

Epibenthic ecology on artificial reefs: community structure in response to material, orientation and predation

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University of New South Wales

Epibenthic ecology on artificial reefs: community structure in response to material, orientation and predation

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Fisheries are an important economic resource globally. The sustainability of fisheries must be maintained to ensure the viability of this resource. Artificial reefs have become increasingly common for use in aiding fisheries as a management strategy. The scientific literature on artificial reefs has debated the effectiveness of artificial reefs in promoting sustainability. Most studies thus far have concentrated on fish populations that are of economic value. It is recognised that more focus is needed on the epibenthic assemblages that grow on the artificial reefs to better understand ecosystem productivity. This thesis examines three basic aspects of epibenthic communities on artificial reefs that are yet to be studied in detail: the effect of reef surface material, exposure to fish predation and surface orientation on epibenthic assemblage development.

A new Offshore Artificial Reef (OAR) was deployed 2 km southeast of the south head of Sydney Harbour, Australia in October 2011. Settlement plates made of four different materials (sandstone, Perspex, turpentine wood and steel) were deployed in three orientations (upward facing, downward facing and vertical surfaces) on the OAR. The settlement plates were retrieved after three months and the effects of surface material and orientation were assessed by census of the epibenthic assemblages. Steel was identified as a less desirable material for the recruitment of sessile invertebrates. Turpentine wood, Perspex and Hawkesbury sandstone surfaces had similar communities and surfaces facing downwards had higher abundances of barnacles.

A fish predator exclusion study was also deployed in three orientations on the OAR to determine the effects of fish predation on epibenthic assemblages. There were no caging artefacts detected during the study. Barnacles were more abundant in the presence of fish predation, suggesting an intermediate trophic interaction potentially involving mesopredators. Barnacles also had rapid recruitment and mortality in the presence of fish predation. This suggests nutrient transfer to higher trophic levels. They were also found to be more abundant on downward facing surfaces and there was greater evidence that they were consumed by mesopredators on vertical surfaces. By incorporating more vertical and downward facing surfaces in artificial reef design, it may be possible to increase productivity but this effect may change with seasons.

More work is required to comprehend the function of epibenthic assemblages in artificial reef ecology. It is important that they are incorporated in a whole-ecosystem approach necessary for assessing the productivity of artificial reefs. This thesis provides evidence that reef construction material and surface orientation will strongly influence the development of epibenthic assemblages and that this may have cascading effects for predatory fish. For artificial reefs to continue evolving as an aid to sustainable fisheries, interdisciplinary approaches are necessary to optimise their design for productivity and the support of native biodiversity.

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Abstract

Thesis abstract

Fisheries are an important economic resource globally. The sustainability of fisheries must be maintained to ensure the viability of this resource. Artificial reefs have become increasingly common for use in aiding fisheries as a management strategy. The scientific literature on artificial reefs has debated the effectiveness of artificial reefs in promoting sustainability. Most studies thus far have concentrated on fish populations that are of economic value. It is recognised that more focus is needed on the epibenthic assemblages that grow on the artificial reefs to better understand ecosystem productivity. This thesis examines three basic aspects of epibenthic communities on artificial reefs that are yet to be studied in detail: the effect of reef surface material, exposure to fish predation and surface orientation on epibenthic assemblage development.

A new Offshore Artificial Reef (OAR) was deployed 2 km southeast of the south head of Sydney Harbour, Australia in October 2011. Settlement plates made of four different materials (sandstone, Perspex, turpentine wood and steel) were deployed in three orientations (upward facing, downward facing and vertical surfaces) on the OAR. The settlement plates were retrieved after three months and the effects of surface material and orientation were assessed by census of the epibenthic assemblages. Steel was identified as a less desirable material for the recruitment of sessile invertebrates. Turpentine wood, Perspex and Hawkesbury sandstone surfaces had similar communities and surfaces facing downwards had higher abundances of barnacles.

A fish predator exclusion study was also deployed in three orientations on the OAR to determine the effects of fish predation on epibenthic assemblages. There were no caging artefacts detected during the study. Barnacles were more abundant in the presence of fish predation, suggesting an intermediate trophic interaction potentially involving mesopredators. Barnacles also had rapid recruitment and mortality in the presence of fish predation. This

suggests nutrient transfer to higher trophic levels. They were also found to be more abundant on downward facing surfaces and there was greater evidence that they were consumed by mesopredators on vertical surfaces. By incorporating more vertical and downward facing surfaces in artificial reef design, it may be possible to increase productivity but this effect may change with seasons.

More work is required to comprehend the function of epibenthic assemblages in artificial reef ecology. It is important that they are incorporated in a wholeecosystem approach necessary for assessing the productivity of artificial reefs. This thesis provides evidence that reef construction material and surface orientation will strongly influence the development of epibenthic assemblages and that this may have cascading effects for predatory fish. For artificial reefs to continue evolving as an aid to sustainable fisheries, interdisciplinary approaches are necessary to optimise their design for productivity and the support of native biodiversity.

Chapter 1

Introduction

Marine fisheries are an integral part of the global economy and our diet. In 2011, 130.8 million tonnes of fish products were consumed with 90.4 million tonnes of this captured from wild fisheries (Food and Agriculture Organization, United Nations 2012). The proportion of wild fisheries that are overexploited continues to increase – albeit more slowly in recent years – to 30 % in 2009 (Food and Agriculture Organization, United Nations 2012). There are over 38 million people worldwide who are directly involved in capture fisheries and are dependent on the sector for income in a fleet of 4.36 million vessels (Food and Agriculture Organization, United Nations 2012). It is therefore important that fisheries are managed sustainably.

In Australia, fisheries are also important to the economy. Fisheries contributed AU\$2.23 billion to the economy in 2011, equivalent to a total volume of 234,164 tonnes. AU\$1.31 billion (162,762 tonnes) of the revenue came from wild fisheries. The fisheries in the state of New South Wales (NSW) were worth AU\$123.5 million in 2011 (Skirtun *et al.* 2012). Recreational fishing in NSW was estimated to be worth in excess of AU\$550 million through purchasing/hiring of boats, fishing tackle and bait (Department of Primary Industries, New South Wales Government 2001). It also represented approximately 30 % of the total commercial catch, while 6 species that are prominently harvested by both recreational and commercial fishers were harvested in greater numbers by recreational fishers (Department of Primary Industries, New South Wales Government 2001). This **emphasises the importance of sustaining the state's fisheries not only** due to their value as a primary resource but also for the value of the services they support.

Although the Food and Agriculture Organization (FAO) reported that only 12 % of fish stocks are classified as overharvested in Australia, many are yet to

be assessed. It is crucial that wild fisheries are managed adequately to ensure the sustainability of this valuable resource (Food and Agriculture Organization, United Nations 2012; Woodhams *et al.* 2012). To achieve this, the NSW Department of Primary Industries has implemented various fisheries management and enhancement tools, the most recent of which included the use of artificial reefs (Reeds 2010).

Introduction to artificial reefs

An artificial reef is defined by The European Artificial Reef Research Network (EARRN) as any submerged structure that is deliberately placed on the seabed to mimic the characteristics of natural reefs (Baine 2001). Artificial reefs were primarily used for aiding fisheries production and fisheries management by altering the spatial and temporal distribution of target species, improving harvesting efficiency and creating new fishing sites (Polovina 1991; Van Treeck and Schuhmacher 1998). They are popular in countries such as Japan and the United State of America where artificial reefs have been used extensively from the early 1900s (Bolding et al. 2004). More recent uses have included the conservation of the natural environment, rehabilitation and protection of coastal environments, adding habitat and supporting recreational fishing (Miller 2002; Van Treeck and Schuhmacher 1998). They are popular with governments because they are a highly visible management activity. It allows governments to display how funds are being used and they are popular amongst anglers who report high catch rates at artificial reefs (Grossman et al. 1997).

From the 1950s to 1970s, waste material such as tyres and wooden pallets were used as opportunistic artificial reefs (Bolding *et al.* 2004). However, these were problematic since waste such as used tyres leached toxic substances into the water that were found to be lethal to certain organisms (Day *et al.* 1993). There has since been an expansion into the types of materials and shapes used for the construction of artificial reefs. Current

artificial reefs are made of modern materials such as fiberglass-reinforced plastic, concrete and steel that are designed to address specific ecological goals (Seaman 2002; Zalmon *et al.* 2012). Artificial reefs are constructed with shapes to accommodate economically important species by providing refuge from predators or niches for juveniles to grow in (Barry and Whickins 1992; Taylor 1998).

Scientific literature on artificial reefs

The literature on artificial reefs has largely revolved around the production versus attraction debate. Initially, the primary function of artificial reefs was to aid fisheries by attracting fish populations to the structure (Bolding *et al.* 2004). More recent artificial reefs have been designed to increase productivity instead of merely attracting existing fish populations (Connell 2001). This was a result of adopting the aim to make fisheries more sustainable by increasing productivity. There have been studies that have since assessed the productivity of artificial reefs to determine if they were in fact increasing productivity or merely attracting existing fish populations (Baine 2001). Bohnsack (1989) suggested that an artificial reef has the potential to increase productivity, if the ecosystem was habitat-limited. However, in recruitmentlimited ecosystems, the increase of available habitat would not necessarily result in an increase in productivity. If production does not increase, the artificial reef attracts existing fish populations, which simply redistributes existing biomass. Additionally, if the existing harvest rate was high for a given fishery, the attraction caused by the artificial reef only increased harvest efficiency and led to overharvesting of an already heavily exploited population (Bohnsack 1989; Polovina 1991). Furthermore, Eklund (1996) showed that an artificial reef with less refuge exposed target species to predation, which resulted in a decrease in their abundance. Therefore, some argued that artificial reefs only increased the susceptibility of fisheries to overfishing (Polovina 1989; Walters et al. 1991; Wege 1981; Wege and Anderson 1979). On the contrary, Svane and Peterson (2001) argued that artificial reefs were

often used in habitat-limited ecosystems and the recruitment of epibenthos was enough to increase the productivity on an artificial reef. Artificial reefs provided potential substrate for epibenthos and therefore provided additional food for other organisms. They provided habitat for fish and epibenthic recruits that otherwise would have been lost from the population (Pickering and Whitmarsh 1997). They also provided shelter for many species, increasing the population size (Connell 2001). Therefore by providing additional habitat, they argued that artificial reefs increased productivity and were producing greater fish biomass.

Taylor (1998) outlined the importance of habitat heterogeneity in the production versus attraction debate. Even if the organic turnover of an artificial reef was low i.e. even if the artificial reef was not colonised by epibenthic assemblages and increased productivity, habitat heterogeneity could still increase production by providing niches for small and juvenile organisms (Eklund 1996; Taylor 1998). Habitat heterogeneity of artificial reefs made comparisons across different artificial reef designs difficult. This is an important aspect to studying artificial reefs and as Svane and Peterson (2001) suggested, the production and attraction debate were not mutually exclusive concepts, but two extremes on a gradient. To effectively study the effects of artificial reefs, a whole-ecosystem approach is necessary and when combined with adequate management of the reef, it is possible to achieve specific predetermined ecological goals (Baine 2001; Bolding *et al.* 2004; Butman 1987; Eckman 1983; Grossman *et al.* 1997).

Scientific literature on epibenthic assemblages

Extensive studies of epibenthic communities have been made in estuarine environments, in particular when assessing the effect of environmental and anthropogenic stressors (Borja *et al.* 2007, Kopp *et al.* 2013, Riera *et al.* 1999, Wharfe 1997). Survey approaches have assessed epibenthic communities in the field using techniques such as photographic quadrats and

SCUBA diver transects (Barrett *et al.* 2009, Dafforn *et al.* 2012), while field manipulation experiments generally use settlement plates and caging to assess recruitment under various treatments (Dafforn *et al.* 2009, Rivero *et al.* 2013). The methods used in this study are therefore well established.

