversity H') is presented for the taxonomic assemblages and also shown for functional groups. Diversity was calculated using only taxa recorded with a number of individuals, organisms that were counted as present/absent have been omitted from these analyses. Functional diversity reflects the diversity of groups assigned by different combinations of the modes associated the traits feeding mode and environmental position (Table 5.1). Algae were not included in functional classifications.

	Controls			**Facilitators**		
	Epoxy	**Bare**	**Rock**	**Mussels**	**Bryozoa**	***Culicia* sp.**
Porifera	1	1	4	1	5	4
Cnidaria	0	0	1	1	1	1
Mollusca	5	8	7	7	8	9
Annelida	5	7	9	4	8	7
Arthropoda	17	12	19	9	25	18
Bryozoa	3	9	6	6	8	5
Tunicata	2	4	3	2	3	2
Rhodophyta	2	1	2	3	2	3
Heterokontophyta	1	1	1	1	1	1
Number of taxa	36	43	52	34	61	50
Taxonomic diversity (H')	2.44	2.21	2.85	1.19	2.57	2.05
Number of functional groups	7	9	9	7	8	8
Functional diversity (H')	1.34	1.16	1.52	0.53	1.56	1.17
Number of feeding groups	4	5	5	5	5	5
Feeding Group diversity (H')	0.83	0.64	0.28	1.27	0.89	0.89

5.3.2. ABUNDANCES OF INVERTEBRATES

The high relief bryozoan treatment had the greatest abundance of macroinvertebrates for molluscs and arthropods. The medium relief mussel treatment had the largest individual abundance number of Annelids (Figure 5.3). The total numbers of individuals across the three mobile phyla were significantly different between the bare control (hardiflex) and both the mussels and bryozoan treatments (Pairwise permANOVA, $P < 0.05$). The total abundances of the other settlement plates were not significantly different to each other when compared using pairwise analysis. The annelids dominating on the mussel plates were spirorbids and serpulids tube worms. The high abundances of molluscs in this treatment reflects the high number of Galeommatidae (Bivalvia) on these plates. The burrowing *Musculus nanus* (Mytilidae) was also found on and amongst the mussels, but was less abundant than the Galeommatidae.

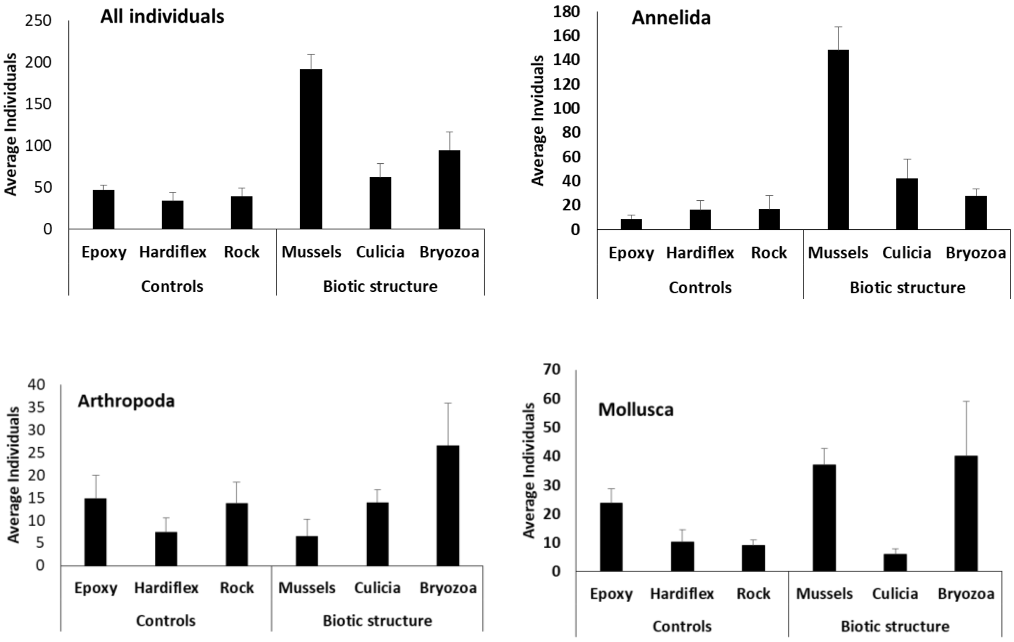


Figure 5.3: Average numbers of all mobile macroinvertebrates, and separately for molluscs, annelids and arthropods per settlement plate for treatments with added facilitator species of different structural complexity, and controls. Error is shown using SE. Note different y-axis scales.

5.3.3 ABUNDANCES OF FUNCTIONAL GROUPS OF MACROINVERTEBRATES

When grouped into functional groups, abundances varied across the treatments. The experimental controls were colonised by most of the functional groups, but they occurred in lower abundances as on the higher relief treatments (Figure 5.4). The largest number of individuals in one functional group (epibenthic filter-feeding) was found on the plates that were covered by mytilid mussels (Figure 5.4B). This group was also the most abundant functional group on the plates treated with *Culicia* sp. This group encompassed all of the tube dwelling annelids (serpulids, spiorbids and sabellids) that were found on the plates. Especially the spionid tube worms were observed in large numbers on the plates in the laboratory examination. Small numbers of *Pomatoceros* sp. (Serpulidae) were also included in this functional group, but were never found with more than six individuals per plate. Whilst this functional group made up the largest number of individuals on both the mussel and rock treatments, variation was high between replicates (Figure 5.4B). The most abundant functional group for the bryozoan treatment was comprised of burrowing bivalve molluscs, mainly *Hiatella* sp. and *Musculus nanus* (Figure 5.4B). The next largest group was the Scavenger Infauna_Top (Figure 5.4 A & B), which was comprised of amphipods and other arthropods.

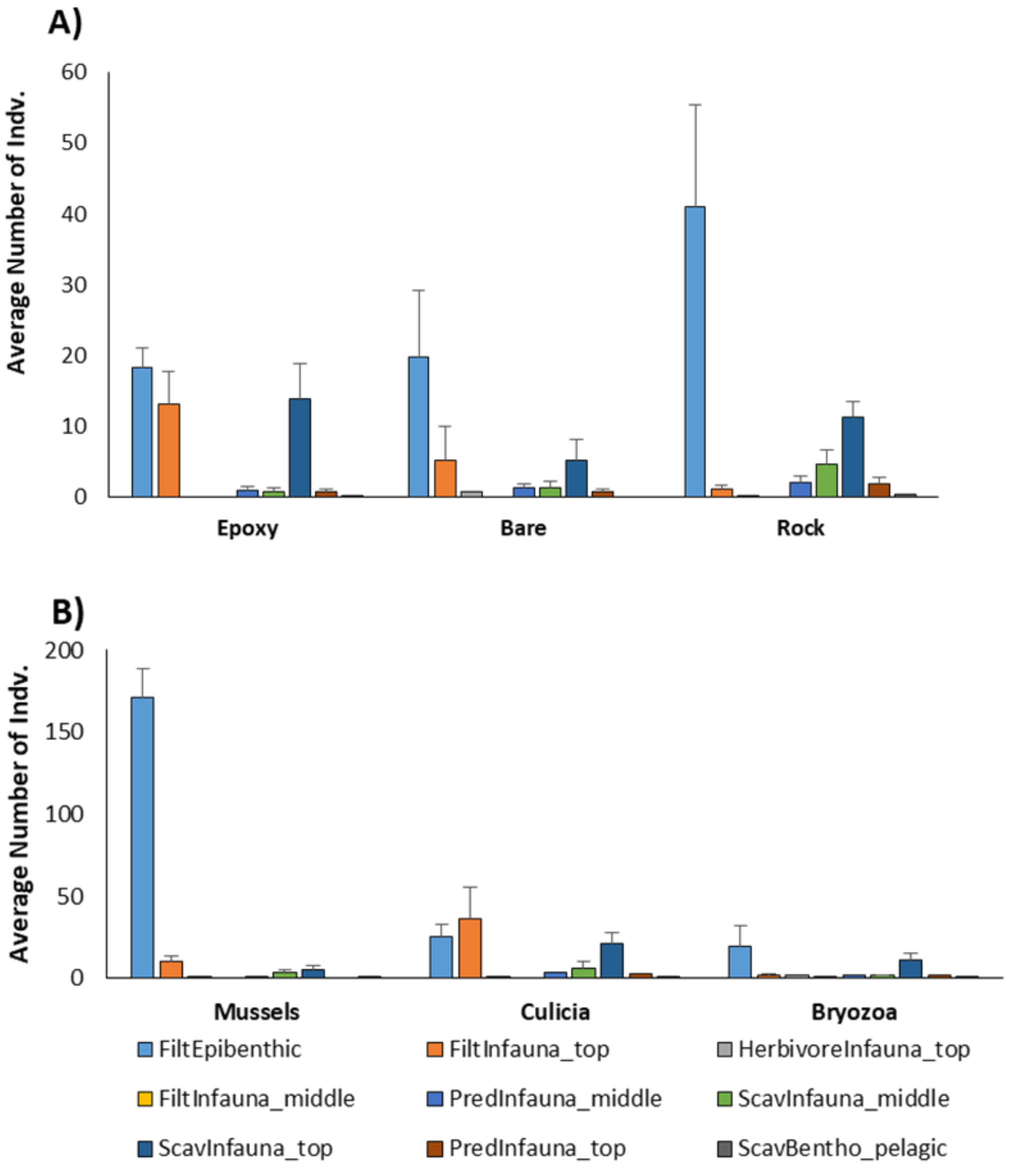


Figure 5.4. Average abundance of organisms in the nine defined (see Table 5.1) functional groups by the treatments **A**) Epoxy, Bare, Rock, and **B**) Mussels, Bryozoa, *Culicia* sp. Functional group abbreviations are as follows; Filter-Feeding Epibenthic (Filt-Epibenthic), Filter-Feeding Infauna_Top (Filt-Infauna_Top), Herbivore Infauna_Top (Herb-Infauna_Top), Filter-Feeding Infauna_Middle (Filt-Infauna_Middle), Predator Infauna_Middle (Pred-Infauna_Middle), Predator Infauna_Top (Pred-Infauna_Top), Scavenger-Infauna_Middle (Scav-Infauna_Middle), Scavenger Infauna_Top (Scav-Infauna_Top), Scavenger Bentho_pelagic (Scav-Bentho-pelagic). Error is shown using +SE.

Table 5.3. Test outcomes for assemblage differences based on taxonomic compositions and functional traits (combined and separately) for single factor (treatment) permANOVAs.

Variable Group	df	MS	F_{perm}	P (perm)
Assemblages Res 24	5	4156	2.53	P <0.001
Feeding mode Res 27	5	745	3.278	P<0.001
Environmental Position Res 24	5	578.2	2.485	P<0.01
Functional groups Res 24	5			P<0.01

Table 5.4: SIMPER analysis of macroinvertebrate assemblages on treatment plates. Macroinvertebrates that are contributing to the similarities within treatment groups are shown (>10% contributors).

Treatment Epoxy	Average sim. 40.4%	Contribution %
Monia sp.		29.9%
Musculus nanus		19.6%
Spirorbidae		10.1%
Treatment Mussels	Average sim. 67.5%	Contribution %
Spirorbidae		28.42%
Galeomatidae		16.75%
Pomatoceros sp.		10.77%
(No treatment) Bare	Average sim 33.1%	Contribution %
Monia sp.		40.29%
Musculus nanus		15.29%
Spirorbidae		10.2%
Tanaid sp.		10.16%
Treatment *Culicia*	Average sim 41.3%	Contribution %
Spionidae		28.1%
Amphipod sp.7		21.2%
Pomatoceros sp.		10.8%
Treatment Bryozoa	Average sim 33.4%	Contribution %
Amphipod sp.7		21.1%
Musculus nanus		18.9%
Spionidae		17.6%
Treatment Rock (abiotic)	Average sim 33.8%	Contribution %
Monia sp.		23%
Corophium sp.		11.1%
Pteridae		10.4%
Cerodocus sp.		10%

5.3.4 ASSEMBLAGES OF NON-COLONIAL INVERTEBRATES

The assemblages on the settlement plates were significantly different between the experimental treatments (Table 5.3). Pairwise comparisons found that only two pairs of assemblages were not significantly different to one another (plates with bryozoans and *Culicia* sp., and bryozoans and rock). The within group similarities were all lower than 50% (except for the mussel treatment) (SIMPER, Table 5.4), indicating that the recruitment onto the plates was also variable within the treatments. The bivalve *Monia* sp. was the greatest contributor to the within group similarities for the rock and the two controls, epoxy and bare (Table 5.4). The bare plates and epoxy treatments both also had spirorbids as the second highest contributor to the within group similarities.

5.3.4 ASSEMBLAGES OF FUNCTIONAL GROUPS

The similarities of the assemblages on the settlement plates, when placed into functional groups, were different to those that were determined considering taxa. Using a 2Stage comparison, the separate resemblance matrices between the two sets of assemblage data showed that they shared 61% similarity. The difference in similarity can be attributed to greater overlap of functional groups between all of the experimental treatments.

The functional group assemblages varied significantly between treatment groups (Table 5.3). The greatest within treatment similarities (SIMPER) for functional groups were found for plates with *Culicia* sp. and mussel transplants (Table 5.5). Shadeplots revealed that the treatment with the non-living rock substrate had almost equal abundance of all the categorised functional groups (Figure 5.5), and SIMPER analysis gave the second lowest within group similarity for this treatment (Table 5.5). On the plates that were treated with mussels, the functional group defined as Filter-feeding Epibenthic was very abundant (Figure 5.5). This group comprised several spionid and serpulid tube worm taxa and contributed to almost 95% of the within treatment similarity (Table 5.5).

Table 5.5. SIMPER Table showing the group similarities (by functional group assemblages) and the functional groups that contributed to that similarity (>10%). Functional groups were created using combinations of the selected Biological Traits (Table 5.1). Functional group names: Filter-Feeding Epibenthic (Filt-Epibenthic), Filter-Feeding Infauna_Top (Filt-Infauna_Top), Herbivore Infauna_Top (Herb-Infauna_Top), Filter-Feeding Infauna_Middle (Filt-Infauna_Middle), Predator Infauna_Middle (Pred-Infauna_Middle), Predator Infauna_Top (Pred-Infauna_Top), Scavenger-Infauna_Middle (Scav-Infauna_Middle), Scavenger Infauna_Top (Scav-Infauna_Top), Scavenger Bentho_pelagic (Scav-Bentho-pelagic).

Bare **Average similarity 31.6%**		**Epoxy** **Average similarity 58.7%**	
Filt-Epibenthic	70.9%	Filt-Epibenthic	51.5%
		Scav-Infauna_Top	25.7%
Rock **Average similarity 40.6%**		**Bryozoa** **Average similarity 45.2%**	
Scav-Infauna_Top	42.1%	Filt-Epibenthic	45.1%
Filt-Epibenthic	39.6%	Scav-Infauna_Top	24.8%
		Filt-Infauna_Top	24.8%
Mussels **Average similarity 80.9%**		***Culicia* sp.** **Average similarity 56.97%**	
Filt-Epibenthic	94.1%	Filt-Epibenthic	65%
		Scav-Infauna_Top	24.84%

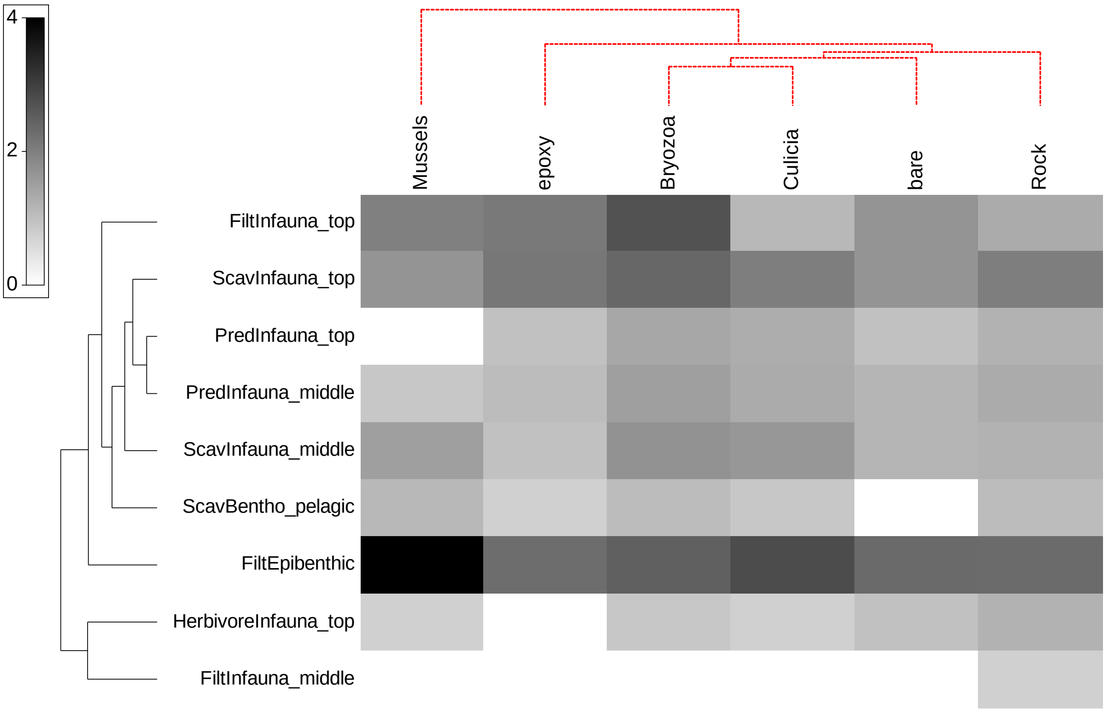


Figure 5.5. Shade plot of Functional Group assemblages across the treatments**.** Functional groups and treatments are ordered by separate cluster analysis, and the treatment cluster is constrained by SIMPROF test outcome with black lines showing significantly different clusters, and red lines cluster denote are not significantly different. Functional group names: Filter-Feeding Epibenthic (Filt-Epibenthic), Filter-Feeding Infauna_Top (Filt-Infauna_Top), Herbivore Infauna_Top (Herb-Infauna_Top), Filter-Feeding Infauna_Middle (Filt-Infauna_Middle), Predator Infauna_Middle (Pred-Infauna_Middle), Predator Infauna_Top (Pred-Infauna_Top), Scavenger-Infauna_Middle (Scav-Infauna_Middle), Scavenger Infauna_Top (Scav-Infauna_Top), Scavenger Bentho_pelagic (Scav-Bentho-pelagic).

