UNIVERSITY OF SOUTHAMPTON

FACULTY OF ENGINEERING, SCIENCE & MATHEMATICS

SCHOOL OF OCEAN & EARTH SCIENCES

Marine Communities of North Sea Offshore Platforms, and the Use of Stable Isotopes to Explore Artificial Reef Food Webs

by

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Thesis for the degree of Doctor of Philosophy

September 2009

UNIVERSITY OF SOUTHAMPTON

ABSTRACT

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MARINE COMMUNITIES OF NORTH SEA OFFSHORE PLATFORMS, AND THE USE OF STABLE ISOTOPES TO EXPLORE ARTIFICIAL REEF FOOD WEBS

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Stable isotope methods offer a powerful means of investigating trophic interactions, allowing assessment of the relative importance of multiple nutrient sources to biological assemblages, as well as estimation of the trophic positions of consumers. Differences in the isotope ratios of consumers between habitats can thus indicate differences in the structures of food webs, or the contributions of different food sources to those food webs.

Isotope methods were used to compare the food web of an artificial reef located off the south coast of England with that of a nearby natural reef system, revealing a similarly complex food web, with similar trophic structure, and similar inputs from the available food sources. Isotope methods should be incorporated into more artificial reef studies, where they have been seldom applied.

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Offshore oil and gas platforms in the North Sea are artificial reefs, hosting substantial assemblages of sessile invertebrates and other associated fauna, and attracting large numbers of fish and motile invertebrates. Structural survey footage provided by the oil and gas industry allowed the investigation of the marine life associated with several of these structures, of varied ages and in various locations in the North Sea.

At least thirty-six taxa of motile invertebrates and fish were observed in association with the structures, most of which were present on all platforms surveyed. While most reef-associated fish were observed around the base of the larger platforms, many thousands of fish were also observed in the water column around these structures at other depths. A small number of sessile taxa dominated the fouling assemblages, in places achieving total coverage of the available surfaces. Fouling composition changed with depth, but this pattern was not identical on all platforms. Platform age and location both affected the fouling assemblages present, but these two factors did not fully explain all the variation.

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DECLARATION OF AUTHORSHIP

I, **Andrew James Guerin**,

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and the work presented in the thesis are both my own, and have been generated by me as the result of my own original research. I confirm that:

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Guerin, A. J., Jensen, A. C., and Jones, D. 2007. Artificial reef properties of North Sea oil and gas production platforms. *In* Oceans 2007-Europe, pp.795-800. Institute of Electrical and Electronics Engineers, Aberdeen, UK.

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ACKNOWLEDGEMENTS

Firstly, I extend my thanks to my principal supervisor, Dr. Antony Jensen, for his support, reassurance, and assistance. His feedback has been invaluable in the preparation of this thesis, and help with field sampling activities was also appreciated. Thanks are also due to Dr. Dan Jones, for feedback on the thesis, advice on some of the statistical analyses, and general help with attempts to get offshore for sampling activities.

I am extremely grateful to the following industry contacts, for providing all the data used in the second part of the thesis: Aly Lyon and Laurence Duffin at IICORR, Neil Duncan and Wendy Brown at Total E&P, Anne-Marie McLaughlin, Sean Young, William Murdoch and Paul Benstead at BP, and Janette Paterson and Alan Buchan at Acergy; without their willing assistance I would have had little to base my work on. Thanks are also due to Dr. Ian Hudson; despite only being involved in the project for a short time, he provided contact details for the above individuals, and obtained the first set of platform survey footage for the project.

Sampling in Poole Bay was only possible thanks to the members of the NOC volunteer dive team. Particular thanks are due to Dr. Ken Collins and Jenny Mallinson, who do a great job running the dive team, but also helped immensely with the organisation of sampling trips, as well as supervising the diving on the day. I would like to thank all the divers who took part in the sampling dives, but especially Ken, Jenny, Antony, Linn Baldock, Alison Bessel, Dr. Chris Hauton, Michael Thompson, and Alastair Brown. I am also grateful to Mike Markey, skipper of *Peveril Myth*.

I thank Dr. Clive Trueman for initial advice on stable isotope work, and Dr. Jason Newton at SUERC for advice and help running my first batch of trial samples. In particular, however, thanks are due to Dr. Rona McGill at SUERC for being extremely welcoming and helpful throughout both my visits there, for patiently walking me through the use of the equipment, explaining how everything worked, and answering my questions.

Thanks to my friends, who have made life more enjoyable over the last few years: Jens, Steve (and Chris), friends and housemates for a very ‘special’ year; Robin; Frank and Jamie, for providing a floor or a bed when I was up in Scotland, and also Claire (and her family), who found me places to stay during several lab visits to East Kilbride. Of course, I must also mention Richard, Paul and Dan; friends for as long as I can easily remember.

Finally, my parents; without their constant support and encouragement, none of this would have happened.

The project was financially supported by the Natural Environment Research Council, with CASE support from Nexen, Inc.

Chapter 1. Introduction to Artificial Reefs

1.1 What is an artificial reef?

The European Artificial Reef Research Network (EARRN; Jensen, 1997), defines an artificial reef as "a submerged structure placed on the substratum deliberately, to mimic some characteristics of a natural reef" (Jensen et al., 2000a). Similarly, Seaman and Jensen (2000) define an artificial reef as "one or more objects of natural or human origin deployed purposefully on the seafloor to influence physical, biological, or socioeconomic processes relating to living marine resources". However, following Svane and Petersen (2001), who define artificial reefs as "submerged structures susceptible to fouling", a broader definition is adopted for this thesis: *a submerged structure, placed either deliberately or accidentally, which functions as habitat for marine biota*. Breakwaters (Stephens and Pondella, 2002), seawalls (Chapman, 2006), piers (Glasby and Connell, 1999), and offshore oil and gas platforms (Wolfson et al., 1979), are all used as habitat by marine organisms, but this is not part of their intended function, and so stricter definitions (such as the EARRN definition above) would explicitly exclude them from consideration as artificial reefs. Furthermore, maritime accidents and military conflicts have resulted in the sinking of thousands of ships; these provide hard habitat on the seabed (Zintzen et al., 2008a, b) but would also be excluded.

1.2 Artificial reefs and epibiota

Artificial reefs provide new habitat for sessile, or 'fouling', organisms (Forteath et al., 1982; Ardizzone et al., 1996; Svane and Petersen, 2001; Brown, 2005) and associated mobile fauna such as small crustaceans, molluscs, and errant polychaetes (Chapman, 2006; Page et al., 2006; People, 2006). Such epibiota on artificial reefs can achieve very high biomass; in extreme cases wet weights of fouling organisms as high as 155 kgm^{-2} have been recorded (Relini et al., 1998).

1.2.1 Factors affecting fouling assemblages on artificial reefs

1.2.1.1 Location

Several key factors driving the nature of biological communities are dependent on location, because it determines the environmental conditions which a reef experiences and the pool of potential colonising species (Sammarco et al., 2004; Zintzen et al., 2008a). Depth affects the composition of fouling assemblages (Perkol-Finkel et al., 2008), because it integrates factors such as light and temperature, and deeper water reefs are generally colonised by fewer species, with lower biomass (Relini et al., 1994; Ardizzone et al., 1997). The local hydrodynamic flow regime also affects the availability of colonists, and of food for filter feeding taxa, exerting an influence over the growth of sessile organisms (Baynes and Szmant, 1989; Guichard et al., 2001).

Aspects of water quality are driven by local and regional effects (natural and anthropogenic). Eutrophic conditions can result in algal overgrowth and exclusion of sessile fauna, and high turbidity also affects artificial reef assemblages (D'Anna et al., 2000), because the resulting low light levels limit algal growth (Falace and Bressan, 2002) and high sediment deposition hampers development of sessile communities (Baynes and Szmant, 1989; Fabi and Fiorentini, 1997).

1.2.1.2 Construction material

A wide range of materials have been used in artificial reef construction (Baine, 2001), including concrete, rock, tyres, stabilised ash wastes, plastics, wood, steel, rope, cars, trains, old vessels and offshore platforms. Some materials (e.g. car bodies) are susceptible to corrosion and burial (Relini, 2000), or can be moved by strong currents and severe weather (Turpin and Bortone, 2002), making them less suitable for artificial reef construction.

Surface texture affects initial colonisation; rougher surfaces promote settlement and softer, more porous materials are better colonised than hard, smooth surfaces (Hixon and Brostoff, 1985). Surfaces may also leach chemicals which can influence

settling larvae (Pawlik, 1992), but this does not always exert a notable effect on the composition of fouling assemblages (e.g. Collins et al., 2002).

As a result of these factors, different materials can acquire different assemblages (Anderson and Underwood, 1994; Brown, 2005). Natural materials such as rock might thus seem to be ideal, and have been used successfully; however, the need to quarry the rock and the lack of control over shape are disadvantageous (Walker et al., 2002), and artificial materials perform comparably to natural rock as habitat (Chapman and Clynick, 2006). Generally, surface texture and chemical properties are increasingly thought to be less important than other factors (Bohnsack et al., 1991; Glasby, 2000), and seemingly unsuitable materials such as plastics do acquire fouling assemblages. However, factors such as long term stability (Spanier, 2000; Turpin and Bortone, 2002) and the possibility of leaching toxic compounds into the environment (Collins and Jensen, 1997) may still preclude the use of some materials.

1.2.1.3 Surface orientation

Surface orientation (both on natural and artificial habitats) is one of the most influential factors affecting the composition of fouling communities (Baynes and Szmant, 1989; Perkol-Finkel and Benayahu, 2004; Chapman and Clynick, 2006; Walker et al., 2007; Zintzen et al., 2008a). Steep or vertical surfaces (and overhangs) experience less sedimentation, which improves conditions for settlement and growth of sessile fauna (Baynes and Szmant, 1989) leading to greater faunal coverage and biodiversity (often with less algae) than on horizontal surfaces (Chapman and Clynick, 2006; Walker et al., 2007).

Surface orientation also affects the level of illumination, and shaded areas generally have less algae and more sessile invertebrates than well lit areas (Glasby, 1999a; Blockley and Chapman, 2006). These variations are partly the result of differences in recruitment: a combination of preferential settlement and post-settlement mortality (Blockley and Chapman, 2006). However, the greater

abundance of sessile invertebrates in shaded areas may also represent release from competition with algal species which do not thrive in shaded conditions.

1.2.1.4 Reef profile

Reefs with high profiles extend up through the water column and consequently experience a range of different environmental conditions; such reefs may accommodate multiple assemblages, increasing overall diversity (Forteath et al., 1982; Perkol-Finkel and Benayahu, 2004).

1.2.1.5 Structural complexity

Increasing the heterogeneity of an environment increases not only the amount of physical space, but also the potential variety of available niches; more complex habitats can thus accommodate greater abundances (Hacker and Steneck, 1990), and greater biological diversity (Guichard et al., 2001; Svane and Petersen, 2001; Pinn et al., 2008). Increasing structural complexity may also offer increased protection from predators (Hixon and Brostoff, 1985), but predators can also aggregate within complex habitat (Hixon and Beets, 1989), potentially *increasing* predator-induced mortality among primary producers and consumers. Increased densities of prey at artificial reefs can translate into increased foraging success for some consumers, (Clark et al., 1999) which might offset the advantages of complexity for their prey at higher predator densities (Grabowski and Powers, 2004).

1.2.1.6 Timing of deployment

The time of year in which a reef is deployed can exert a significant effect on the composition of the assemblage, at least in temperate waters, as a result of seasonal variation in larval supply (Bram et al., 2005; Brown, 2005).

1.2.2 Colonisation of artificial reefs by epibiota

Colonisation by fouling species usually occurs via settlement of pelagic larvae, and this accounts for more than 90% of the initial colonists arriving at reef habitat

(Osman, 1977). Over time, assemblage organisation and complexity increase (Sammarco et al., 2004) along with total biomass (Relini and Relini, 1997) and biodiversity (Ardizzone et al., 1996). However, colonisation and succession may not follow the same 'trajectory' as on natural reefs. As already described, differences in location, construction material and surface orientation (for example) mean that artificial reefs often have a different ecological 'starting position' to natural reefs, and small differences in initial settlement conditions may lead to observable differences in the resulting assemblages (Brown, 2005; Perkol-Finkel et al., 2005; Perkol-Finkel et al., 2006). Other factors, such as the abundance of local predators (Nydam and Stachowicz, 2007), can also strongly influence the development of fouling communities. Despite this short- to medium-term differentiation, there is some evidence that over long periods of time artificial reefs within a locality may converge towards a single 'climax' assemblage (Anderson and Underwood, 1994; Brown, 2005), although differences (such as those resulting from structural factors such as surface orientation) can sometimes still be detected over a century after first settlement (Perkol-Finkel et al., 2006). As artificial reef assemblages mature, they may also come to resemble communities on natural reefs (Anderson and Underwood, 1994; Perkol-Finkel and Benayahu, 2004; Perkol-Finkel et al., 2006).

However, it might be expected that there would be biological differences between artificial and natural reefs, resulting from the factors described in Section 1.2.1. For example, artificial reefs often have greater proportions of highly inclined and vertical faces than natural reefs (Chapman, 2006). Indeed, several studies have identified differences in species composition, biodiversity, biomass, and spatial variability, between mature artificial reefs and nearby natural habitats (Badalamenti et al., 2002; Bulleri et al., 2005; Chapman, 2006; Perkol-Finkel et al., 2006; Page et al., 2007).

1.3 Artificial reefs and fish

1.3.1 Fish populations at artificial reefs

Fish occur in consistently greater numbers around seabed features such as natural reefs than in featureless areas (Bohnsack, 1989). Unsurprisingly, large numbers of

fish are also found at artificial reefs (Santos et al., 1997), sometimes at higher densities than around natural reefs (Ambrose and Swarbrick, 1989; Bohnsack, 1989). Aggregated fish generally position themselves within 15-20m of small artificial reefs (Sargent et al., 2006); for larger constructions elevated fish densities extend as much as 50-80m out (Stanley and Wilson, 1996; Fabi et al., 2002a). Smaller fish generally associate more closely with structures, while larger fish range over a wider area in the vicinity of the reef (Dempster, 2005). Fish on artificial reefs can be thought of as belonging to one of three categories (Bohnsack et al., 1991): 'reef-associated'/'reef-resident' (obligate reef dwelling fish species which spend a large part of their life-cycle at reef habitat, and usually feed on reef-based resources); 'reef-aggregated'/'visitors' (species that are not always associated with reef habitat, nor dependent on reef-based resources, but may be attracted to structures in large numbers); or 'reef-indifferent'/'transient' (species which may be seen at reefs, but with no indication that they are particularly attracted to reef habitat).