Epibenthic studies generally range from monthly to 12-month sampling periods depending on the aim of the study. Those focusing on issues such as spatial competition or the effect of primary recruitment on subsequent benthic recruitment use samples left in the field for longer periods (4-12 months) (Connell 2001, Glasby and Connell 2001). Those focusing on the initial recruitment of epibenthic species leave samples in the field for a shorter duration (1-3 months), as the primary recruitment will be matured but subsequent competition will not obscure settlement preferences (Boaventura *et al.* 2006, Dafforn *et al.* 2012, Sagasti *et al.* 2000). Studies looking at the effect of substrate – which would focus mostly on the primary recruitment – on epibenthic species should therefore be of a shorter duration, to allow the primary recruits to mature while avoiding the confounding effects of a secondary recruitment layer (Boaventura *et al.* 2006).

The use of cages has been used to determine the effects of fish predation on epibenthic assemblages (Connell 1999a, Connell 1997, Hedge and Johnston 2012, Rivero *et al.* 2013). Connell (1997) has validated the methodology of using cages without affecting other factors such as shading and hydrodynamics to exclude fish predation. The use of two different mesh sized cages has also been shown to be useful in determining predation effects by fish of different size (Connell 1999a). This is especially useful in environments where the exact trophic relationships between the epibenthic assemblage and the various fish predators are not clearly identified. Scientific literature on the specific diets of fish commonly found on artificial reefs such as *Pelates sexlineatus* and *Acanthopagrus australis* are not always available (Connolly 2003, Folpp *et al.* 2011). Hence, the use of different mesh size cages can help distinguish the relationships between epibenthic assemblages and various

sized fish predators, which may feed directly on the epibenthos or the mobile invertebrates that it supports. A mesh size of 50 mm excludes larger fish (greater than 200 mm body length), while a smaller mesh size excludes most fish except juveniles and smaller fish such as Blenniidae and Gobiidae (Connell 1999a, Connell 1997).

The substrate material and the surface orientation have also been documented to affect epibenthic assemblages in environments such as marinas where anthropogenic modifications provide artificial substrate for invertebrates (Dafforn *et al.* 2012, Knott *et al.* 2004). Different taxonomic groups and species have been shown to vary in abundance by substrate and orientation: certain ascidian species, hydrozoans and serpulids vary by orientation, while bryozoans such as *Schizoporella* and *Bugula*, as well as ascidians such as *Styela* and *Diplosoma* vary by substrate (Azevedo *et al.* 2006, Fitzpatrick and Kirkman 1995, Glasby and Connell 2001, Miura and Kajihara 1984). The interactions between different substrates and orientations however, are relatively unknown due to the scarcity of studies focusing on these two factors.

These methods – though utilised frequently in coastal environments – are yet to be fully applied in the context of studying the epibenthic assemblages on artificial reefs to assess the role these new structures play in the greater ecosystem of an artificial reef

Epibenthic assemblages on artificial reefs – the knowledge gap (Knowledge gap – epibenthic assemblages)

Difficulties in studying artificial reefs may arise from a lack of control with natural reefs, degree of isolation, age and heterogeneity of the artificial reefs. One solution to these problems was to adopt a whole-ecosystem approach to assess artificial reefs (Butman 1987; Carr and Hixon 1997; Eckman 1983; Pratt 1994; Svane and Peterson 2001). Previous studies have primarily

focused on economically targeted species (Bohnsack 1989). There is a need for more studies looking at the epibenthic assemblage on artificial reefs, but very few studies have actually conducted such experiments (Connell 2001; Lindquist *et al.* 1994; Relini *et al.* 2002; Svane and Peterson 2001). There is a substantial knowledge gap on how artificial reefs influence the development of epibenthic assemblages, and the consequences that changes in the assemblage may have on higher trophic levels found in the ecosystem.

While there are many aspects to an artificial reef that may affect epibenthic assemblages, there are three that require particular attention. The first is the effect of reef material on epibenthic settlement. There are numerous studies that have suggested differences in epibenthic assemblages are a result of artificial substrates found in various environments (Atilla et al. 2003; Dafforn et al. 2012; Glasby 1999a; Glasby and Connell 1999; Knott et al. 2004). Few have concentrated on materials and environments directly relevant to artificial reefs. Seaman (2002) explained that the reason for this was 'corporate memory' where most of the history and knowledge of artificial reefs and their materials were known only by practitioners and scholars, and not published as peer-reviewed articles. Seaman outlines the need for a global database of studies and the improvement of appropriate usage and practices. The lack of scientific literature and the effect of this 'corporate memory' is evident from the large number of patents for artificial reef design and manufacturing compared to peer-reviewed literature (Chul 2012; Heon et al. 2012; Jae 2012; Kim 2012; Okazaki 2012; Sawada et al 2012).

Predator-prey interactions of artificial reefs also require attention, as these are the basis of fish production. In particular, the trophic interactions of fish and other predators with benthic invertebrates and algae that form the epibenthic assemblage on artificial reefs, have been identified as an important factors in influencing the epibenthic assemblage in many previous studies (Barrett *et al.* 2009; Sih *et al.* 1985; Whiting *et al.* 2011; Xu *et al.* 2012). This interaction is important not only because of the direct effects to predator and

prey, but also because of the indirect effects to other organisms, known as trophic cascades (Carpenter *et al.* 1985). The epibenthic assemblage is expected to form an important part of the artificial reef ecosystem, as it is a source of food as well as shelter for a range of organisms (Connell 2001; Relini *et al.* 2002; Svane and Peterson 2001). By assessing these interactions, it can be determined if the benthic productivity could lead to an increase in fish production. The scientific knowledge of the predator-prey interactions on artificial reefs is just as unexplored as the effects of reef materials on the development of the epibenthic assemblage.

Additionally, surface orientation can alter abundances of epibenthic species of bryozoans, sponges and barnacles in estuarine environments (Dafforn *et al.* 2012; Glasby and Connell 2001; Knott *et al.* 2004; Knott *et al.* 2006; Moura *et al.* 2008; Walker *et al.* 2007). It is unknown whether these effects translate to an artificial reef environment and how they will interact with surface material and fish predation.

Study system

A study system had to be defined to adequately address the knowledge gaps identified above. Previous studies have outlined the need for adequate controls between artificial and natural reefs, although it is difficult to compare reefs without quantifying structural heterogeneity (Bohnsack 1989; Polovina 1991; Taylor 1998). Other factors such as the biological history, substratum characteristic differences, reef size and degree of isolation complicate direct comparisons between artificial and natural reefs (Carr and Hixon 1997; Glasby and Connell 1999; Pratt 1994). These factors are difficult to keep constant but measures such as hydrodynamic properties on and around the artificial reefs can be measured and should be taken into account (Butman 1987; Eckman 1983).

Due to the lack of literature on epibenthic assemblages on artificial reefs, a fundamental study was required to address basic aspects of the epibenthic assemblage on artificial reefs. Such an opportunity arose when a new Offshore Artificial Reef (OAR) was deployed off Sydney Harbour in New South Wales, Australia in October 2011. A 42 tonne steel artificial reef with dimensions of 12 m (height) and 15 m x 15 m at the base was sunk 2 km southeast of the south head of Sydney Harbour at a depth of 38 m (S 33°50.797', E 151°17.988'). Four 60 tonne concrete blocks were chained to each corner to secure it to a sandy bottom. This OAR deployment allowed experiments to be set up on the structure to address some of the basic aspects of artificial reef ecology. By working *in situ* on the OAR, confounding factors could be minimised by providing our own treatments within the artificial structure. Assessments could then be made of the effects of materials, predation and surface orientation on epibenthic assemblage on an artificial reef.

Epibenthic studies in the region have often found taxonomic groups such as barnacles (*Balanus trigonus, Balanus variegatus*), various bryozoans (*Beania magellanica, Celleporaria* sp, *Microporella* sp) and serpulid worms to dominate recruitment on natural substrates as well as experimental settlement plates (Anderson and Underwood 1994, Connell 2001). Therefore it is likely that the epibenthic assemblage on the study site will be composed of similar taxonomic groups. These studies have also observed the recruitment of algae (Connell 2001, Glasby 1999a, Glasby 1999b). However, because of the difference in depth and therefore light availability, as well as the hydrodynamics that could alter the transport of spores, the study site is predicted to have reduced algal abundance to previous studies in the area that are in shallower environments (Phillips and Blackshaw 2011, Underwood *et al.* 1991). The OAR had close to 100% cover of epibenthic invertebrates and macroalgae at the time of deployment, observed through live video footage from SCUBA divers.

Previous observations at the OAR showed a variety of fish species inhabiting the OAR. Atypichthys strigatus (mado), Nelusetta ayraud (chinamanleatherjacket), Nemadactylus douglasii (blue morwong), Trachurus novaezelandiae (yellowtail scad) and Seriola lalandi (yellowtail kingfish) have all been observed around the OAR. While species such as T. novaezelandiae are mainly planktivores and S. lalandi prey on them, A. strigatus, N. ayraud and N. douglasii are species that are known to feed on a variety of invertebrates, including benthic invertebrates (Glasby and Kingsford 1994, Peristiwady and Geistodoerfer 1991, Stewart and Hughes 2009). N. douglasii mainly feed on macroinvertebrates but have also been documented to feed on sessile invertebrates such as bryozoans, ascidians, sponges and serpulids as well as mobile invertebrates in the epibenthic assemblage such as gastropods, flatworms, copepods and amphipods (Stewart and Hughes 2009). Both A. strigatus and N. ayraud similarly consume both sessile and mobile invertebrates, they also feed on algae in the epibenthic assemblages (Glasby and Kingsford 1994, Peristiwady and Geistodoerfer 1991). Barnacles are one taxonomic group that are a dominant component of the epibenthic assemblage that has not been documented as being common prey items for these fish species.

Thesis outline

This thesis addresses the effect of surface material, predation and surface orientation on the epibenthic assemblage on an artificial reef. Chapter 2 addresses the issue of surface material and orientation on epibenthic assemblage using settlement plates of various materials deployed in different orientations. By comparing the epibenthic assemblages across the two treatments, differences and interactions were identified by surface material and orientation. The assemblage was expected to differ by surface material as well as by orientation due to substratum characteristic differences and settlement preferences (Carr and Hixon 1997; Glasby and Connell 1999; Glasby and Connell 2001).

Chapter 3 addresses the issue of fish predation and surface orientation on epibenthic assemblages using Perspex settlement plates caged and deployed in different orientations. Comparisons of epibenthic assemblages across the two orthogonal treatments (predator exclusion and surface orientation) allowed us to identify differences and interactions in the assemblage by predator-prey interactions and surface orientation. The assemblage was expected to differ by predation treatments as well as surface orientation from results of previous predation and orientation studies (Connell 2001; Glasby and Connell 2001). This thesis will quantify the effect of these factors on the epibenthic assemblage in a novel offshore environment. The experiments for both chapters were deployed for 3 months on the OAR in 2012 to fill the specified knowledge gaps in the artificial reef literature. They were deployed over the same period using similar techniques. Therefore there are some similarities in the methodology described for both chapters.