Canonical analysis of principle coordinates (CAP) were used to test the hypothesis that the foundation species on the plate will predict the type of assemblage that was found on the plate at sampling. Alternatively, the hypothesis that the relief (low, high and none) provided by the treatment could predict the type of assemblage (Table 5.6) was also tested. The treatment type provided a spatial representation that reflected some differentiation of functional groups (Figure 5.6A), but mostly overlap in assemblages (Figure 5.6A). The type of relief that the facilitator species provided also showed little difference in the predicted similarities between and within functional groups by treatment (Figure 5.6B). The plate treatment, when used to predict the assemblages misclassified 16.7% (5/30 Correct) of the samples whilst the relief, defined at none, low, medium, and high, misclassified 63.3% (11/30 Correct) of the samples (Table 5.6). For the taxonomically grouped non-colonial macroinvertebrates (Figure 5.6 C and D), more distinction by treatment emerged, and relief was the best predicator of assemblages (misclassification 63.3%, 21/30 correct).

Table.5.6 CAP diagnostics for CAP analysis (Figures 5.6A, B, C, D) to test whether Functional group and non-colonial macro invertebrate assemblages could be best allocated by factors Treatment and Relief.

	Functional Groupings	
	Treatment	**Relief**
Number of samples	30	30
m	2	3
Total correct	5	11
Misclassification error	16.7%	63.3%
Trace statistic	P=0.1413	P=0.082
Delta statistic	P=0.1792	P=0.1255
	Non-colonial Macro invertebrate assemblages	
	Treatment	**Relief**
Number of samples	30	30
m	10	10
Total correct	16	21
Misclassification error	46.7%	30%
Trace statistic	**P=0.0001**	**P=0.0002**
Delta statistic	P=0.0016	P=0.0002

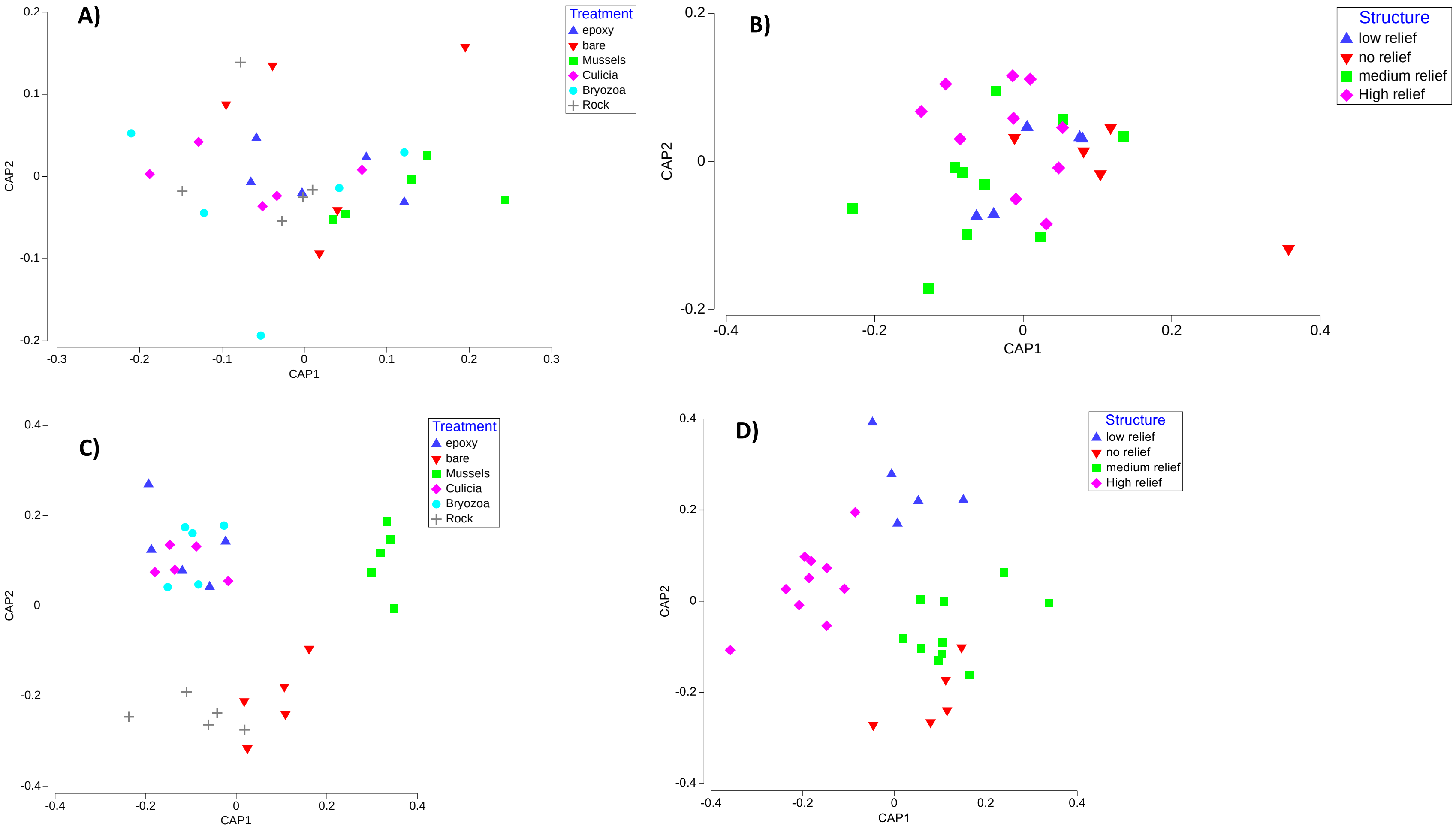


Figure 5.6. CAP analysis plots of **A)** Functional group assemblages of macroinvertebrates predicted by treatment type, **B)** Functional group assemblages of macroinvertebrates predicted by relief type, **C)** Non-colonial macroinvertebrate assemblages predicted by treatment type, **D)** Non-colonial macroinvertebrate assemblages predicted by relief. CAP testing results appear in Table 5.6

5.4. DISCUSSION

The presence of foundation species and the associated positive processes of facilitation can structure assemblages on artificial reefs. The provision of additional structures on an artificial surface in this experiment did show higher diversity and abundances for macroinvertebrates on plates with more structurally complex foundation species, such as hard encrusting cheilostomata bryozoans and corals. There was no greater facilitation of defined functional group types of organisms onto any treatments, with nearly all defined groups represented amongst the provided structural types and control plates.

5.4.1 RECRUITMENT AND FACILITATION

The plates had similar species compositions but the abundances varied between treatments. This suggests that the supply of larvae and mobile juveniles and adults were the same for all plates, but facilitator species promoted higher abundances of some taxa over others. Plates with larger, physical structure providing facilitators evaluated in this experiment, such as bryozoans and corals, had larger abundances of the mobile macroinvertebrates than plates with the low relief abiotic (rock) and experimental controls. These two facilitators may also feed on or compete with some of the mobile invertebrates (larvae or adult form). The physical structure or relief provided by facilitators was the most important determinant of assemblages in this experiment, with the selected structural types. This is often the cause of variations in mobile macroinvertebrate assemblages across sampled patches on artificial reefs (Schroeter et al. 2015, Suzeiro et al. 2011). Greater effects on the mobile species diversity were anticipated, as sessile invertebrates are less affected by manipulating the densities of facilitators (Bros et al. 1987).

Different assemblages were found on the different treatments. The particular responses of a community to ecosystem engineers or facilitating species will depend on the requirements of different recruiting species (Suzeiro et al. 2010). Most of the taxa were found across the treatments, but the foundation species either facilitated greater recruitment of some taxa or provided more resources for individuals moving between patches of substrate. Some of this was seen in the small-bodied bivalves (Galeommatidae) found on the mussel plates, or the large numbers of spionid worms on some of the other treatment groups. The large number of burrowing bivalves found on the mussel plates, can possibly be explained by accumulation of sediment observed on these plates, which can increase mobile taxa numbers (Yakovis et al. 2005), but no sediment accumulations were noticed on the vertically deployed plates in this experiment.

The role of biotic structures in providing habitat and recruitment opportunities is an added level of ecological complexity within a fouling community on an artificial reef (Schroeter et al. 2015). Large physical structure providing species, like the bryozoans and coral, had greater diversity within the assemblages. Species richness and density will increase with habitat complexity (Schroeter et al. 2015), however total density can decline within assemblages when there is increased competition by the new arrivals (Suzeiro et al. 2011). The different macroinvertebrate assemblages that were found on the treatments included not only recruiting organisms, but also mobile visitors from other patches of nearby substrate. The number of treatments tested on the experimental frame set up may have resulted in a localised increase in species diversity, as the number of foundation species within habitat patches can increase diversity (Yakovis et al. 2008).

All of the foundation species that were provided in the treatments were filter feeders. The addition of filter feeding physical ecosystem engineering species such as bryozoans can increase diversity, however these can also inhibit recruitment by larval predation (Cruz Suzeiro et al. 2011). Artificial reefs are known to themselves facilitate the settlement of large numbers of filter feeders, often in areas where there are no natural hard substrates and above benthic soft sediments (Zintzen et al. 2008, Maar et al. 2009). Increased predation of planktonic larvae will influence the available supply and potentially alter the facilitation cascades (Halpern et al. 2007) of different assemblages as well as in the surrounding habitats.

5.4.2 FUNCTIONAL GROUPS ON ARTIFICIAL REEFS

Artificial reefs can have very different assemblages to those that are found on natural reefs both in terms of taxa and their abundances (Maar et al. 2009). Habitat heterogeneity and facilitation provided by physical ecosystem engineers lead to more variations in assemblages, between patches on the same reef. Understanding the interactions between facilitating taxa and overall diversity and succession in reef assemblages is important, but the numerous permutations possible because of post and pre-settlement processes, make this an often impossible task for resource managers. Transplanting, removing or sponsoring the growth of foundation species can give managers options to meet some of the reef objectives by fast tracking desirable facilitation cascades to produce more similar communities (Halpern et al. 2007). As governments and non-government organisations (NGOs) build reefs or coastal infrastructure (unintentional reefs), focus is shifting to the need to identify and use materials or surfaces that will promote settlement (Dafforn et al. 2015B, Strain et al. 2018). With this in mind, it would be important to emphasise the importance to create structures that either are seeded or constructed with surfaces that will promote the settlement of high relief facilitating species.

Identifying physical ecosystem engineers that facilitate the recruitment of invasive species, can be important for biosecurity management, as climate related invasion pressure increases in coastal seas (Altieri et al. 2010). Patches of high relief facilitating species can be immediately collected *in-situ* and examined for small interstitial invasive species, which could minimise search times and monitoring cost. This method would not need the deployment and maintenance of specialised collection devices such as settlement plates or larval traps. Selected habitats could be targeted for specific invaders of concern. The few invasive taxa that were found in this experiment were amphipods (*Corophium* sp., *Jassa* sp.) and some of the colonial ascidians (*Botrylloides* spp.), all of which are now widespread in the region and were most probably first introduced centuries ago (Hewitt et al. 2004). None showed preference for any of the facilitating taxa provided in the experiment. Large encrusting and complex shaped cheilostomata bryozoans would be a candidate for monitoring for invasive amphipods, whilst the plate with mytilid mussels could be useful for detecting other invasive mussels.

A better approach to artificial reef monitoring from a functional group perspective would be to determine if artificial reefs can provide the same ecosystem services using different assemblages. Indeed, functional groups may already be a better way to measure community responses to disturbance (Mouillot et al. 2013), with many artificial reefs being long term disturbances to the surrounding soft benthos. Strong positive relationships have already been found between functional diversity and species diversity for fish (Micheli & Halpern 2005).

The biological traits exhibited by benthic organisms can provide a wealth of information on their role in ecosystem functioning (Bremner et al. 2006). The traits that were selected in this study were chosen to best represent important characteristics in the early recruitment stages of an assemblage, such as larval stage and mobility. Most functional groups defined in this study, were found on all treatment groups but the numbers of organisms within the functional groups did vary between treatments. Less abundant organisms within some of the functional groups found on a treatment, could mean that resilience within that patch is weaker than in treatments that had multiple representatives from that functional group.

Reefs are not homogenous communities and the creation of different patches by different foundation species may be responsible for spatial and temporal community variations (Yakovis et al. 2008). The assemblages of functional groups found on the different settlement plates had very different similarities both within and between treatment types. Functional group similarities between patches on a reef in Europe also had significantly different assemblages (Munari 2013); most probably caused by habitat heterogeneity, as was found between the treatment groups in this study. Each of the different treatment groups was not defined by a singular functional group as was expected. Most of the within group similarity across the treatments, was caused by the numbers of a particular functional group, that was mostly comprised of encrusting tubeworms. The two control treatments did not have significantly less functional groups present, indicating that structure alone might not be necessary for functional diversity. Facilitation cascades are common in aquatic arthropods and when grouped together can create functional groups that will outperform species monocultures (Cardinale et al. 2002).

Comparisons between the treatments will inherently depend on the definitions of functional groups adopted in this study. Utilising a multi-trait approach is useful as various traits or characteristics can be combined (Bremner et al. 2006), creating more encompassing and/or directed functional groups. Organisms grouped only by trophic categories can simplify and downplay, what is usually a positive relationship between species and functional diversity (Micheli & Halpern 2005). However, biological trait analysis (Bremner et al. 2006) will depend on the information available for the taxa, and the combinations may not reflect relationships within the functional groupings, such as competition.

5.4.3 CONCLUSIONS

Facilitation is an important process that can lead to very different assemblages of taxa on the same artificial substrate. Increasing the settlement of species that are providing biotic structure can especially help to increase and maintain diversity on artificial or restored reefs should be factored into the planning stages of artificial reefs (Ido & Perkol-Finkel 2015). Biotic structure can also help to increase the complexity on the surface of artificial reefs that lack the surface topography of natural coastal hard substrates (Firth et al. 2014). The facilitator species that were used in this study were colonised by very different assemblages of taxa and functional groups. Analysing how facilitation can affect the diversity, assemblage type and functional groups present within patches, can help to explain some of the variation within reefs. However, the selection of facilitators needs to be carefully considered as new potential facilitators may colonise and shift the development of the fouling assemblage. The variability of facilitator species or physical ecosystem engineers increases assemblage heterogeneity within fouling communities on hard substrates. The identification of functional groups that provide valuable ecosystem services and then the promotion of those groups may be a good management outcome for some artificial reefs (Pratt 1994).

More in-situ and longer term studies are needed to examine whether targeted removal for some facilitating species, would be effective in detecting early invasive cryptic species. Long term studies are also needed to look at how functional groups are facilitated by facilitator species and later succession stages of a fouling community. The classification of the functional groups would also need to be further refined to match the objectives, which need to explore the ecosystem functions and services that artificial reefs can provide or align with those that are provided by natural reefs. There are many practical advantages to understanding the role of facilitators in structuring communities, especially on artificial reefs where some of the objectives may be to restore damaged or destroyed natural populations.

CHAPTER 6. GENERAL DISCUSSION OF RESULTS AND FUTURE DIRECTIONS

This chapter summarises some of the key findings from this thesis and provides recommendations on future artificial reef research and monitoring studies. The increasing urbanisation of coastlines around the world (Bulleri & Chapman 2010, Dafforn et al. 2015a, Firth et al. 2016b), coupled with new frontiers in resource extraction such as deep sea oil (Forteath et al. 1982, Butler & Connolly 1996) , marine renewables (Andersson et al. 2009) and fisheries enhancements (Pickering et al. 1999), have meant an escalation of artificial hard substrates available for settlement. Many of these substrates are located in close proximity to population centres (Bulleri & Chapman 2010, Dafforn et al. 2015a, Firth et al. 2016b), this may increase invasion susceptibility because of the maritime transport corridors. New hard substrates are creating unintentional and intentional artificial reefs, hosting communities that can be very different to those found on natural hard substrates, as this study has shown. This study has sought to investigate some of the post-settlement processes that affect succession, leading to different assemblages on artificial substrates. Artificial reefs are not usually made up of equivalent materials and structure as natural reefs and this results in fouling communities that are different from the surrounding reefs (Perkol–Finkel et al. 2006, Badalmenti et al. 2002). The scale of the ecological change may be less visible than it is on land, but the creation of ‘hard substrate islands’ amongst soft sediment habitats or the creation of ‘alien’ dominated fouling communities adjacent to natural reefs will have long term effects on the resilience of marine communities.

Active substrate selection (Connell 2000) and therefore derived differences in assemblages were expected in all parts of this project, but findings showed that a complex chain of events or variability in recruitment may determine the assemblages found on artificial reefs. As the scale of post-settlement processes or the extent of the supply-side ecology may be limited by the distance to adjacent hard substrates, regional similarities in fouling communities have been demonstrated to be stronger than those between the same substrates on larger spatial scales (Osman 2015).