Diel trends have been observed in the patterns of fish residency around artificial reefs; both reef-resident and reef-aggregated fish appear to be present at higher densities during the day (Santos et al., 2002; Sargent et al., 2006), although different occupancy patterns may occur on different reef systems (Fabi and Sala, 2002). These trends may have different explanations for different fish taxa: reef-resident fish may become quiescent and hide within the reef at night (Sargent et al., 2006), while reef-aggregated fish may simultaneously disperse outwards to forage in other habitats (Fowler et al., 1999; Sargent et al., 2006). Fish assemblages can also show seasonal changes (Godoy et al., 2002; Relini et al., 2002b; Dempster, 2005) and some fish also show a high degree of site fidelity (Jorgensen et al., 2002; Workman et al., 2002).

1.3.2 Colonisation of reefs by fish

While fish may colonise reefs as larvae, they can also arrive via immigration of juveniles and adults (as can larger species of invertebrate). The rapid appearance of large fish around artificial reefs indicates that immigration of adults is significant

(Walsh, 1985; Bohnsack et al., 1994). Ongoing re-supply (recruitment) of motile species (particularly fish) onto a reef is generally from external sources, using largely the same mechanisms as initial colonisation, and also through some reproduction on the reef itself. Once fully developed, artificial reefs can themselves become a source of fish larvae (Stephens and Pondella, 2002).

1.3.3 Potential benefits of artificial reefs to fish

1.3.3.1 Food

Artificial reef epibiota may represent an additional source of food for aggregated and resident fish (Johnson et al., 1994; Falace and Bressan, 1997; Sanchez-Jerez et al., 2002; Santos et al., 2002; Page et al., 2007), and foraging efficiency can be higher on artificial reefs (Clark et al., 1999), providing more energy for growth (Harmelin and Bellan-Santini, 1997; Relini and Relini, 1997). Where different prey species are available on artificial reefs, these can in some cases represent more favourable prey for aggregated fish (Clark et al., 1999; Page et al., 2007), and this can contribute to improved condition in foraging fish (Berumen et al., 2005).

However, removal of epibiota from artificial habitats does not always reduce the numbers of aggregated fish (Moreau et al., 2008), and large numbers of fish are aggregated by reefs long before fouling assemblages have time to develop. Some studies have found no difference in the diets of certain fish species between artificial reefs and control sites (Bohnsack et al., 1991). Although some aggregated fish may utilise reef-based food resources, this is clearly not the only factor driving their aggregation, and they are able to obtain food elsewhere.

1.3.3.2 Shelter

Reefs are thought to provide shelter and cover for some species (Hixon and Beets, 1989; Moreau et al., 2008). While there is evidence of greater fish abundances around reefs with more shelter provision (Hixon and Beets, 1989), there is little direct evidence that this is a result of improved prey survival (Svane and Petersen, 2001). Furthermore, reefs do not only provide cover for prey species; they can also

hide predators (Hixon and Beets, 1989; Spanier, 1997), and the aggregation of fish around artificial reefs may result in (or be coincident with) the aggregation of transient predators of those fish, resulting in an ultimately negative effect on their survival (Leitao et al., 2008).

1.3.3.3 Other possible benefits

Artificial reefs may provide ample sites for egg-laying, nest building, and other reproductive behaviours (Harmelin and Bellan-Santini, 1997), while these features and their surroundings may provide nursery areas for juvenile fish, and also shelter and refuge sites from predation (but see Section 1.3.3.2 above). For some species, high densities of conspecific adults found at artificial reefs may enhance larval recruitment (Sweatman, 1985).

Finally, fish may derive energetic savings from use of current shadows and lee waves which are generated by the presence of a structure, or may use structures as reference points for orientation and navigation (Bohnsack, 1989).

1.3.4 Factors affecting fish populations at artificial reefs

Some of the key factors influencing fish populations at artificial reefs are the same as those affecting epibiota. Location substantially affects colonisation of artificial reefs by fish (Walsh, 1985; Herrera et al., 2002; Moreno, 2002). Higher profile reefs attract a greater abundance and diversity of fish than lower profile reefs; offshore oil and gas production platforms are extreme examples, as they extend from the sea surface to the sea floor, sometimes in more than 200m depth of water, attracting fish at all depths (Aabel et al., 1997a). Structural complexity exerts a similar influence over fish assemblages as it does over fouling organisms; greater structural complexity generally provides more habitat space, niche diversity, and shelter (but see Section 1.3.3.2), leading to greater fish abundance and diversity (Charbonnel et al., 2002; Kawasaki et al., 2003). However, there are factors affecting fish assemblages which are not relevant to fouling communities.

1.3.4.1 Reef size

The density and diversity of fish species at an artificial reef is related to the surface area and the volume of a reef. Generally, larger reefs attract more fish overall (Ardizzone et al., 1997), but small reefs may have a *proportionally* greater fish aggregation effect than larger reefs, perhaps because they have a higher perimeter to area ratio or 'edge effect' (Jan et al., 2003). Therefore, given a fixed quantity of material for reef-building, a number of smaller reef units may in some cases attract more fish than a single large one.

1.3.4.2 Epibiota

The fouling community itself influences the nature of the fish community (Hueckel and Buckley, 1989; Hueckel et al., 1989), as significant fouling of a reef increases structural complexity and provides food and shelter. While this may be important to some fish species (Clynick et al., 2007), it has less (or no) effect on others, which remain at reefs in large numbers even after experimental removal of all epibiota (Moreau et al., 2008).

1.3.4.3 Arrangement of reef units

Reefs are often built in multiple 'units'. The way in which the units of a reef development are arranged can be important. For example, the simple distance between units affects fish aggregation (Bohnsack et al., 1991). If it is assumed that each reef or reef unit has a certain 'area of influence' it makes most sense for these areas to be as close together as possible without overlapping. If they overlap, food availability may be limited, or overgrazing of sediment communities may occur. Optimal reef spacing for small reef units is claimed to be about 30-40 metres (Sargent et al., 2006).

1.4 Effects of artificial reefs on surrounding environment

1.4.1 Attraction versus Production

Fish and other organisms are indisputably attracted to reefs, and growth of fouling biomass on these structures clearly represents enhanced local production (Svane

and Petersen, 2001; Steimle et al., 2002). However, an important issue is the extent to which this can translate into increased production of fish biomass, particularly for fish species (and some motile invertebrate species) of commercial importance. This has become known as the 'attraction versus production' debate.

1.4.1.1 The importance of the attraction-production debate

The attraction-production debate is important, because large aggregations of fish around artificial reefs are likely to be targeted by fishers. A number of scenarios can be envisaged (Polovina, 1991; Relini and Relini, 1997):

1. ATTRACTION, NO PRODUCTION
    a. Artificial reefs attract fish, consequently local fish biomass is redistributed, but no additional biomass becomes exploitable.
    b. Artificial reefs attract fish, biomass is redistributed, resulting in more fish becoming exploitable
2. ATTRACTION WITH PRODUCTION

    New habitat results in stock increases, as well as stock redistribution

If fish biomass is simply redistributed (ATTRACTION, NO PRODUCTION) it may become easier to harvest (Relini and Relini, 1997). This could allow fishing effort to be reduced with no reduction in catch, or continued at the same levels for greater catches (which could result in overfishing or worsening of overfishing in already heavily fished environments). However, this increase of biomass at the reef might result from a reduction in biomass in surrounding habitats (Harmelin and Bellan-Santini, 1997). Furthermore, species or populations which were not previously exploitable because they were only present at low densities may become harvestable (scenario 1b above), exposing them to potential overfishing. If those fish removed from reefs by fishing are quickly replaced by new immigrants, then overfishing at artificial reefs could drive exploited populations towards extinction. However, if the rate at which fish repopulate the reef is slow (for example, lower than the population growth rate) then fishing at reefs will not result in population extinction (Polovina, 1991). More positively, if installation of artificial reefs results

in new production as well as stock redistribution (scenario 2) then careful increases in fish extraction could be made without threatening exploited populations.

1.4.1.2 Is there evidence of production?

While there is some evidence of stock redistribution resulting from the construction of artificial reefs (Walker et al., 2002), conclusively demonstrating production of new biomass is more difficult.

Many invertebrates are 'habitat-limited', such that provision of suitable new habitat space would support greater population growth, and therefore increased production. This has been demonstrated for numerous crustacean species (Hacker and Steneck, 1990; Eggleston and Armstrong, 1995; Butler and Herrnkind, 1997; Loher and Armstrong, 2000), cephalopods (Polovina and Sakai, 1989), and even some reef-associated fish (Halpern, 2004). In some cases, adults of certain species do not appear to be habitat-limited, but earlier life-cycle stages are (Butler and Herrnkind, 1997; Halpern, 2004); artificial habitats could still benefit populations of these species. However, many fish species are not habitat-limited (Robertson et al., 1981; Sweatman, 1985; Bohnsack, 1989; Powers et al., 2003; Halpern, 2004). It is much harder to show increased production of reef-aggregated (but not habitat-limited) species. A few studies have claimed to show increased regional biomass for some species (Walker et al., 2002), but these results have not been repeated.

One means of resolving the debate is to determine whether or not any of the potential benefits of artificial reefs listed in Section 1.3.3 are realised for any particular species of interest, in the form of either increased growth of adults (somatic production) or increased reproduction/recruitment of juveniles (Harmelin and Bellan-Santini, 1997).

1.4.2 Effects on the surrounding benthos

An artificial reef may not provide sufficient new food resources to support the potentially large numbers of associated consumers. This might lead to the creation of a 'feeding halo': a region of lowered biomass in the vicinity of the reef, resulting from the foraging activities of reef-based organisms (Badalamenti and D'Anna,

1997). Decreases in soft-bottom species diversity or density have been observed around some artificial reefs (Guichard et al., 2001; Danovaro et al., 2002), but not others (Davis et al., 1982; Jensen et al., 1994; Fabi et al., 2002b; Fukunaga and Bailey-Brock, 2008). However, artificial reefs also alter the hydrodynamic regime in their vicinity, which influences sediment structuring and therefore the benthic community around the reef (Herrera et al., 2002). Disentangling the physical and ecological components of any 'reef effect' on the surrounding benthos has been attempted in a few cases, leading to general agreement that it is the physical effects of the reef on sediment structuring that are more significant (Guichard et al., 2001; Danovaro et al., 2002; Fabi et al., 2002b).

1.4.3 Artificial reefs and biological invasions

While healthy native communities tend to be resistant to invasion, native species have no clear advantage over invasive species when settling on newly available surfaces (Glasby et al., 2007). Disturbance of native assemblages also provides potential opportunities which invasive species can exploit (Bulleri and Airoldi, 2005). Consequently, artificial structures can have higher abundances of invasive or exotic species than natural habitat (Cohen et al., 2005; Page et al., 2007; Tyrrell and Byers, 2007). Invasive species are sometimes present on coastal defence structures, which, given their wide distribution, may provide invasion 'corridors' through areas which would otherwise have no suitable habitat (Bulleri and Airoldi, 2005). Anthropogenic habitat provision (intentional or otherwise) may thus facilitate biological invasions (Bulleri and Airoldi, 2005; Glasby et al., 2007). Biological invasions are a large and growing problem (Grosholz, 2002); planning of artificial reefs should include consideration of invasion risk, and reefs should be designed (if possible) in ways that reduce the advantage to invasive taxa.

1.5 Artificial reef applications

1.5.1 Exploitation reefs (fishing reefs)

It is common knowledge among fishermen that greater catches can be attained around objects on the seafloor such as wrecks (Santos et al., 1997). It was an

obvious step from making this observation to actively creating artificial reefs and other types of fish aggregating device (FAD) for fishing purposes. The Catch Per Unit Effort (CPUE) can be significantly higher around artificial reefs than on featureless natural seabed, and even greater than at natural reefs, reducing running costs and increasing profit (Whitmarsh et al., 2008). However, if poorly managed, this can result in overfishing (Section 1.4.1.1).

Fishing reefs can be intended to benefit artisanal, recreational or commercial fisheries (Seaman and Sprague, 1991), or a combination of these. Artisanal fishermen use artificial reefs and FADs in small inshore fisheries, usually constructed from lightweight natural materials with a limited lifespan. These small reefs sometimes succeed in creating small-scale, sustainable fisheries for local fishermen (Collins et al., 2000). Artificial reefs for commercial fishermen mainly aim to increase catches and reduce operating costs and tend to be larger and more complex than those for artisanal fishermen (Santos et al., 1997). The logical extension of this type of fishing reef strategy is the use of artificial reefs to create commercial fishing grounds where none existed. This kind of ambitious undertaking has been attempted on a massive scale in Japan (Stone et al., 1991; Simard, 1997).

Since artificial reefs provide new surfaces for epibiota, they show great potential as aquaculture sites. Reefs can be designed and built to maximise their suitability for settlement of commercially important organisms. Harvesting exposes the surfaces for a new round of settlement and growth. Reefs for this purpose can create viable shellfish farming industries where no suitable natural habitat exists, for example on open, sandy bottoms (Fabi and Fiorentini, 1997).

1.5.2 Reefs for fisheries management

Distinct from their use as exploitation reefs, artificial reefs can also be used more generally as a fisheries management tool. Many areas of sensitive habitat are threatened by illegal trawling and networks of ‘anti-trawling’ reefs are an effective way of excluding trawlers from these grounds (Bombace, 1997). The exclusion of particular gears (such as trawls) from an area can also allow easier use by different

fishing gears (such as hand-lining and static gears) which can reduce conflict between different fisheries (Revenga et al., 2000).

Placement of artificial reefs within Marine Protected Areas (MPAs) might enhance their beneficial effects by not only acting as anti-fishing structures, but also by aggregating fish within MPAs, increasing the protection they offer and perhaps accelerating fish population growth and recovery (Pitcher et al., 2002). Furthermore, such reefs can be useful regardless of the outcome of the attraction-production debate for that biological system, since even in the worst-case scenario of attraction only (alternatives 1a and 1b in Section 1.4.1.1), attracted fish will still be retained within the MPA, and thus protected from fishing (Pitcher and Seaman, 2000; Pitcher et al., 2002).

1.5.3 Reefs for habitat enhancement and restoration

Artificial reefs are sometimes deployed for the stated purpose of environmental mitigation, by providing habitat to compensate for that lost during a construction project or industrial incident. There is significant uncertainty over the outcome of mitigation reefs and confidence in their effectiveness is low (Powers et al., 2003), as mitigation projects must provide appropriate habitat, must not risk further impacts, and must be large enough to mitigate the impacts sufficiently (Burton et al., 2002).

1.5.4 Recreational reefs

Recreational fishermen often use artificial reefs because of the abundance of fish in a discrete location, and they can also often catch fish of larger sizes than at natural sites. Furthermore, artificial reefs can be very popular with recreational divers (Ditton et al., 2002), as the aggregation effect increases the chances of encountering larger or more interesting fish in greater numbers. Usage of such sites can be intensive, and locally economically important (McGinnis et al., 2001). In areas without natural coral reefs, ARs can provide diving and fishing attractions, and in other locations they can take the pressure off over-used natural dive sites and recreational fishing grounds. Another recreational use is the ‘surfing reef’, an

offshore structure intended to generate waves suitable for surfing (Mead and Black, 1999).