Chapter 2

Implications of structural materials for epibenthic communities on an Offshore Artificial Reef

Abstract

Artificial reefs provide shelter for targeted fish species and may also provide a source of food depending on the epibenthic community that grows on the reef structure. Little is known about the effects of artificial reef construction materials on the development of epibenthic communities. Settlement plates of four materials (Perspex, Hawkesbury sandstone, turpentine wood and mild steel) were deployed in three orientations (upwards facing, downwards facing) and vertical) at 31 m depth on a 42 tonne Offshore Artificial Reef (OAR) between December and March (2012/13) near Sydney, Australia. After 3 months, settlement surfaces were retrieved and epibenthic assemblages were censused. Steel surfaces had lower species richness, total abundance and diversity compared to Perspex, sandstone and wood surfaces. Steel is not an ideal material for the initial recruitment and growth of benthic invertebrates and a longer period would be required for a mature benthic invertebrate community to develop. Once an initial biogenic substrate is established, the negative effects on the recruitment and development of the steel may be reduced as epibenthic growth has been observed on the OAR since its deployment over 2 years earlier. The surface orientation showed similar trends to those from other studies where the serpulid worm, *Pomatocerous* sp. were more abundant on downward facing surfaces and bryozoans showed species-specific results. The bryozoan Celleporaria nodulosa was more abundant on upwards facing surfaces whereas Beania magellanica was more abundant on downward facing surfaces. Both surface material and orientation are important factors for developing epibenthic assemblages. This could influence the fish assemblage and is therefore an important factor that must be considered in artificial reef design. Hence it is vital that epibenthic

communities are incorporated into artificial reef ecology as it has broader implications on the artificial reef ecosystem.

Introduction

Artificial reefs are an increasingly common feature in the world's oceans and they are deployed for a range of reasons. Increasingly, artificial reefs are designed and deployed specifically to increase fish abundances (Miller 2002; Van Treeck and Schuhmacher 1998). These purpose-built artificial reefs are designed to imitate fish habitat, so are functionally different to scuttled vessels or piles of used tyres. New materials such as fibreglass-reinforced plastic, concrete and steel are now commonly used. The use of such artificial reefs has become common such that purpose-built reefs made of steel and concrete are regularly deployed in Japan, the United States of America and Australia (Bolding *et al.* 2004; Branden *et al.* 1994). These reefs provide shelter and food for numerous economically important species, thereby increasing the size of their population and enhancing fisheries (Connell 2001). The epibenthic assemblage is an important part in providing food for these species (Svane and Peterson 2001).

Although the design and construction of artificial reefs has developed in the past few decades, the scientific literature has not kept pace with these developments (Svane and Peterson 2001). Published studies of artificial reefs tend to focus on the functional role of artificial reefs in fish populations. In **particular, there has been a strong focus on the 'production/attraction' debate** in which some have argued that artificial reefs simply redistribute existing biomass by attracting existing fish populations to the artificial reef, increasing harvesting efficiency and leading to overharvesting (Bohnsack 1989; Polovina 1991). While others argue that artificial reefs provided a substrate in the area for the production of organism that sustain fish populations, therefore producing additional fish in the area (Connell 2001; Pickering and Whitmarsh 1997). Fewer studies have looked at what factors associated with the design would optimise the reef for purposes such as increasing economically targeted fish populations. Seaman (2002) described this lack of publication as **'corporate memory' where the kn**owledge of artificial reefs was known by

practitioners and scholars but were not published. Compounding the problem of this 'corporate memory' is the increasing number of patents on artificial reef designs (Chul 2012; Heon *et al.* 2012; Kim 2012; Oakazaki 2012, Sawada *et al.* 2012) compared to scientific literature. The consequence of this is a collection of studies that have focused on monitoring fish populations and behaviour, and a lack of focus on invertebrates that studies have continuously outlined as being important in determining fish abundances (Boaventura *et al.* 2006; Svane and Peterson 2001).

The surface material and the orientation found on these artificial reefs are likely to be an important aspect of reef productivity. Past studies have shown that substrate type and surface orientation play an important role in determining the epibenthic assemblage, thus these factors have an effect on economically targeted species through trophic cascades (Carpenter *et al.* 1985; Dafforn *et al.* 2012). It is therefore imperative that we study the effect different materials have on the epibenthic assemblages (Svane and Peterson 2001). Studies are yet to comprehensively test the effects of materials used for building artificial reefs (such as steel) and compare them with natural substrates (such as sandstone) by assessing the epibenthic assemblage on those surfaces. These effects need to be identified to further optimise the design of artificial reefs to increase the productivity of the reef.

Studies on surface material and its effects on the epibenthic assemblage have mostly been conducted in shallow estuarine environments (Dafforn *et al.* 2012; Knott *et al.* 2006). These studies have found that natural substrates (usually Hawkesbury sandstone in southeast Australia) promote the settlement of algae (Dafforn *et al.* 2012; Glasby 1999a; Glasby 1999b; Glasby and Connell 1999), while algae were found to be less abundant on artificial substrates (Glasby 1999b). Artificial substrates had higher abundances and diversity of epibenthos, although they were also more susceptible to invasive species (Connell and Glasby 1999; Dafforn *et al.* 2012). Despite this, invasive species were never more abundant than the native species even on artificial

substrates (Dafforn *et al.* 2012). Previous research has also shown that epibenthic assemblages on artificial substrates were more abundant on concrete and wood compared to metals and fibreglass (Anderson and Underwood 1994; Azevedo *et al.* 2006). The effects of these materials on epibenthic assemblages are yet to be determined in offshore environments where more substantial artificial reefs are utilised.

Previous studies have also shown the importance of surface orientation on epibenthic assemblages. Some studies have suggested that orientation is more important than the surface material in determining the epibenthic assemblage that settles on a surface (Knott *et al.* 2004). This is due to a number of factors that effect surfaces on different orientations such as light, hydrodynamics and sedimentation (Airoldi 2003; Glasby 1999a; Knott *et al.* 2004). Algae, serpulid worms, sponges and bryozoans are some of the epibenthic groups that have been shown to respond to surface orientation (Duggins *et al.* 1990; Glasby 1999a; Knott *et al.* 2006; Levring 1966). These effects are yet to be assessed in detail in offshore environments where Offshore Artificial Reefs (OAR) are used.

Settlement plates were deployed on an OAR, to better understand the importance of surface material and orientation on epibenthic assemblages. The surfaces were made of steel (used to construct the OAR), Hawkesbury sandstone (natural reference substrate), turpentine wood (commonly used in Sydney Harbour for wharves and piles) and Perspex (commonly used for recruitment studies) and were deployed on upward facing, downward facing and vertical surfaces. The OAR was deployed in October 2011, in the waters off Sydney, Australia. The 42 tonne steel artificial reef was positioned **approximately 2 km southeast of Sydney's south head, with a goal to increase** the population of recreationally targeted fish species (Reeds 2010). This research had two main aims. The first was to determine how the development of epibenthic assemblages changes with surface material type. It was expected that the fouling communities would differ between materials,

particularly the natural (sandstone) and artificial (steel) materials. The second aim was to determine the effects of surface orientation on epibenthic development on an OAR. It was expected that surface orientation would have a great influence on the fouling community.

Methods

Offshore Artificial Reef – general description

The location for this study was an Offshore Artificial Reef (OAR), located approximately 2 km southeast of the south head of Sydney Harbour (S 33°50.797', E 151°17.988') (**Fig. 2.1**). The OAR is a purpose built steel construction with dimensions of 12 m in height by 15 m x 15 m at the base. It weighs 42 tonnes and is situated on a natural sandy bottom at a depth of 38 m. Four 60 tonne concrete blocks are chained to each corner for stability (**Fig. 2.1**). The reef was deployed in October 2011. This experiment took place on the reef from December 2012 to March 2013.

Recruitment study

An experimental study of benthic recruitment on different surface materials and orientations was conducted on the OAR. Backing panels made of grey polyvinyl chloride (PVC) were attached to the OAR. Panels were designed to fit over the steel beams used to construct the OAR and were held in place with Kevlar smart bands. Eight backing panels were fitted to the beams on the top deck of the main structure of the OAR, at 31 m depth (**Fig. 2.1**). They were all fitted to face the same direction to reduce confounding effects from current direction.

Panels contained an upward facing, downward facing and vertical surface (**Fig. 2.2**). Attached to each surface orientation were four settlement plates (11 cm x 11 cm x 1 cm), spaced 8 cm apart, made of one of four materials. Perspex was used as a reference material commonly used for studying benthic invertebrate assemblages (Birdsey *et al.* 2012; Clark and Johnston 2009; Dafforn *et al.* 2009; Knott *et al.* 2009; Rivero *et al.* 2013). Hawkesbury sandstone was selected as the natural reference substrate as it constitutes the rocky reef substrate in the region (Chapman and Underwood 2009; Green

et al. 2012). Turpentine wood (*Syncarpia glomulifera*) was chosen, as a reference artificial substrate, which is commonly used to construct piers in Sydney Harbour (Connell and Glasby 1999). The outer part of the bark was used, as it is resistant to marine borers, which makes it desirable for use in piers (Cookson and Barnacle 1987). Brushed mild steel (no grade) was the final of four settlement surfaces, which is the material that the OAR is made of. One settlement plate of each surface material type was randomly allocated to one of four positions in each orientation on each backing panel to make a total of 8 replicates of each surface material by orientation combination.

Panels were attached by SCUBA divers on the 28th, 29th of November and 7th of December, 2013 and left submerged for 3 months, at which point they were retrieved for census. Upon collection, settlement plates were immediately fixed in 7 % buffered formaldehyde solution for 24 h then drained, washed and preserved in 80 % ethanol.

Epibenthic assemblage

Epibenthic recruits on each settlement plate were censused under a dissecting microscope. A 100-point (10 x 10) grid was used to subdivide the plate, with all organisms within a random subsample of 16 grid squares counted to give a measure of abundance. Organisms were classified to species or the lowest possible taxonomic level. The organisms were also classified into three size classes: small, medium and large. The size of serpulids and amphipod tubes were assessed by the operculum or tube opening, respectively. An opening of 0.1 cm or less was classified as small, 0.1-0.2 cm was classified as medium, and greater than 0.2 cm was classified as large. All other organisms were classified by their diameter at the widest point. A diameter of less than 0.2 cm was classified as small, 0.2-0.5 cm as medium and greater than 0.5 cm as large.

The 100-point grid was also used to calculate percent cover of organisms on each surface. Organisms found directly under each intersect were counted and given a value of 1 % cover. The same size classes were applied.

After the abundance and percent cover data were recorded, a full plate scan was performed to ensure that any species present on the plate but not found in the subsample were accounted for. Any species found in the scan were given a value of 0.5 for abundance measure or 0.5 % for percent cover.

Statistical analysis

Community assemblage

The percent cover and abundance measure were analysed in the same way. To test the effect of surface material and orientation on epibenthic abundance, permutational multivariate analyses of variance (PERMANOVA) with two fully crossed fixed factors (surface material and orientation) were conducted on the abundance measure and percent cover data using the statistical package PRIMER v6.0 (Plymouth Routines in Multivariate Ecological Research) with PERMANOVA+ (PRIMER-E Ltd, UK). The data were log₁₀ transformed and a resemblance matrix was created using Bray-Curtis similarity with a dummy variable of 1. The PERMANOVA was run using 99,999 permutations of the data. The PERMDISP function was used to determine if the differences in dispersion between groups occurred.

PERMANOVA was also used on the abundance data to test whether the amount of time the panels were submerged affected the abundance and thus may be considered a confounding factor. There was no significant effect. A PERMANOVA was also conducted to test for confounding effects between the eight backing panels (blocking effects). There were no confounding effects. Principal coordinate ordinations (PCO) were used to visualise the similarities or dissimilarities between material and orientation. To better visualise trends, vector lines for species with a Pearson correlation of 0.5 were overlaid on the PCO to show their correlation with various treatments. PCOs were done in PRIMER v6.0.