6.1 SUMMARY OF RESULTS

The first data chapter (Chapter 2) evaluated the sampling methods commonly used in fouling assemblage studies. Both photo-quadrats and specimen collections through scrapings were analysed. The increased use of photo-analysis and percentage cover estimation makes temporal monitoring easy and is a non-destructive collection method that minimises or prevents disturbance to the reef (Meese & Tomich 1992). Digital analysis has the advantage that substrates are not damaged, but clear limitations of the method became apparent. The level of taxanomic resolution is low as it is restricted to large obvious taxa, as was previously found (Macedo et al. 2006). The number of non-sessile and cryptic species missed by the photo analysis meant that this method, when used on its own can underestimate differences between sites and different reef substrates. My comparative assessment corroborated that cryptic or smaller organisms can be missed by photo-quadrats (Zintzen et al. 2008), which can obscure analyses of subsequent succession pathways. Furthermore, the two dimensional percentage cover estimates are not adequately assessing the complex three dimensional structures provided by some epibiota, such as certain colonial filamentous ectoprocts. These complex structures were shown to facilitate a diverse fouling assemblage (Chapter 5). Photo-quadrats also miss some of the burrowing fauna such as mussels (*Musculus nanus)* found inside sponges, often in high abundances when the samples were physically examined. This may influence the estimates of ecosystem services such as filter feeding, that the reef is providing to the surrounding environment. Photo-quadrats are also dependant on the presence of easily identified large macroinvertebrates or algae, while cryptic algae or invertebrates are grouped into an ‘other’ category, limiting the variables which can be analysed (Somerfield & Clarke 1995). However, advances in cameras and image processing software will increase the capabilities for lower taxonomic resolution.

The overall assessment of fouling assemblages on established artificial and natural reefs was thus found to differ subject to the sampling method used. This method also affected the evaluation of similarities between the fouling assemblages on the different artificial reefs. This finding illuminates problems when studies that have used different methodologies are compared, i.e. the conclusions or level of similarities of community on a concrete reef that has been sampled using photo-quadrats would not be comparable with another study that directly sampled the surface using scraping. Additional consideration arises for studies looking at NIS, as without resorting to actual physical sampling, cryptic and interstitial species can be missed. However, the disturbance to the fouling assemblage during physical scraping may inhibit long term monitoring outcomes.

This chapter contributes to raising the awareness of benefits and constraints from each of the two most commonly used methods to assess fouling assemblages. However a decision on the methods used will also depend on the targeted biological components/systems and the temporal and spatial scale of a project.

The comparison of fouling assemblages on long established artificial and natural reefs assessed with both methods was taken further to look at substrate specific assemblages and relationships between the epibiota (from photo-quadrats) and associated mobile invertebrates obtained from scrapings (Chapter 3). All the reefs that were sampled in Chapter 3 had very different fouling assemblages from each other. The dissimilarity was also found for the reefs that had the same underlying substrate, suggesting that factors other than substrate may play a greater role in the development of fouling assemblages. Assemblages on artificial substrates may be more or less similar to natural reefs, depending on the adjacent hard substrates, regional hydrology, larval supply (Underwood & Fairweather 1989) and frequency of disturbance.

Despite a long history of purpose built artificial reef creation, there are too few peer reviewed Australian studies comparing fouling communities on artificial to natural reefs (Baine 2001). However, Kay & Keough (1981) compared the recruitment of fouling communities onto cleared patches on pier (jetty) pylons and the shells of bivalve *Pinna bicolor.* Walker and Schlacher (2014) found that even after 3 years, the fouling community on a large artificial reef was still very different to that which was found on nearby natural reefs. In my study many of the organisms sampled were only found on the artificial reefs and the site with the highest species richness was the steel ship wreck. The large number of taxa at the *SS. Hougomont* were mostly comprised of mobile epifauna, sponges and bryozoan species. Large abundances of tubeworm filter feeders has been recorded on other steel wrecks in Belgium (Zitzen et al 2008) and Australia (Walker & Schlacer 2014). Steel is a relatively modern artificial material that is being used as a reef material through surplus/sunken vessels (Walker et al. 2007, Walker & Schlacher 2014, Appendix II), oil rigs (Macreadie et al. 2011) and purpose built fish habitats (Grove et al. 1989). Shipwrecks have been previously found to host extremely diverse and unique assemblages (Zintzen et al. 2006). The large diversity found on what is a relatively old vessel (>80 years) shows that the steel can support more diverse assemblages than natural reefs, even after long submersion times. A similar long-lived distinction between fouling assemblages on steel and natural reefs has been shown by Perkol-Finkel et al. (2006) (See Appendix II).

The purpose deployed artificial reef sampled in this study was a tyre reef that was part of a state wide project of similar reefs built thirty years ago (Branden et al. 1994). The tyre reef did not have abundant large macroalgae or colonial macroinvertebrate species cover like the other sites. However, while large epibiota were absent from the reef, the numbers of some non-colonial macroinvertebrates, in particular small bivalves and solitary ascidians were higher than any other reef sampled. The lack of epibiota may be explained by the ability of the underlying tyre reef substrate to flex (Reimers & Branden 1994) and thus shed off large hard encrusting species or even soft encrusting species. This structural attribute of tyre reefs create a potentially unintended regular disturbance regime reducing the diversity of epibiota on these artificial reefs (Connel & Slatyer 1977). The negative physical effects of tyre reefs has previously reported (Collins et al. 2002), but the further effect of the ‘flexing’ on the associated fouling assemblage was not yet known. Tyres reefs are also suspected to be leaching heavy metals into the marine environment, in particular zinc (Collins et al. 1995). This study revealed that the physical disturbance induced the absence of facilitator species in the epibiota, leading to very distinct assemblages of mobile and non-colonial invertebrates on the tyre reef. This was characterised by higher abundances of small bivalve species such as *Musculus* sp. and *Hiatella* sp.

The species richness for the rocky reef sites varied between two sampled sites, but mobile fauna, such as arthropods and molluscs, were found at both. In the Gulf St Vincent (GSV), small rocky reefs or broken rock/rubble bottoms are dominated by macroalgal cover (Bryars & Rowling 2009); and the two surveyed rocky reefs were not an exception. Second Valley had more Heterokontophyta present, including *Cystophora* spp. and *Sargassum* spp., Frenchmans Cap had none in the sampled area. The cover can be attributed to adjacent algae, although the holdfasts were not present in the scraping samples of the same quadrats.

The two introduced tunicates, *Botrylloides leachii* and *Ciona intestinalis* are commonly found on coastal artificial reefs in the GSV region. *B. leachii* was observed on plates in the settlement experiment in chapter 4 and the facilitation experiment in Chapter 5. Networks of new artificial reefs could act as stepping stones for non-indigenous species (NIS) to link unconnected areas (Zintzen et al. 2006, Sheehy & Vik 2010, Bishop et al. 2017) or add to the local larvae pool. The artificial reefs sampled in this study did not have a higher occurrence of these long-established NIS than the natural reefs. European fan worms, *Sabella spallanzani*, have been previously observed in high numbers on the Kingscote jetty site but had been removed before this study took place by local divers.

The design of the sampling in this chapter and the preceding one (2) were influenced by logistics. The scattered locations (Branden et al. 1994) and varied weather and sea conditions for the reefs limited the design, as there was often only so much the divers could do once on site. Many of the remote reefs, such as the Kingscote Tyre Reef and the wreck of the *SS Hougomont* had either never been surveyed or not dived on for decades. The *SS Hougomont* and the *ex-HMAS Hobart* (See Appendix 2) are both protected shipwrecks that required extensive negotiation with both state and commonwealth shipwreck maritime archaeologists. The heritage and social value of the reefs vs their ecological value is yet another approach that needs to be further explored. The heritage considerations and sampling trip conditions provided additional challenges to the goal of comparing the existing reef communities between reefs for this study, however the same would apply to any other comparative study.

Strong substrate and seasonal recruitment trends (Reimers & Branden 1994, Glasby & Connell 1999) were expected in the settlement plate comparison (chapter 4), highlighting the paramount importance of substrate choice. However, this study showed that for recruitment onto settlement plates, the site was more important than the substrate. Shifts in the mobile macroinvertebrate assemblages over regional distances were also found by Foster et al. (2014). As with chapter 3, the location of the reef affected what larvae were available to settle, followed by seasonal and then substrate effects. Steel and rubber settlement plates had high percentages of empty space. The empty space on the rubber plates was similar to the empty space found on the old established tyre reef in Chapter 3. The large amount of empty space on the steel settlement plates was, however, unexpected as the steel reef that was the former *SS Hougomont* was covered in dense algae, sponges and tunicates. The surface corrosion on the steel plates could have caused much of the assemblage that grew on the plate to be lost or inhibited from recruiting over the timeframe of the experiment. Some colonial encrusting bryozoans were found on the steel plates, but these had only a few ovoids (individuals) in each colony. The diversity of the assemblage on the *SS Hougomont* does mean that the rate of surface corrosion at this scuttled vessel did not prevent settlement. The surface of steel substrate can be stabilised by the cover of some of the early pioneer species like the *Didenumn* spp., facilitating succession. The relationship between pioneer encrusting species and the corrosion of surfaces is an area that requires future research, especially as steel reefs are becoming more common (See Appendix II).

By understanding the ecological processes in fouling communities on artificial substrate, marine managers will be able to design reefs or provide secondary structural complexity that encourages the growth of a fouling community that will minimise the impact of the artificial substrate on the surroundings (Dyson & Yocom 2015, Dafforn et al. 2015b) or maximise its ability to be analogous to the surrounding natural substrates. Chapter 5 sought to identify biogenic habitats or foundation species that could be used to facilitate the recruitment of a diverse set of functional groups, which could increase resilience or reduce establishment of invasive species (Dafforn et al. 2015b, Greenfield et al. 2016). While there was no greater facilitation of any defined functional groups of organisms onto any treatments, with nearly all defined groups represented amongst the provided structural types, the provision of additional structures showed that the presence of more structurally complex foundation species led to higher diversity and abundances for macroinvertebrates recruiting on to the experimental plates. The use of biotic structures not only increases surface complexity (Firth et al. 2014, Ido & Perkol-Finkel 2015), but this study has shown the relief provided will increase functional diversity and perhaps ultimately resilience. Focusing on promoting positive interactions with facilitators, will also enable reef managers to minimise risks of artificial reefs having very different functional group assemblages (Munari 2013).

Functional groups offer a relatively easy way to measure the health of an ecosystem (Norkko et al. 2015), however identifying these will still require more basic initial reef assessments. For fouling communities, this method can be applied with the use of non-destructive sampling, although in this experiment, the interstitial fauna was also considered, which required the destruction of the sample on the settlement plate. Using functional groups may be a new realistic perspective for the management and comparative analyses of artificial reefs on larger geographic scales (Firth et al. 2014), given communities upon them remain so distinct from natural reefs. The presence of most of the functional groups across the treatments does demonstrate that facilitating species or ecosystem engineers, can lead to alternative assemblage states existing between patches

(Baskett & Salomon 2010) or reef types (Munari 2013). The addition of facilitating species may help modern marine infrastructure to be designed to incorporate and foster biodiversity, adding to resilience (Dafforn et al. 2015b, Greenfields et al. 2016). Addition of biogenic or artificial (Coombes et al. 2015) structure can help to make up for simple or less complex and uniform surfaces, which are usually provided on artificial reefs (Firth et al. 2014).

6.2 CONCLUSIONS AND RECOMMENDATIONS

Artificial substrates have been a habitat for thousands of years, but their prominence is increasing as Ocean Sprawl intensifies (Firth et al. 2016b). The focus on fishery enhancement benefits and questions regarding the concept of attraction vs. production have dominated artificial reef research since the 1980s (Bohnsack 1989). However, as demonstrated by this study and others (especially those on artificial substrates) for invertebrates and algae, artificial reefs can produce assemblages that are often very dissimilar to those on surrounding hard substrates, even after a considerable time spent submerged (Perkol-Finkel et al. 2008, Walker & Schlacher 2014). There is still much left to investigate to understand how pre- and post-settlement processes affect invertebrate fouling assemblages on artificial substrates. The attraction vs. production concept needs to be looked at with specific reference to the invertebrate fouling assemblages on an artificial substrate. The usefulness of artificial reefs for habitat restoration is limited, due to the uncertainty that remains about the substrate preference and selectivity for settling larvae. A lot of reefs are deployed for enhancement or the establishment of an alternative ecosystem (Pratt 1994), with the potential for unique, unpredictable and long lasting assemblages of species to form (Walker et al. 2007). Smart coastal development such as the adaption or repurposing of offshore structures to other uses, or combining multiple uses on one structure could limit the impact and offer new opportunities for restoration (Dafforn et al. 2015a).

For any reef with ecosystem restoration objectives, management must be equipped to monitor the site, including measures of structure and function (Pratt 1994). Artificial reefs have an important role to play in future habitat restoration, but more work is needed to better understand their implications on the development of fouling assemblages and how to best intervene and use natural processes to achieve outcomes. There is a need for more comparative designs looking at recruitment onto artificial substrates with sufficient replication over space and time. The recruitment processes operating at different spatial and temporal scales can lead to significant seasonal variations in flora and fauna settling upon disturbed and undisturbed patches of substrate. The ecological processes make it hard to predict or generalise the assemblages of species in fouling assemblages at certain points of time and space. It is especially concerning that many artificial substrates are found in harbours where they are ideal habitats for sessile and non-sessile invasive species (Airoldi et al. 2015). New technology and methods on the horizon such as eDNA (Bohmann et al. 2014) and high frequency sonars (Arney et al. 2017) will also allow cryptic reef assemblages like fish and interstitial invertebrates to be quickly and cheaply sampled with higher accuracy.

Future research is needed to look at the similarity of an artificial reef to the surrounding hard substrates and the prevailing currents. From the work undertaken in this thesis, the distance to adjacent hard substrates emerged as important for the early stages of a community. This could be the inverse in later stages, as a larger number of recruits from the artificial substrates may contribute to the diversity of the adjacent reefs whilst increase the reefs resilience to small scale disturbances. Proximity of adjacent hard substrates may reflect the resilience of the assemblage on an artificial reef. Future research is needed to look at the similarity of an artificial reef to the surrounding hard substrates and the prevailing currents. The use of scuttled navy vessels (Appendix 2) as reefing materials presents opportunities to study a large replicated substrate (eg. Ex-HMAS *Hobart*, *Brisbane* and *Perth*) in different locations. These vessels as well as the South Australian Tyre Reefs are nearly always deployed in seagrass meadows/sandy bottoms. An experiment deploying settlement plates at different distances to the vessel down and up currents coupled with potentially eDNA (Bohmann et al. 2014) could investigate spatial recruitment effects. The focus on recreating or seeking ‘perfect’ natural fouling communities on artificial reefs is perhaps chasing an impossible or unrealistic expectation, when many artificial reefs have been deployed without a thought as to the habitat they offer. Achieving a level of functional similarity may be the only way artificial reefs can best be integrated into a regional network or complex of hard substrates.

7. REFERENCES

AGUILERA, M. A., BROITMAN, B. R. & THIEL, M. 2016. Artificial breakwaters as garbage bins: Structural complexity enhances anthropogenic litter accumulation in marine intertidal habitats. Environmental Pollution, 214, 737-747.

AIKINS, S. & KIKUCHI, E. 2001. Studies on habitat selection by amphipods using artificial substrates within an estuarine environment. Hydrobiologia, 457, 77-86.

AIROLDI, L., TURON, X., PERKOL-FINKEL, S. & RIUS, M. 2015. Corridors for aliens but not for natives: effects of marine urban sprawl at a regional scale. Diversity and Distributions, 21, 755-768.

ALTIERI, ANDREW H., BREGJE K. VAN WESENBEECK, MARK D. BERTNESS, AND BRIAN R. SILLIMAN, 2010. Facilitation cascade drives positive relationship between native biodiversity and invasion success. Ecology 91, no. 5 1269-1275

AMBROSE, R. & ANDERSON, T. 1990. Influence of an artificial reef on the surrounding infaunal community. Marine Biology, 107, 41-52.

AMBROSE, R. F. & SWARBRICK, S. L. 1989. Comparison of fish assemblages on artificial and natural reefs off the coast of southern California. Bulletin of Marine Science, 44, 718-733.

ANDERSSON, M. H., BERGGREN, M., WILHELMSSON, D. & ÖHMAN, M. C. 2009. Epibenthic colonization of concrete and steel pilings in a cold-temperate embayment: a field experiment. Helgoland Marine Research, 63, 249-260.

ANDERSON, M. & UNDERWOOD, A. 1994. Effects of substratum on the recruitment and development of an intertidal estuarine fouling assemblage. Journal of Experimental Marine Biology and Ecology, 184, 217-236.

ARNEY, R.N., FROEHLICH, C.Y. AND KLINE, R.J., 2017. Recruitment Patterns of Juvenile Fish at an Artificial Reef Area in the Gulf of Mexico. Marine and Coastal Fisheries, 9(1), pp.79-92.

ASELTINE-NEILSON, D. A., BERNSTEIN, B. B., PALMER-ZWAHLEN, M. L., RIEGE, L. E. & SMITH, R. W. 1999. Comparisons of turf communities from Pendleton Artificial Reef, Torrey Pines Artificial Reef, and a natural reef using multivariate techniques. Bulletin of Marine Science, 65, 37-57.

AVIDSON, I. C., MCCANN, L. D., FOFONOFF, P. W., SYTSMA, M. D. & RUIZ, G. M. 2008. The potential for hull-mediated species transfers by obsolete ships on their final voyages. Diversity and Distributions, 14, 518-529.

BADALAMENTI, F., CHEMELLO, R., D'ANNA, G., RAMOS, P. H. & RIGGIO, S. 2002. Are artificial reefs comparable to neighbouring natural rocky areas? A mollusc case study in the Gulf of Castellammare (NW Sicily). ICES Journal of Marine Science: Journal du Conseil, 59, S127-S131.

BAILEY-BROCK, J. H. 1989. Fouling community development on an artificial reef in Hawaiian waters. Bulletin of Marine Science, 44, 580-591.

BAINE, M. 2001. Artificial reefs: a review of their design, application, management and performance. Ocean & Coastal Management, 44, 241-259.