1.5.6 Other functions

A recently explored prospect is the use of artificial reefs for nutrient removal (Laihonen et al., 1997; Angel and Spanier, 2002). The substantial growth of algae and filter feeders on reefs may allow them to remove nutrients from the water column, but this is likely to be inefficient and only useful in relatively small bodies of water with little flow. Similarly, artificial reefs have been proposed as a way of ameliorating the impact of fish farms on benthic communities (Gao et al., 2008). This would require regular harvesting (and therefore presumably disposal) of fouling organisms in order to truly remove the nutrients from the system, which could create aquaculture opportunities (Gao et al., 2006).

Finally, artificial reefs have been used extensively for research purposes, including general research into ecological processes on reefs (Hixon and Beets, 1989), investigation of fisheries enhancement potential of artificial reefs, (Jensen et al., 1994; Jensen and Collins, 1997) and evaluation of the suitability of various materials as artificial reef substrate (Collins and Jensen, 1995; Collins et al., 2002).

1.5.7 *de facto* artificial reefs

This category includes infrastructure such as harbour walls (Stephens and Pondella, 2002), offshore platforms (Wolfson et al., 1979), sea defences (Chapman, 2006), shipwrecks (Zintzen et al., 2008a), and almost any man-made item on the sea bed (Caselle et al., 2002). These are all used by marine organisms as habitat, and can be thought of as *de facto* artificial reefs. Many such *de facto* reefs are fairly durable and resistant to maritime conditions. However, their design is not optimised for habitat provision, and growth of marine life may be regarded as a nuisance, with measures taken to remove growth or prevent it (Picken, 1986). Furthermore, such structures may eventually be removed entirely.

Despite this, *de facto* artificial reefs are likely to be, globally, the most significant type of artificial reef; they can be dominant habitat types in some regions, with

significant consequences for regional biodiversity. For example, over 50% of the foreshore of Sydney Harbour is thought to be made up of man-made structures (Bulleri et al., 2005), and as much as 90% of the hard bottom habitat in the waters off the US state of Louisiana may be composed of oil platforms (Polovina, 1991).

1.6 Structure of thesis

The thesis is divided into two parts. **Part I** is an exploration of the use of stable isotope methods to investigate the food web structure associated with artificial reefs, and consists of 5 chapters. **Chapter 2** introduces artificial reef food web studies and the principles behind stable isotope ratio analysis as a tool in ecological research. **Chapter 3** details the comparison of stable isotope ratios of reef taxa from an artificial reef with those from a nearby natural reef, while **Chapter 4** extends the comparison by attempting to use isotope ratio data to investigate the trophic structure of the same two reef systems. **Chapter 5** is a self-contained investigation into the effect of sample preparation techniques on stable isotope ratios of carbonate-rich samples. **Chapter 6** summarises the results from **Part I**.

Part II is concerned with the role of offshore oil and gas platforms in the North Sea as artificial reefs. **Chapter 7** describes the state of knowledge about the biology of offshore platforms around the world, and introduces the concept of 'rigs-to-reefs'. **Chapter 8** details the marine life associated with eight North Sea structures, while **Chapter 9** compares the fouling assemblages of these platforms in greater detail, relating the differences to their location and the time since their installation. The results and conclusions of **Part II** are summarised in **Chapter 10**

PART I

EXPLORING ARTIFICIAL REEF FOOD WEBS USING STABLE ISOTOPE ANALYSIS

Chapter 2. Introduction to Part I: Investigating artificial reef food webs

2.1 Food webs on artificial reefs

2.1.1 Trophic transfer to reef fish

One means of demonstrating production of fish biomass at artificial reefs, as an alternative to simple behavioural aggregation (Bohnsack, 1989), is to demonstrate energy transfer from reef epibiota to local fish populations. This can be established by sampling the stomach contents of fish at artificial reefs, and looking for differences in their diets compared to fish at other sites (Relini et al., 2002a; Sanchez-Jerez et al., 2002; Page et al., 2007) or for the presence in the diet of 'indicator species' which are only present on artificial reefs (Relini et al., 2002a).

Some species of reef-associated fish, such as *Scorpaena notata* and *Scorpaena cabrilla* in the Mediterranean (Relini et al., 2002a), or *Centropristis striata* (Lindquist et al., 1994) and *Oxylebius pictus* (Page et al., 2007) in North America, have been found to derive a significant proportion of their diet from artificial reef-based resources, whilst still exploiting food resources in neighbouring habitats to some degree. Other fish may exploit reefs when available; fish sampled from reef habitats can have different diets to conspecifics sampled from areas without reefs (Relini et al., 2002a; Sanchez-Jerez et al., 2002). Even some species apparently not linked to reef habitat may derive some of their diet from reef-based resources (Relini et al., 2002a). Fish diets can also differ between natural and artificial reefs, particularly where there are differences in the relative abundances of prey taxa (Vose and Nelson, 1994; Page et al., 2007).

However, the number of detailed studies which explore fish diet at artificial reefs is comparatively small (Lindquist et al., 1994; Vose and Nelson, 1994; Relini et al., 2002a; Page et al., 2007). Furthermore, conventional methods of diet assessment suffer from numerous difficulties (Hyslop, 1980). Stomach contents only provide a 'snapshot' of food consumption over a relatively short period of time prior to sampling, which is not guaranteed to represent typical diet, and individual fish may often be sampled with empty stomachs (either as a result of a lack of recent

feeding activity, or because they have regurgitated their stomach contents during capture). These two factors alone dictate that comparatively large numbers of individuals need to be sampled, which can risk impacting the population being studied. The need for extensive sampling also compounds the already time-consuming task of sorting, identifying and quantifying fish stomach contents (Hyslop, 1980). Quantification of diet can itself prove problematic, particularly since there are several approaches: frequency of occurrence, numerical abundance, proportional contribution by volume, and proportional contribution by mass; all of which can give conflicting impressions of the importance of prey species in the diet. Partial digestion of prey items complicates identification and can result in underestimation of individual prey sizes. Additionally, different types of prey pass through the gut at different rates (hard parts of prey, for example, are known to be retained in the gut for longer periods of time) which introduces further biases into estimates of dietary importance (Hyslop, 1980).

2.1.2 Structure of reef food webs

The 'attraction-production' debate has been mainly concerned with fish, partly because fish are often the focus of commercial fisheries (and thus often the impetus for artificial reef creation), but also because for many invertebrate taxa on artificial reefs, production of biomass is obvious and uncontroversial (Svane and Petersen, 2001). Trophic structure of fouling assemblages, however, is of interest in its own right. Numerous studies have explored the taxonomic composition of artificial reef fouling assemblages and how they vary in response to various factors (Section 1.2.1), such as location (Zintzen et al., 2008a), depth (Relini et al., 1994), light levels (Glasby, 1999a), surface orientation (Perkol-Finkel et al., 2006), and surface composition (Brown, 2005). Differences have been recorded between assemblages on artificial substrates and on natural reefs (Badalamenti et al., 2002; Bulleri et al., 2005; Chapman, 2006; Perkol-Finkel et al., 2006; Page et al., 2007). However, little work has been undertaken on trophic interactions among fouling organisms on artificial reefs, apart from some experimental studies investigating the effects of removal of consumer species on assemblage composition (e.g. Bulleri

et al., 2000). Consequently, it has not been established whether or not differences in species composition between natural and artificial reefs might result in different trophic structures, or if superficial similarities between assemblages on some reefs might be masking differences in food web structures. Given that much effort has gone into investigating the extent to which artificial reef assemblages approximate those on natural reefs (Chapman, 2003; Bulleri et al., 2005; People, 2006; Perkol-Finkel and Benayahu, 2007), trophic structure is a neglected topic.

The lack of artificial reef trophic studies is not simply due to a focus on fish, but is also the result of the difficulties involved in establishing the diet of reef invertebrates. Dissecting out the gut contents of small invertebrates (where possible) is very delicate and detailed work, and identification of food can be difficult, especially for herbivorous taxa (Alfaro, 2008). Fortunately, modern analytical techniques such as lipid biomarker analysis and stable isotope analysis allow inferences about diet to be made for any organisms from which tissue can be sampled.

2.2 Stable isotopes in ecological research

2.2.1 Introduction to isotope approach

Stable isotope analysis involves measurement of the ratios of naturally occurring stable isotopes of chemical elements in biological samples (Fry, 2006). The most commonly considered elements are carbon and nitrogen, both of which have two naturally occurring stable isotopes ($^{13}C/^{12}C$ and $^{15}N/^{14}N$). In both cases, the lighter isotope is the more common, and the ratio of the heavy to light isotopes is given using 'δ' notation, which is expressed in 'permil' (represented by the symbol ‰). The carbon stable isotope ratio of a sample is referred to as the '$\delta^{13}C$', and the nitrogen stable isotope ratio as the '$\delta^{15}N$'.

These ratios are of interest, because stable isotope ratios in consumers reflect the stable isotope ratios of their food (DeNiro and Epstein, 1978; Minagawa and Wada, 1984). However, as a result of slight differences in the reaction speeds of different isotopes, consumer tissues are generally enriched in the heavier isotopes (^{13}C and

^{15}N) relative to their diet (Peterson, 1999). This enrichment (known as 'trophic fractionation') is usually taken to be +1.0‰ for carbon (DeNiro and Epstein, 1978), and +3.4‰ for nitrogen (Minagawa and Wada, 1984).

The larger trophic fractionation for nitrogen makes it ideal for analysis of the trophic position of organisms within food webs. Since $\delta^{15}N$ increases by 3.4‰ with each trophic step (Minagawa and Wada, 1984), the trophic position of a consumer can be calculated by comparing its $\delta^{15}N$ with a value for a 'trophic baseline', such as the $\delta^{15}N$ of primary consumers (Cabana and Rasmussen, 1996). The small increase in $\delta^{13}C$ per trophic level (+1.0‰; DeNiro and Epstein, 1978), on the other hand, makes it less useful for estimating trophic position, but much more useful for tracking the influence of different dietary sources through food webs (Peterson, 1999). For example, benthic and pelagic primary production sources typically have different $\delta^{13}C$ values, which means that by measuring the $\delta^{13}C$ of consumers, the relative contributions of benthic and pelagic production to food webs can be assessed (Jennings et al., 1997).

2.2.2 Advantages of isotope approach

Stable isotope ratio methods have several advantages over traditional methods such as stomach content analysis. The isotope ratio of consumer tissues represents the diet integrated over time, rather than a recent snapshot (O'Reilly et al., 2004). This method does not suffer from the biases associated with stomach content methods, as the isotope values of consumer tissues only reflect the components of the diet that are actually assimilated. Since isotope analysis requires only very small tissue samples (DeNiro and Epstein, 1978), it can also be conducted on very small organisms for which gut content analysis is not feasible, such as amphipods (Crawley et al., 2007).

2.2.3 Applications

Stable isotope methods have been applied to a wide range of ecological topics, including: identification of feeding sites (Leakey et al., 2008) and food sources (Stenroth et al., 2006); detection of spatial (Jennings et al., 1997), seasonal (Kang et

al., 2007), and ontogenetic (Hoeinghaus and Davis, 2007; Menard et al., 2007; Reich et al., 2007; Parry, 2008) diet shifts; determination of food chain length (Cabana and Rasmussen, 1996), food web structure (Iken et al., 2001; Bergquist et al., 2007; Richoux and Froneman, 2007), and how these can change over time (Wainright et al., 1993); and tracking of anthropogenic nutrient input into natural habitats (Cabana and Rasmussen, 1996; Dolenec et al., 2007; Fukumori et al., 2008). These are simply a selection of topics that have been addressed in marine environments; the range of ecological topics to which isotope methods have been applied is vast (Peterson, 1999; Fry, 2006).

However, despite the obvious utility of the approach, stable isotopes have not been widely used in the study of artificial habitats. To date it appears that only two published studies into the structure of food webs in marine artificial habitats have used stable isotope methods (Riera et al., 2004; Schaal et al., 2008).

2.3 Aims and objective of Part I

The following chapters explore the use of stable isotope ratio analysis in the investigation of food webs associated with an artificial reef and nearby natural reef system. The principal aims are:

- To evaluate the use of stable isotope methods to explore artificial reef food webs
- To compare the structure of food webs on artificial and natural reefs

Additionally, Chapter 5 describes a short analysis of one of the preparation techniques (sample acidification) which is applied to certain types of sample prior to isotope ratio analysis.

2.4 The Poole Bay Artificial Reef

The Poole Bay artificial reef is one of a small number of licensed artificial reef projects in the UK (Jensen, 2002). The reef was originally deployed in June 1989 (Jensen et al., 2000b) as an experiment to assess the environmental suitability of two materials, cement-stabilised pulverised fuel ash and flue gas desulphurisation

gypsum (both waste products from power generation), for the construction of artificial reefs (Collins and Jensen, 1995). Eight reef units (made using different mixtures of concrete and the above materials) were deployed on a flat sandy seabed at a depth of 10m below Chart Datum in Poole Bay, off the south coast of England (Fig. 2.1). Since deployment, it has also been used as a site for research on various topics, including fish behaviour (Fowler et al., 1999), colonisation by epibiota (Hatcher, 1997), lobster activity patterns (Smith et al., 1998) and effects of reefs on infauna (Jensen et al., 1994). In July 1998 the reef system was expanded by the addition of a further eight units to evaluate the use of scrap tyres as artificial reef material (Collins et al., 2002), giving a total of 16 reef units spread over an approximately 40 x 40m area. The reef is regularly visited by divers from the National Oceanography Centre, making it an ideal site to undertake an investigation into food webs on artificial reefs. A nearby natural reef system (known as 'Wrasse Reef') is located approximately 2km to the Northeast of the artificial reef in similar water depth, and covers an area of approximately 80 x 60m; sampling on 'Wrasse reef' thus allows comparison with a typical natural reef community.