Diversity

The diversity measures of species richness and Shannon's diversity index as well as total abundance were calculated. Univariate PERMANOVA was used to test the effect of surface material and orientation on these measures. Data were square root transformed and a Euclidean distance resemblance matrix was constructed. PERMANOVA was run with 99,999 permutations of the data. The PERMDISP function was used to determine if the differences in dispersion **between groups occurred. Tukey's HSD test** was performed to further examine differences amongst groups, using the statistical program SPSS (IBM Corporation, USA). Prism 6 (GraphPad Software Corporation, USA) was used to graph any differences observed from the PERMANOVA results.

Species-level responses

To avoid issues of low values, species with a mean abundance below 2 were excluded from individual analysis. Univariate PERMANOVAs were run on all other species to determine differences between surface material and orientation. Data were square root transformed and a Euclidean distance resemblance matrix was constructed. PERMANOVA was run with 99,999 permutations of the data. The PERMDISP function was used to determine if **the differences in dispersion between groups occurred. Tukey's HSD test was** used to interpret significant single species effects, and were done using the statistical program SPSS. Prism 6 was used to graph any differences observed from the PERMANOVA results.



Figure 2.1. (a) A map of Sydney Harbour, New South Wales with the location of the Offshore Artificial Reef (OAR) marked by the symbol •. The OAR is located at S 33°50.797', E 151°17.988'. (b) A photograph of the OAR taken on the 29th of September 2011 at Sydney Ports before deployment. (c) A model diagram of the OAR including its concrete weights with the top deck where the experimental panels were fixed outlined by the black rectangle (image: NSW DPI). Approximate depth of the top deck was 31 m.



Figure 2.2. (a) A schematic diagram of the experimental panel used made of polyvinyl chloride (PVC). Four 11 cm x 11 cm settlement plates made of Perspex, Hawkesbury sandstone, mild steel and turpentine wood were secured on three orientations, upward facing, vertical and downward facing. It was made to fit over steel beams of 15 cm width and secured with Kevlar smart bands. (b) A photo of the actual experimental panel that shows the randomly allocated position of each surface material on each surface orientation.

Results

Recruitment – epibenthic assemblage

Individuals of the same species from different size classes behaved similarly; hence abundance was totalled across size classes.

The mean percent cover for all plates was 93%. The mean percent cover for sandstone, Perspex, turpentine and steel was 96%, 98%, 95% and 82% respectively. Percent cover had similar trends as the abundance measure. Therefore only the abundance measures are presented further.

44 species of epibenthic invertebrates and 8 mortality parameters were identified from the plate counts. The dominant taxonomic groups were barnacles, bryozoans, serpulid worms and hydrozoans. A full list of the species found can be found in the Appendix (**Table A1**).

The summary of the size data are also presented in the Appendix. The three most abundant taxa, the barnacle species *Balanus trigonus*, the serpulid worm *Pomatocerous* sp. and total abundance of bryozoans were plotted against surface material (**Figure A1**) and orientation (**Figure A2**). The abundance shows that there was consistent recruitment of these species as well as growth over the period of deployment. To attain more details about the effect of surface material and orientation on invertebrate size, a more detailed size study would be required. This was outside the scope of this study.

Epibenthic assemblages differed with settlement surface material and orientation, although there was no interaction between the two factors (**Table 2.1**).

The PCO shows strong segregation between assemblages on steel versus the three other surface materials. Perspex, turpentine and sandstone assemblages were more similar to each other, compared to the assemblage that is found on steel (**Fig. 2.3a**). Pearson correlation vectors indicate that all species correlating strongly with differences between surface materials had a lower abundance on steel surfaces (**Fig. 2.3c**).

The PCO also displayed a difference between assemblages on different orientations. There is a large separation between assemblages on upwards facing surfaces, and assemblages on downwards facing and vertical surfaces (**Fig. 2.3b**). The correlation vectors show that the sediment matrix was more abundant on surfaces facing upwards whereas sycon sponge was more abundant on vertical and downward facing surfaces (**Fig. 2.3c**).

Diversity

There was an effect of surface material on species richness, Shannon's diversity index and total abundance (**Table 2.2**). All three were reduced on steel surfaces (**Fig. 2.4a**, **b**, **c**) although there was no difference between steel and Perspex for Shannon's diversity (**Fig. 2.4c**). Shannon's diversity index was greater on upwards facing surfaces than on downwards facing surfaces (**Fig. 2.4d**). Differences in Shannon's diversity index with surface orientation may have been due to dispersion (PERMDISP P < 0.05) as the means were similar across surface orientations (**Fig. 2.4d**).

Species-level response – surface material

The type of surface material deployed affected the abundance of a wide range of different groups of organisms: the barnacle *Austrobalanus imperator*, newly settled barnacle recruits (not possible to identify to species yet); dead barnacles (empty barnacle tests); the bryozoans *Beania discodermidae* and *Parasmittina* sp.; and hydroids (**Table 2.3**). The effects

for *A. imperator* and *B. discodermidae* may have been caused by differences in dispersion (PERMDISP P < 0.05).

Dead barnacles (**Fig. 2.5a**) and *Parasmittina* sp. (**Fig. 2.5b**) were less abundant on steel. Barnacle recruits were more abundant on Perspex and turpentine while least abundant on steel (**Fig. 2.5c**). Although the PERMANOVA indicated that hydroids differed in abundance on the different **surface materials, the Tukey's test showed no difference between the surface** materials (**Fig. 2.5d**). Although there was a difference in dispersion, the barnacle *A. imperator* was less abundant on steel (**Fig. 2.5e**) while the bryozoan *B. discodermidae* was more abundant on turpentine (**Fig. 2.5f**).

Species-level response – surface orientation

The bryozoans *Beania magellanica* and *Celleporaria nodulosa*, sycon sponge and the sediment matrix differed with surface orientation (**Table 2.3**). The difference found for sycon sponges and sediment matrix may have derived from differences in dispersion (PERMDISP P < 0.05).

C. nodulosa and the sediment matrix were more abundant on upwards facing surfaces (**Fig. 2.6a**, **b**). *B. magellanica* was more abundant on downward facing surfaces (**Fig. 2.6c**), whereas sycon sponges were more abundant on both vertical and downward facing surfaces (**Fig. 2.6d**).

Species level response – surface material and orientation

The barnacle Balanus trigonus, bryozoan *Arachnopusia unicornis* and serpulid worm *Pomatocerous* sp. differed across both surface material and orientation (**Table 2.3**). Dispersion may have caused the difference of settlement patterns of *A. unicornis* between surface materials and *Pomatocerous* sp. between surface orientations (PERMDISP P < 0.05).

B. trigonus was less abundant on steel and more abundant on downwards facing surfaces (**Fig. 2.7a**). Although there was a difference in dispersion, *A. unicornis* was more abundant on steel than on sandstone (**Fig. 2.7b**). While analysis indicated that *A. unicornis* differed in abundance by surface material, **the Tukey's test showed no difference between the orientations (Fig. 2.7b**). *Pomatocerous* sp. was less abundant on turpentine (**Fig. 2.7c**). Although there was a difference in dispersion, the abundance of *Pomatocerous* sp. was higher on downwards facing surfaces (**Fig. 2.7c**).
Table 2.1. Multivariate PERMANOVA results of the epibenthic abundance for surface material, orientation and interaction between the two factors. P-values are derived from 99,999 permutations of the data and bold figures indicate a P-value of less than 0.05.

Source	df	MS	Р
Material	3	1843	<0.001
Orientation	2	799.0	<0.001
Material x Orientation	6	93.91	0.770
Residual	80	117.3	
Total	91		



Figure 2.3. (a) PCO of the multivariate epibenthic data displayed by surface material and (b) orientation. (c) The vector lines show species that were strongly correlated (Pearson correlation of 0.5) to the two factors.

Table 2.2. PERMANOVA results of species richness, Shannon's diversity index and total abundance for surface material, orientation and interaction between the two factors. P-values are derived from 99,999 permutations of the data and bold figures indicate a P-value of less than 0.05.

	Species richness				annon's diver	sity index	Total abundance			
Source	df	MS	Р	df	MS	Р	df	MS	Р	
Material	3	283.8	<0.001	3	0.1087	0.007	3	60280	<0.001	
Orientation	2	7.555	0.238	2	0.1432	0.004	2	6259	0.140	
Material x Orientation	6	4.813	0.477	6	0.0204	0.557	6	2428	0.599	
Residual	80	5.170		80	0.0248		80	3112		
Total	91			91			91			





c) Shannon's diversity index by material

d) Shannon's diversity index by orientation



Figure 2.4. (a) Species richness plotted with standard errors against surface material. (b) Total abundance plotted with standard errors against surface material. (c) Shannon's diversity index plotted with standard errors against surface material and (d) orientation. Means not sharing the same letter are significantly different (Tukey HSD, P < 0.05).

Table 2.3. Univariate PERMANOVA results of species abundance for species with a mean abundance greater than 2. PERMANOVA tested the two factors: surface material and orientation as well as the interaction between the two factors. P-values are derived from 99,999 permutations of the data and bold figures indicate a P-value of less than 0.05.

	Barnacle													
		Balanus tri	gonus	A	Austrobalanus imperator			Barnacle red	cruit	Dead barnacle				
Source	df	MS	P	df	MS	Р	df	MS	P	df	MS	Р		
Material	3	62.59	< 0.001	3	3.845	< 0.001	3	2.970	0.002	3	46.30	<0.001		
Orientation	2	15.94	0.001	2	0.7270	0.415	2	1.528	0.068	2	3.533	0.407		
Material x Orientation	6	2.997	0.600	6	0.7141	0.478	6	0.4178	0.603	6	2.519	0.761		
Residual	80	3.461		80	0.7376		80	0.5467		80	3.557			
Total	91			91			91			91				
				1		Bry	ozoan			1				
	A	rachnopusia	unicornis	16	Beania discode	rmidae	Beania magellanica			Celleporaria nodulosa				
Source	df	MS	Р	df	MS	Р	df	MS	Ч	df	MS	Р		
Material	3	8.448	<0.001	3	8.477	<0.001	3	0.6746	0.924	3	1.951	0.278		
Orientation	2	8.047	0.001	2	1.042	0.188	2	14.30	<0.001	2	8.355	<0.001		
Material x Orientation	6	1.382	0.786	6	0.4726	0.943	6	1.658	0.524	6	1.880	0.287		
Residual	80	1.953		80	0.9478		80	1.782		80	1.573			
Total	91			91			91			91				
		D		1			1	6		1				
	1	Bryozoa	an	Serpulid			Sponge				Hydroid			
Courses	df	Parasmilli	na sp.	df	POINALOCEIOL	is sp.	df	Sycon sponge		Hydroid		D		
Source	ui o	IVIS	P 10.001	ui 2	12.20		ui 2	1.05	P	ui 2	IVIS	P 0.007		
	3	6.1/6	<0.001	3	12.20	0.005	3	1.695	0.084	3	2.611	0.007		
Urientation Matarial & Originatation	2	1.648	0.355	2	16.46	0.002	2	7.362		2	0.2139	0.868		
Material x Orientation	6	1.389	0.532	6	2.010	0.897	6	0.8730	0.551	6	0.6439	0.575		
	80	1.504		80	3.668		80	0.9546		80	0.7568			
TOTAL	91			91			91			91				
	1			1										

		Sediment r	natrix
Source	df	MS	Р
Material	3	2.845	0.221
Orientation	2	121.9	<0.001
Material x Orientation	6	1.135	0.873
Residual	80	2.046	
Total	91		



Figure 2.5. Species abundance for (**a**) dead barnacles, (**b**) *Parasmittina* sp., (**c**) barnacle recruits, (**d**) hydroids, (**e**) *Austrobalanus imperator* and (**f**) *Beania discodermidae* by surface material with standard error bars. Means not sharing the same letter are significantly different (Tukey HSD, P < 0.05) except for hydroids where there was no difference observed between surface materials.