BARRETT, N., OH, E., MEYER, L., JONES, D. & EDGAR, G. 2010. A biological monitoring survey of reef biota within Bathurst Channel, Southwest Tasmania. University of Tasmania, Hobart.

BARROS, F., UNDERWOOD, A. & LINDEGARTH, M. 2001. The influence of rocky reefs on structure of benthic macrofauna in nearby soft-sediments. Estuarine, Coastal and Shelf Science, 52, 191-199.

BASKETT, M.L. & SALOMON, A.K. 2010. Recruitment facilitation can drive alternate states on temperate reefs. Ecology, 91, 1763-1773

BAX, N., WILLIAMSON, A., AGUERO, M., GONZALEZ, E. & GEEVES, W. 2003. Marine invasive alien species: a threat to global biodiversity. Marine Policy, 27, 313-323.

BISHOP, M.J., MAYER-PINTO, M., AIROLDI, L., FIRTH, L.B., MORRIS, R.L., LOKE, L.H., HAWKINS, S.J., NAYLOR, L.A., COLEMAN, R.A., CHEE, S.Y. & DAFFORN, K.A. 2017. Effects if ocean sprawl on ecological connectivity: impacts and solutions. Journal of Experimental Marine Biology and Ecology. 492. 7-30.

BLYTHE, J.N. & PINEDA, J., 2009. Habitat selection at settlement endures in recruitment time series. *Marine Ecology Progress Series*, *396*, pp.77-84.

BOBADILLA, M. & SANTELICES, B. 2005. Variations in the dispersal curves of macroalgal propagules from a source. Journal of Experimental Marine Biology and Ecology, 327, 47-57

BOHMANN, K., EVANS, A., GILBERT, M.T.P., CARVALHO, G.R., CREER, S., KNAPP, M., DOUGLAS, W.Y. & DE BRUYN, M., 2014. Environmental DNA for wildlife biology and biodiversity monitoring. Trends in Ecology & Evolution, 29(6), pp.358-367.

BOHNSACK, J. A. 1979. Photographic quantitative sampling of hard-bottom benthic communities. Bulletin of Marine Science, 29, 242-252.

BOHNSACK, J. A. 1989. Are high densities of fishes at artificial reefs the result of habitat limitation or behavioral preference? Bulletin of Marine Science, 44, 631-645.

BORGES, L.M.S. 2014, Biodegredation of wood exposed in the marine environment: Evaluation of the hazard posed by marine wood-borers in fifteen European sites, International Biodeterioration & Biodegradation, 97-104

BORTONE, S. A. & KIMMEL, J. J. 1991. Environmental Assessment and Monitoring of Artificial Habitats. In: W. Seaman Jr (ed). Artificial Habitats for Marine and Freshwater Fisheries. San Diego: Academic Press. P 177-236

BRANDEN, K. L., POLLARD, D. A. & REIMERS, H. A. 1994. A review of recent artificial reef developments in Australia. Bulletin of Marine Science, 55, 982-994.

BREMNER, J., RODGERS, S.I. & FRID, C.L.J. 2006. Methods for describing ecological functioning of marine benthic assemblages using biological traits analysis (BTA). Ecological Indicators, 6.3, 609-622

BRICKHILL, M.J., LEE, S.Y. & CONNOLLY, R.M. 2005. Fishes associated with artificial reefs: attributing changes to attraction or production using novel approaches. Journal of Fish Biology, 67, pp.53-71.

BRICKHILL, M.J., LEE, S.Y. & CONNOLLY, R.M. 2006. Fishes associated with artificial reefs: attributing changes to attraction or production using novel approaches. Journal of Fish Biology, 67, 53-71

BROS, W.E. 1987. Effects of removing or adding structure (barnacle shells) on recruitment to a fouling community in Tampa Bay Florida. Journal of Experimental Marine Biology and Ecology, 105. 275-296.

BRYARS, S. & ROWLING, K. 2009. Benthic habitats of eastern Gulf St Vincent: major changes in benthic cover and composition following European settlement of Adelaide. Transactions of the Royal Society of South Australia, 133, 318-338.

BULLERI, F. & CHAPMAN, M.G., 2010. The introduction of coastal infrastructure as a driver of change in marine environments. Journal of Applied Ecology, 47(1), pp.26-35.

BURT, J., BARTHOLOMEW, A., USSEGLIO, P. BAUMAN, A. & SALE P.F. 2009. Are artificial reef surrogates of natural habitats for corals and fish in Dubai, United Arab Emirates? Coral Reefs, 28, 663-675

BUTLER, A. 1986. Recruitment of sessile invertebrates at five sites in Gulf St. Vincent, South Australia. Journal of Experimental Marine Biology and Ecology, 97, 13-36.

BUTLER, A. 1991. Effect of patch size on communities of sessile invertebrates in Gulf St Vincent, South Australia. Journal of Experimental Marine Biology and Ecology, 153, 255-280.

BUTLER, A. J. & CONNOLLY, R. 1999. Assemblages of sessile marine invertebrates: still changing after all these years? Marine Ecology Progress Series, 182, 109-118.

BUTLER, A. J. & CONNOLLY, R. M. 1996. Development and long term dynamics of a fouling assemblage of sessile marine invertebrates. Biofouling, 9, 187-209.

BYE, J. 1976. Physical Oceanography of Gulf St Vincent and Investigator Strait. In: TWIDALE, C.R., M.J. TYLER & B.P. Webb (eds) Natural History of the Adelaide Region. Royal Society of South Australia, 143-160.

CAHILL, A.E., DE JODE, A., DUBOIS, S., BOUZAZA, Z., AURELLE, D., BOISSIN, E., CHABROL, O., DAVID, R., EGEA, E., LEDOUX, J.B. AND MÉRIGOT, B., 2017. A multispecies approach reveals hot spots and cold spots of diversity and connectivity in invertebrate species with contrasting dispersal modes. Molecular ecology, 26(23), pp.6563-6577.

CALL, K.A., HARDY, J.T. & WALLIN, D.O. 2003. Coral reef habitat discrimination using multivariate spectral analysis and satellite remote sensing. International Journal of Remote Sensing. 24. 2627-2639

CANNING-CLODE, J. & SUGDEN, H. 2014 “Assessing Fouling communities” In: DOBRETSOV, S., WILLIAMS, D.N. & THOMASON, J.C. (eds) Biolfouling Methods, John Wiley & Sons Inc, pg. 252, Proquest Ebook Central.

CARDINALE, B.J., PALMER, M.A. & COLLINS, S.L. 2002. Species diversity enhances ecosystem functioning through interspecific facilitation. Nature, 415, 426-429

CARR, M. H. & HIXON, M. A. 1997. Artificial reefs: the importance of comparisons with natural reefs. Fisheries, 22, 28-33.

CARTER, R. W. G. 1988. Chapter Eleven - The Management of Coastal Waters. Coastal Environments. An Introduction to the Physical, Ecological and Cultural Systems of Coastlines. London: Academic Press. Pp 383 – 430

CARVALHO, S., MOURA, A., CURDIA, J., CANCELA DA FONSECA, L. & SANTOS, M.N. 2013. How complementary are epibenthic assemblages in artificial and nearby rocky reefs? Marine Environmental Research. 92, 170-177

CHANG, C.Y. & MARSHALL, D.J., 2017. Quantifying the role of colonization history and biotic interactions in shaping communities–a community transplant approach. Oikos, 126(2).

CHAPMAN, M. & BULLERI, F. 2003. Intertidal seawalls—new features of landscape in intertidal environments. Landscape and Urban planning, 62, 159-172.

CLAAR, D. C., EDWARDS, K. F. & STACHOWICZ, J. J. 2011. Positive and negative effects of a dominant competitor on the settlement, growth, and survival of competing species in an epibenthic community. Journal of experimental marine biology and ecology, 399, 130-134.

CLARKE, K. R., SOMERFIELD, P. J., AIROLDI, L. & WARWICK, R. M. 2006. Exploring interactions by second-stage community analyses. Journal of Experimental Marine Biology and Ecology, 338, 179-192.

CLARKE, K.R., GORLEY, R.N., SOMERFIELD, P.J. & WARWICK, R.M. (2014) Change in Marine Communities: An Approach to Statistical Analysis and Interpretation, 3rd edition edn. PRIMER-E Ltd, Plymouth, UK

COATES, D. A., DESCHUTTER, Y., VINCX, M. & VANAVERBEKE, J. 2014. Enrichment and shifts in macrobenthic assemblages in an offshore wind farm area in the Belgian part of the North Sea. Marine Environmental Research, 95, 1-12.

COBB, J.S., BOOTH, J.D. & CLANCY, M. 1997, "Recruitment strategies in lobsters and crabs: a comparison", Marine and Freshwater Research, vol.48, pp.797-806

COLLINS, K.J., JENSEN, A.C. & ALBERT, S., 1995. A review of waste tyre utilisation in the marine environment. Chemistry and Ecology, 10(3-4), pp.205-216.

COLLINS, K., JENSEN, A., MALLINSON, J., ROENELLE, V. & SMITH, I. 2002. Environmental impact assessment of a scrap tyre artificial reef. ICES Journal of Marine Science: Journal du Conseil, 59, S243-S249.

COOMBES, M,A., LA MARCA, E., NAYLOR, L.A., THOMPSON, R.C. 2015. Getting into the Groove: Opportunities to enhance the ecological value of hard coastal infrastructure using fine scale surface textures. Ecological Engineering, 77, 314-323

CONNELL, J. H. 1978. Diversity in tropical rain forests and coral reefs. Science, 199, 1302-1310.

CONNELL, J. H. 1985. The consequences of variation in initial settlement vs. post-settlement mortality in rocky intertidal communities. Journal of experimental marine biology and ecology, 93, 11-45.

CONNELL, J. H. & SLATYER, R. O. 1977. Mechanisms of succession in natural communities and their role in community stability and organization. American naturalist, 1119-1144.

CONNELL, S. & GLASBY, T. 1999. Do urban structures influence local abundance and diversity of subtidal epibiota? A case study from Sydney Harbour, Australia. Marine Environmental Research, 47, 373-387.

CONNELL, S. D. 2001. Urban structures as marine habitats: an experimental comparison of the composition and abundance of subtidal epibiota among pilings, pontoons and rocky reefs. Marine Environmental Research, 52, 115-125.

COOMBES, M. A., LA MARCA, E. C., NAYLOR, L. A. & THOMPSON, R. C. 2015. Getting into the groove: Opportunities to enhance the ecological value of hard coastal infrastructure using fine-scale surface textures. Ecological Engineering, 77, 314-323.

CRUZ-RIVERA, E. & HAY, M.E., 2001. Macroalgal traits and the feeding and fitness of an herbivorous amphipod: the roles of selectivity, mixing, and compensation. Marine Ecology Progress Series, 218, pp.249-266.

DAFFORN, K. A., GLASBY, T. M. & JOHNSTON, E. L. 2012. Comparing the invasibility of experimental “reefs” with field observations of natural reefs and artificial structures. PloS one, 7, e38124.

DAFFORN, K.A., GLASBY, T.M., AIROLDI, L., RIVERO, N.K., MAYER-PINTO, M. & JOHNSTON, E. 2015A. Marine Urbanization: an ecological framework for designing multifunctional artificial structures. Frontier in Ecology and the Environment, 13 (2), 82-90.

DAFFORN, K.A., MAYER-PINTO, M., MORRIS, R. & WALTHAM, N.J. 2015B. Application of management tools to integrate ecological principles with the design of marine infrastructure. Journal of Environmental Management, 153, 61-73

DAFFORN, K. A., JOHNSTON, E. L., & GLASBY, T. M. (2009). Shallow moving structures promote marine invader dominance. Biofouling, 25(3), 277-287.

DALBY JR, J. E. & YOUNG, C. M. 1993. Variable effects of ascidian competitors on oysters in a Florida epifaunal community. Journal of Experimental Marine Biology and Ecology, 167, 47-57.

DAVIS, A. R. & BUTLER, A. J. 1989. Direct observations of larval dispersal in the colonial ascidian Podoclavella moluccensis Sluiter: evidence for closed populations. Journal of Experimental Marine Biology and Ecology, 127, 189-203.

DAY, V., PAXINOS, R., EMMETT, J., WRIGHT, A. & GOECKER, M., 2008. The Marine Planning Framework for South Australia: A new ecosystem-based zoning policy for marine management. Marine Policy, 32(4), 535-543.

DEAN, T.A. & HURD, L.E., 1980. Development in an estuarine fouling community: the influence of early colonists on later arrivals. Oecologia, 46(3), pp.295-301.

DIAL, R. & ROUGHGARDEN, J. 1998. Theory of marine communities: the intermediate disturbance hypothesis. Ecology, 79, 1412-1424.

DIAMANT, A., TUVIA, A. B., BARANES, A. & GOLANI, D. 1986. An analysis of rocky coastal eastern Mediterranean fish assemblages and a comparison with an adjacent small artificial reef. Journal of Experimental Marine Biology and Ecology, 97, 269-285.

DITTON, R. B., OSBURN, H. R., BAKER, T. L. & THAILING, C. E. 2002. Demographics, attitudes, and reef management preferences of sport divers in offshore Texas waters. ICES Journal of Marine Science: Journal du Conseil, 59, S186-S191.

DOBRETSOV, S., WILLIAMS, D.N. & THOMASON, J.C. eds. 2014. Biofouling Methods. John Wiley and Son. 2014.

DOWLING, R. K. & NICHOL, J. 2001. The HMAS Swan artificial dive reef. Annals of Tourism Research, 28, 226-229.

DUDGEON, S. & PETRAITIS, P. S. 2001. Scale-dependent recruitment and divergence of intertidal communities. Ecology, 82, 991-1006.

DUMAS, P., BERTAUD, A., PEIGNON, C., LEOPOLD, M. & PELLETIER, D. 2009. A “quick and clean” photographic method for the description of coral reef habitats. Journal of Experimental Marine Biology and Ecology, 368, 161-168.

DURR, S. & WAHL, M. 2004. Isolated and combined impacts of blue mussels (Mytilus edulis) and barnacles (Balanus imporvisus) on structure and diversity of a fouling community. Journal of Experimental Marine Biology and Ecology. 306, 181-195

DYSON, K. & YOCOM, K. 2015. Ecological design for urban waterfronts. Urban Ecosystems, 18, 189-208.

EDELIST, D. & SPANIER, E., 2009. Influence of Levantine Artificial Reefs on the fish assemblage of the surrounding seabed. Mediterranean Marine Science, 10(1), pp.35-54.

ELLINGSEN, K., CLARKE, K., SOMERFIELD, P. & WARWICK, R. 2005. Taxonomic distinctness as a measure of diversity applied over a large scale: the benthos of the Norwegian continental shelf. Journal of Animal Ecology, 74, 1069-1079.

ENGLUND, G. & COOPER, S. D. 2003. Scale effects and extrapolation in ecological experiments. Advances in Ecological Research. Academic Press. 161-213

FARIÑAS-FRANCO, J. M., ALLCOCK, L., SMYTH, D. & ROBERTS, D. 2013. Community convergence and recruitment of keystone species as performance indicators of artificial reefs. Journal of Sea Research, 78, 59-74.

FIRTH, L., THOMPSON, R., BOHN, K., ABBIATI, M., AIROLDI, L., BOUMA, T., BOZZEDA, F., CECCHERELLI, V., COLANGELO, M. &

EVANS, A. 2014. Between a rock and a hard place: environmental and engineering considerations when designing coastal defence structures. Coastal Engineering, 87, 122-135.

FIRTH, L. B., MIESZKOWSKA, N., GRANT, L. M., BUSH, L. E., DAVIES, A. J., FROST, M. T., MOSCHELLA, P. S., BURROWS, M. T., CUNNINGHAM, P. N., DYE, S. R. & HAWKINS, S. J. 2015. Historical comparisons reveal multiple drivers of decadal change of an ecosystem engineer at the range edge. Ecology and Evolution, 5, 3210-3222.

FIRTH, L.B., BROWNE, K.A, KNIGHTS, A.M., HAWKINS, S.J. & NASH, R. 2016A. Eco-engineered rock pools: A concrete solution to biodiversity loss and urban sprawl in the Marine Environment. Environmental Research Letters. 11, 9

FIRTH, L.B., KNIGHTS, A.M., BRIDGER, D., EVANS, A.J., MIESZKOWSKA, MOOREM P.J., O’CONNOR, N.E., SHEEHAN, THOMPSON, R.C. & HAWKINS S.J. 2016B. Ocean Sprawl: Challenges and Opportunities for biodiversity management in a changing world. Oceanography and Marine Biology, An Annual Review, 54, 193-firth269

FITZHARDINGE, R.C. & BAILEY-BROCK, J.H., 1989. Colonization of artificial reef materials by corals and other sessile organisms. *Bulletin of Marine Science*, *44*(2), pp.567-579.

FLOERL, O. & INGLIS, G.J., 2005. Starting the invasion pathway: the interaction between source populations and human transport vectors. Biological Invasions, 7(4), pp.589-606.

FORTEATH, G., PICKEN, G., RALPH, R. & WILLIAMS, J. 1982. Marine growth studies on the North Sea oil platform Montrose Alpha. Marine Ecology Progress Series, 8, 61-68.

FOSTER, M., HARROLD, C. & HARDIN, D. 1991. Point vs. photo quadrat estimates of the cover of sessile marine organisms. Journal of Experimental Marine Biology and Ecology, 146, 193-203.

FOSTER, S., SMALE, D.A., HOW, J., DE LESTANG, S., BREARLEY, A. & KENDRICK, G.A., 2014. Regional-scale patterns of mobile invertebrate assemblage structure on artificial habitats off Western Australia. Journal of experimental marine biology and ecology, 453, pp.43-53.

FRAZER, T. K. & LINDBERG, W. J. 1994. Refuge spacing similarly affects reef-associated species from three phyla. Bulletin of Marine Science, 55, 388-400.