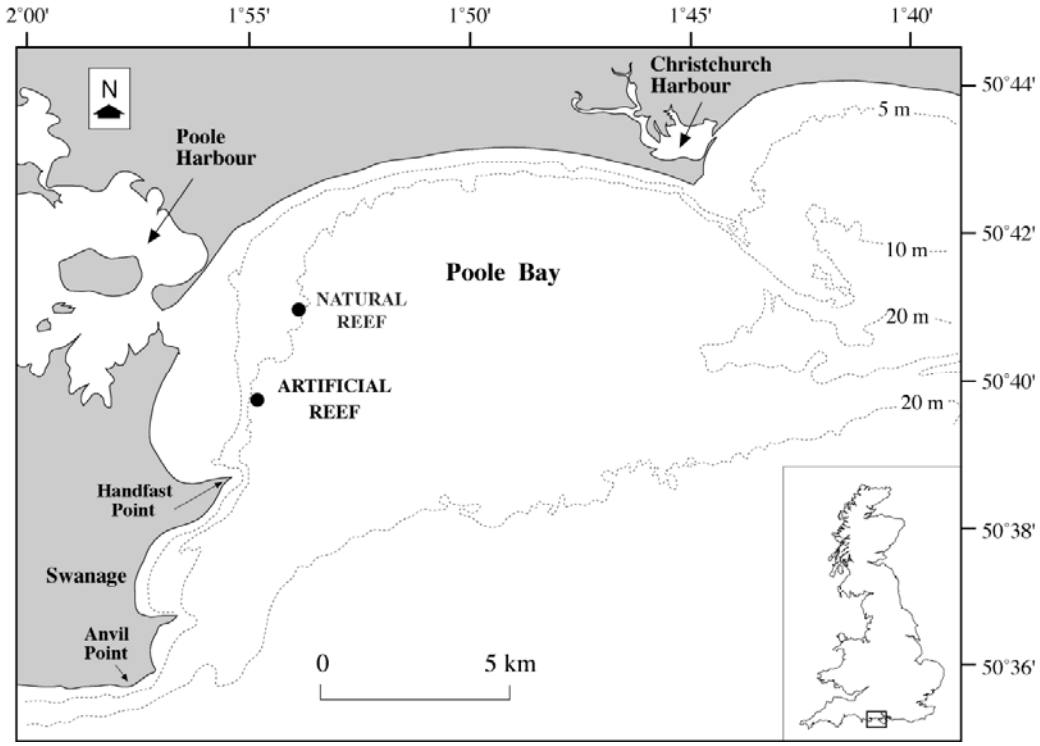


Figure 2.1 The location of the Poole Bay artificial reef. Adapted from Jensen et al. (2000b), with permission.

Chapter 3. Stable isotope ratios of consumers on natural and artificial reefs

3.1 Introduction

Differences in consumer stable nitrogen isotope ratios between sites can indicate differences in the length of the food chain (since $\delta^{15}N$ is strongly enriched at each trophic step, a lower $\delta^{15}N$ can indicate fewer steps; Jennings et al., 1997) or differences in the 'trophic baseline' (the $\delta^{15}N$ of primary consumers) which can be caused by localised effects such as nutrient enrichment from anthropogenic sources (Cabana and Rasmussen, 1996). Differences in carbon isotope ratios between sites can indicate shifts in diet, changes in food web structure, or changes in the relative inputs of different primary production sources to the food web (Jennings et al., 1997; Richoux and Froneman, 2007). Although simply comparing the isotope ratios of consumers at two sites does not allow us to distinguish between these possible causes of variation or to make any authoritative statements about food web structure, it does provide a useful indication of possible differences. Large differences between the isotope ratios of consumers at two sites will need to be explained if present, and if there are no differences, then this is a useful initial indication of trophic similarity between sites.

Consequently, looking for differences in consumer isotope ratios is a relatively common means of investigating trophic differences between locations (Richoux and Froneman, 2007; Yokoyama and Ishihi, 2007), or at different times. However, very few studies have compared artificial and natural reefs using this method (Schaal et al., 2008).

This chapter applies the approach to an artificial reef in Poole Bay (Fig 2.1), by comparing the carbon and nitrogen stable isotope ratios of consumer taxa which were sampled both on the artificial reef itself and on a neighbouring natural reef system ('Wrasse Reef').

3.2 Methods

3.2.1 Sampling

Samples were first collected from the two reef sites in 2007 (October/November). This initial sampling aimed to collect representatives of most major groups of flora and fauna. Sampling was carried out using SCUBA, and teams of divers visited the reefs over the course of two sampling days to collect the necessary material. Diving teams moved around the reefs searching for mobile fauna (crustaceans and gastropod molluscs) and sampling reef epifauna from the surfaces of rocks/reef blocks using metal paint scrapers. Algae were collected, including not only algae growing on the reefs, but also algae which were snagged on the artificial reef. Samples were placed in pre-labelled bags at the point of sampling and returned to the surface. Mesh traps were baited with broken mussels, crabs, and mackerel, and deployed at the artificial reef for several hours between dives, in order to catch fish and crabs. 'Bottle traps' constructed from 2l plastic soft drinks bottles (the top part of the bottle was cut off, inverted, and pushed back into the bottle) were also used to collect small motile invertebrates.

Traps generally failed to collect fish, so an additional static gear was deployed. A 10m length of 2m high monofilament net was wound around a length of plastic tube, such that it could be slowly unravelled underwater. Divers progressively unwound the net at the sea bed, while periodically attaching lead diving weights to the bottom. Along the top of the net were attached a series of small plastic floats, approximately one every 700mm, which served to keep the net upright. The gear was secured to the reef at each end, and deployed such that it was curved around one side of one artificial reef unit. After a period of deployment of between one and two hours, divers returned to recover the net. The net and any contents were stuffed into a large bag, which was then sent to the surface using an inflatable lifting bag. Any fish caught in the net or traps were immediately killed.

Further sampling was carried out during 2008 (Mid-August to November). Reef epifauna such as barnacles, polychaetes and amphipods were targeted by using metal scrapers to remove fauna from small areas of reef surface, and by collecting

samples of growing and snagged algae. In addition, several small rocks were collected and brought back to the lab for the removal of additional epifauna. Divers were specifically tasked with seeking out the following fauna: mobile crustacea (especially *Necora puber, Cancer pagurus*, and hermit crabs, family Paguroidea), gastropods (especially *Buccinum undatum*, *Ocenebra erinacea* and *Crepidula fornicata*) and any other mobile fauna. Traps were deployed on both reef systems, as was the static net. When deployed on the natural reef, the net was set parallel to the reef, along one edge.

Once recovered to the surface, all samples were kept cool in buckets of seawater and returned to the laboratory on the day of collection for processing. Samples were kept in clean seawater in a cold room (~5°C) prior to sorting, which was usually carried out within 12-24 hours of collection (the sorting of live organisms from sediment samples on one date in 2007 took an additional 24 hours).

3.2.2 Initial sorting and preservation of samples

3.2.2.1 Scraped samples

Samples of scraped reef material were placed in white trays filled with clean seawater for sorting. Algae and encrusting fauna were shaken to dislodge any hidden fauna, such as amphipods or polychaetes. Barnacles were extracted from their carapaces, and sedentary polychaetes were extracted from their tubes. All individuals were rinsed in Milli-Q water to remove sediments and other debris, before being individually wrapped in metal foil and frozen at -20°C. During 2007 sampling, attempts were made to identify species as much as possible prior to freezing, but the volume of material collected did not allow time for detailed identification. During 2008, however, a camera-equipped binocular microscope was available, and individuals were usually photographed for later identification.

3.2.2.2 Gastropods

All gastropods were measured using callipers, recording their maximum linear dimension. Individuals were extracted from their shells, rinsed in distilled water, wrapped in metal foil, and frozen at -20°C.

Table 3.1 Biometric data recorded for sampled organisms from artificial and natural reef sites.

Taxon	Measures recorded	Units
Fish	Total length	mm
	Standard length	mm
	Wet mass (whole)	g
	Wet mass (eviscerated)	g
	Wet mass (liver)	g
Brachyura (*Necora puber*) (*Cancer pagurus*)	Carapace width	mm
	Wet mass	g
Anomura (Paguroidea)	Dried mass (whole, removed from shell)	g
Gastropoda	Maximum linear dimension (in shell)	mm
	Dried mass (shell removed)	g
Bivalvia	Maximum linear dimension (in shell)	mm
	Dried mass (in shell)	g
Amphipoda	Dried mass (whole)	g

3.2.2.3 Large crustacea (Crabs, hermit crabs)

All large crustaceans were photographed, measured (measures recorded in Table 3.1), dabbed dry and weighed whole (wet weight). One leg was removed from each individual, from which a sample of muscle tissue was dissected, ensuring it was free of shell fragments. Muscle samples were then wrapped in metal foil and frozen at -20°C. Hermit crabs were removed from their gastropod shells, briefly rinsed in Milli-Q water, then wrapped in metal foil and frozen whole.

3.2.2.4 Fish

All fish were photographed, measured, and wet weighed (Table 3.1). All viscera were removed, and eviscerated weight was recorded. Livers of individual fish were separately weighed. A sample of white muscle tissue was then dissected out of the dorsal musculature, ensuring that it was free from bones and scales. These muscle samples were wrapped in foil and frozen.

3.2.2.5 Other taxa

Any other sampled taxa were rinsed in Milli-Q water, wrapped in foil and frozen at this stage, and measured as described in Table 3.1.

3.2.3 Sample preparation and pre-treatment

All frozen samples were lyophilised (freeze-dried) for at least 24 hours until completely dry (this was usually sufficient for complete drying of small samples, but larger samples sometimes required longer periods). After freeze-drying, samples were weighed and stored in sealed glass vials. Samples were then further treated depending on the nature of the sample.

Table 3.2 Preparation and pre-treatment of samples for stable isotope analysis, detailing what tissues were sampled for analysis (or if whole animals were used), what body parts were removed when whole animals were sampled, and whether or not samples were acidified in order to remove carbonates.

Taxon	Sample	Parts removed?	Acid treatment?
Fish	Dorsal white muscle	n/a	No
Ascidians	Whole animal	None	No
Polychaetes	Whole animal	Feeding tentacles, tubes	No
Gastropods	Foot muscle	n/a	No
Paguroidea	Whole animal	None	Yes
Large crustacea	Leg muscle	n/a	No
Ostrea edulis	Adductor muscle	n/a	No
Barnacles	Whole	Feeding appendages, shell	No
Amphipods	Whole	None	No

3.2.3.1 Fish

Samples of dried fish muscle were ground into a homogenous powder (using a ball mill) without further treatment. Fish white muscle tissue is low in lipids and does not contain significant carbonate, and consequently lipid and carbonate removal is unnecessary for this sample type (Bunn et al., 1995; Soreide et al., 2006).

3.2.3.2 Large crustacea (Crabs, hermit crabs)

Where muscle samples had been dissected out, these were simply ground into a homogenous powder (using a mortar and pestle) once dry. Crustacean muscle tissue is regarded as being low in lipids, and therefore lipid removal is unnecessary (Bodin et al., 2007). Similarly, sampling of muscle avoids the inclusion of carbonates, obviating the need for acid treatment.

Hermit crabs (Paguroidea) were generally too small for dissection of muscle samples to be practical. Therefore whole dried individuals were ground into

homogenous powder, which was acid treated to remove carbonates. Acid treatment of hermit crab samples is considered in detail in Chapter 5. However, for this part of the investigation the 'Acidification with Rinsing' treatment was used. This consisted of placing a small amount of sample material in a centrifuge tube, acidifying it with 1M HCl until effervescence ceased, spinning the tube in a centrifuge at 5000 rpm for 5 minutes, and discarding the supernatant. Milli-Q water was then added to the tube, which was agitated for 30 seconds, and spun in the centrifuge for another 5 minutes at 5000rpm, before discarding the water. This rinsing treatment was repeated twice more, by which time enough acid had been removed for the sample to register neutral pH (verified by testing with pH indicator paper). In order to obtain accurate results for $\delta^{15}N$, untreated material for each individual was also analysed.

3.2.3.3 Amphipoda

Amphipods were ground to produce a homogenous powder, although some individuals were a suitable mass to be analysed whole. Where individuals were too small, several were pooled in order to generate large enough samples.

3.2.3.4 Cirripedia

Individual barnacles were removed from their carapace, separated from their feeding appendages, and were otherwise untreated. Larger barnacles were ground into homogenous powder, but this was unnecessary for some individuals which were already of an appropriate mass for analysis.

3.2.3.5 Gastropods

A portion of foot muscle was removed from each individual dried gastropod, with care taken to avoid including fragments of the shell or the operculum. Muscle samples were ground to homogenous powder and otherwise untreated.

3.2.3.6 Bivalves (*Ostrea edulis*)

After freeze-drying, samples of adductor muscle tissue were removed and ground to homogenous powder. No other treatments were carried out; adductor muscle is

not significantly affected by lipid removal (Yokoyama et al., 2008) and inclusion of carbonate was avoided.

3.2.3.7 Polychaetes

Generally, polychaetes were analysed without any treatment other than grinding, after removal of any feeding appendages or remaining fragments of tube material.

3.2.4 Stable isotope ratio mass spectrometry procedure

All isotope analysis was carried out at the Natural Environment Research Council Life Sciences Mass Spectrometry Facility (East Kilbride node) at the Scottish Universities Environmental Research Centre. Carbon and Nitrogen isotope ratios were measured simultaneously by continuous-flow isotope ratio mass spectrometry (CF-IRMS) using a Costech (Milan, Italy) ECS elemental analyser coupled to a Thermo-Finnigan (Bremen, Germany) Delta Plus XP mass spectrometer. Subsamples of 600 to 800 µg of material from each dried and ground sample were weighed into tin capsules using a Mettler Toledo MX5 microbalance, and were then placed into an automated carousel. After every 10 samples, a pair of standards were analysed; either two gelatine standards or a pair of alanine standards (one ^{15}N labelled). This allowed the data to be corrected for any drift which may have occurred over time in the readings of the mass spectrometer. In addition, at the beginning of each sample run, a number of gelatine standards of different masses were analysed in order to allow correction for 'linearity' – changing mass spectrometer readings with sample weight.

Stable carbon and nitrogen isotope ratios are reported using δ notation, which represents the deviation in parts per thousand (‰) of the sample isotope ratio from that of defined international standards, Vienna Pee Dee Belemnite (for carbon) and atmospheric air (for nitrogen). This figure is calculated using the following equation:

$$\delta = [(R_{SAMPLE} / R_{STANDARD}) - 1] * 1000$$

where *R* is the measured isotope ratio (in this case either $^{13}C/^{12}C$ or $^{15}N/^{14}N$) in the sample or the appropriate international standard.

3.2.5 Data Analysis

Statistical analyses were carried out using SPSS and SigmaStat. Mean stable isotope ratios for taxa were compared between sites and sampling years using t-tests and ANOVA procedures where data conformed to the assumptions of normality and homogeneity of variance. Where they did not, and where data transformation did not produce normal distribution, non-parametric equivalents (Mann-Whitney U and Kruskal-Wallis tests) were used. Correspondence to normal distribution was tested using the Kolmogorov-Smirnov and/or Shapiro-Wilk tests, and homogeneity of variances was tested using Levene's test. Given the variation in testing across taxa because of data considerations (number of factors, number of factor levels) the specific tests used are described in the results section for each taxon in turn.

3.3 Results

3.3.1 Large Crustacea

3.3.1.1 Paguroidea

In total, 41 hermit crabs from the two reef sites over both sampling years were analysed to determine their carbon and nitrogen stable isotope ratios (Table 3.3). A larger number (19) from the natural reef in 2008 were analysed (relative to other site/year combinations), which allowed the investigation of potential effects of individual size on $\delta^{13}C$ and $\delta^{15}N$ independent of any year and site effects. $\delta^{15}N$ and dry mass data were not normally distributed ($p < 0.05$, Table 3.3) and were therefore $\log_{(e)}$ transformed (transformed data were normally distributed; $p > 0.05$, Table 3.3).