Figure 2.6. Species abundance for (**a**) *Celleporaria nodulosa*, (**b**) sediment matrix, (**c**) *Beania magellanica* and (**d**) sycon sponge by surface orientation with standard error bars. Means not sharing the same letter are significantly different (Tukey HSD, P < 0.05).



Figure 2.7. Species abundance for (**a**) *Balanus trigonus*, (**b**) *Arachnopusia unicornis* and (**c**) *Pomatocerous* sp. by surface material and orientation with standard error bars. Means not sharing the same letter are significantly different (Tukey HSD, P < 0.05) except for *A. unicornis* where there was no difference observed between surface orientations.

Discussion

This study found a significant effect of both surface material and orientation on the epibenthic assemblage. This highlights the importance of taking these factors into consideration when designing artificial reefs. It was found that steel had the lowest species richness, diversity and total abundance compared to other materials. This suggests that steel may not be the most suitable material to use for building artificial reefs to encourage the initial recruitment of epibenthos. Upwards facing surfaces were found to have a different community composition, dominated by the sediment matrix, but the effect of surface orientation was species-specific. Artificial reef designs need to incorporate these factors to tailor their structure for epibenthic assemblages.

Surface material

Steel was the material that supported the least species richness and total abundance of epibenthos. This supports the study by Anderson and Underwood (1994) where they found metal to have lower abundance when compared to concrete and plywood. The only anomaly was *Arachnopusia unicornis*, which was found to be most abundant on steel. Steel is comparatively difficult for most epibenthic species to settle on and will therefore take longer to be fouled to a similar extent as sandstone. The potential consequence of this is that the steel OAR will spend a longer period as an attracting structure for fish rather than growing an epibenthos and being productive. This is not ideal for the OAR as it was designed to produce a surplus fish population for recreational fishing. While it is in this attraction phase, the OAR might be increasing harvesting efficiency, contrary to the ecological goals for this OAR.

The fouling properties of turpentine were similar to sandstone and Perspex except that it had the highest abundance of the bryozoan *Beania*

discodermidae and the lowest abundance of the serpulid worm *Pomatocerous* sp. While turpentine is popular for use as piles and wharfs for their resistant properties against marine borers, it does not appear to deter fouling invertebrates with the exception of *Pomatocerous* sp. (Cookson and Barnacle 1987). Therefore turpentine wood is resistant to marine borers in optimum environmental conditions, but is not particularly resistant to epibenthic organisms. Thus it has potential as a material for artificial reefs, particularly compared to steel, which had a comparatively lower species richness and total abundance of epibenthos. Considering the similar life expectancy of turpentine of approximately 30 years (Cookson and Barnacle 1987) and the OAR of 30 years (Paik, R 2011, pers. comm., 29 September), turpentine is a possible alternative material for building artificial reefs. However, as with any material, its suitability will depend on careful scrutiny in regards to cost and structural properties.

Perspex was similar in its epibenthic characteristics to Hawkesbury sandstone (the natural substrate reference material). Species richness, Shannon's diversity index and total abundance, and the multivariate species composition data all showed similar responses to both Perspex and sandstone. This similarity justifies the common usage of Perspex as settlement plates in epibenthic studies (Birdsey *et al.* 2012; Clark and Johnston 2009; Dafforn *et al.* 2009; Knott *et al.* 2009; Rivero *et al.* 2013). Due to the similarities observed in this study, Perspex is an appropriate alternative to using sandstone for these studies. Perspex surfaces are more durable and lighter than sandstone, which has implications for experimental logistics and safety in the field. These advantages of using Perspex justify its popularity for use in many epibenthic surveys.

Accounting for the observed interactions between surface material and epibenthic assemblage could enhance artificial reef design. In the case of the Sydney OAR, steel is probably the least suitable material to use for construction in order to encourage initial recruitment of epibenthos, and thus

increase the overall productivity of the OAR. Sandstone and turpentine were colonised more readily, which is a favourable characteristic for an artificial reef that is aimed to increase biotic productivity.

There are other materials that are currently being used to construct artificial reefs, such as concrete (Seaman 2002). Concrete has been studied as an artificial substrate in estuarine environments (Anderson and Underwood 1994; Connell 2001). Studies are yet to specifically address the effects of concrete artificial reefs on epibenthic assemblages in offshore environments. Anderson and Underwood (1994) suggested that concrete had more abundant epibenthos compared to metal. Concrete may promote a similar epibenthic community to sandstone, hence there is a need to assess the use of concrete as a material for large, purpose-built OARs. Concrete is often used for smaller artificial reef structures (Bailey-Brock 1989; Carr and Hixon 1997; Folpp et al. 2011), and is now manufactured specifically for building artificial reefs. Reef Ball Australia Pty Ltd in conjunction with Boral Ltd have developed marine concrete for building artificial reefs. It is made of different components that reduce the toxic chemicals that leach from normal concrete in marine environments (Lennon, D 2012, pers. comm., 15 February). It shows potential for use as an artificial reef material. This material is yet to be thoroughly tested through a comprehensive study of the epibenthic assemblage, fish residency and movement, and trophic interactions. These studies will prove valuable as this new material is gaining popularity. This particular concrete was not available during this study, so its interaction with the epibenthic assemblage could not be tested.

Surface orientation

Surface orientation had species-specific effects on the epibenthic assemblage. The results agreed with previous studies where the upwards facing surface had a high abundance of sediment matrix (Knott *et al.* 2004). The serpulid worm *Pomatocerous* sp. was more abundant on downward facing surfaces,

which has also been observed previously (Miura and Kajihara 1984; **O'Donnell** 1984). The barnacle *Balanus trigonus* was more abundant on downwards facing surfaces, which was not expected as previous studies have found barnacles to be more abundant on upwards facing surfaces (Glasby and Connell 2001). Bryozoans had species-specific variation as seen previously (Duggins *et al.* 1990; Fitzpatrick and Kirkman 1995). *Beania magellanica* preferred downwards facing surfaces whereas *Celleporaria nodulosa* preferred upwards facing surfaces.

As the effects of surface orientation are species-specific, the optimum orientation will be determined by species that are important for productivity. From the results of Chapter 3, *B. trigonus* is one of the species that are important in transferring benthic productivity to higher trophic levels. From this result, it is logical for artificial reefs to include more downwards facing surfaces to maximise the orientation that *B. trigonus* prefers, allowing for an increase in abundance and therefore an increase in potential nutritional supply for fish populations. The effect of surface orientation on the interaction between barnacles and fish predation is discussed further in Chapter 3.

A surprising result from this study was the lack of algae found in the epibenthic assemblage. Studies in the region have commonly found algae in their assemblages (Glasby 1999a; Glasby 1999b; Glasby and Connell 1999). Although these studies were done in shallow estuarine environments, algae have still been observed in depths similar to the OAR (Underwood *et al.* 1991). Therefore it is unlikely that depth or light availability was the sole factor for the lack of algae. One influencing factor could be the isolation of the OAR from natural reefs. Some macroalgal species are known to have spores that only travel a few metres from the adult and the nearest macroalgal source is at least 1 km away (Phillips and Blackshaw 2011). It could also be a result of seasonal variation, as temporal variance can affect epibenthic communities as discussed by Anderson and Underwood (1994). The lack of algae may have contributed to a dominant coverage of

invertebrates as algae and invertebrates are well-documented competitors in epibenthic assemblages (Glasby 1999a; Levring 1966). An alternative hypothesis is that fish herbivory and the high abundance of food particles for the invertebrates brought to the OAR by the current could have given sessile invertebrates the competitive edge over macroalgae. The consequence of this lack of algae is a void in the trophic relationships of the artificial reef that would otherwise support herbivores and its predators. In a whole ecosystem approach, this is an important aspect that requires further examination.

Importance of epibenthic assemblages

This study highlights the importance of bottom-up ecology in artificial reef research, as the epibenthos is crucial to determining whether artificial reefs have the ability to be productive. The attraction and production debate can thus gain clarity by looking at the epibenthos of artificial reefs. Previous studies on artificial reefs have tended to focus on target fish species (Bolding et al. 2004). These studies are only useful in identifying factors affecting those specific species. It is acknowledged that the complex nature of artificial reefs requires a whole-ecosystem approach to determine the suitability of artificial reefs for the set ecological goals (Svane and Peterson 2001) and this needs an in-depth understanding of the epibenthos. The Sydney OAR was deployed with the aim of increasing recreationally important fish species, and by assessing the epibenthos on the OAR, it was determined that the construction material of the OAR was not the most suitable for initial epibenthos development. These results can be combined with those of Chapter 3 to show the importance of the epibenthos at the OAR to the fish assemblage. Further examination of the productivity of the epibenthos will greatly benefit artificial reef research, as the epibenthos form an important base of the food web, especially in the absence of macroalgae, which was the case in this study.

Future studies

The reduced recruitment of organisms onto steel in this study suggests that an alternative material would have been more suitable, and concrete is an ideal candidate that should be evaluated. Particularly given the new concrete mixes that are being custom made for the marine environment. An important point to be made with new materials that are manufactured for building artificial reefs is that there needs to be a paradigm shift from the 'corporate memory' that exists now to a more transparent, scientifically driven process. This applies to the concrete mix developed by Reef Ball Australia Pty Ltd and Boral Ltd. An increased scientific interest into this concrete material will increase its availability for epibenthic studies. The ecology-based approach to material evaluation applies to all materials now specifically being developed for building artificial reefs. While it is understandable that the specific details of these materials remain an industry secret, this should not be an obstacle in assessing their effectiveness as a productive artificial reef material. Since artificial reefs are being used more commonly to aid the sustainability of fisheries (Miller 2002), this is a crucial step in ensuring that the design of purpose-built artificial reefs incorporates up-to-date ecological information.

While steel was determined to be the poorest performer of the materials tested, there needs to be further exploration into assemblage development over time. Although steel was the slowest to be fouled, the assemblage maturation once an epibenthic assemblage is established is unknown. Fainburg *et al.* (2012) showed how the initial biogenic substrate could have an effect on the subsequent invertebrates that settle on this substrate. Further study into assessing that secondary biogenic substrate is required, as video footage taken by SCUBA divers in December 2012 and July 2013 showed a great increase in fouling on the OAR that extended over the initial biogenic substrate.

Finally, studies focusing on the trophic relationships on artificial reefs are required. They will assist in further linking the invertebrates to its predators and to other trophic levels. This will help identify which prey species are of particular importance for supporting the targeted fish populations, which will determine which reef material and orientations are most important. The epibenthic assemblages in this study were subject to predation and this relationship needs attention. This aspect is addressed in detail in Chapter 3.

Conclusion

This study has shown the importance of the type of material used to construct the artificial reefs as it influences the epibenthic assemblage that grows on the structure. This is an important aspect that should be taken into account for the construction of artificial reefs, especially when they are purpose-built to increase productivity and form part of a solution to fishery sustainability. The epibenthic assemblage forms an important part of the whole-ecosystem approach in assessing artificial reefs. A paradigm shift is required to advance the use of artificial reefs by moving from using opportunistic materials driven by manufacturing corporations to scientifically based materials that are beneficial for the ecological management strategies. This opens up a plethora of possibilities for further studies that address the need for a whole-ecosystem approach, which is essential for adequately determining the success and future of artificial reefs.