FULTON, E. A., PARSLOW, J. S., SMITH, A. D. M. & JOHNSON, C. R. 2004. Biogeochemical marine ecosystem models II: the effect of physiological detail on model performance. Ecological Modelling, 173, 371-406.

GALLAGHER, E.D., JUMARS, P.A. & TRUEBLOOD, D.D. 1983. Facilitation of soft-bottom benthic succession by tube builders. Ecology, 64 (5), 1200-1216

GLASBY, T. 2000. Surface composition and orientation interact to affect subtidal epibiota. Journal of Experimental Marine Biology and Ecology, 248, 177-190.

GLASBY, T. & CONNELL, S. 1999. Urban structures as marine habitats. Ambio, 28, 595-598.

GLASBY, T. M., CONNELL, S. D., HOLLOWAY, M. G. & HEWITT, C. L. 2007. Nonindigenous biota on artificial structures: could habitat creation facilitate biological invasions? Marine Biology, 151, 887-895.

GREENFIELD, B.L., KRAAN, C., PILDITCH, C.A. & THRUSH, S.F. (2016). Mapping functional groups can provide insight into ecosystem functioning and potential resilience of intertidal sandflats. Marine Ecology Progress Series, 548, 1-10

GROSSMAN, G. D., JONES, G. P. & SEAMAN JR, W. J. 1997. Do artificial reefs increase regional fish production? A review of existing data. Fisheries, 22, 17-23

GROVE, R.S. & SONU, C.J., 1985. Fishing reef planning in Japan. Artificial reefs: Marine and freshwater applications, pp.187-251.

GROVE, R.S., SONU, C.J. AND NAKAMURA, M., 1989. Recent Japanese trends in fishing reef design and planning. Bulletin of Marine Science, 44(2), pp.984-996

HÄDER, D. P., KUMAR, H. D., SMITH, R. C. & WORREST, R. C. 1998. Effects on aquatic ecosystems. Journal of Photochemistry and Photobiology B: Biology, 46, 53-68.

HADFIELD, M.G. 2011, Biofilms and Marine Invertebrate Larvae: What Bacteria Produce That Larvae Use to Choose Settlement Sites. 3, 1, 453-470

HALPERN, B.S., SILLIMAN, B.R., OLDEN, J.D., BRUNO, J.P. & BERTNESS, M.D. 2007. Incorporating positive interactions in aquatic restoration and conservation. Frontiers in Ecology and the Environment, 5, 153-160

HARDIMAN, N. & BURGIN, S. 2010. Recreational impacts on the fauna of Australian coastal marine ecosystems. Journal of Environmental Management, 91, 2096-2108.

HARVEY, E. & SHORTIS, M., 1995. A system for stereo-video measurement of sub-tidal organisms. Marine Technology Society Journal, 29(4), pp.10-22.

HAY, M.E., PARKER, J.D., BURKEPILE, D.E., CAUDILL, C.C., WILSON, A.E., HALLINAN, Z.P. & CHEQUER A.D. 2004. Mutualisms and aquatic community structure: the enemy of my enemy is my friend. Annual Review of Ecology, Evolution and Systematics, 175-197

HEERY, E.C., BISHOP, M.J., CRITCHLEY, L.P., BUGNOT, A.B., AIROLDI, L., MAYER-PINTO, M., SHEEHAN, E.V., COLEMAN, R.A., LOKE, L.H., JOHNSTON, E.L. AND KOMYAKOVA, V., 2017. Identifying the consequences of ocean sprawl for sedimentary habitats. Journal of Experimental Marine Biology and Ecology.

HEWITT, C.L. 2002. Distribution and biodiversity of Australian tropical marine bioinvasions. Pacific Science. 56. 213-222

HEWITT, C.L., CAMPBELL, M.L., THRESHER, R.E., MARTIN, R.B., BOYD, S., COHEN, B.F., CURRIE, D.R., GOMON, M.F., KEOUGH, M.J., LEWIS, J.A., LOCKETT, M.M., MAYS, N., MCARTHUR, M.A., O'HARA, T.D., POORE, G.C.B., ROSS, D.J., STOREY, M.J., WATSON, J.E. & WILSON, R.S. 2004 Introduced and cryptogenic species in Port Philip Bay, Victoria, Australia. Marine Biology, 144, 183-202

HISCOCK, K., SHARROCK, S., HIGHFIELD, J. & SNELLING, D. 2010. Colonization of an artificial reef in south-west England—ex-HMS ‘Scylla’. Journal of the Marine Biological Association of the United Kingdom, 90, 69-94.

HOBBS, R. J., ARICO, S., ARONSON, J., BARON, J. S., BRIDGEWATER, P., CRAMER, V. A., EPSTEIN, P. R., EWEL, J. J., KLINK, C. A. & LUGO, A. E. 2006. Novel ecosystems: theoretical and management aspects of the new ecological world order. Global Ecology and Biogeography, 15, 1-7.

HUNT, H. L. & SCHEIBLING, R. E. 1997. Role of early post-settlement mortality in recruitment of benthic marine invertebrates. Marine Ecology Progress Series, 155, 269-301.

HUNTER, W. & SAYER, M. 2009. The comparative effects of habitat complexity on faunal assemblages of northern temperate artificial and natural reefs. ICES Journal of Marine Science: Journal du Conseil, p 58.

HYNES, M. V., PETERS, J. E. & RUSHWORTH, D. 2004. Artificial Reefs: A disposal option for Navy and MARAD Ships. DTIC Document.

IDJADI, J.A. & EDMUNDS, P.J. 2006. Scleractinian corals as facilitators for other invertebrates on a Carribean reef. Marine Ecology Progress Series 319, 117-127

IDO, S. & PERKOL-FINKEL, S. 2015. Blue is the new green – Ecological enhancement of concrete based coastal and marine infrastructure. Ecological Engineering, 84, 260-272.

JAMESON, R., SMITH, M. L. & FAIRWEATHER, P. 1995. Sieve mesh-size and taxonomic resolution needed to describe natural spatial variation of marine macrofauna. Marine Ecology Progress Series. 118, 187-198.

JENSEN, A. 2002. Artificial reefs of Europe: perspective and future. ICES Journal of Marine Science: Journal du Conseil, 59, S3-S13.

JONES, C.G., LAWTON, J.H. & SHACHAK, M., 1994. Organisms as ecosystem engineers. In Ecosystem management (pp. 130-147). Springer, New York, NY.

JONES, A. T. & WELSFORD, R. W. 1997. Artificial reefs in British Columbia, Canada. OCEANS'97. MTS/IEEE Conference Proceedings, 1997. IEEE, 415-418.

KAY, A. M. & BUTLER, A. J. 1983. ‘Stability’of the fouling communities on the pilings of two piers in South Australia. Oecologia, 56, 70-78.

KAY, A. M. & KEOUGH, M. J. 1981. Occupation of patches in the epifaunal communities on pier pilings and the bivalve Pinna bicolor at Edithburgh, South Australia. Oecologia, 48, 123-130.

KEOUGH, M. & BUTLER, A. 1979. The role of asteroid predators in the organization of a sessile community on pier pilings. Marine Biology, 51, 167-177.

KEOUGH, M.J. & BUTLER, A.J. 1983, Temporal Changes in species number in an assemblages of sessile marine invertebrates, Journal of Biogeography, 10, 4. 317-330

KEOUGH, M. J. & DOWNES, B. J. 1982. Recruitment of marine invertebrates: the role of active larval choices and early mortality. Oecologia, 54, 348-352.

KIRKBRIDE-SMITH, A. E., WHEELER, P. M. & JOHNSON, M. L. 2013. The Relationship between Diver Experience Levels and Perceptions of Attractiveness of Artificial Reefs-Examination of a Potential Management Tool. PloS one, 8

KOEHL, M.A.R. & HADFIELD, M.G. 2004. Soluble settlement cue in slowly moving water within coral reefs induces larval adhersion to surfaces, Journal of Marine Systems. 49, 75-88

KOHLER, K. E. & GILL, S. M. 2006. Coral Point Count with Excel extensions (CPCe): A Visual Basic program for the determination of coral and substrate coverage using random point count methodology. Computers & Geosciences, 32, 1259-1269.

KRIWY, P. & UTHICKE, S. 2011. Microbial diversity in marine biofilms along a water quality gradient on the Great Barrier Reef. Systematic and applied microbiology, 34, 116-126.

KROHLING, W., BROTTO, D. S. & ZALMON, I. R. 2006. Functional role of fouling community on an artificial reef at the northern coast of Rio de Janeiro State, Brazil. Brazilian Journal of Oceanography, 54, 183-191.

LAM, K., SHIN, P. K., BRADBEER, R., RANDALL, D., KU, K. K., HODGSON, P. & CHEUNG, S. G. 2006. A comparison of video and point intercept transect methods for monitoring subtropical coral communities. Journal of Experimental Marine Biology and Ecology, 333, 115-128.

LANGLOIS, T. J., ANDERSON, M. J. & BABCOCK, R. C. 2005. Reef-associated predators influence adjacent soft-sediment communities. Ecology, 86, 1508-1519.

LAVENDER, J. T., DAFFORN, K. A. & JOHNSTON, E. L. 2014. Meso-predators: A confounding variable in consumer exclusion studies. Journal of Experimental Marine Biology and Ecology, 456, 26-33.

LAVENDER, J.T., DAFFORN, K.A., BISHOP. M.J. & JOHNSTON, E.L. 2017. Small scale habitat complexity of artificial turf influences the development of associated invertebrate assemblages, Journal of Experimental Marine Biology and Ecology. 2017, 105-112

LE CORRE, N., JOHNSON, L.E., SMITH, G.K. & GUICHARD, F. 2015, Patterns and scales of connectivity: temporal stability and variation within a marine metapopulation. Ecology, Vol. 96, 8 pp.2245-2235

LEEWORTHY, V., MAHER, T. & STONE, E. 2006. Can artificial reefs alter user pressure on adjacent natural reefs? Bulletin of Marine Science, 78, 29-38.

LEUJAK, W. & ORMOND, R. 2007. Comparative accuracy and efficiency of six coral community survey methods. Journal of Experimental Marine Biology and Ecology, 351, 168-187.

LEWIN, R. 1986. Supply side ecology, Science , 234, 25-27

LIVERSAGE, K., COLE, V., COLEMAN, R. & MCQUAID, C. 2017. Availabilty of microhabitats explains a widespread pattern and informs theory on ecological engineering of boulder reefs. Journal of Experimental Marine Biology and Ecology. 489, 36-42

LOKE, L.H.L & TODD, P.A. 2016. Structural Complexity and component type increase intertidal biodiversity independently of area. Ecology, 97, 2, 383-393

MAAR, M., BOLDING, K., PETERSEN, J.K., HANSEN, J.L. AND TIMMERMANN, K., 2009. Local effects of blue mussels around turbine foundations in an ecosystem model of Nysted off-shore wind farm, Denmark. Journal of Sea Research, 62(2-3), pp.159-174.

MACEDO, I. M., MASI, B. P. & ZALMON, I. R. 2006. Comparison of rocky intertidal community sampling methods at northern coast of Rio de Janeiro State, Brazil. Brazilian Journal of Oceanography, 54, 147-154.

MACLEOD, I., MORRISON, P., RICHARDS, V. & WEST, N. 2004. Corrosion monitoring and the environmental impact of decommissioned naval vessels as artificial reefs. Metal 04: international conference on metals conservation. Canberra, Australia, 2004. 4-8.

MACREADIE, P. I., FOWLER, A. M. & BOOTH, D. J. 2011. Rigs-to-reefs: will the deep sea benefit from artificial habitat? Frontiers in Ecology and the Environment, 9, 455-461.

MAGORRIAN, B. & SERVICE, M. 1998. Analysis of underwater visual data to identify the impact of physical disturbance on horse mussel (*Modiolus modiolus*) beds. Marine Pollution Bulletin, 36, 354-359.

MarLIN, 2006. BIOTIC - Biological Traits Information Catalogue. Marine Life Information Network. Plymouth: Marine Biological Association of the United Kingdom. Available from <www.marlin.ac.uk/biotic>

MARTINS, G. M., NETO, A. I. & CACABELOS, E. 2016. Ecology of a key ecosystem engineer on hard coastal infrastructure and natural rocky shores. Marine Environmental Research, 113, 88-94.

MCAFFEE J. & CONNELL, S.2017. Huge restored reef aims to bring Suth Australia's oysters back from the brink, The Conversation, June 28th, 2017 **http://theconversation.com/huge-restored-reef-aims-to-bring-south-australias-oysters-back-from-the-brink-77405**

MCCAULEY, D.J., PINSKY, M.M, PALUMBI, S.R., ESTES, J.A., JOYCE, F.H. & WARNER, R.R. 20015. Marine defaunation: Animal loss in the global ocean. Science, 347, 6219

MCCOOK, L., 2001. Competition between corals and algal turfs along a gradient of terrestrial influence in the nearshore central Great Barrier Reef. Coral Reefs, 19(4), pp.419-425

MCGLENNON, D. & BRANDEN, K. L. 1994. Comparison of catch and recreational anglers fishing on artificial reefs and natural seabed in Gulf St. Vincent, South Australia. Bulletin of Marine Science, 55, 510-523.

MEESE, R. J. & TOMICH, P. A. 1992. Dots on the rocks: a comparison of percent cover estimation methods. Journal of Experimental Marine Biology and Ecology, 165, 59-73.

MICHELI, F. & HALPERN, B.S., 2005. Low functional redundancy in coastal marine assemblages. Ecology Letters, 8(4), pp.391-400.

MILBRANDT, E. C., BARTLESON, R. D., COEN, L. D., RYBAK, O., THOMPSON, M. A., DEANGELO, J. A. & STEVENS, P. W. 2012. Local and regional effects of reopening a tidal inlet on estuarine water quality, seagrass habitat, and fish assemblages. Continental Shelf Research, 41, 1-16.

MINCHINTON, T. E. & SCHEIBLING, R. E. 1991. The influence of larval supply and settlement on the population structure of barnacles. Ecology, 72, 1867-1879.

MINEUR, F., COOK, E. J., MINCHIN, D., BOHN, K., MACLEOD, A. & MAGGS, C. A. 2012. Changing coasts: marine aliens and artificial structures. Oceanography and marine biology, 50, 189-234.

MOOK, D. 1980. Seasonal variation in species composition of recently settled fouling communities along an environmental gradient in the Indian River Lagoon, Florida. Estuarine and Coastal Marine Science, 11, 573-581.

MOOK, D. H. 1981. Effects of disturbance and initial settlement on fouling community structure. Ecology, 62, 522-526.

MORGAN, O. A., MASEY, D. M. & HUTH, W. L. 2009. Diving demand for large ship artificial reefs. Marine Resource Economics, 24, 43-59.

MORRISON, P. F. 2003. Biological monitoring of the HMAS Swan. A report to the Geographe Bay Artificial Reef Society. Perth.

MOUILLOT, D., GRAHAM, N.A.J., VILLEGER, S., MASON, N.W.H. & BELLWOOD, D.R. 2013, A functional approach reveals community responses to disturbances. Trends in Ecology and Evolution, 28, 167-177

MUNARI, C. 2013. Benthic community and biological trait composition in respect to artificial coastal defence structures: A study case in the northern Adriatic Sea. Marine Environmental Research, 90, 47-54

NORKKO, J. GAMMAL, J., HEWITT, J.E., JOSEFSON, A.B., CARSTENSEN, J. & NORKKO, A. 2015. Seafloor ecosystem function relationships: In Situ Patterns of Change Across Gradients of Increasing Hypoxic Stress. Ecosystems, 18, 1424-1439.

NYDAM, M. & STACHOWICZ, J. J. 2007. Predator effects on fouling community development. Marine Ecology Progress Series, 337, 93-101.

OLAFSSON, E. B., PETERSON, C. H. & AMBROSE, W. Does recruitment limitation structure populations and communities of macro-invertebrates in marine soft sediments. UCL Press, London, 1994.

OSENBERG, C. W., MARY, C. M. S., WILSON, J. A. & LINDBERG, W. J. 2002. A quantitative framework to evaluate the attraction–production controversy. ICES Journal of Marine Science: Journal du Conseil, 59, S214-S221.

OSMAN, R. W. & WHITLATCH, R. B. 1995. The influence of resident adults on recruitment: a comparison to settlement. Journal of Experimental Marine Biology and Ecology, 190, 169-198.

OSMAN, R.W. 2015. Regional variation in the colonization of experimental substrates by sessile marine invertebrates: Local vs. regional control of diversity. Journal of Experimental Marine Biology and Ecology, 473, 227-286.

PAWLIK, J. R., HENKEL, T. P., MCMURRAY, S. E., LÓPEZ-LEGENTIL, S., LOH, T.-L. & ROHDE, S. 2008. Patterns of sponge recruitment and growth on a shipwreck corroborate chemical defense resource trade-off. Marine Ecology Progress Series, 368, 137-143.

PAWLIK, J.R. 1992, Chemical Ecology of the settlement of benthic marine invertebrates, Oceanography Marine Biology Annual Review, 30, 273-335

PECHENIK, J. A. 1990. Delayed metamorphosis by larvae of benthic marine invertebrates: does it occur? Is there a price to pay? Ophelia, 32, 63-94.

PECHENIK, J.A., 1999. On the advantages and disadvantages of larval stages in benthic marine invertebrate life cycles. Marine Ecology Progress Series, pp.269-297.

PERKOL-FINKEL, S. & BENAYAHU, Y. 2004. Community structure of stony and soft corals on vertical unplanned artificial reefs in Eilat (Red Sea): comparison to natural reefs. Coral Reefs, 23, 195-205.

PERKOL-FINKEL, S. & BENAYAHU, Y. 2005. Recruitment of benthic organisms onto a planned artificial reef: shifts in community structure one decade post-deployment. Marine Environmental Research, 59, 79-99.