There was no significant correlation between $\delta^{13}C$ and log-transformed dry mass (Pearson's Correlation coefficient = -0.123, d.f. = 19, $p > 0.05$). Log-transformed dry mass and log-transformed $\delta^{15}N$ were, however, significantly correlated (Pearson's Correlation coefficient = 0.588, d.f. = 19, $p < 0.01$; Fig. 3.1). While the correlation was statistically significant, the r^2 value was 0.346, indicating that the relationship between size and $\delta^{15}N$ was not particularly strong, accounting for approximately only one third of the variation in $\delta^{15}N$.

Table 3.3 $\delta^{13}C$, $\delta^{15}N$ and dried mass data for hermit crabs (family Paguroidea) collected from both reef sites in 2007 and 2008. Departure from normal distribution for raw and $log_{(e)}$ transformed data tested using Shapiro-Wilk test; *$p < 0.05$, ns = Not Significant ($p > 0.05$).

						Shapiro-Wilk W	
			Mean	S.E.	n	Raw	$Log_{(e)}$
$\delta^{13}C$ (‰)	2007	Artificial Reef	-20.80	0.27	9	0.936 ns	-
		Natural Reef	-20.42	0.38	9	0.933 ns	-
	2008	Artificial Reef	-19.72	0.15	4	0.892 ns	-
		Natural Reef	-20.47	0.23	19	0.905 ns	-
$\delta^{15}N$ (‰)	2007	Artificial Reef	10.43	0.11	9	0.896 ns	0.901 ns
		Natural Reef	10.08	0.11	9	0.913 ns	0.904 ns
	2008	Artificial Reef	9.92	0.11	4	0.978 ns	0.980 ns
		Natural Reef	10.58	0.13	19	0.899*	0.908 ns
Mass (g)	2007	Artificial Reef	0.161	0.025	9	0.908 ns	0.910 ns
		Natural Reef	0.102	0.023	9	0.914 ns	0.944 ns
	2008	Artificial Reef	0.092	0.066	4	0.727*	0.915 ns
		Natural Reef	0.098	0.014	19	0.866*	0.915 ns

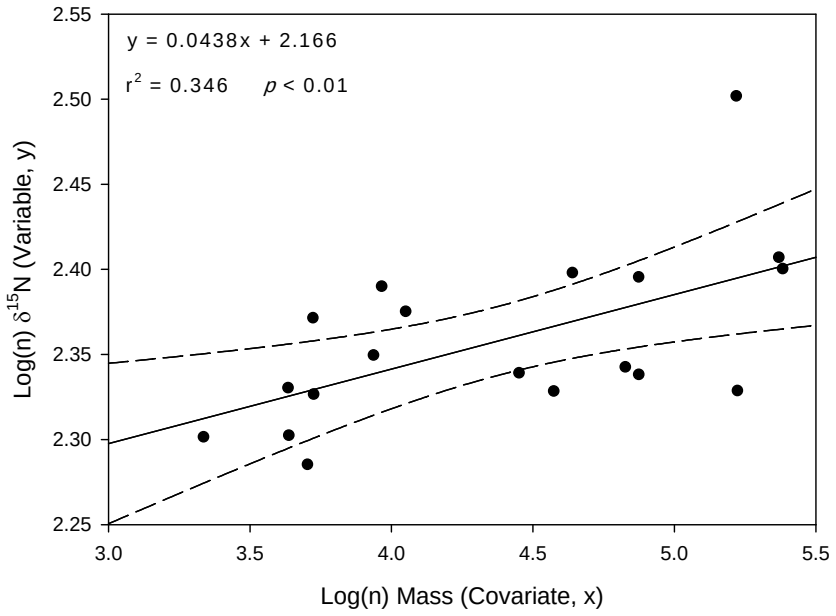


Figure 3.1 Relationship between log-transformed mass (g) and log-transformed $\delta^{15}N$ data for hermit crabs (family Paguroidea) collected on the natural reef site in 2008. Dashed lines are 95% confidence intervals of the regression line. The relationship is statistically significant (Pearson's Correlation coefficient = 0.588, d.f. = 19, $p < 0.01$).

Given the existence of a significant relationship between mass and $\delta^{15}N$ for this taxon at this site in 2008, mean $\delta^{15}N$ values for hermit crabs from both sites in both years were compared using a two-way ANOVA procedure with site and year as the factors and mass as a covariate (ANCOVA). Despite the absence of a clear correlation between mass and $\delta^{13}C$, mass was also included as a covariate for a

similar procedure testing for significant variation in $\delta^{13}C$. Mass and $\delta^{15}N$ data were $\log_{(e)}$ transformed to provide normally distributed data, but transformation of $\delta^{13}C$ data was not necessary (Table 3.3).

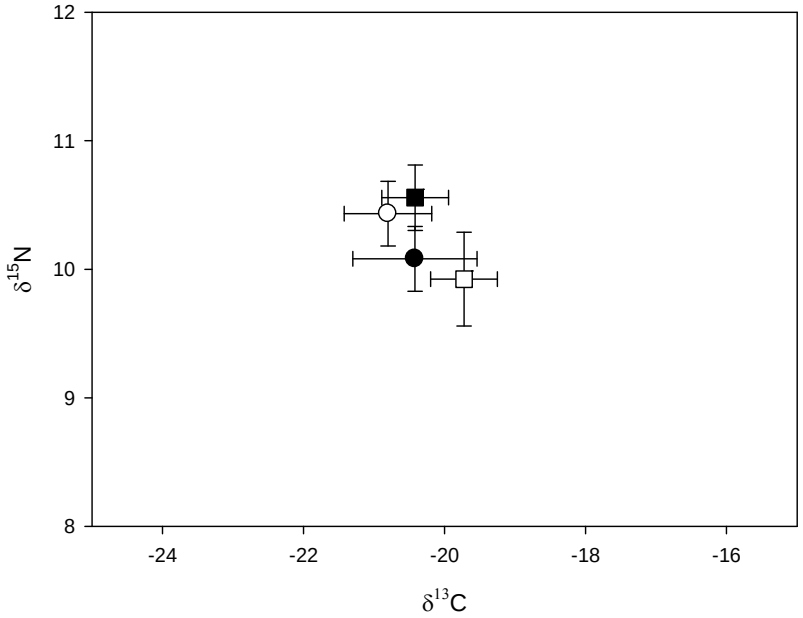


Figure 3.2 $\delta^{13}C$ and $\delta^{15}N$ (‰) for hermit crabs (Paguroidea) collected from natural and artificial reef sites in 2007 and 2008. Black symbols: natural reef, white symbols: artificial reef, circles: 2007 samples, squares: 2008 samples. Error bars are ±95% confidence intervals.

Mean $\delta^{13}C$ did not vary significantly between artificial and natural reef sites, nor between samples collected in 2007 and 2008 (Year and Site are not significant contributors to the ANOVA model; Table 3.4, Fig. 3.2). In agreement with the correlation analysis carried out on 2008 natural reef samples, individual mass was also not found to have a significant influence on $\delta^{13}C$ (Table 3.4).

Table 3.4 Two-way ANCOVA of hermit crab $\delta^{13}C$ between natural and artificial reef sites across both sampling years, with $\log_{(e)}$ mass as a covariate. ns = Not Significant ($p > 0.05$).

	df	Mean Square	F
Model	5	3432.508	3586.885
$\log_{(e)}$ mass	1	0.003	0.003 ns
Year	1	1.819	1.901 ns
Site	1	0.251	0.263 ns
Year * Site	1	2.175	2.273 ns
Error	36	0.957	

$\delta^{15}N$ was influenced by the mass of individuals; $\log_{(e)}$-transformed mass was a significant term in the ANCOVA model ($p < 0.01$, Table 3.5). However, while its

influence was *statistically* significant, the contribution of mass to the model was relatively small. Site and year had no significant effect on $\delta^{15}N$ ($p > 0.05$, Table 3.5), but the Site*Year interaction term was significant, indicating that the effect of site varied between the two sampling years. However, the contribution to the model of this factor was again extremely small, despite being statistically significant. The profile plot for the model (Fig. 3.3) shows the small magnitude of the effect: the largest difference between marginal means was only 0.5‰ (natural reef 2007 vs. 2008 samples), and the difference between the two years on the artificial reef was only 0.16‰.

Table 3.5 Two-way ANCOVA comparison of hermit crab $\delta^{15}N$ between natural and artificial reef sites across both sampling years, with $\log_{(e)}$ mass as a covariate. *$p < 0.05$, **$p < 0.01$, ns = Not Significant ($p > 0.05$)

	df	Mean Square	F
Model	5	44.846	33945.420
$\log_{(e)}$ mass	1	0.016	12.041**
Year	1	0.002	1.307 ns
Site	1	0.001	1.101 ns
Year * Site	1	0.007	5.166*
Error	36	0.957	

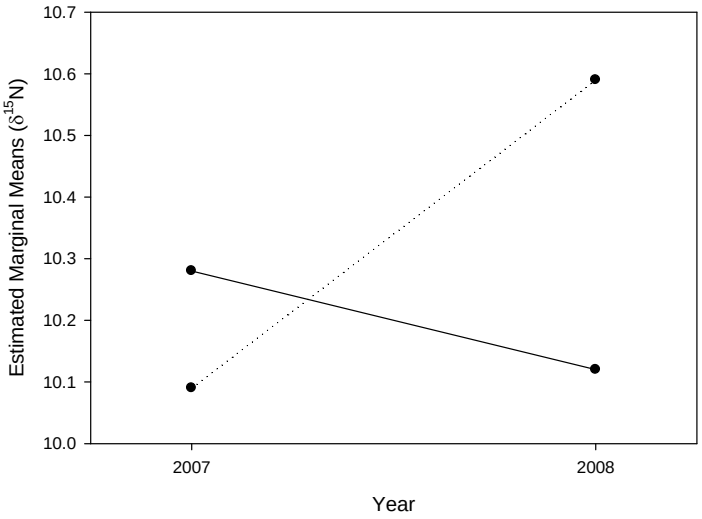


Figure 3.3 Profile plot for two-way ANCOVA examining the effects of year, site, and mass (g) on $\delta^{15}N$ (‰) of hermit crabs (Paguroidea). $\delta^{15}N$ values have been back-transformed from the $\log_{(e)}$-transformed data used during the analysis. Marginal means evaluated at $\log_{(e)}$mass = -2.4592, corresponding to a dry mass of 0.0855g. Solid line: artificial reef, dotted line: natural reef.

3.3.1.2 *Necora puber*

Three individuals of this species were collected from each reef in 2007, but in 2008 only two were obtained, both from the artificial reef (Table 3.6). No significant variation (Fig 3.4) was detected among these groups (2007 artificial reef, 2007 natural reef, 2008 artificial reef), in either mean $\delta^{13}C$ (Kruskal-Wallis, $H = 1.139$, d.f. = 2, $p > 0.05$) or mean $\delta^{15}N$ (Kruskal-Wallis, $H = 0.472$, d.f. = 2, $p > 0.05$).

Table 3.6 $\delta^{13}C$ and $\delta^{15}N$ data (‰) for *Necora puber* collected from both reef sites in 2007 and 2008. Departure from normal distribution tested using Shapiro-Wilk test; ns = Not Significant ($p > 0.05$). Test not executed for 2008 artificial reef crabs, due to insufficient sample number.

			Mean	S.E.	n	Shapiro-Wilk W
$\delta^{13}C$	2007	Artificial Reef	-17.45	0.37	3	0.988 ns
		Natural Reef	-17.16	0.14	3	0.982 ns
	2008	Artificial Reef	-17.43	0.20	2	n/a
$\delta^{15}N$	2007	Artificial Reef	12.59	0.42	3	1.000 ns
		Natural Reef	12.70	0.58	3	0.991 ns
	2008	Artificial Reef	13.24	0.01	2	n/a

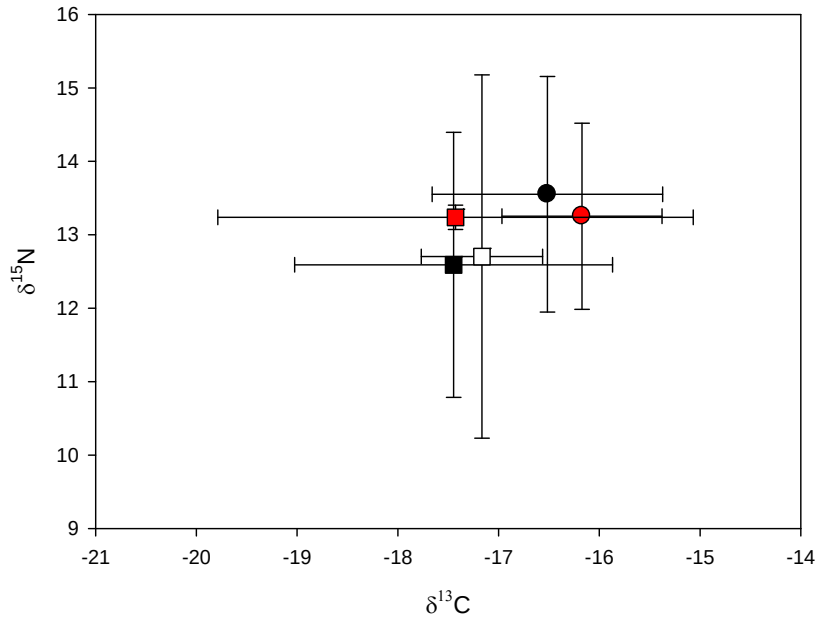


Figure 3.4 $\delta^{13}C$ and $\delta^{15}N$ (‰) for *Necora puber* and *Cancer pagurus* collected from natural and artificial reef sites in 2007 and 2008. Circles are *C. pagurus,* square symbols are *N. puber.* Black symbols are 2007 artificial reef samples, white symbols are 2007 natural reef, and red symbols are 2008 artificial reef. Error bars are ±95% confidence intervals

3.3.1.3 *Cancer pagurus*

A total of 7 individual crabs were collected, 4 in 2007 and 3 in 2008, all from the artificial reef site (Table 3.7). No significant difference was found between the mean $\delta^{13}C$ (t-test, t = -0.759, d.f. = 5, $p > 0.05$) or the mean $\delta^{15}N$ (t-test, t = 0.465, d.f. = 5, $p > 0.05$) of individuals collected in 2007 and 2008 (Fig 3.4).

Table 3.7 $\delta^{13}C$ and $\delta^{15}N$ data (‰) for *Cancer pagurus* collected from the artificial reef. Departure from normal distribution tested using Shapiro-Wilk test; ns = Not Significant ($p > 0.05$).