Chapter 3

Predation and orientation effects on epibenthic community development on an Offshore Artificial Reef

Abstract

Purpose-built artificial reefs aim to increase fish abundance by providing shelter for some fish as well as a source of food. The extent to which artificial structures support local fish productivity depends partly on food availability in the form of the epibenthic community that develops on the reef structure. This study examined the relationship between fish predation and epibenthic community development in three orientations of substrate deployed at 31 m depth on an Offshore Artificial Reef (OAR) near Sydney, Australia. Settlement plates with four caging treatments (25 mm mesh cage, 50 mm mesh cage, uncaged and cage control) were deployed in three orientations (upwards facing, downwards facing and vertical) on the OAR between December and March (2012/13) and epibenthic assemblages were censused. Barnacles were the dominant member of epibenthic assemblages and had a higher abundance on uncaged surfaces regardless of their orientation. The number of dead barnacles (empty tests) was also higher on uncaged surfaces. This suggests that the turnover of barnacles was higher in the presence of fish predators potentially mediated by an intermediate trophic level of mesopredators. Surface orientation also had effects on the epibenthic assemblage that were similar to previous studies. Serpulid worms Pomatocerous sp. and Salmacina australis were more abundant on downward facing surfaces. The sediment matrix was more abundant on upwards facing surfaces. Bryozoans had species-specific responses. This study highlights the potential for epibenthic assemblages on artificial reefs to contribute to local productivity. Further studies are needed to investigate complex trophic interactions between targeted fish species, mesograzers and the epibenthos on artificial reefs.

Introduction

Artificial reefs are increasingly being utilised to enhance local fish abundances and promote sustainable commercial and recreational fisheries (Miller 2002; Van Treeck and Schuchmacher 1998). Artificial reefs have evolved in design to best match the habitat, water conditions and ecosystems of targeted locations (Baine 2001). Purpose-built artificial reefs may aim to increase refuges, food and ultimately the abundance of targeted species (Connell 2001; Eklund 1996; Pickering and Whitmarsh 1997). The effectiveness of reef designs has tended to focus entirely on target species and have ignored the ecosystem mechanisms that are associated with changes in species abundances. Reefs provide both habitat and food for targeted fish species, yet few studies have examined predator-prey interactions on artificial reefs or the potential for reef epibenthos to contribute to fish productivity (Carpenter et al. 1985; Dafforn and Glasby 2012). The epibenthos and their relationship with fish predators need to be studied further to optimise the design of future artificial reefs, as an increase in productivity of the epibenthos could increase fish production.

Predation studies in marine environments are a regular feature of trophic transfer studies (Connell and Anderson 1999; Guariento *et al.* 2011), and have been shown to have variable effects; some studies have shown fish predation to affect the epibenthic assemblage most during recruitment (Osman and Whitlatch 2004), while others have observed no effects of fish predation on epibenthic recruits (Connell 2001). The knowledge on the relationships between fish and epibenthic assemblages on artificial reefs remain limited. The few studies that have focused on predator-prey interactions on artificial reefs have highlighted a reliance of targeted fish species on epibenthic assemblages that grow on the surface of artificial reefs (Leitao *et al.* 2007; Relini *et al.* 2002). As trophic interactions between fish and epibenthic astemblages are complex, a greater understanding is required of these interactions and how reef design might affect fish productivity (Xu *et*

al. 2012). Fundamental information such as identifying epibenthic species that are predated on by fish is required.

Fish feeding is constrained not only by the presence or absence of prey but also by the accessibility of that prey (Westhoff et al. 2013). Surface orientation influences the development of epibenthic assemblages (Knott et al. 2004), and recent evidence suggests that fish feeding also varies with orientation (Bolton *et al.* in review). However, studies on predator-prey interactions remain focused on prey refuge and habitat heterogeneity (Power 1992) and few focus on how surface orientation affects accessibility of the epibenthos. There are a number of reasons why factors such as light, hydrodynamics and sedimentation that show variation in response to orientation will affect epibenthic communities (Airoldi 2003; Glasby 1999a; Knott et al. 2004). Algae are typically more abundant on upward and vertically orientated surfaces due to increased light availability (Glasby 1999a; Levring 1966). Sedimentation on upwards facing surfaces can change epibenthic assemblages by smothering and preventing recruitment (Airoldi 2003; Azevedo et al. 2006). No studies of Offshore Artificial Reefs (OAR) have experimentally examined how orientation may affect the epibenthos, and the extent to which fish consume these assemblages.

The effects of fish predation on the epibenthos are often studied with the use of mesh cages to exclude target predators from the developing epibenthic assemblage (Connell 1997; Connell and Anderson 1999). Although the use of cages has been observed to cause confounding effects, appropriate cage controls can minimise these effects (Connell 1997). Different sized fish predators have also been found to have different effects on the epibenthic assemblage, and it is possible to distinguish these effects using two different mesh sizes (Connell and Anderson 1999). With the large variety of fishes and niches present at the OAR, a smaller mesh size cage of 25 mm was used to only allow access to juvenile fish and species of blennies and gobies that are not usually associated with sessile epibiota predation (Connell, 1999) and a

larger mesh size cage of 50 mm was used to exclude large fish (>200 mm total length) (Connell, 1997).

To assess the effects of predation and surface orientation on epibenthic assemblage development we deployed experiments on a newly deployed OAR. The OAR was deployed off Sydney Harbour in New South Wales, Australia in October of 2011 and the 42 tonne steel artificial reef was positioned approximately 2 km southeast of the south head of Sydney Harbour with the purpose of aiding fishing by increasing recreationally targeted fish species (Reeds 2010). Experimental panels were deployed with a two factor fully orthogonal experiment on the OAR to test three questions. These were:

- Excluding fish from settlement surfaces affects epibenthic assemblage development on an offshore artificial reef;
- 2) Surface orientation affects epibenthic assemblage development on an offshore artificial reef; and
- 3) Excluding fish will have different effects depending on the orientation of surfaces for the development of epibenthic assemblages.

Methods

Offshore Artificial Reef – general description

This study was deployed on an Offshore Artificial Reef (OAR) located approximately 2 km southeast of the south head of Sydney Harbour (S 33°50.797', E 151°17.988') (**Fig. 3.1**). The OAR is a purpose built steel construction with dimensions of 12 m in height by 15 m x 15 m at the base. It weighs 42 tonnes and is situated on a natural sandy bottom at a depth of 38 m. Four 60 tonne concrete blocks are chained to each corner for stability (**Fig. 3.1**). The reef was deployed in October 2011. This experiment took place on the reef from December 2012 to March 2013.

Recruitment study

An experimental study of epibenthic recruitment under different predation pressures and surface orientations was conducted on the OAR. Backing panels made of grey polyvinyl chloride (PVC) were designed to fit over the steel beams used to construct the OAR and were held in place with Kevlar smart bands. Eight backing panels were fitted to the beams on the top deck of the main structure of the OAR, at 31 m depth (**Fig. 3.1**). They were all fitted to face the same direction to reduce confounding effects from current direction.

Panels contained an upward facing, downward facing and vertical surface (**Fig. 3.2**). Attached to each surface orientation were four Perspex settlement plates (11 cm x 11 cm x 0.4 cm), which were spaced 8 cm apart and randomly assigned to one of four caging treatments for each surface orientation. Large fish were excluded using 50 mm mesh cages, which permitted small fish access to the settlement plates. Small 25 mm mesh cages were used to exclude both large and small fish. Uncaged plates were completely exposed to fish predation. Half-caged 25 mm mesh plates were

used as cage controls to determine whether there were any artefacts associated with caging. The smaller mesh size was used for the cage control as any caging artefacts that may arise from altered hydrodynamics or shading would be more prominent in the smaller mesh size. There were a total of 8 replicates of each caging treatment by orientation combination.

Panels were attached by SCUBA divers on the 28th, 29th of November and 7th of December, 2013 and left submerged for 3 months, at which point they were retrieved for census. Upon collection, settlement plates were immediately fixed in 7 % buffered formaldehyde solution for 24 h then drained, washed and preserved in 80 % ethanol.

Epibenthic assemblage

Epibenthic recruits on each settlement plate were censused under a dissecting microscope. A 100-point (10 x 10) grid was used to subdivide the plates, with all organisms within a random subsample of 16 grid squares counted to give a measure of abundance. Organisms were classified to species or the lowest possible taxonomic level. The organisms were also classified into three size classes: small, medium and large. The size of serpulids and amphipod tubes were assessed by the operculum or tube opening, respectively. An opening of 0.1 cm or less was classified as small, 0.1-0.2 cm was classified as medium, and greater than 0.2 cm was classified as large. All other organisms were classified by the diameter at the widest point; a diameter of less than 0.2 cm was classified as small, 0.2-0.5 cm as medium and greater than 0.5 cm as large.

The 100-point grid was also used to calculate percent cover of organisms on each plate. Organisms found directly under each intersect were counted and given a value of 1 % cover. The same size classes were applied.

After the abundance and percent cover data were recorded, a full plate scan was performed to ensure that any species present on the plate but not found in the subsample were given a value of 0.5 for abundance measure or 0.5 % for percent cover.

Statistical analysis

Community assemblage

Percent cover and abundance measure were analysed in the same way. A permutational multivariate analysis of variance (PERMANOVA) with two fully crossed fixed factors (caging treatment and orientation) was conducted on the abundance measure between uncaged and cage control treatments to determine if there were any caging artefacts. The statistical package PRIMER (Plymouth Routines in Multivariate Ecological v6.0 Research) with PERMANOVA+ (PRIMER-E Ltd, UK) was used. Data were log₁₀ transformed and a resemblance matrix was created using Bray-Curtis similarity with a dummy variable of 1. The PERMANOVA was run using 99,999 permutations of the data. There were no caging artefacts, thus subsequent analyses only included three caging treatments: 25 mm mesh cages (small cage), 50 mm mesh cages (large cage) and uncaged. To test the effect of caging and surface orientation on epibenthic abundance, PERMANOVA was conducted on the percent cover and abundance data. The PERMDISP function was used to determine if the differences in dispersion between groups occurred.

PERMANOVA was also used on the abundance data to test whether the amount of time the panels were submerged affected abundance and thus may be considered a confounding factor. There was no confounding effect. A PERMANOVA was also conducted to test for confounding effects between the eight settlement panels (blocking effects). There were no confounding effects.

Principal coordinate ordinations (PCO) were used to visualise the similarities or dissimilarities between caging treatment and orientation. To better visualise trends, vector lines for species with a Pearson correlation of 0.6 were overlaid on the PCO to show their correlation with various treatments. PCOs were done in PRIMER v6.0.

Diversity

The diversity measures of species richness and Shannon's diversity index as well as total abundance were calculated. Univariate PERMANOVA was used to test the effect of caging and surface orientation on these measures. Data were square root transformed and a Euclidean distance resemblance matrix was constructed. PERMANOVA was run with 99,999 permutations of the data. The PERMDISP function was used to determine if the differences in dispersion **between groups occurred. Tukey's HSD** test was performed to further examine differences amongst groups, using the statistical program SPSS (IBM Corporation, USA).

Species-level responses

To avoid issues of low values, species with a mean abundance below 4 were excluded from individual analysis. Univariate PERMANOVAs were run on all other species to determine differences between caging treatment and orientation. Data were square root transformed and a Euclidean distance resemblance matrix was constructed. PERMANOVA was run with 99,999 permutations of the data. The PERMDISP function was used to determine if **the differences in dispersion between groups occurred. Tukey's HSD test was** used to interpret significant single species effects, and were done using the statistical program SPSS. Prism 6 (GraphPad Software Corporation, USA) was used to graph any differences observed from the PERMANOVA results.