PERKOL-FINKEL, S., SHASHAR, N., BARNEAH, O., BEN-DAVID-ZASLOW, R., OREN, U., REICHART, T., YACOBOVICH, T., YAHEL, G., YAHEL, R. & BENAYAHU, Y. 2005. Fouling reefal communities on artificial reefs: does age matter? Biofouling, 21, 127-140.

PERKOL-FINKEL, S., SHASHAR, N. & BENAYAHU, Y. 2006. Can artificial reefs mimic natural reef communities? The roles of structural features and age. Marine environmental research, 61, 121-135.

PERKOL-FINKEL, S. AND BENAYAHU, Y., 2007. Differential recruitment of benthic communities on neighbouring artificial and natural reefs. Journal of Experimental Marine Biology and Ecology, 340(1), pp.25-39.

PERKOL-FINKEL, S. & BENAYAHU, Y., 2009. The role of differential survival patterns in shaping coral communities on neighboring artificial and natural reefs. Journal of Experimental Marine Biology and Ecology, 369(1), pp.1-7.

PETRAITIS, P.S. & LATHAM, L.E. 1999. The importance of scale in testing the origins of alternative community states. Ecology, 80, 429-442

PETRAITIS, P.S. & DUDGEON, S.R. 2004. Detection of alternative stable states in marine communities. Journal of Experimental Marine Biology and Ecology. 300, 343-371

PICKERING, H., WHITMARSH, D. & JENSEN, A. 1999. Artificial reefs as a tool to aid rehabilitation of coastal ecosystems: investigating the potential. Marine Pollution Bulletin, 37, 505-514.

PICKETT, S. T. & THOMPSON, J. N. 1978. Patch dynamics and the design of nature reserves. Biological Conservation, 13, 27-37

PONTI, M., FAVA, F., PERLINI, R. A., GIOVANARDI, O. & ABBIATI, M. 2015. Benthic assemblages on artificial reefs in the northwestern Adriatic Sea: Does structure type and age matter? Marine Environmental Research, 104, 10-19.

PRATT, J.R., 1994. Artificial habitats and ecosystem restoration: managing for the future. Bulletin of Marine Science, 55(2-3), pp.268-275.

REIMERS, H. & BRANDEN, K. 1994. Algal colonization of a tire reef—influence of placement date. Bulletin of Marine Science, 55, 460-469.

RELINI, G., RELINI, M., PALANDRI, G., MERELLO, S. & BECCORNIA, E. 2007. History, ecology and trends for artificial reefs of the Ligurian Sea, Italy. Hydrobiologia, 580, 193-217.

RODRIGUEZ, S. R., OJEDA, F. P. & INESTROSA, N. C. 1993. Settlement of benthic marine invertebrates. Marine Ecology Progress Series, 97, 193-207.

SAMS, M.A. & KEOUGH, M.J. 2012. Contrasting effects of variable species recruitment on marine sessile communities. Ecology, 93, 1153-1163.

SAMS, M.A. & KEOUGH, M.J. 2013. Early recruitment variation and an established dominant alter the composition of a template fouling community. Marine Ecology Progress Series, 486, 79-91

SEAMAN, W., 2007. Artificial habitats and the restoration of degraded marine ecosystems and fisheries. Hydrobiologia, 580(1), pp.143-155.

SCHROETER, S. C., REED, D. C. & RAIMONDI, P. T. 2015. Effects of reef physical structure on development of benthic reef community: a large-scale artificial reef experiment. Marine Ecology Progress Series, 540, 43-55.

SHEEHY, D. J. & VIK, S. F. 2010. The role of constructed reefs in non-indigenous species introductions and range expansions. Ecological Engineering, 36, 1-11.

SIMPSON, T.J.S., SMALE, D.A., MCDONALD, J.I., & WERNBERG, T. 2017. Large scale variability in the structure of sessile invertebrate assemblages in artificial habitats reveals the importance of local-scale processes. Journal of Experimental Marine Biology and Ecology. 294, 10-19

SMALE, D.A. 2012. Extreme spatial variability in sessile assemblage development in subtidal habitats off southwest Australia (southeast Indian Ocean). Journal of Experimental Marine Biology and Ecology, 438, 76-83.

SMALE. D.A. 2013. Multi-scale patterns of spatial variability in sessile assemblage structure do not alter predictably with development time. Marine Ecology Progress Series, 482, 20-41.

SMITH, A. M., KREGTING, L., FERN, S. & FRASER, C. I. 2011. Sedimentology of a wreck: The Rainbow Warrior revisited. Marine Pollution Bulletin, 62, 2412-2419.

SOMERFIELD, P. & CLARKE, R.K. 1995. Taxonomic levels, in marine community studies, revisited. Marine Ecology Progress Series, 127, 113-119.

SOMERFIELD P. & CLARKE, R,K. 2013.Inverse analysis in non-parametric multivariate analyses: Distinguishing groups of associated species which convey coherently across samples. Journal of Experimental Marine Biology and Ecology. 449, 261-273

SOUSA, W. P. 1979. Disturbance in marine intertidal boulder fields: the non equilibrium maintenance of species diversity. Ecology, 60, 1225-1239.

SOUSA, W. P. & DANGREMOND, E. M. 2011. Trophic Interactions in Coastal and Estuarine Mangrove Forest Ecosystems. Treatise on Estuarine and Coastal Science. Waltham: Academic Press. 6-40

SPANIER, E., LAVALLI, K. L. & EDELIST, D. 2011. An Overview of Their Application for Fisheries Enhancement, Management, and Conservation. Artificial Reefs in Fisheries Management, 77.

STACHOWICZ, J.J. 2001. Mutualism, facilitation and the structure of ecological communities: positive interactions play a critical, but underappreciated role in ecological communities by reducing physical or biotic stresses in existing habitats and by creating new habitats on which many species depend. Bioscience, 51, 235-246

STACHOWICZ, J.J. & BYRNES, J.E. 2006. Species diversity, invasion success, and ecosystem functioning: disentangling the influence of resource competition, facilitation, and extrinsic factors. Marine Ecology Progress Series 311, 251-262

STEWART, R. I. A., DOSSENA, M., BOHAN, D. A., JEPPESEN, E., KORDAS, R. L., LEDGER, M. E., MEERHOFF, M., MOSS, B., MULDER, C., SHURIN, J. B., SUTTLE, B., THOMPSON, R., TRIMMER, M. & WOODWARD, G. 2013. Chapter Two - Mesocosm Experiments as a Tool for Ecological Climate-Change Research. In: GUY, W. & EOIN, J. O. G. (eds.) Advances in Ecological Research. Academic Press. 48, 71-182.

STOLK, P., MARKWELL, K. & JENKINS, J. M. 2007. Artificial reefs as recreational scuba diving resources: a critical review of research. Journal of Sustainable Tourism, 15, 331-350.

STRAIN, E.M., OLABARRIA, C., MAYER-PINTO, M., CUMBO, V., MORRIS, R.L., BUGNOT, A.B., DAFFORN, K.A., HEERY, E., FIRTH, L.B., BROOKS, P. AND BISHOP, M.J., 2017. Eco-engineering urban infrastructure for marine and coastal biodiversity: which interventions have the greatest ecological benefit?. Journal of Applied Ecology. 1, 426-441.

SUDGEN, H., PANUSCH, R., LENZ, M., WAHL, M. & THOMASON, J. C. 2007. Temporal variability of disturbances: is this important for diversity and structure of marine fouling assemblages? Marine Ecology, 28, 368-376.

SUZEIRO, M.C., BORTOLUS, A. & SCHWINDT, E., 2011. Habitat complexity and community composition: relationships between different ecosystem engineers and the associated macroinvertebrate assemblages. Helgoland Marine Research, 65(4), p.467.

SVANE, I. & PETERSEN, J. K. 2001. On the problems of epibioses, fouling and artificial reefs, a review. Marine Ecology, 22, 169-188.

THRUSH, S. & WHITLATCH, R. 2001. Recovery dynamics in benthic communities: balancing detail with simplification. Ecological comparisons of sedimentary shores. Springer.

TODD, C. D. & TURNER, S. J. 1986. Ecology of intertidal and sublittoral cryptic epifaunal assemblages. I. Experimental rationale and the analysis of larval settlement. Journal of Experimental Marine Biology and Ecology, 99, 199-231.

TODD, C.D. 1998, Larval supply and recruitment of benthic invertebrates: do larvae always disperse as much as we believe? Hydrobiologia, 375, 1-21

TOFT, J. D., OGSTON, A. S., HEERHARTZ, S. M., CORDELL, J. R. & FLEMER, E. E. 2013. Ecological response and physical stability of habitat enhancements along an urban armored shoreline. Ecological Engineering, 57, 97-108.

TURNER, C. H., EBERT, E. E. & GIVEN, R. R. 1969. Man-made reef ecology, State of California, Department of Fish and Game.

UNDERWOOD, A. & ANDERSON, M. 1994. Seasonal and temporal aspects of recruitment and succession in an intertidal estuarine fouling assemblage. Journal of the Marine Biological Association of the United Kingdom, 74, 563-584.

UNDERWOOD, A. & FAIRWEATHER, P. 1989. Supply-side ecology and benthic marine assemblages. Trends in Ecology & Evolution, 4, 16-20.

VAN DOLAH, R. F., WENDT, P. H., KNOTT, D. M. & WENNER, E. L. 1988. Recruitment and community development of sessile fouling assemblages on the continental shelf off South Carolina, U.S.A. Estuarine, Coastal and Shelf Science, 26, 679-699.

VANDERKLIFT, M., WARD, T. J. & JACOBY, C. 1996. Effect of reducing taxonomic resolution on ordinations to detect pollution-induced gradients in macrobenthic infaunal assemblages. Marine Ecology Progress Series, 136, 137-145.

VIEIRA, E.A., DUARTE, L.F.L. & DIAS, G.M. 2012, How timing of predation affect composition and diversity of species in a marine sessile community? Journal of Experimental Maine Biology and Ecology, 412, 126-133

WAITE, I. R., HERLIHY, A. T., LARSEN, D. P., URQUHART, N. S. & KLEMM, D. J. 2004. The effects of macroinvertebrate taxonomic resolution in large landscape bioassessments: an example from the Mid-Atlantic Highlands, USA. Freshwater Biology, 49, 474-489.

WALKER, S. J., SCHLACHER, T. A. & SCHLACHER-HOENLINGER, M. A. 2007. Spatial heterogeneity of epibenthos on artificial reefs: fouling communities in the early stages of colonization on an East Australian shipwreck. Marine Ecology, 28, 435-445.

WALKER, S.J. & SCHLACHER T.A. 2014. Limited habitat and conservation value of a young artificial reef. Biodiversity and Conservation, 23, 433-447.

WENDT, P.H., KNOTT, D.M. & VAN DOLAH, R.F., 1989. Community structure of the sessile biota on five artificial reefs of different ages. Bulletin of Marine Science, 44(3), pp.1106-1122.

WESTPHALEN, G. 2010. Surveys across reefs in the Adelaide and Mt Lofty Ranges Natural Resource Management Region 2009-2010. Report to the Conservation Council of South Australia and Reef Watch.

WETZEL, M. A., SCHOLLE, J. & TESCHKE, K. 2014. Artificial structures in sediment-dominated estuaries and their possible influences on the ecosystem. Marine environmental research, 99, 125-135.

WHITE, P. S. & PICKETT, S. Natural disturbance and patch dynamics. Academic Press, 1985.

WIECZOREK, S. K. & TODD, C. D. 1998. Inhibition and facilitation of settlement of epifaunal marine invertebrate larvae by microbial biofilm cues. Biofouling, 12, 81-118.

WILDING, T. A. & SAYER, M. D. 2002. Evaluating artificial reef performance: approaches to pre-and post-deployment research. ICES Journal of Marine Science: Journal du Conseil, 59, S222-S230.

WORM, B., LOTZE, H.K. & SOMMER, U. 2001, “Algal propagule banks modify competition, consumer and resource control on Baltic rock shores”, Oecologia, vol. 128, pp.281-293

YAKOVIS, E.L., ARTEMIEVA, A.V., FOKIN, M.V., GRISHANKOV, A.V. & SHUNATOVA, N.N., 2005. Patches of barnacles and ascidians in soft bottoms: associated motile fauna in relation to the surrounding assemblage. Journal of Experimental Marine Biology and Ecology, 327(2), pp.210-224.

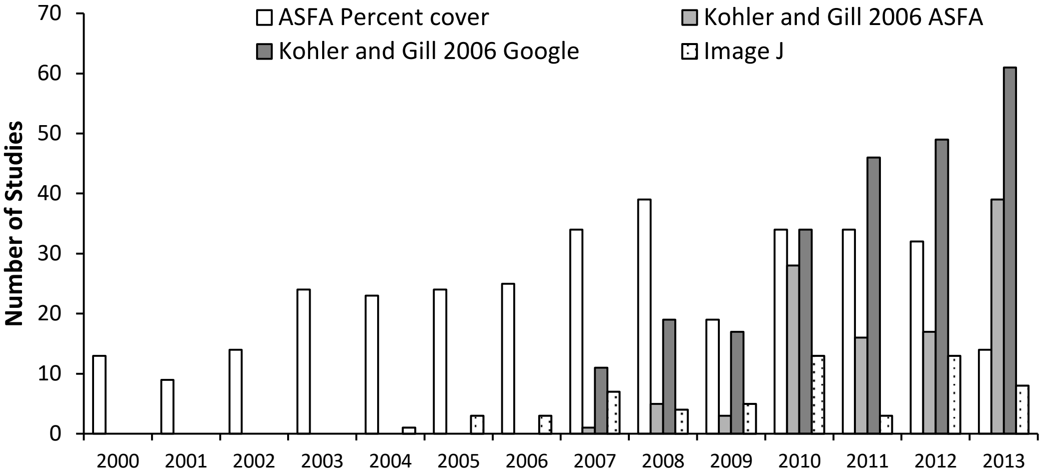
YAKOVIS, E.L., ARTEMIEVA, A.V., SHUNATOVA, N.N. & VARFOLOMEEVA, M.A., 2008. Multiple foundation species shape benthic habitat islands. Oecologia, 155(4), pp.785-795.

ZHAO, B., ZHANG, S. & QIAN, P.-Y. 2003. Larval settlement of the silver-or goldlip pearl oyster Pinctada maxima (Jameson) in response to natural biofilms and chemical cues. Aquaculture, 220, 883-901.

ZINTZEN, V., MASSIN, C., NORRO, A. & MALLEFET, J. 2006. Epifaunal inventory of two shipwrecks from the Belgian Continental Shelf. Hydrobiologia, 555, 207-219.

ZINTZEN, V., NORRO, A., MASSIN, C. & MALLEFET, J. 2008. Spatial variability of epifaunal communities from artificial habitat: Shipwrecks in the Southern Bight of the North Sea. Estuarine, Coastal and Shelf Science, 76, 327-344.

APPENDIX. I



SUPPLEMENTARY FIGURE 2.1: Systematic review of studies using percent cover and image analysis software. Results were obtained using either Google Scholar and Aquatic Sciences and Fisheries Abstracts (ASFA) searches. The number of studies from ASFA that had percent cover in the keywords is shown since 2000. The citations of the Coral Point Count with excel extensions (CPCe) software paper (Kohler and Gill 2006) is shown from both ASFA and Google Scholar. For contrast, the number of studies with the keyword “ImageJ” (another percent cover analysis software package) is also shown.

Supplementary Table 2.1: Summary table showing the two tested methods, scraping (physical sampling) versus, Photo-analysis (percent cover) and whether they qualitatively or quantitatively sample components of the fouling communities on artificial reefs. N/A is not applicable or not able to be quantified, the term ‘conspicuous’ refers to organisms visible in the fouling community in-situ/photographs.

	Method	
Community Component assessed	**Photo-analysis**	**Scraping**
Large Macroalgae	Quantitative (percentage cover)	Quantitative (displacement volume)
Small and turfing algae	Quantitative(% cover) Conspicuous	Qualitative (All) (presence/absence)
Colonial invertebrates	Quantitative (% cover) Conspicuous	Qualitative (presence/absence)
Macroinvertebrates (interstitial fauna)	N/A	Quantitative (individuals)
Community assessment suitability		
Monitoring patches over time	Yes (non-destructive)	No (destructive)
Diversity estimates	Macroalgae, Colonial invertebrates-yes	Algae, colonial invertebrates, interstitial fauna-yes

Supplementary Table 2.2: Composite presence/absence species list for photoanalysis (*) and scraping methods (x). Site abbreviations, SS: *SS. Hougomont*, EB: Edithburgh Jetty, RB: Rapid Bay Jetty, KJ: Kingscote Jetty, TR: Kingscote Tyre Reef, SV: Second Valley, FC: Frenchmans Cap.