		Mean	S.E.	n	Shapiro-Wilk W
$\delta^{13}C$	2007	-16.51	0.36	4	0.925 ns
	2008	-16.17	0.19	3	1.000 ns
$\delta^{15}N$	2007	13.55	0.50	4	0.954 ns
	2008	13.25	0.30	3	0.972 ns

3.3.2 Gastropoda

3.3.2.1 *Crepidula fornicata*

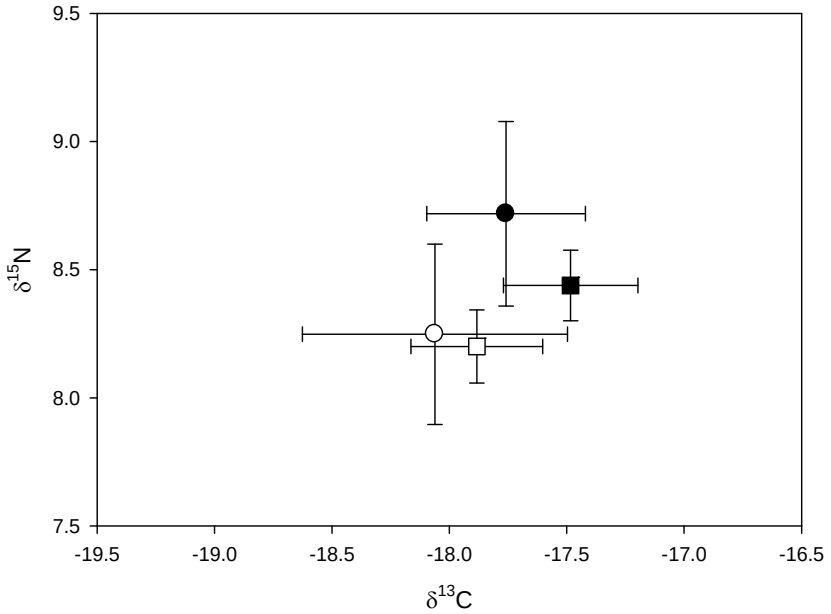


Figure 3.5 $\delta^{13}C$ and $\delta^{15}N$ (‰) for *Crepidula fornicata* collected from natural and artificial reef sites in 2007 and 2008. Black symbols: natural reef, white symbols: artificial reef, circles: 2007 samples, squares: 2008 samples. Error bars are ±95% confidence intervals.

A total of 38 individuals were collected from the two reef sites, and all data ($\delta^{13}C$, $\delta^{15}N$ or mass) were normally distributed for both sites in 2007 and 2008 (Table 3.8). Using pooled data from both sites in both years, a significant correlation was found between $\delta^{13}C$ and mass (g) of individual *C. fornicata* (Pearson's Correlation Coefficient = 0.496, n = 36, $p < 0.01$; Fig. 3.6), but there was no correlation between mass (g) and $\delta^{15}N$ (Pearson's Correlation Coefficient = -0.039, n = 36, $p > 0.05$).

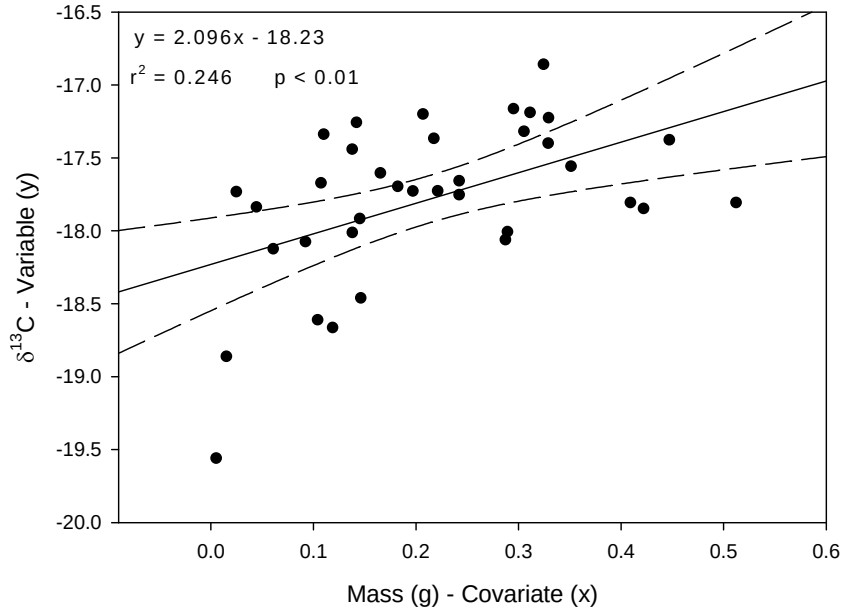


Figure 3.6 Relationship between mass (g) and $\delta^{13}C$ (‰) data for all *C. fornicata*. Dashed lines are 95% confidence intervals of the regression line. The relationship is statistically significant (Pearson's Correlation coefficient = 0.496, n = 36, $p < 0.01$).

Table 3.8 $\delta^{13}C$ and $\delta^{15}N$ data (‰) for *C. fornicata* collected from both sites in 2007 and 2008. Departure from normal distribution tested using Shapiro-Wilk test; ns = Not Significant ($p > 0.05$).

			Mean	S.E.	n	Shapiro-Wilk W
$\delta^{13}C$	2007	Artificial Reef	-18.06	0.24	9	0.899 ns
		Natural Reef	-17.75	0.15	9	0.920 ns
	2008	Artificial Reef	-17.88	0.12	10	0.892 ns
		Natural Reef	-17.48	0.13	10	0.916 ns
$\delta^{15}N$	2007	Artificial Reef	8.25	0.15	9	0.945 ns
		Natural Reef	8.72	0.16	9	0.901 ns
	2008	Artificial Reef	8.20	0.06	10	0.914 ns
		Natural Reef	8.44	0.06	10	0.959 ns
Mass (g)	2007	Artificial Reef	0.081	0.028	7	0.912 ns
		Natural Reef	0.159	0.023	9	0.981 ns
	2008	Artificial Reef	0.272	0.047	7	0.926 ns
		Natural Reef	0.299	0.023	9	0.884 ns

Mean $\delta^{13}C$ and $\delta^{15}N$ were compared between sites in both years using a two-way ANOVA procedure with mass as a covariate (ANCOVA).There was no significant difference between the mean $\delta^{13}C$ of *C. fornicata* from different sites or sampled in different years; both year and site were not significant terms in the model ($p > 0.05$; Table 3.9). Mass had a small but statistically significant effect on $\delta^{13}C$ ($p < 0.05$) but no effect on $\delta^{15}N$ ($p > 0.05$; Table 3.10), in agreement with the results of the correlation analyses above.

Table 3.9 Two-way ANCOVA analysis of $\delta^{13}C$ for *C. fornicata* from natural and artificial reef sites across both sampling years, with mass as a covariate. *$p < 0.05$, ns = Not Significant ($p > 0.05$)

	df	Mean Square	F
Model	5	2277.082	10169.482
mass	1	1.702	7.601*
Year	1	0.099	0.441 ns
Site	1	0.482	2.154 ns
Year * Site	1	0.074	0.332 ns
Error	31	0.224	

Both year and site had a statistically significant effect on $\delta^{15}N$ ($p < 0.05$; Table 3.10), while mass did not ($p > 0.05$). The effects of site and year were independent (Year*Site interaction term not significant; $p > 0.05$; Table 3.10), with lower $\delta^{15}N$ in 2008 on both sites, and lower $\delta^{15}N$ on the artificial reef in both years (Fig. 3.7). Both factors made only very small contributions to the model, indicating that their effect was relatively small. The effect of year was only 0.35‰ on the natural reef and 0.28‰ on the artificial reef, while the site effect was 0.29‰ in 2007 and 0.23‰ in 2008 (at mass = 0.214118g).

Table 3.10 Two-way ANCOVA comparison of $\delta^{15}N$ for *C. fornicata* from natural and artificial reef sites across both sampling years, with mass as a covariate. *$p < 0.05$, ns = Not Significant ($p > 0.05$)

	df	Mean Square	F
Model	5	512.215	4664.248
mass	1	0.082	0.745 ns
Year	1	0.504	4.591*
Site	1	0.545	4.961*
Year * Site	1	0.010	0.765 ns
Error	31		

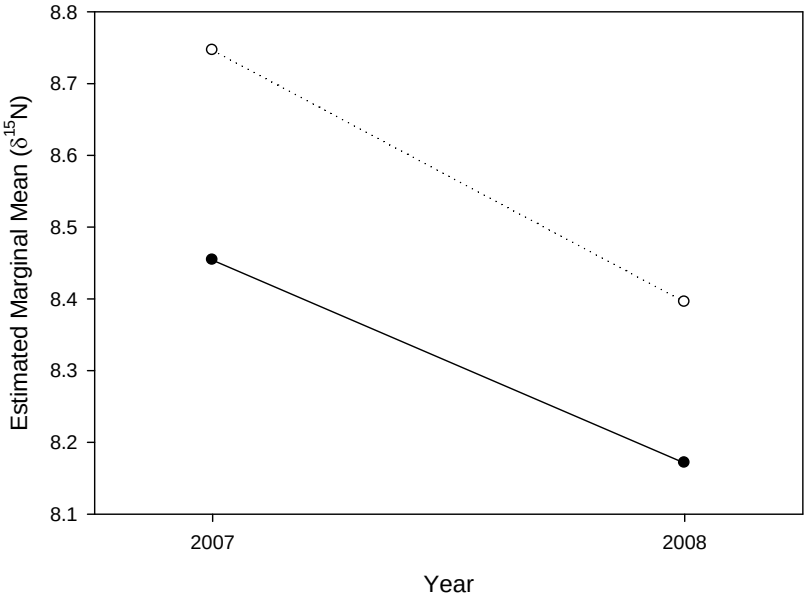


Figure 3.7 Profile plot for two-way ANCOVA examining the effects of year, site, and mass on $\delta^{15}N$ (‰) in *C. fornicata*. Marginal means evaluated at dry mass = 0.214118g. Solid line: artificial reef, dotted line: natural reef.

Table 3.11 $\delta^{13}C$ and $\delta^{15}N$ data (‰) for gastropods, excluding *C. fornicata*, collected from the artificial and natural reefs. Departure from normal distribution tested using Shapiro-Wilk test; ns = Not Significant ($p > 0.05$).

				Mean	S.E.	n	Shapiro-Wilk W
Gibbula cineraria	2008	$\delta^{13}C$	Artificial Reef	-19.91	0.23	5	0.849 ns
			Natural Reef	-20.04	0.28	2	n/a
		$\delta^{15}N$	Artificial Reef	10.77	0.37	5	0.975 ns
			Natural Reef	9.59	0.04	2	n/a
Buccinum undatum	2008	$\delta^{13}C$	Artificial Reef	-16.52	0.20	3	0.991 ns
			Natural Reef	-16.83	0.10	3	0.954 ns
		$\delta^{15}N$	Artificial Reef	12.76	0.66	3	0.960 ns
			Natural Reef	12.31	0.19	3	0.878 ns
Ocenebra erinacea	2007	$\delta^{13}C$	Artificial Reef	-16.53	0.04	2	n/a
			Natural Reef	-15.66	0.14	7	0.900 ns
		$\delta^{15}N$	Artificial Reef	11.14	0.20	2	n/a
			Natural Reef	11.74	0.24	7	0.951 ns
	2008	$\delta^{13}C$	Artificial Reef	-15.35	0.15	7	0.987 ns
			Natural Reef	-15.78	n/a	1	n/a
		$\delta^{15}N$	Artificial Reef	12.57	0.18	7	0.914 ns
			Natural Reef	11.28	n/a	1	n/a

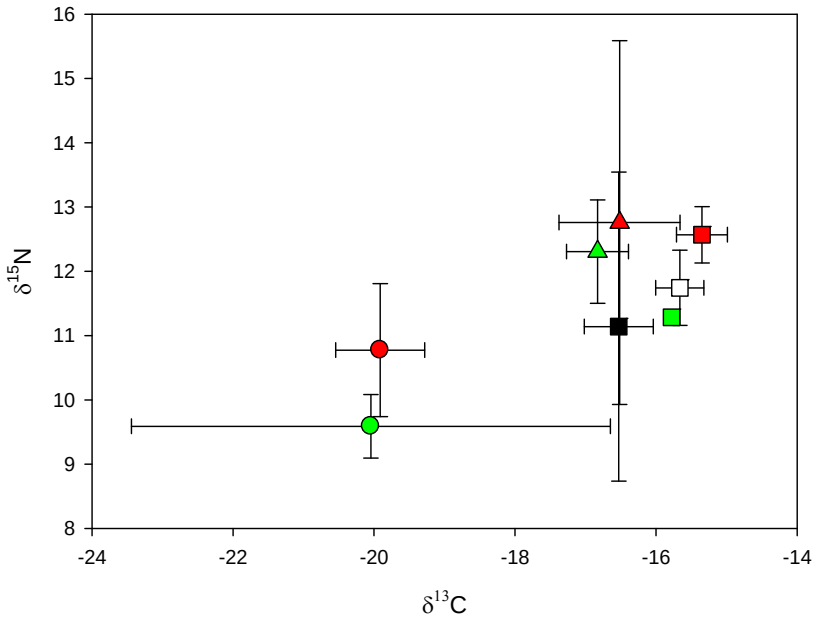


Figure 3.8 $\delta^{13}C$ and $\delta^{15}N$ (‰) for other gastropods collected from natural and artificial reef sites in 2007 and 2008. Circles: *Gibbula cineraria*, squares: *Ocenebra erinacea*, triangles: *Buccinum undatum*. Black: artificial reef 2007, white: natural reef 2007, red: artificial reef 2008, green: natural reef 2008. Error bars are ±95% confidence intervals.

3.3.2.2 *Gibbula cineraria*

This species was only collected in 2008, and was only obtained from each site in small numbers (Table 3.11). No significant differences were found between the two sites in either the mean $\delta^{13}C$ (Mann-Whitney test; z = -0.387, $p > 0.05$) or the mean $\delta^{15}N$ (Mann-Whitney test; z = -1.936, $p > 0.05$).

3.3.2.3 *Buccinum undatum*

Individual *Buccinum undatum* were only collected from the two reef sites in 2008 (Table 3.11). No significant differences were found between the two sites in either the mean $\delta^{13}C$ (t-test; t = 1.394, d.f. = 4, $p > 0.05$) or the mean $\delta^{15}N$ (t-test; t = 0.659, d.f. = 4, $p > 0.05$).

3.3.2.4 *Ocenebra erinacea*

Ocenebra erinacea were collected from both sites in both years, although only two were obtained from the artificial reef in 2007 and only one from the natural reef in 2008 (Table 3.11).