Figure 3.1. (a) A map of Sydney Harbour, New South Wales with the location of the Offshore Artificial Reef (OAR) marked by the symbol •. The OAR is located at S 33°50.797', E 151°17.988'. (b) A photograph of the OAR taken on the 29th of September 2011 at Sydney Ports before deployment. (c) A model diagram of the OAR including its concrete weights with the top deck where the experimental panels were fixed outlined by the black rectangle (image: NSW DPI). Approximate depth of the top deck was 31 m.



Figure 3.2. (a) A schematic diagram of the experimental panel used made of polyvinyl chloride (PVC). Four 11 cm x 11 cm settlement plates with 25 mm mesh cage, 50 mm mesh cage, uncaged and cage control treatments were secured on three orientations, upward facing, vertical and downward facing. It was made to fit over steel beams of 15 cm width and secured with Kevlar smart bands. (b) A photo of the actual experimental panel that shows the randomly allocated position of each caging treatment on each surface orientation.

Results

Recruitment - epibenthic assemblage

Individuals of the same species from different size classes behaved similarly; hence abundance was totalled across size classes. Similarly, percent cover had similar patterns as the abundance measure. Therefore only the abundance measures are presented here.

Although effects were present for both caging (df = 2, MS = 1144, P < 0.001) and surface orientation (df = 2, MS = 3362, P < 0.001) on multivariate community, this pattern appears to be driven by a few key species.

Diversity

There was a difference in species richness by caging (df = 2, MS = 90.18, P < 0.001) and surface orientation (df = 2, MS = 58.76, P = 0.002), as well as **an interaction (df = 4, MS = 25.58, P = 0.024).** Shannon's diversity index also differed by surface orientation (df = 2, MS = 0.4181, P < 0.001). These differences were likely driven by a number of rare species with low abundances. These effects were also not as strong as the effects found at species-level. Therefore only the results of species recruitment are shown.

Species-level response – caging treatment

The barnacle *Balanus trigonus*, barnacle recruits (not possible to identify to species yet) and dead barnacles (empty barnacle tests) had different abundances between caging treatments (**Table 3.1**). Both *B. trigonus* and dead barnacle abundance was higher on uncaged plates compared to small caged surfaces (**Fig. 3.3a, b**), while barnacle recruits were more abundant on uncaged surfaces compared to both cage sizes (**Fig. 3.3c**).

Species-level response - surface orientation

The bryozoans, *Arachnopusia unicornis* and *Celleporaria nodulosa*, the serpulid worms, *Pomatocerous* sp. and *Salmacina australis*, and dead barnacles differed with surface orientation (**Table 3.1**). *A. unicornis* was less abundant on vertical surfaces (**Fig. 3.4a**), while *C. nodulosa* was more abundant on upward facing surfaces (**Fig. 3.4b**) although the Tukey's test did not detect a difference between the orientations. *Pomatocerous* sp. was more abundant on downward facing surfaces compared to upwards facing surfaces (**Fig. 3.4c**). *Salmacina australis* was more abundant on downward facing surfaces was more abundant on downward facing surfaces (**Fig. 3.4d**) however, post-hoc Tukey's tests showed no differences between the surface orientations. Dead barnacles were more abundant on vertical surfaces (**Fig. 3.4e**).

The sediment matrix showed an interaction between caging treatments and surface orientation (**Table 3.1**). Although there was a difference in dispersion (PERMDISP P < 0.05), surface orientation seemed to have a stronger effect where it was more abundant on upward facing surfaces (**Fig. 3.4f**).

Table 3.1. Univariate PERMANOVA results of species abundance for species with a mean abundance greater than 4. PERMANOVA tested the two factors: caging and surface orientation as well as the interaction between the two factors. P-values are derived from 99,999 permutations of the data and bold figures indicate a P-value of less than 0.05.

	Barnacle										Bryozoan		
	Bá	alanus trige	onus	Barnacle recruit		Dead barnacle			Arachnopusia unicornis				
Source	df	MS	Р	df	MS	Р	df	MS	Р	df	MS	Р	
Caging	2	22.33	0.001	2	3.878	0.015	2	12.16	0.007	2	2.538	0.290	
Orientation	2	6.982	0.111	2	1.730	0.142	2	28.97	<0.001	2	6.713	0.007	
Caging x Orientation	4	3.373	0.433	4	0.3980	0.757	4	4.443	0.202	4	1.763	0.578	
Residual	63	3.441		63	0.8525		63	3.254		63	2.054		
Total	71			71			71			71			

		Bryozoar	ı									
	Celle	eporaria no	ndulosa	<i>Pomatocerous</i> sp.			Salmacina australis			Sediment matrix		
Source	df	MS	Р	df	MS	Р	df	MS	Р	df	MS	Р
Caging	2	1.084	0.623	2	5.349	0.213	2	0.9248	0.982	2	8.054	0.003
Orientation	2	3.726	0.037	2	13.46	0.007	2	152.7	0.003	2	94.69	<0.001
Caging x Orientation	4	1.311	0.580	4	1.703	0.908	4	16.14	0.622	4	4.333	0.017
Residual	63	1.543		63	3.746		63	24.11		63	1.779	
Total	71			71			71			71		



Figure 3.3. Species abundance for (a) *Balanus trigonus*, (b) barnacle recruits and (c) dead barnacles by caging treatment with standard error bars. Means not sharing the same letter are significantly different (Tukey's HSD, P < 0.05).



Figure 3.4. Species abundance for (**a**) *Arachnopusia unicornis*, (**b**) *Celleporaria nodulosa*, (**c**) *Pomatocerous* sp., (**d**) *Salmacina australis* and (**e**) dead barnacles by surface orientation with standard error bars. (**f**) Abundance of sediment matrix by caging treatment and surface orientation with standard error bars. Means not sharing the same letter are significantly different (Tukey's HSD, P < 0.05) except for C. nodulosa and *S. australis* where there were no differences observed between surface orientations.

Discussion

Epibenthic assemblage is affected by both fish predation and surface orientation in this study. This indicates that trophic interactions and the effect of orientation on these interactions require careful consideration in the design of artificial reefs. Barnacles were potentially consumed by mesopredators, which are then possibly consumed by fish predators. These trophic interactions suggests a transfer of productivity to fish assemblages. Surface orientation also affected epibenthic community composition where the upwards-facing surfaces were dominated by the sediment matrix, but the effect was species-specific. Artificial reef designs need to incorporate these factors to optimise their structure to increase reef productivity.

Caging treatment

Barnacles had higher abundances on uncaged surfaces, or in the presence of fish predators. This suggests that there is a predator-prey interaction that benefits barnacles such as *Balanus trigonus*, which is contrary to those previously observed by Leitao *et al.* (2007) where fish were found to prey on barnacles, decreasing their abundance. One mechanism for our non-intuitive result is the presence of an intermediate mesopredator such as flatworms or whelks that prey on barnacles but is in turn preyed upon by fish. Fish have often been observed to feed on mobile invertebrates that inhabit the epibenthos (Leitao *et al.* 2007; Relini *et al.* 2002). In the presence of fish predation, mesopredators that prey on barnacles may be consumed themselves, thus reducing predation pressure on barnacles, increasing their abundance. Larger fish could be responsible for this effect as the difference was greatest between the uncaged and small caged surfaces. Methods such as fish gut content analysis is invaluable in identifying the exact species that contributed to this result.

This study indicates that in the presence of fish predation, the recruitment and mortality of barnacles occurs more rapidly. Barnacles were the dominant settling organisms during our study and new settlers were observed in greater abundance on uncaged plates (along with mature barnacles and dead barnacles). This suggests that there is an increased turnover for barnacles on uncaged surfaces. This may reflect a potential for increased fish productivity if fish are eating the mesopredators and the energy is transferred to higher trophic levels. Further research to identify epibenthic and mesopredator abundances in fish guts would help to confirm our hypothesis.

If barnacles are an important source of nutrition on the reef then specific interactions between barnacles and their mesopredators should be examined and quantified. The mobile invertebrates inhabiting the epibenthos should also be censused. Studies in estuarine environments have found that mobile invertebrates were not closely related to the epibenthos type (Birdsey *et al.* 2012). Whether this translates to an offshore environment is unknown. This is an important relationship to consider due to the results of studies such as Relini *et al.* (2002) that highlights up to 91% of food intake of certain fish species was mobile invertebrates that are found in the epibenthic assemblage on artificial substrates. By identifying these other trophic relationships, it is possible to fully assess the functionality of the epibenthos in increasing the overall productivity of artificial reefs, which increases fish production.

Surface orientation

Consistent with previous studies, surface orientation had a strong influence on the development of epibenthic assemblages. Upward facing surfaces had a higher proportion of surface covered by sediment matrix, similar to observations by Knott *et al.* (2004). Serpulid worms *Pomatocerous* sp. and *Salmacina australis* were more abundant on downward facing surfaces, consistent with previous findings for other serpulids (Miura and Kujihara **1984; O'Donnell 1984). Dead barnacles were more abundant on vertical** surfaces – a result that has not been reported although other studies have not censused empty barnacle tests. Bryozoan response to surface orientation was species-specific, as has been observed in Chapter 2 as well as other studies (Duggins *et al.* 1990; Fitzpatrick and Kirkman 1995).

In this study, barnacles were the dominant component of the epibenthos and their abundances were highest on vertical and downward facing orientations. In addition, we observed greater evidence of mesopredator predation on barnacles on vertically oriented surfaces (more empty barnacle tests). These results suggest that the optimum orientation for increasing epibenthic productivity on this artificial reef is to increase the amount of vertical and downward facing surfaces. If mesopredators are eating barnacles, and fish are eating mesopredators, then these surface orientations will increase the nutritional transport from the epibenthos up through the trophic levels.

The recommendation to increase vertical and downward facing surfaces is contrary to much temperate reef ecology literature, which suggests that productivity and diversity are heavily dependent on a thriving macroalgal forest (Gribben et al. 2009). The absence of algae on our settlement plates is an interesting result. Studies in the region have commonly found algae in the epibenthic assemblages (Glasby and Connell 1999; McKenzie et al. 2011), but they have also been observed at depths similar to the OAR (Underwood et al. 1991). Therefore it is unlikely that depth or light availability were the sole factors determining the lack of macroalgae. Potentially contributing to the lack of algae is the isolation of the OAR to the nearest natural reef. Some macroalgal species are known to have spores that only travel a few metres and the nearest macroalgal source of spores is at least 1 km away (Phillips and Blackshaw 2011). There may also be a seasonal component to our study as temporal variance can affect recruitment patterns (Anderson and Underwood 1994). A temporal study could determine persistence of productivity on the OAR and the presence or absence of algae throughout the year. The lack of algae may have contributed to a dominant coverage of

invertebrates as algae and invertebrates are well-documented competitors in epibenthic assemblages (Glasby 1999a, Levring 1966). An alternative hypothesis is that fish herbivory and the high abundance of food particles for the invertebrates brought to the OAR by the current could have given sessile invertebrates the competitive edge over macroalgae. These are other trophic interactions that are yet to be identified at the OAR. The OAR is clearly not supporting a vital part of the food web that includes herbivores and macroalgae. This could be a potential productivity void that the artificial reef is missing and requires further examination.