Phyla/Order/Family	Taxa	SS	EB	SV	RB	KJ	TR	FC
Porifera								
Ancorinidae	*Stelletta* sp.			x				
Callyspongiidae	*Callyspongia* sp.		*	x/*	x/*			
Chondrillidae	*Chondrilla* sp.	*	*	*	*	*	x/*	
Chondropsidae	*Chondropsis* sp.	*	*	*	x/*	x	x	
Darwinellidae	*Darwinella* sp.	x	*	x	*	*	*	*
	Dendrilla sp.	x/*	*		x/*		x/*	x
Dysideidae	*Euryspongia* sp .		*	*	*	*	*	*
	Dysidea sp.	x						
Irciniidae	*Psammocinia* sp.	x						
Leucosoleniidae	*Leucosolenia* sp.	x	x	x	x			x
Microcionidae	*Clathria* sp.		x/*	x	*			x
	Holopsamma laminaefavosa	x						
Mycalidae	*Mycale* sp.	x	x	x	x	x		x
Spongiidae	*Spongia* sp.	x			x			x
Soleneiscidae	*Leucetta* sp.			x				
Sycettidae	*Sycon* sp.	x/*		x/*				*
Tethylidae	*Tethya* sp.	*						
Thorectidae	*Thorecta* sp.	x						
Valoniaceae	*Dictyosphaeria sericea*			x				
Cnidaria								
Agalmatidae	Agalmatidae indet sp.	x						

Phyla/Order/Family	Taxa	SS	EB	SV	RB	KJ	TR	FC
Campanulariidae	*Obelia australis*	x						x
	Obelia geniculata	x			x			
	Hydroid indet sp.				*			
Corallimorphidae	*Corallimorphus* sp.				x			
Lobophylliidae	*Parascolymia* sp.	*	*					
Rhizangiidae	*Culicia* sp.	*	*	x	x	x/*	x/*	x/*
Sertulariidae	*Sertularella* sp.	x						
Platyhelminthes								
Platyhelminthes	indet sp 1 (Yellow)	x						
	indet sp 2 (Green)	x						
Nematoda								
Nematode	Indet sp.1	x						
Annelida								
Oligochaeta	Indet. Oligochaete sp. 1	x					x	
Amphinomidae	Amphinomidae indet sp.		x					
Eunicidae	*Eunice* sp.	x	x		x	x		
Nereididae	Nereididae indet sp. 1	x	x		x	x	x	
	Nereididae indet sp. 2	x	x			x		
	Nereididae indet sp.3	x	x	x		x		
	Nereididae indet sp. 4	x	x			x		
	Nereididae indet sp. 5		x	x		x		
	Nereididae indet sp. 6					x		
Phascolionidae	*Phascolsoma* sp.	x	x		x	x	x	
Polynoidae	*Lepidonotus* sp.	x	x	x	x	x		
Sabellidae	*Sabellastarte australiensis*	x		x	x	x		

Phyla/Order/Family	**Taxa**	**SS**	**EB**	**SV**	**RB**	**KJ**	**TR**	**FC**
	Sabellidae indet sp. 1				*			
	Sabellidae indet sp. 2							
Serpulidae	Serpulidae indet sp.			x	x		x	
	Pomatoceros sp.	x						
	Serpulid indet sp. 1	x		x				
Spirobidae	Spirobidae indet sp.			x				
Syllidae	Syllidae indet sp.		x	x	x	x		
Terebellidae	*Terebellida* sp.	x				x		x
	Amphitrite sp.	x						
	Euploymnia sp.	x	x					
Mollusca								
Cryptoplacidae	*Cryptoplax* sp.	x						
Ischnochitonidae	*Ischnochiton* sp.1	x						
	Ascanthochitona bednalli	x						
Bivalvia								
Arcidae	*Barbatia* sp.				x			
Hiatellidae	*Hiatella australis*	x	x	x	x	x	x	
Malleidae	*Malleus* sp.	x			x	*	x/*	
Mytilidae	*Mytilus galloprovincialus*		x					
	Musculus nanus	x	x		x	x	x	
	Xenostrobus inconstans						x	
	Trichomya hirsuta						x	
Ostreidae	*Ostrea angasi*	x	x		x	*	x/*	
Pectinidae	*Chlamys asperrimus*						x	
Pteriidae	*Vulsella spongiarum*	x						
Veneridae	*Venerupis galactites*	x						

Phyla/Order/Family	Taxa	SS	EB	SV	RB	KJ	TR	FC
Gastropoda								
Buccinidae	*Cominella* sp.				x			
Mitridae	Mitridae indet sp.	x						
Nassariidae	*Nassarius particeps*	x		x				
	Nassarius pauperatus		x	x				
Olividae	*Amalda etithane*						x	
Phasianellidae	*Phasianella ventricosa*	x		x			x	
Plakobranchidae	*Elysia* sp.	x						
Ranellidae	*Argobuccinium* sp.						x	
Rissoidae	*Rissoina fasciata*	x						
Turbinidae	*Astralium aureum*			x				
Trochidae	*Cantharidella* sp.		x					
	Clanculus sp.	x		x				
	Thalotia conica	x						
	Phasianotrochus sp.			x				
	Conus sp.	x						
Indet Mollusc	Indet sp. 1						x	
	Indet sp. 2						x	
Arthropoda								
Cirripedia								
Balanidae	*Balanus* sp.		x				x	
Calanticidae	*Smilium* sp.						x	
Stenetriidae	*Stenetrium armatum*	x	x	x	x	x		x
Tetraclitidae	*Epopella simplex*		x					
indet Cirripedia	Cirripedia indet sp.						x	
Isopoda								

Phyla/Order/Family	**Taxa**	**SS**	**EB**	**SV**	**RB**	**KJ**	**TR**	**FC**
Anthuridae	*Mesanthura* sp.	x			x	x		x
Gnathiidae	*Gnathia* sp.	x	x	x		x		x
Sphaeromatidae	*Cilicaea* sp.	x			x	x		
	Cilicaea latereillei				x			
	Amphoroidea elliptera			x				
	Amphoroidea arigustata	x		x	x	x		
	Cymodopsis sp.	x				x	x	
	Neosphaeroma laticauda			x				
	Dynamella sp. 1	x						
	Dynamella sp. 2	x						
	Cerceis sp.	x						
	Cerceis trilobata	x						
	Argathona sp.			x				
Amphipoda								
Corophiidae	*Corophium* sp. 1	x	x	x	x	x	x	x
	Corophium sp. 2	x				x		
	Corophium sp. 3	x		x				
	Achelia assimilis			x				
Gammaridae	indet sp.1	x		x	x			
	indet sp. 2		x		x	x		
	indet sp. 3	x	x	x	x			x
	indet sp. 4	x	x	x				
	indet sp.5	x	x					
	indet sp.6	x	x		x	x		
	indet sp.7	x	x		x			
	indet sp. 8	x	x			x		

Phyla/Order/Family	**Taxa**	**SS**	**EB**	**SV**	**RB**	**KJ**	**TR**	**FC**
	indet sp. 9	x		x				x
Leucothoidae	*Leucothoe spinicarpis*	x	x	x		x		x
Maeridae	*Ceradocus* sp.		x					
Phliantidae	*Quasimodia* sp.	x		x				
Tanaidacea	indet sp. 1	x	x	x	x	x	x	x
	indet sp. 2		x					x
	Tanais dulongii	x						
Mysidacea	Mysid indet sp.1		x		x		x	
	Mysid indet sp. 2	x						
Decapoda								
Alpheidae	*Alpheus* sp.	x						
	Alpheus hailstonei	x	x		x	x		
	Alpheus villosus				x			x
	Synalpheus streptodactylus		x					
Dromiidae	*Fultodromia nodipes*							
Galatheidae	*Galathea australiensis*	x						
Hippolytidae	*Hippolyte* sp.	x						
Hymenosomatidae	*Halicarcinus* sp.1	x						
	Halicarcinus ovatus	x		x		x	x	x
	Halicarcinus innominatus	x						
Inachidae	*Dumea latipes*	x						
Paguridae	*Pagurixus handrecki*	x				x		
Pilumnidae	*Pilumnus etheridgei*	x				x		
	Actumnus setifer	x	x					
Echinodermata								
Antedonidae	*Antedon incommoda*	x						

Phyla/Order/Family	**Taxa**	**SS**	**EB**	**SV**	**RB**	**KJ**	**TR**	**FC**
Asteriidae	*Coscinasterias muricata*	x						
	Asteriidae indet sp. 1	x						
Cucumariidae	*Australocnus* sp.	x						
Goniasteridae	*Tosia australis*		x	x	x			
Ophiacidae	Ophiacidae indet sp.		x					
Ophidiasteridae	*Ophiactis tricolor*	x						
	Ophicomina australis	x						
	Ophionereis schayeri	x						
	Ophiothrix caespitosa						x	
	Clarkcoma sp.	x			x		x	
Bryozoa								
Bitectiporidae	*Schizomavella triangula*	x						
Buskiidae	*Cryptopolyzoon wilsoni*	x	x					x
	Cryptopolyzoon sp. 1	x						
Candidae	*Caberea* sp.	x						
	Tricellaria monotrypa	x						
	Candidae indet sp. 1	x						
Cheiloporinidae	*Cheiloporina* indet sp. 1	x		x				
	Cyclostomata indet sp.	x						
Didymosellidae	*Didymosella larvalis*	x						
Hippothoidae	*Hippothoa apurosa*	x						
Lepraliellidae	*Celleporaria* sp. 1	x	x	x	x	x	x	x
	Celleporaria sp. 2				x			
	Celleporaria canaliculata	x						
	Celleporaria bispinata		x				x	
Lichenoporidae	*Lichenopora* sp.	x						

Phyla/Order/Family	Taxa	SS	EB	SV	RB	KJ	TR	FC
Phidoloporidae	*Triphyllozoon* sp.	x	x	x				x
	Triphyllozoon monififerum	x						
Rhabdozoidae	*Rhabdozoum wilsoni*	x						
Smittinidae	*Smittina papillifera*	x						
	Indet Encrusting Bryozoa		*		*			
Tunicata								
Ascidiidae	*Phallusia* sp.					*		
Cionidae	*Ciona intestinalis*					x	x	
Clavelinidae	*Clavelina* sp.		x	x/*	*		x	x
Didemidae	*Didemnum* indet sp.	x	x	x	x	x	x	
	Didemnum sp.	*	*	*	*	*	*	*
Holozoidae	*Sycozoa cerebriformis*	x	x	x				
Polycitoridae	*Eudistoma* sp.			x				
Pyuridae	*Pyura* sp.	x	x	x/*	x/*	x	x	x
Styelidae	*Botrylloides leachi*	x	x		x	x		x
Styelidae	*Botrylloides perspicum*					x		
	Botrylloides sp.				*	*		*
ALGAE								
Chorophyta								
Caulerpaceae	*Caulerpa longifolia*	x				x		
	Caulerpa sp.	x						
	Caulerpa geminata	x						x
	Caulerpa flexilis	x						
	Indet *Caulerpa* sp.	*				*		*

Phyla/Order/Family	**Taxa**	**SS**	**EB**	**SV**	**RB**	**KJ**	**TR**	**FC**
Codiaceae	*Codium* sp.	x/*		x				
Ulvaceae	*Ulva* sp.							*
Indet Green Algae	Indet Green Algae	*		*				
Rhodophyta								
Champiaceae	*Champia viridis*	x		x	x	*	x	x/*
Corallinaceae	*Jania* sp.	x						
	Metagoniolithon sp.	x				*		
	Haliptilon sp. 1	x						
	Haliptilon sp. 2	x						
Gracilariaceae	*Gracilaria* sp.	x				*	x	*
Halymeniaceae	*Thamnoclonium* sp.				x			
	Grateloupia filicina			*				
	Semnocarpa minuta	x						
Hypneaceae	*Hypnea* sp.	x				*		*
Peyssonneliaceae	*Peyssonnelia* sp.	x	x	x			x	x
	Sonderopelta sp.			x				
Plocamiaceae	*Plocamium* sp.	x/*			*			x/*
Phyllophoraceae	*Stenogramme* sp.				x			
	Peyssonnelia sp					*		*
Rhodymeniaceae	*Botryocladia sonderi*	x						
	Rhodymenia sp	x						
Indet Red Algae	Indet Red Algae	*	*		*	*		*
Heterokontophyta								
Alariaceae	*Eklonia radiata*		x					
Cystoseiraceae	*Platythalia augustifolia*	x						
	Indet Cystophora sp.	*	*	*			*	*

Phyla/Order/Family	**Taxa**	**SS**	**EB**	**SV**	**RB**	**KJ**	**TR**	**FC**
	Cystophora sp.	x	x	x	x			
Dictyotaceae	*Zonaria* sp.		x	x				
	Padina sp.	x/*		x/*	x			*
	Lobophora variegata	x	x	x				
Sargassaceae	*Sargassum* sp.	x/*	*	x/*	x			*
Seirococcaceae	*Scytothalia doryocarpa*	x						
Sporochnaceae	*Encyothalia cliftoni*	x						
Indet Brown Algae	Indet Brown algae	*	*	*	*			*

Appendix 1 Supplementary Table 2.3: Biological Trait Analysis (bta) categories used to create functional groups for attached fauna in chapter 5.

	Taxa	**Environmental position**	**Feeding habit**	**Functional Group**
Mollusca	Monia sp.	Epibenthic	Filter	FiltEpibenthic
Mollusca	Indet Ptteridae spp.	Epibenthic	Filter	FiltEpibenthic
Mollusca	*Ostrea* sp.	Epibenthic	Filter	FiltEpibenthic
Mollusca	*Chlamys* sp.	Epibenthic	Filter	FiltEpibenthic
Mollusca	*Electroma* sp.	Epibenthic	Filter	FiltEpibenthic
Mollusca	Galeomatidae spp.	Epibenthic	Filter	FiltEpibenthic
Mollusca	*Musculus nanus*	Infauna_top	Filter	FiltInfauna_top
Mollusca	*Hiatella* sp.	Infauna_top	Filter	FiltInfauna_top
Mollusca	*Phasianella* sp.	Infauna_top	Herbivore	HerbivoreInfauna_top
Annelida	Spirorbidae spp.	Epibenthic	Filter	FiltEpibenthic
Annelida	Spionidae spp.	Epibenthic	Filter	FiltEpibenthic
Annelida	*Metalaospira* sp.	Epibenthic	Filter	FiltEpibenthic
Annelida	*Pomatoceros* sp.	Epibenthic	Filter	FiltEpibenthic
Annelida	*Sabellastarte australiensis*	Infauna_middle	Filter	FiltInfauna_middle
Annelida	Indet Eunice sp.	Infauna_middle	Predator	PredInfauna_middle
Annelida	Indet Nereid sp.	Infauna_middle	Scavenger	ScavInfauna_middle
Annelida	Indet Syllid sp.	Infauna_middle	Scavener	ScavInfauna_middle
Annelida	Indet Polynoidae spp.	Infauna_middle	Predator	PredInfauna_middle
Annelida	Indet Oligochaete spp.	Infauna_middle	Scavenger	ScavInfauna_middle
Annelida	*Phascosoloma* spp.	Infauna_middle	Scavenger	ScavInfauna_middle

	Taxa	**Environmental position**	**Feeding habit**	**Functional Group**
Arthropoda	*Balanus trigonisis*	Epibenthic	Filter	FiltEpibenthic
Arthropoda	*Tetraclitella purpurasens*	Epibenthic	Filter	FiltEpibenthic
Arthropoda	Indet Amphipoda sp.13	Infauna_top	Scavenger	ScavInfauna_top
Arthropoda	Indet Amphipoda sp.14	Infauna_top	Scavenger	ScavInfauna_top
Arthropoda	Indet Amphipoda sp.1	Infauna_top	Scavenger	ScavInfauna_top
Arthropoda	Indet Amphipoda sp. 2	Infauna_top	Scavenger	ScavInfauna_top
Arthropoda	Indet Amphipoda sp. 3	Infauna_top	Scavenger	ScavInfauna_top
Arthropoda	Indet Amphipoda sp.4	Infauna_top	Scavenger	ScavInfauna_top
Arthropoda	Indet Amphipoda sp.5	Infauna_top	Scavenger	ScavInfauna_top
Arthropoda	Indet Amphipoda sp. 7	Infauna_top	Scavenger	ScavInfauna_top
Arthropoda	Indet Amphipoda sp.6	Infauna_top	Scavenger	ScavInfauna_top
Arthropoda	Indet Amphipoda sp.8	Infauna_top	Scavenger	ScavInfauna_top
Arthropoda	Indet Amphipoda sp.9	Infauna_top	Scavenger	ScavInfauna_top
Arthropoda	Indet Amphipoda sp.10	Infauna_top	Scavenger	ScavInfauna_top
Arthropoda	Indet Amphipoda sp.11	Infauna_top	Scavenger	ScavInfauna_top
Arthropoda	Indet Amphipoda sp. 12	Infauna_top	Scavenger	ScavInfauna_top
Arthropoda	Indet Ceradocus spp.	Infauna_top	Scavenger	ScavInfauna_top
Arthropoda	*Leucathoe spincarpis*	Infauna_top	Scavenger	ScavInfauna_top
Arthropoda	Indet Corophium sp.1	Infauna_top	Scavenger	ScavInfauna_top
Arthropoda	Indet Corophium sp. 2	Infauna_top	Scavenger	ScavInfauna_top
Arthropoda	Tanaid sp.1	Infauna_top	Scavenger	ScavInfauna_top
Arthropoda	Tanaid sp.2	Infauna_top	Scavenger	ScavInfauna_top

	Taxa	**Environmental position**	**Feeding habit**	**Functional Group**
Arthropoda	*Cercies* spp.	Infauna_top	Scavenger	ScavInfauna_top
Arthropoda	*Stentrium* spp.	Infauna_top	Scavenger	ScavInfauna_top
Arthropoda	Indet Mysid sp.	Bentho_pelagic	Scavenger	ScavBentho_pelagic
Arthropoda	*Processa australis*	Bentho_pelagic	Scavenger	ScavBentho_pelagic
Arthropoda	*Synalpheus streptodactlyus*	Infauna_top	Predator	PredInfauna_top
Arthropoda	*Palaemon serenus*	Infauna_top	Predator	PredInfauna_top
Arthropoda	*Galathea australiensis*	Infauna_top	Predator	PredInfauna_top
Arthropoda	*Halicarcinus ovatus*	Infauna_top	Predator	PredInfauna_top
Arthropoda	*Notomithrax ursus*	Infauna_top	Predator	PredInfauna_top
Tunicata	*Clavelina* spp.	Epibenthic	Filter	FiltEpibenthic
Tunicata	Didemnum spp.	Epibenthic	Filter	FiltEpibenthic
Tunicata	Indet Stylidae sp.	Epibenthic	Filter	FiltEpibenthic
Tunicata	Indet Pyura sp.	Epibenthic	Filter	FiltEpibenthic

APPENDIX II: MAKE FISH NOT WAR. THE USE OF LARGE NAVAL SHIPS AS ARTIFICIAL REEF MATERIALS.