Table 3.12 (a) Two-way ANOVA comparison of $\delta^{13}C$ for *Ocenebra erinacea* from natural and artificial reef sites across both sampling years. *$p < 0.05$, **$p < 0.01$, ns = Not Significant ($p > 0.05$)

	df	Mean Square	F
Model	4	1040.480	7786.012
Year	1	0.632	4.728 *
Site	1	0.106	0.790 ns
Year * Site	1	0.942	7.053 *
Error	13	0.134	

(b) Pairwise comparisons

Comparison	Difference of means	Test Statistics (p,q)
Site within 2007: Artificial vs. Natural reef	0.866	2, 4.177 *
Site within 2008: Artificial vs. Natural reef	0.431	2, 1.561 ns
Year within Artificial Reef: 2007 vs. 2008	1.180	2, 5.692 **
Year within Natural Reef: 2007 vs. 2008	0.118	2,0.425 ns

There was a marginally significant ($p = 0.049$) effect of year on $\delta^{13}C$ and a significant site*year interaction (Table 3.12). Inspecting the mean $\delta^{13}C$ values for each site/year combination (Table 3.11), the values for the natural reef in both years and the artificial reef in 2008 were all similar (ranging from -15.78 to -15.35: a small difference), while the mean $\delta^{13}C$ for this species on the artificial reef in 2007 was clearly more different. *Post hoc* multiple comparisons (Tukey's test) confirm that the 2007 artificial reef samples were significantly different ($p < 0.05$) from the other site/year combinations, among which there was no further significant variation (Table 3.12).

With regard to mean $\delta^{15}N$, neither year nor site appeared to have a significant effect, however there was a significant year*site interaction (Table 3.13). Inspecting the mean values for each site/year combination (Table 3.11) the values for the natural reef in both years were not much separated (only 0.46‰ different), and the value for the artificial reef in 2007 was only a further 0.14‰ different. However, samples from the artificial reef in 2008 showed a higher value. *Post hoc* multiple comparisons (Tukey's test) confirm that the 2008 artificial reef samples were significantly different ($p < 0.05$) from the other site/year combinations, among which there was no further significant variation (Table 3.13).

Table 3.13 (a)Two-way ANOVA comparison of $\delta^{15}N$ for *Ocenebra erinacea* from natural and artificial reef sites across both sampling years. *$p < 0.05$, **$p < 0.01$, ns = Not Significant ($p > 0.05$)

	df	Mean Square	F
Model	4	611.711	2073.184
Year	1	0.525	1.780 ns
Site	1	0.261	0.885 ns
Year * Site	1	1.998	6.773 *
Error	13	0.295	

(b) Pairwise comparisons

Comparison	Difference of means	Test Statistics (p,q)
Site within 2007: Artificial vs. Natural reef	0.603	2, 1.598 ns
Site within 2008: Artificial vs. Natural reef	1.286	2, 3.132 *
Year within Artificial Reef: 2007 vs. 2008	1.429	2, 4.639 **
Year within Natural Reef: 2007 vs. 2008	0.460	2, 1.121 ns

3.3.3 Cirripedia

Barnacles (*Balanus spp.*) were collected from both reefs in both years (Table 3.14; Fig. 3.9). No significant differences were detected between sites or years for mean $\delta^{13}C$ (Table 3.15) or mean $\delta^{15}N$ (Table 3.15).

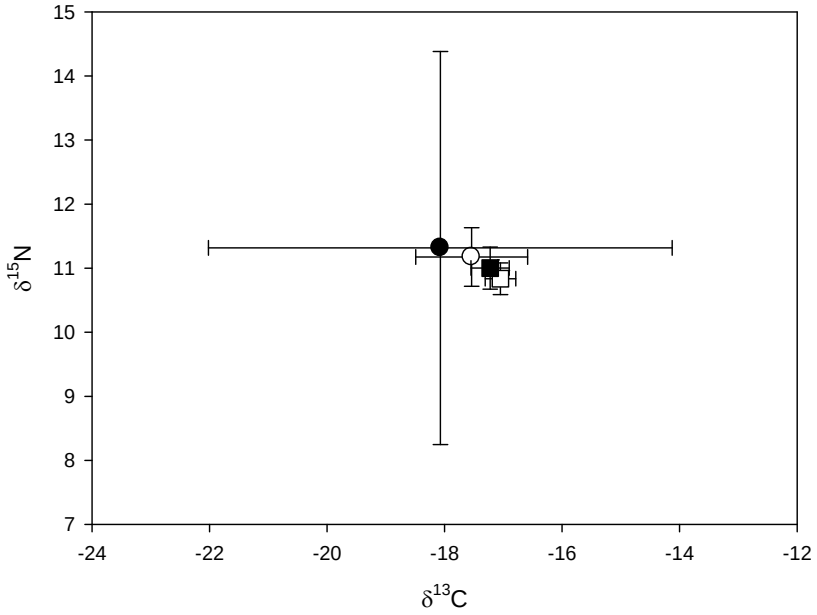


Figure 3.9 $\delta^{13}C$ and $\delta^{15}N$ (‰) for barnacles (*Balanus* spp.) collected from natural and artificial reef sites in 2007 and 2008. Black symbols: natural reef, white symbols: artificial reef, circles: 2007 samples, squares: 2008 samples. Error bars are ±95% confidence intervals.

Table 3.14 $\delta^{13}C$ and $\delta^{15}N$ data (‰) for barnacles (*Balanus spp.*) collected from both reef sites in 2007 and 2008. Departure from normal distribution tested using Shapiro-Wilk test; ns = Not Significant ($p > 0.05$).

			Mean	S.E.	n	Shapiro-Wilk W
$\delta^{13}C$	2007	Artificial Reef	-17.54	0.41	9	0.978 ns
		Natural Reef	-18.07	0.92	3	0.909 ns
	2008	Artificial Reef	-17.05	0.11	10	0.962 ns
		Natural Reef	-17.22	0.15	11	0.933 ns
$\delta^{15}N$	2007	Artificial Reef	11.17	0.20	9	0.933 ns
		Natural Reef	11.32	0.71	3	0.898 ns
	2008	Artificial Reef	10.83	0.11	10	0.972 ns
		Natural Reef	11.00	0.15	11	0.955 ns

Table 3.15 Two-way ANOVA comparison of $\delta^{13}C$ for barnacles (*Balanus* spp.) from natural and artificial reef sites across both sampling years. ns = Not Significant ($p > 0.05$)

	df	Mean Square	F
Model	4	2479.482	3442.521
Year	1	2.810	3.901 ns
Site	1	0.793	1.101 ns
Year * Site	1	0.200	0.278 ns
Error	29	0.720	

Table 3.16 Two-way ANOVA comparison of $\delta^{15}N$ for barnacles (*Balanus* spp.) from natural and artificial reef sites across both sampling years. *$p < 0.05$, ns = Not Significant ($p > 0.05$)

	df	Mean Square	F
Model	4	1003.298	3110.520
Year	1	0.670	2.077 ns
Site	1	0.149	0.461 ns
Year * Site	1	0.001	0.003 ns
Error	29	0.323	

3.3.4 Bivalves – *Ostrea edulis*

Oysters (*Ostrea edulis*) were collected from both sites in 2007, but only one oyster was analysed, from the natural reef site, in 2008 (Table 3.17; Fig. 3.10). Therefore only samples from 2007 were compared between sites; no significant differences between sites in mean $\delta^{13}C$ (t-test; t = -0.981, d.f. = 6, $p > 0.05$) or mean $\delta^{15}N$ (t = 1.455, df = 3.978, $p > 0.05$) were detected. The one oyster analysed from the 2008

samples had carbon and nitrogen stable isotope values very close to the mean values for those sampled in 2007 (Table 3.17; Fig. 3.10).

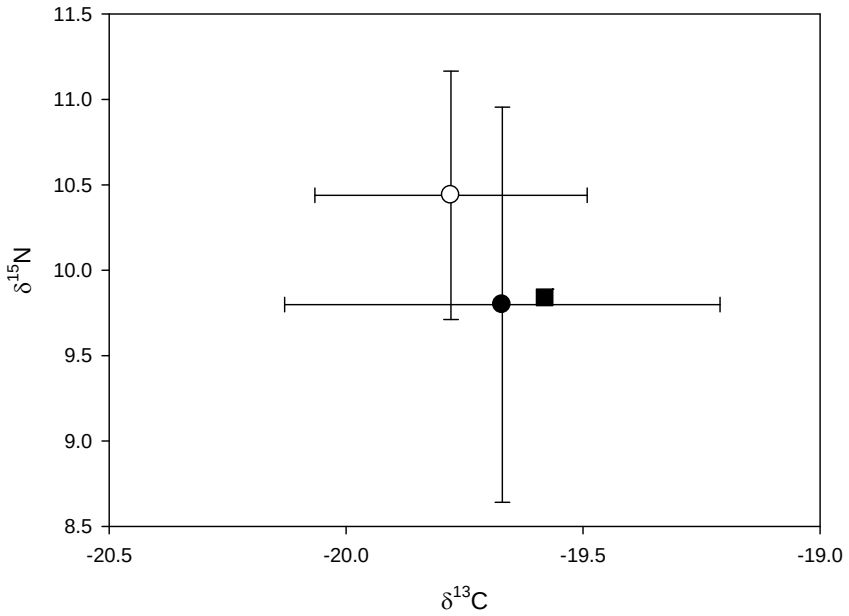


Figure 3.10 $\delta^{13}C$ and $\delta^{15}N$ (‰) for *Ostrea edulis* collected from natural and artificial reef sites in 2007 and 2008. Black symbols: natural reef, white symbols: artificial reef, circles: 2007 samples, squares: 2008 samples. Error bars are ±95% confidence intervals.

Table 3.17 $\delta^{13}C$ and $\delta^{15}N$ data (‰) for *Ostrea edulis* collected from both reef sites in 2007 and 2008. Departure from normal distribution tested using Shapiro-Wilk test; ns = Not Significant ($p > 0.05$).

			Mean	S.E.	n	Shapiro-Wilk W
$\delta^{13}C$	2007	Artificial Reef	-19.78	0.09	4	0.972 ns
		Natural Reef	-19.61	0.14	4	0.986 ns
	2008	Natural Reef	-19.58	n/a	1	n/a
$\delta^{15}N$	2007	Artificial Reef	10.43	0.23	4	0.869 ns
		Natural Reef	10.08	0.09	4	0.874 ns
	2008	Natural Reef	9.84	n/a	1	n/a

3.3.5 Tunicates: *Styela clava*

Styela clava were collected from both reefs in 2007. However, there were large size discrepancies among those sampled from the natural reef site. Accurate mass data were not recorded, but 3 of the individuals were much larger than the others (small individuals all had dry weights ranging from 200 to 800mg). Therefore samples were split into 'Large' and 'Small' size groups. Large individuals (sampled on the natural reef) differed significantly (Fig. 3.11) from small samples (sampled on the artificial reef) in both mean $\delta^{13}C$ (t-test; t = -4.144, d.f. = 5, $p < 0.01$) and

mean $\delta^{15}N$ (t = 14.228, d.f. = 5, $p < 0.001$). The one small individual sampled on the natural reef had $\delta^{13}C$ and $\delta^{15}N$ values close to those of the small individuals collected from the artificial reef (Fig. 3.11, Table 3.18).

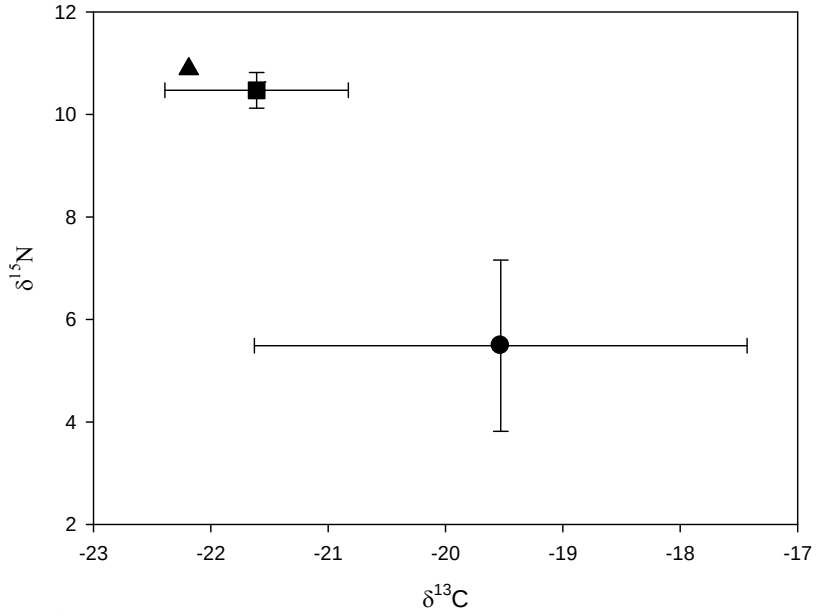


Figure 3.11 $\delta^{13}C$ and $\delta^{15}N$ (‰) for *Styela clava* collected from natural and artificial reef sites in 2007. Circle: large (natural reef), square: small (artificial reef), triangle: small (natural reef). Error bars are ±95% confidence intervals.

Table 3.18 $\delta^{13}C$ and $\delta^{15}N$ data (‰) for *Styela clava* collected from both reef sites in 2007. Departure from normal distribution tested using Shapiro-Wilk test; ns = Not Significant ($p > 0.05$).

			Mean	S.E.	n	Shapiro-Wilk W
$\delta^{13}C$	Small	Artificial Reef	-21.61	0.25	4	0.899 ns
		Natural Reef	-22.19	n/a	1	n/a
	Large	Natural Reef	-19.53	0.49	3	0.999 ns
$\delta^{15}N$	Small	Artificial Reef	10.47	0.11	4	0.875 ns
		Natural Reef	10.89	n/a	1	n/a
	Large	Natural Reef	5.49	0.39	3	1.000 ns

3.3.6 Polychaetes

3.3.6.1 *Platynereis* spp.

This taxon was sampled from both reefs in 2008 (Table 3.19, Fig 3.12). There was no significant difference in the mean $\delta^{13}C$ between reefs (t-test; t = 2.169, d.f. = 10, $p > 0.05$), but the mean $\delta^{15}N$ was higher on the natural reef (t = -2.485, d.f. = 10, $p < 0.05$) although only by 0.46‰.