Importance of epibenthic assemblages

A large number of the studies on artificial reefs have focused on target fish species (Bolding et al. 2004; D'Anna et al. 2011, Leitao et al. 2008) and only assessed species of interest. The complex nature of artificial reefs requires a whole-ecosystem approach to determine the suitability of artificial reefs for the set ecological goals (Svane and Peterson 2001). The OAR was deployed with the aim of increasing recreationally important fish species and by assessing the epibenthos and its interactions with its predators, we can now suggest that the OAR is indeed contributing to local productivity albeit in a more complex manner than previously considered. Studies assessing fish populations are important in assessing the success of artificial reefs. These studies however, need to link them with its prey such as those by Brickhill et al. (2005) looking at gut content of fish species. Studies such as these will link fish species of interest to the epibenthic assemblage and its associated inhabitants. This is particularly useful for identifying trophic interactions that are important in the whole-ecosystem approach that may include benthic or pelagic productivity. Other methods that identify trophic links are of great importance as they reveal the interactions that are crucial to understanding the functions of artificial reefs. Stable isotope analysis of carbon and nitrogen, and ecosystem modelling are examples that can be used to determine these links within an artificial reef system. These methods are valuable in

connecting and identifying parts of the system that will allow greater understanding of how artificial reefs can be designed to increase fish productivity. Epibenthic assemblages should be included as an important part of any artificial reef assessment as part of a whole ecosystem approach. Their use has great potential for further studies.

Conclusion

This study has highlighted the importance of studying epibenthic assemblages in the pursuit of understanding fish productivity on artificial reefs. This is an important aspect to consider when using the whole-ecosystem approach to suitably analyse artificial reefs and their benefits. Particularly when the purpose of an artificial reef is to increase overall productivity as part of a solution to maintaining the sustainability of a fishery. There is a great need for further exploration into the epibenthic assemblages, as well as other trophic relationships that link the productivity from the base of the food web to the higher trophic levels that are economically targeted. Studying targeted fish species alone cannot uncover the functional role of artificial reefs. There is great potential and information to come from studying epibenthic communities and the focus now needs to turn to them to further contribute to understanding and developing artificial reef designs.

Chapter 4

Discussion

The trophic interactions between epibenthic assemblages on artificial reefs and their fish predators are a technically difficult area to study, and this thesis has alluded to indirect interactions. Surface material, predation and surface orientation were all found to affect abundances of epibenthic species on artificial reefs. These factors may be important in determining the vital part of the reef's productivity. The role of mobile invertebrates as both the predators of epibenthos and the prey of fish requires further study. Novel and multidisciplinary approaches are needed to quantify the effect of artificial reefs on the production of fish and its contribution to coastal fisheries. Experimental studies on the Offshore Artificial Reef (OAR) provided substantial insight into potential drivers of epibenthic community structure. Surface material was important for recruitment and steel was the material that had the least epibenthic recruits and is therefore not considered an ideal material for building artificial reefs. Particularly in the initial stages of deployment, a steel artificial reef may only act as a fish attractant before it starts to be productive through an increase in epibenthic biomass. Barnacles were identified as a potentially important species for transferring benthic productivity to higher trophic levels and their recruitment should be considered in artificial reef design. Barnacles were more abundant on downward facing surfaces and were apparently consumed in greater numbers on vertical surfaces. By maximising downward facing and vertical surfaces on artificial reefs it may be possible to increase the abundance of barnacles that are available to predators. The importance of the epibenthic assemblage is underrated when assessing reef productivity and when optimising artificial reef design. While the epibenthic communities were relatively well developed, further studies could look at the epibenthic communities of subsequent development of a longer deployment and settlement beyond the primary settlement layer on the surface of the artificial reef.

Whole-ecosystem approach

A whole-ecosystem approach is necessary for assessing the effectiveness of artificial reefs as productive aids to sustainable fisheries (Svane and Peterson 2001). Such an approach is required to reduce confounding effects that emerge when conducting direct comparisons of fish abundances on natural and artificial reefs (Hunter and Sayer 2009; Taylor 1998). As part of a whole ecosystem approach, there is a need for further epibenthic community studies. Despite the important role of epibenthic assemblages in natural reef ecosystems, they are not well studied on artificial reefs and this knowledge gap must be addressed in order to continue developing sustainable solutions to fisheries management.

A broader assessment of the Offshore Artificial Reef (OAR) is ongoing and this study forms but one component. In conjunction with the epibenthic study in this thesis, there are other studies currently being conducted on a variety of measures. Fish movement is being monitored through the use of acoustic tags and receivers. Recreational fishing effort is being monitored through the use of cameras and surveys. Baited and unbaited underwater remote videos are being used to record fish populations. Chlorophyll in the water column is being monitored on the structure using fluorometers, and the current direction and strength is being recorded by using mechanical and acoustic flow meters. All these factors are important in comprehensively understanding the processes that govern the OAR to better understand how it can be optimised for fish productivity. When available, multiple lines of evidence will be combined to assist with the interpretation of the epibenthic data.

Future direction

There are many potential directions for artificial reef research that can be built on the results of this thesis. In relation to surface material, concrete is gaining popularity in the manufacturing of artificial reefs (Lan and Hsui 2006). This surface will require testing for potential effects on epibenthic recruitment. In order to understand predator-prey interactions it may be necessary to quantify mesopredators and their activity. This may further explain the productivity chain that is present at the OAR. In addition, further studies might examine the role of structural heterogeneity of surface in a range of orientations. Studies have repeatedly demonstrated the importance of habitat heterogeneity for epibenthic assemblages thus by identifying these effects it is possible to optimise the design of artificial reefs (Eklund 1996; Hunter and Sayer 2009; Svane and Peterson 2001; Taylor 1998). The initial biogenic layer of epibenthos, which may facilitate other organisms, provides one aspect of heterogeneity and this would be a useful direction for further research.

There are other types of studies not specifically mentioned in previous chapters that may be of benefit to continuing the effective development of artificial reefs. Seasonal studies would be useful (Anderson and Underwood 1994; Gilg *et al.* 2010) as would studies on a range of temporal scales. Seasonal studies may reveal a yearly (or other) temporal cycle that governs the artificial reef and determines the optimum times for deployment or harvesting in order to meet ecological goals. As an example, if the temporal variance was better understood, it might be possible to deploy reefs when specific prey species will recruit best for the target recreational fish species. Temporal studies are also essential when comparing artificial reefs to natural reefs as controls since both the variance and the types of reefs have to be accounted for and compared.

Assessing trophic relationships between native and non-native epibenthic invertebrates will also contribute to the understanding of how different invertebrates may interact within the ecology of artificial reefs (Connell and Glasby 1999; Dafforn *et al.* 2012).

Biomass and other biometric parameters are another novel method to incorporate both epibenthic assemblages and fish populations. The biomass of the epibenthic assemblages as well as specific prey species will be useful in determining the best artificial reef designs to maximise fish productivity. More specific biometrics such as carbohydrates, lipids and proteins would enable the determination of the nutritional value of prey species. Factors such as predation and nutrient availability are known to impact lipid content of some marine worms (Busarova *et al.* 2011; Gardner *et al.* 1985). By identifying these effects, we will be able to design artificial reefs to support a more nutritionally productive structure to support greater productivity.

Other invertebrate communities that are present in the vicinity of the artificial reef environment should also be assessed. Artificial reefs are often deployed on sandy bottom habitats to increase hard substratum in the region (Baine 2001). Therefore the study of sediment infauna such as the one conducted by Zalmon *et al.* (2012) will be central in assessing the effects of an artificial reef **on another habitat. A 'halo' effect of reduced infaunal abundance might result** from deploying a structure in a sandy bottom habitat by increasing predation on infauna. Zalmon *et al.* (2012) found that the effect of the artificial reef on infauna surrounding the reef was relatively weak, although this was considered to be a consequence of strong bottom sea currents. This highlights the need to assess interdisciplinary parameters.

Water flow is another aspect that requires multiple approaches. The water flow on a natural reef is expected to be very different from artificial reefs as these structures are usually taller, therefore providing a more threedimensional flow model in the water column. Flow has been shown in estuaries to influence epibenthic assemblages (Palardy and Whitman 2011). Palardy and Whitman (2011) did not control for the effect of fish predation. This factor has to be isolated, especially on artificial reefs where predatorprey interactions are considered to be strong. Hydrodynamic observations such as those currently being undertaken on the OAR will also be of great
significance. This provides information on the current direction and strength over the artificial reef and will better indicate water flow that affects plankton, nutrients and fish populations (Suthers *et al.* 2011).

An increasingly common tool for ecosystem assessment is the analysis of stable isotopes. By comparing the stable isotope characteristics of invertebrates and their predators, a clearer picture of the food web structure of an artificial reef may emerge. This approach can apply to all species that are present on artificial reefs to assess the interspecific relationships that are **key to an artificial reef's productivity.**

Finally, fish population surveys that have been common on artificial reefs should continue. The assessment of targeted species is a basic requirement for assessing artificial reefs. However, assessments should be incorporated into a wider framework that includes other relevant species on the reef. In addition to fish abundance, movement and reproduction, there are other methods such as fish gut content surveys that link fish species to their prey. The results from these surveys may vary by location. Some studies have found up to 91 % of a fish predator's diet consisted of invertebrates found in the epibenthos whereas others found that 50 % of another fish predator's diet consisted of other fish species (Brickhill *et al.* 2005; Relini *et al.* 2002). These links need to be further explored and expanded by combining data with data from other methods cited above.

Conclusion

Studies that compare one aspect of an artificial reef in an attempt to determine production and attraction characteristics are, although crucial, lacking in conviction. The future of artificial reef ecology lies with interdisciplinary approaches that encompass the entirety of the artificial reef ecosystem. The global popularity of artificial reefs is increasing and our approach to assessing them will need to evolve. Reefs designed to achieve

specific ecological goals are the key to a successful future and they require substantial supporting evidence.

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Appendix

Table A1. Species list of all epibenthic invertebrates and mortality parametersobserved on the settlement plates.

Taxonomic Group	Species	Taxonomic Group	Species
Ascidians	<i>Botrylloides leachii Botrylloides schlosseri Didemnidae sp. Diplosoma sp. Pyura sp.</i> Solitary ascidian	Serpulid worms	Pomatocerous sp. Hydroides elegans Galeolaria sp. Salmacina australis Sabella sp. Spirorbis sp.
Barnacles	Austrobalanus imperator Balanus amphitrite	Sponges	<i>Sycon sp.</i> Unknown sponge
	Balanus trigonus Balanus variegatus Megabalanus coccopoma Barnacle recruit	Mortality parameters	Barnacle scar Dead barnacle (empty test) Pomatocerous scar <i>Saccostrea</i> scar
Bivalves	<i>Saccostrea glomerata Theora sp.</i> <i>Theora sp. 2</i> Juvenile bivalves		<i>Theora sp.</i> scar <i>Theora sp. 2</i> scar Amphipod tubes Sediment matrix
Bryozoans	Arachnopusia unicornis Beania discodermidae Beania magellanica Celleporaria nodulosa Celleporaris sp. Chaperiopsis cristata Conopeum sp. Disporella sp. Microporella lunifera Mucropetraliella ellerii Osthimosia glomerata Parasmittina sp. Smittina sp. Tricellaria sp. Tubulipora sp. Unknown bryozoan		
Corals	Plesiastrea versipora		
<u>Foraminifera</u> Hydrozoans	Unknown toraminifera Hydrozoa sp.		



Figure A1. Abundance of the barnacle *Balanus trigonus*, the serpulid worm *Pomatocerous* sp. and bryozoans of three size classes by surface material. Barnacle recruits were used to represent the small barnacles as at that size they are not distinguishable between species.



Figure A2. Abundance of the barnacle *Balanus trigonus*, the serpulid worm *Pomatocerous* sp. and bryozoans of three size classes by surface orientation. Barnacle recruits were used to represent the small barnacles as at that size they are not distinguishable between species.