BACKGROUND

The population growth in coastal regions around the world has meant that there are now a wide variety of submerged artificial structures available for colonisation. Artificial reefs are created for many reasons; enhancing fish stocks, recreational scuba diving, and promoting biodiversity and as a way of restoring degraded ecosystems (Hiscock at al. 2010). The reefs are constructed from a wide variety of materials, but increasingly there is a trend in using surplus or obsolete vessels and structures as reefing materials. Scuttled steel structures such as ships or oil production platforms (rigs) can create very different habitats for fouling communities than those on natural rocky reefs. The scale of the *rigs to reef* program and the size of the structures involved is immense, as there are over 7500 rigs to be decommissioned in The Gulf of Mexico (Macreadie et al. 2011). Utilising oil rigs or parts of the structure as reefing materials may help to conserve deep-sea ecosystems in the Gulf, by preventing trawling and providing space for larval settlement where hard substrate is limited (Macreadie et al. 2011). In shallow coastal seas, many artificial reefs are likely created from steel vessels, through the unintentional loss or deliberate preparation and sinking. Whilst common features in coastal shelf systems there has been few peer reviewed studies on the use of surplus vessels as reefing materials.

Governments in the USA, Canada, Australia (Stolk et al. 2007, Walker et al. 2007), New Zealand (Smith et al. 2011) and Britain (Hiscock et al. 2010) and many more countries (Table 1), have all disposed of surplus naval and government vessels as reefs. The trend in utilising steel vessels is increasing

(Figure 1), as more approach the ends of their useful life. The increasing usage of vessels as reefing material follows the deactivation of previously large naval reserve fleets after the Cold War and fiscal realities of defence budgets (Hynes et al, 2004), coupled with the growing demand from recreational divers and coastal tourism operators (Ditton et al. 2002). Ex-Naval vessels are popular with divers and are thus desirable for coastal communities (Ditton et al. 2002, Kirkbride-Smith et al. 2013), as they are seen to support non-consumptive dive tourism and could increase revenue (Stolk et al. 2007). The demand for vessels is sometimes so strong, that community lobbying begins whilst the vessels are still in service, as is the case for the Australian ship, *HMAS Tobruk* (http://www.qldscubadive.com.au/Tobruk.html). In the United States there are an estimated 350 vessels that will require disposal over the next 20 years, however there is 400 locations or organisations that have expressed desire to obtain a vessel for use as an artificial reef (Hynes et al. 2004). In 2004, the United States Navy concluded artificial reef deployment to be the cheapest disposal option, as environmental guideline prohibit these ships being sent overseas for recycling, as toxic chemicals are found on board (PCBs) (Hynes et al. 2004). In America, the use of large surplus vessels as reef material has been underway since the 1970s when the Texas government bid for 7 surplus naval cargo ships. The aircraft carrier *USS Oriskany* was sunk off Pensacola Florida in May 2006, the world's largest artificial reef (in terms of a single component) specifically for use by SCUBA divers (Morgan et al. 2006). Many of the ex- government vessels selected for use as artificial reefs are obsolete and have spent long periods at anchor in harbour, potentially facilitating the spread of invasive species (Davidson et al. 2008). The United States government now bans the use of all pre-

1985 build vessels for use as artificial reefs, because of the toxic materials and resources that need to be recovered and disposed of properly (Hynes et al. 2004). Sinking large vessels is generally a popular proposition, with sinking's being televised live and publicised by media (eg. *Ex-HMAS Adelaide* at Avoca Beach or *USS Oriskany* in Florida, see for links). The acquisition and sinking process can mean that projects are often past the point of no return early on, in terms of community sentiment and investment (Figure 6). The cost associated with sinking a vessel in the California was an estimated $46 million (Pendleton 2005) and the need to offset the initial outlay with the economic benefits to coastal communities, can mean the reefs 'success' is not measured by ecological outcomes.The disposal of ships as artificial reefs is an attractive economic and populist proposition for governments as it limits the cost for disposal (Hynes et al. 2004, Smith et al. 2011) while providing commodity to communities.

FOULING COMMUNITIES ON SHIP REEFS

Little is known of the impacts of the long term structural differences associated with the reefing material as there is a lack of comparative studies using a natural benchmark site. We have summarised the main findings of the few studies that have looked at reefs created from surplus vessels (Table 4). With the exception of Pawlik et al. (2004), the fouling communities on the vessels were either very different to comparative sites or significantly changed the surrounding soft benthos (MacLeod et al. 2004, Smith et al. 2011).The ecological benefits of steel ship artificial reefs are often based around the assumption that providing additional habitat will lead to greater productivity (Bohnsack 1989) and boost abundances of desired taxa. The ex-HMS *Scylla*, although developed a diverse community, was different to surrounding hard substrates both natural and artificial and missing many species considered rare, scarce or threatened (Hiscock et al. 2010). The first vessel sunk in Australia was the ex-HMAS Swan in Western Australia (Dowling & Nichols 2001), supporting a large biomass of fish, although the species composition on the wreck remained distinctly different to natural reefs in the region (Morrison 2003).

A history of antifouling treatments can mean that even the remnants of paint can structure the fouling community on steel wrecks. On some reefs only barnacles were being found on the painted lower sections of the hulls of two vessels years after sinking (Walker et al. 2007; Hiscock et al. 2010). The fouling communities are also heterogeneous over an artificial structure, depending on the orientation or structure of the substrate (Walker et al. 2007). Spatial heterogeneity is one of the traits of artificial reefs (Svane & Petersen 2001, Walker et al. 2007) and is especially true for scuttled vessels (Perkol-Finkel et al. 2006). The orientation of the surface can affect the light availability; the shading can structure the fouling community (Glasby 2007), together with depth. The reef itself, whilst providing a habitat for a fouling community, can also be seen as long term impacts on the soft sediment it is almost always deployed upon. The footprint of a scuttled vessel is large and can cover 5000 m^2 of seafloor, increasing foraging pressure on the surrounding soft sediment infauna from the reefs inhabitants (Barros et al. 2001) and also changing the long term sediment characteristics (Richards et al. 2003).

Appendix II Table 4: Summarised results from studies that have compared communities on ship based artificial reefs or have discussed ecological effects of using vessels as reefs. See reference list for full citations.

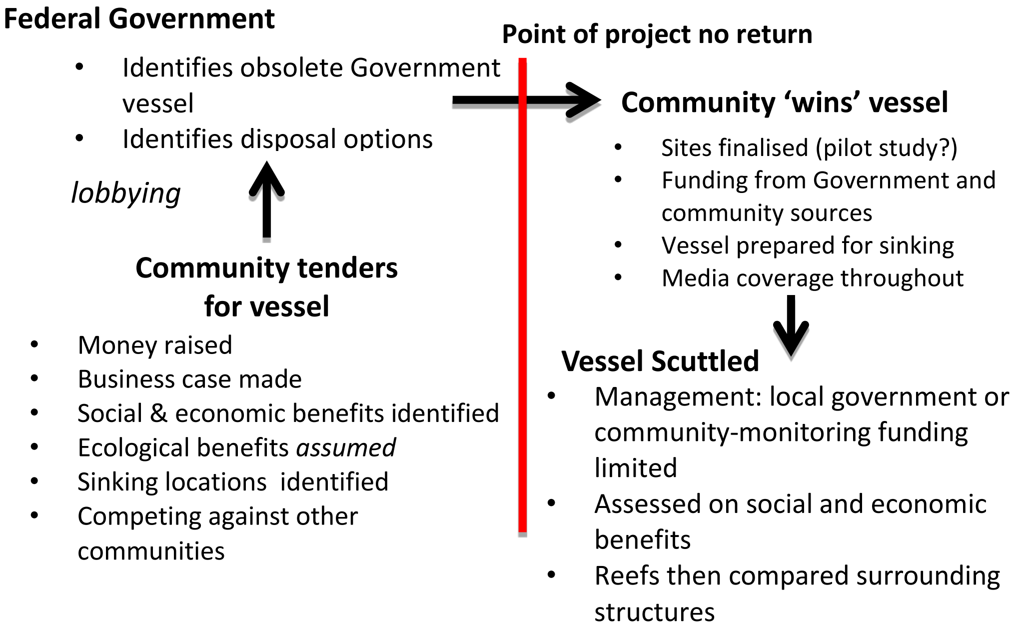
Study	**Vessel**	**Location**	**Results/Conclusions**
Walker et al. 2014	*Ex-HMAS Brisbane*	Australia	Fouling Community different to nearby natural reef communities after 3 years
Walker et al. 2007	*Ex-HMAS Brisbane*	Australia	Fouling community on the vessel was heterogeneous
MacLeod et al. 2004	*Ex-HMAS Swan, ex-HMAS Brisbane, ex-HMAS Hobart*	Australia	Increases in TBT and metal concentrations in surrounding soft sediments around vessels need to be monitored
Morrisson 1991	*Ex-HMAS Swan*	Australia	Fish communities different to other natural reefs
Jones & Welsford 1997 (conference abstract)	*G.B. Church,*	Canada	Only reported colonization data on the *G.B Church*, No comparative sites. Arthropods were the largest group.
Smith et al. 2011	*MV. Rainbow Warrior*	New Zealand	Bryozoan community can change sedimentary record around wreck
Perkol-Finkel et al. 2005	*SS Carnatic*	Red Sea	Fouling Community remained distinct after 100 years

Fricke et al. 2007	*Ex-HMSAS Good Hope*	South Africa	Fouling Community never reached stable state and diversity declined
Hiscock et al. 2010	*Ex-HMS Scylla*	UK	Fouling Community different to both nearby rock and artificial reefs- after 5 years
Pawlik et al. 2008	*USS. Spiegel Grove*	USA	Sponge communities approaching those on neighbouring reefs after 5 years
Leeworthy et al. 2006	*USS Spiegel Grove*	USA	Surrounding natural reefs were visited less by divers but diving trips increased
Sheehy & Vik 2010	*USS Spiegel Grove, USS Oriskany*	USA	Risk of long distance transport of NIS if ships are donated for reef project
Devault et al. 2016	*USS Oriskany* and others	Global	Review of issues associated with disposing of contaminated shipwrecks

The fouling communities on artificial reefs made from vessels can remain very different to that of an adjacent natural reef even after 100 years of submersion, because of the structural differences (Perkol-Finkel et al. 2005). As corrosion weakens the internal structure and oceanographic forces exert forces onto the wreck surface may in fact create a habitat suitable for fouling communities by reducing the vertical surfaces of the wreck. The amidships section collapses first, whilst the bow and stern typically remain recognisable for a longer period of time (Amer Khan, pers. com.). The *SS. Hougomont* was scuttled in 1936 and the collapsed amidships section had a fouling community that is more similar to that on a rocky shelf reef (Chapter 2). The bow and stern sections, by providing structure that is very different to surrounding habitats have maintained community differences over almost a century and it is expected that the differences would continue (Perkol-Finkel & Benayahu 2006). The use of ex-Naval ships vessels, which are the most desirable vessels for reef programs (Shani et al. 2012, Ditton et al. 2002) may mean that structural differences can persist even longer. The more stringent structural guidelines (Lloyds guidelines for naval vessels) and maritime heritage preservation through active corrosion mitigation post-sinking (Richards et al. 2003), could ensure structural differences remain centuries after sinking.

CONCLUSIONS AND FUTURE RESEARCH DIRECTIONS

Coastal communities need to consider the ecological impacts of such large vessels early in the planning process, as the structural differences will mean the community on steel wrecks will remain different to adjacent natural reefs decades later (Perkol-Finkel & Benayahu 2006). The majority of the Australian population (64%) resides in eight capital cities, of which seven are on the coast (Hardiman & Burgin 2010). More vessels are expected to be scuttled in Australia and around the world as they reach obsolescence to meet the demand for purpose built artificial reefs for fishing or diving. The strong demand for artificial reefs made by sinking military ships (Ditton et al. 2002), a focus on anthropogenic benefits (economic and social) and a worldwide supply of obsolete vessels will mean that steel ship reefs will only become more common in the marine environment. Future research opportunities need to assess the long term development of fouling assemblages on the vessels and compare to similar sized patched of natural and artificial hard substrates. Naval vessels also present interested spatial comparison opportunities as they are the largest replicated reefing materials available and are designed to be easily accessible for divers. The use of large steel vessels for reefing material is an example of a novel ecosystem (Hobbs et al. 2006), created to primarily to fulfil social and economic objectives (Turner 1969), rather than ecological ones and the long term impacts on surrounding ecosystems are not fully understood.



Appendix II Figure 6: General outline of pathway for sinking Vessels for use as reefs.

Appendix II Figure 3: Worldwide number of surplus naval ships *specifically* scuttled for the use as artificial reefs (For fishing or diving) (excluding those used for target practice or accidental losses) 1972 to 2013 from around the world. (See table in Appendix 1 for full details of vessels).

Appendix II Table 1. List of ex-Government Vessels, types, year and location sunk for use as specially prepared artificial reefs. List does not take into account any vessels expended in live fire exercises or lost accidently or through

Vessel Name and Country	**Location**	**Type**	**Year**
Australia			
exHMAS Adelaide	NSW	FFG	2011
exHMAS Canberra	Vic	FFG	2009
ex HMAS **Brisbane**	QLD	DDG	2005
exHMAS Hobart	SA	DDG	2002
ex HMAS **Perth**	WA	DDG	2001
ex HMAS **Swan**	WA	DDE	1997
ex **HMAS Derwent**	WA	DDE	1994
Canada			
HMCS Annapolis	Port **Graves**	DE	2011
HMCS Nipigon	Quebec	Destroyer	2003
HMCS Cape **Breton**	Vancouver island	Maintenance **ship**	2001
HMCS Saskatchewan	British Columbia	DE	1997
HMCS Columbia	British Columbia	Frigate	1996
HMCS Mackenzie	British Columbia	DE	1995
HMCS Chaudiere	British Columbia	Frigate	1992
HMCS **Saguenay**	Nova **Scotia**	Frigate	1990
Mexico			
HMCS Restigouche	Acapulco	Frigate	2001
HMCS Kootenay	Acapulco **?**	Frigate	2001
ARM General Miguel Negrete	Veracruz	Minesweeper	2001
ARM **General Felipe Xicotencatl**	Cozumel	Minesweeper	1999
Cayman Islands			
USS **Kitiwake**	Cayman **Is.**	Submarine **support ship**	2011
New Zealand			
HMNZS Canturbury		Frigate	2005
HMNZS Wellington		Frigate	2005
HMNZS Waikato		Frigate	2000
HMNZS **Tui**		Hydrographic **ship**	1999
United Kingdon			
HMAS **Scylla**		frigate	2004
United States			

USCGC Mohawk	Florida	Coastguard Cutter	2012
USS Kittiwake	Grand Cayman	Submarine **rescue ship**	2011
USNS General Hoyt **S. Vanderberg**	Florida	Satellite Tracking	2010
USS Arthur **W. Radford**	Atlantic **coast**	Destroyer	2010
USS Texas **Clipper**	Texas	Cargo ship	2007
USS Cruise	Delaware	Minesweeper	2007
USS Oriskany	Florida	Carrier	2006
USS William **H. Standley**	Coral Sea	missle cruiser	2005
USS Elliot	coral **sea**	Destroyer	2005
USGC Spar	Morehead City	Buoy **Tender**	2004
USS Jublant	Veracruz	Minesweeper	2001
HMCS **Yukon**	San **Diego**	DE	2000
USS Spiegel **Grove**	Florida	Dock Landing Ship	1998
Yo-257	Hawaii	Yard **oiler**	1996
USS Indra	North Carolina	Landing **craft tank**	1992
USS Algol	New Jersey	Cargoship	1991
USS Yancey	North **Carolina**	Cargoship	1990
USCGS Cyane	San **Diego**	Coast **Guard Cutter**	1989
USS Blenny	Maryland	Submarine	1989
USS Muliphen	Florida	Cargoship	1989
USS Rankin	Florida	Cargoship	1988
USS Unimak	Virginia	Coastguard **Cutter**	1988
USS Vermillion	South Carolina	Cargoship	1988
USS Aeolus	North **Carolina**	Cargoship	1988
USS Accokeek	Gulf **of Mexico**	Tug	1987
USCGC Bibb	Florida	Coastguard **Cutter**	1987
USCGC Duane	Florida	Coastguard **Cutter**	1987
USS Mindanao	Florida	Cargoship	1980
USS **Mona Island**	Viginia	Cargoship	1975
USS Fred T. Berry	Florida	Destroyer	1972

Taiwan

USS **Fort Marion**	Taiwan	Landing **ship**	2000
USS Leonard F. Mason	Taiwan	Destroyer	2003

Thailand

HTMS Kledkaeo	Phi Phi **Island**	Supply ship	2014
T11	Ko Chang	Coastal Patrol Ship	2013
HTMS Chang	Ko Chang	Landing craft tank	2012
HTMS Phetra	Ko Man **Nok**	Landing craft **tank**	2012
HTMS Mataphon	Ko **Larn**	Landing craft **tank**	2012
HTMS Sattakut	Koh **Tao**	Landing Barge	2012
HTMS Prab	Chumphon	Landing Barge	2011
HTMS **Kut**	Pattaya	Landing **Barge**	2006
HTMS Khram	Pattaya	Landing **craft tank**	2003

Malta

P31 **Patrol Boat**	Malta	Patrol **Boat**	2009
P29 Patrol Boat	Malta	Patrol **Boat**	2007

Cuba			
Frigate 356	Cayman **Brac**	Frigate	1996
Portugal			
NRP Hermenegildo Capelo	Algarve	Frigate	2013
NRP Almeida **de Carvalho**	Algarve	Oceanographic Vessel	2013
NRP **Oliveira e Carmo**	Algarve	Corvette	2012
NRP Zambeze	Algarve	Patrol **Vessel**	2012
South Africa			
SAS Pietermaritzburg	**Simonstown**	**Minesweeper**	**1994**