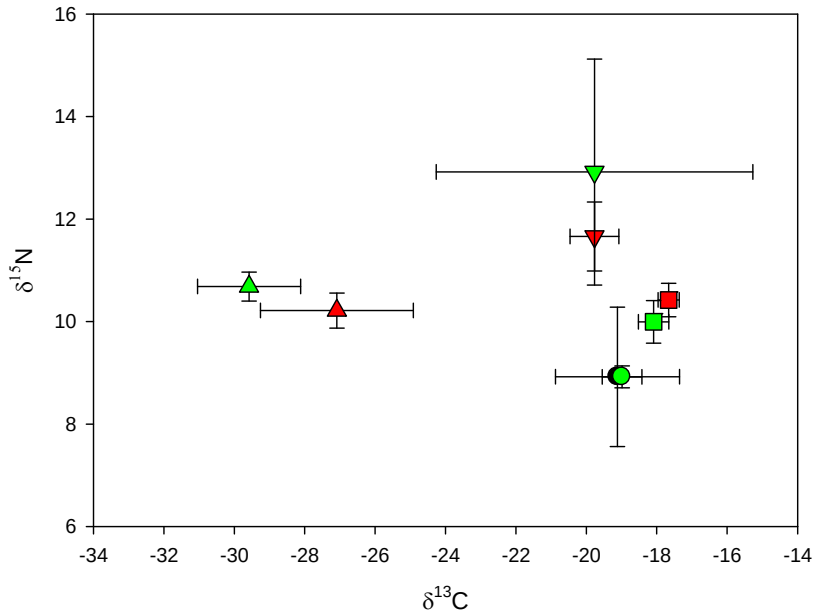


Figure 3.12 $\delta^{13}C$ and $\delta^{15}N$ (‰) for polychaetes collected from natural and artificial reef sites in 2007 and 2008. Circles: *Bispira volutacornis*, squares: *Sabellaria spinulosa*, triangles: *Platynereis* spp., inverted triangles: *Lysidice ninetta*. Black: artificial reef 2007, red: artificial reef 2008, green: natural reef 2008. Error bars are ±95% confidence intervals.

3.3.6.2 *Lysidice ninetta*

Sampled on both reefs in 2008 only (Table 3.19), no significant differences existed between sites in mean $\delta^{13}C$ (Mann-Whitney U; Z = 0.000, $p > 0.05$) or $\delta^{15}N$ (Z = -1.934, $p > 0.05$) for this species (Fig 3.12).

3.3.6.3 *Sabellaria spinulosa*

Sabellaria spinulosa were only sampled during 2008, from both sites (Table 3.19, Fig 3.12). No significant effects of site on mean $\delta^{13}C$ (t-test; t = 1.829, d.f. = 18, $p > 0.05$) or $\delta^{15}N$ (Mann-Whitney U; Z = -1.739, $p > 0.05$) were detected.

3.3.6.*4 Bispira volutacornis*

This species was sampled from both sites, but was only collected from each site in different years (Table 3.19, Fig 3.12). No significant differences in mean $\delta^{13}C$ (t-test; t = -0.330, d.f. = 6, $p > 0.05$) or $\delta^{15}N$ (t-test; t = 0.004, d.f. = 6, $p > 0.05$) were found between the samples collected from the artificial reef in 2007 and those collected from the natural reef in 2008.

Table 3.19 $\delta^{13}C$ and $\delta^{15}N$ data (‰) for polychaetes collected from the artificial and natural reefs. Departure from normal distribution tested using Shapiro-Wilk test; $^{**}p < 0.01$; ns = Not Significant ($p > 0.05$).

				Mean	S.E.	n	Shapiro-Wilk W
Platynereis spp.	2008	$\delta^{13}C$	Artificial Reef	-27.08	0.89	7	0.955 ns
			Natural Reef	-29.57	0.53	5	0.911 ns
		$\delta^{15}N$	Artificial Reef	10.22	0.14	7	0.891 ns
			Natural Reef	10.68	0.10	5	0.976 ns
Lysidice ninetta	2008	$\delta^{13}C$	Artificial Reef	-19.77	0.31	10	0.949 ns
			Natural Reef	-19.77	0.37	2	n/a
		$\delta^{15}N$	Artificial Reef	11.66	0.30	10	0.923 ns
			Natural Reef	12.92	0.18	2	n/a
Sabellaria spinulosa	2008	$\delta^{13}C$	Artificial Reef	-17.66	0.13	10	0.888 ns
			Natural Reef	-18.09	0.19	10	0.962 ns
		$\delta^{15}N$	Artificial Reef	10.42	0.14	10	0.700 **
			Natural Reef	9.99	0.18	10	0.854 ns
Bispira volutacornis	2007 2008	$\delta^{13}C$	Artificial Reef	-19.11	0.41	3	0.913 ns
			Natural Reef	-18.98	0.20	5	0.902 ns
	2007 2008	$\delta^{15}N$	Artificial Reef	8.92	0.32	3	0.999 ns
			Natural Reef	8.92	0.08	5	0.830 ns

3.3.7 Amphipoda (Gammaroidea)

Amphipods were sampled from all sites in both years, though in greater numbers in 2008 (Table 3.20, Fig 3.13). No attempt was made to divide them into groups based on taxonomy or other criteria. While site and year had no effect individually upon $\delta^{13}C$, there was a significant interaction in the ANOVA model between these two factors ($p < 0.01$; Table 3.21). During 2007 there was no difference between the mean $\delta^{13}C$ of amphipods sampled from the two reefs, but there was such a difference in 2008 ($p < 0.01$; Table 3.21). Similarly, while artificial reef samples were no different in mean $\delta^{13}C$ between 2007 and 2007, the 2008 natural reef samples differed from those collected in 2007 ($p < 0.05$; Table 3.21). Mean $\delta^{15}N$ did not vary between sites or years (Table 3.22).

Table 3.20 $\delta^{13}C$ and $\delta^{15}N$ data (‰) for amphipods collected from both reef sites in 2007 and 2008. Departure from normal distribution tested using Shapiro-Wilk test; ns = Not Significant ($p > 0.05$).

			Mean	S.E.	n	Shapiro-Wilk W
$\delta^{13}C$	2007	Artificial Reef	-22.43	0.80	8	0.826 ns
		Natural Reef	-19.93	0.90	6	0.940 ns
	2008	Artificial Reef	-20.76	0.86	13	0.901 ns
		Natural Reef	-23.95	0.88	18	0.949 ns
$\delta^{15}N$	2007	Artificial Reef	9.81	0.17	8	0.931 ns
		Natural Reef	10.55	0.38	6	0.924 ns
	2008	Artificial Reef	10.02	0.24	13	0.874 ns
		Natural Reef	9.94	0.15	18	0.981 ns

Table 3.21 (a) Two-way ANOVA comparison of $\delta^{13}C$ for amphipods from natural and artificial reef sites across both sampling years. $^{**}p < 0.01$, ns = Not Significant ($p > 0.05$)

	df	Mean Square	F
Model	4	5585.296	558.768
Year	1	13.128	1.313 ns
Site	1	1.117	0.112 ns
Year * Site	1	76.682	7.671 **
Error	41	9.996	

(b) Pairwise comparisons

Comparison	Difference of means	Test Statistics (p,q)
Site within 2007: Artificial vs. Natural reef	2.507	2, 2.077 ns
Site within 2008: Artificial vs. Natural reef	3.196	2, 3.927 **
Year within Artificial Reef: 2007 vs. 2008	1.672	2, 1.664 ns
Year within Natural Reef: 2007 vs. 2008	4.031	2, 3.825 *

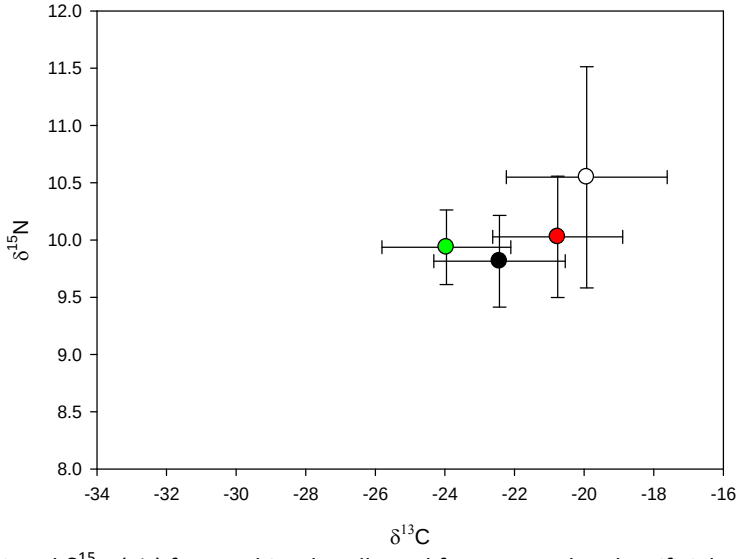


Figure 3.13 $\delta^{13}C$ and $\delta^{15}N$ (‰) for amphipods collected from natural and artificial reef sites in 2007 and 2008. Black: artificial reef 2007, white: natural reef 2007, red: artificial reef 2008, green: natural reef 2008. Error bars are ±95% confidence intervals.

Table 3.22 Two-way ANOVA comparison of $\delta^{15}N$ for amphipods from natural and artificial reef sites across both sampling years. NS = Not Significant ($p > 0.05$)

	df	Mean Square	F
Model	4	1130.691	2069.677
Year	1	0.374	0.685 ns
Site	1	0.976	1.786 ns
Year * Site	1	1.602	2.932 ns
Error	41	0.546	

3.3.8 Fish: *Labrus bergylta*

Fish were only successfully sampled on the artificial reef, but the ballan wrasse, *Labrus bergylta*, was caught in both years (Table 3.23). While $\delta^{15}N$ did not differ significantly between years, (t-test; t = -1.333, d.f. = 4.367, $p > 0.05$), mean $\delta^{13}C$ was higher in 2008 (t-test; t = -4.037, d.f. = 5, $p < 0.05$), though the difference in means was only 0.52‰.

Table 3.23 $\delta^{13}C$ and $\delta^{15}N$ data (‰) for *Labrus bergylta* collected from the artificial reef. Departure from normal distribution tested using Shapiro-Wilk test; ns = Not Significant ($p > 0.05$).

		Mean	S.E.	n	Shapiro-Wilk W
$\delta^{13}C$	2007	-18.26	0.10	4	0.888 ns
	2008	-17.73	0.07	3	0.928 ns
$\delta^{15}N$	2007	15.09	0.27	4	0.816 ns
	2008	15.55	0.14	3	0.952 ns

Table 3.24 Summary of taxon-by-taxon comparison results, including differences between sample means, where statistically significant ($p < 0.05$). Grey cells indicate instances where samples did not allow for the relevant comparison (where a taxon was only sampled from one site or in one year). AR = Artificial Reef, NR = Natural Reef. Bracketed number is the magnitude of the difference in means between sites/years as appropriate.

	$\delta^{13}C$		$\delta^{15}N$		Other comments
	Site effect?	Year effect?	Site effect?	Year effect?	
Paguroidea	NO	NO	2007: AR > NR (0.19) 2008: NR > AR (0.47)	AR: 2007 > 2008 (0.16) NR: 2008 > 2007 (0.50)	$\delta^{15}N$ (weakly) correlated with individual dry mass. Mean differences < 1‰
Necora puber	NO	NO	NO	NO	
Cancer pagurus		NO		NO	
Crepidula fornicata	NO	NO	2007: NR > AR (0.30) 2008: NR > AR (0.23)	AR: 2007 > 2008 (0.28) NR: 2007 > 2008 (0.35)	$\delta^{13}C$ (weakly) correlated with individual dry mass. Mean differences < 1‰
Gibbula cineraria	NO		NO		
Buccinum undatum	NO		NO		
Ocenebra erinacea	2007: NR > AR (0.87) 2008: NO EFFECT	AR: 2008 > 2007 (1.18) NR: NO EFFECT	2007: NO EFFECT 2008: AR > NR (1.29)	AR: 2008 > 2007 (1.43) NR: NO EFFECT	$\delta^{13}C$ - 2007 AR differs from all others $\delta^{15}N$ – 2008 AR differs from all others
Cirripedia	NO	NO	NO	NO	
Ostrea edulis	NO		NO		
Styela clava	(NO)		(NO)		Complicated by size-based clustering
Platynereis spp.	NO		2008: NR > AR (0.46)		Mean difference < 1‰
Lysidice ninetta	NO		NO		
Sabellaria spinulosa	NO		NO		
Bispira volutacornis	(NO)	(NO)	(NO)	(NO)	2008 NR and 2007 AR compared: no difference.
Gammaroidea	2007: NO EFFECT 2008: AR > NR (3.20)	AR: NO EFFECT NR: 2007 > 2008 (4.03)	NO	NO	$\delta^{13}C$ - 2008 NR differs from all others
Labrus bergylta		AR: 2008 > 2007 (0.52)		NO	Mean difference < 1‰

3.4 Discussion

3.4.1 A brief note on statistical power

The term 'statistical power' refers to the ability of a given statistical test to detect a genuine difference between sample means; a test with low power may fail to identify a real difference between sample means. Because of the nature of field sampling, a number of the tests conducted during this analysis were calculated using very small sample sizes, and therefore some tests may have had very low power. Most statistical software packages now have the ability to analyze the power of a test, for example SigmaStat automatically outputs the power of tests carried out by the user, and when it is low, warns that "Less than desired power indicates you are less likely to detect a difference when one actually exists. Negative results should be interpreted cautiously."

However, 'retrospective power analysis' of this type is dangerous, and has been strongly cautioned against (Hoenig and Heisey, 2001). The goal of statistical testing is to determine whether or not the null hypothesis – that two or more sample means do not differ – can be rejected; if the data do not allow the hypothesis to be rejected, then that is all that can be reported. Indeed, in reality, *all* negative results should be interpreted cautiously, since failure to statistically reject a null hypothesis never constitutes absolute *proof* that there is no difference in the sample means.

Nevertheless, the small sample sizes for some of the taxa analysed here do necessitate careful interpretation, but while it is perfectly reasonable to be circumspect about interpreting results based on small sample sizes, low statistical power is never an excuse to 'rescue' statistically non-significant results.

3.4.2 Comparing taxa found on artificial and natural reefs

For the majority of taxa analysed in this chapter, no statistically significant differences were found in their mean stable carbon and nitrogen isotope ratios between the two sites, or between the two sampling years (Table 3.24). Small sample sizes for some taxa mean that potentially significant differences may have

been missed (but see Section 3.4.1 above), however the results here support the hypothesis that organisms on artificial reefs are exploiting the same basic nutrient sources, at similar trophic levels, as those on natural reefs.

Some taxa were unfortunately only sampled in one year. Given the lack of isotopic differences between the two reefs and the two years, it might be reasonable to tentatively assume that where a particular taxon did not differ between reefs, and was only sampled in one year, that that taxon would also not differ between the two years.

It should also be noted that for several taxa where a statistically significant difference in either one or both isotope ratios was detected between sites (or years), the actual difference in means is comparatively small (< 1‰). Generally, differences this small are not considered to be of particular ecological relevance, since not only is isotope fractionation approximately this magnitude for carbon, but also this is easily within the range of individual variation in isotopic composition, which can be up to 2‰ (DeNiro and Epstein, 1978).

There is also a possibility that any differences detected might result from sampling biases, particularly where sample sizes were small. Several taxa were not uniformly distributed across reef habitat, which raises the possibility of small-scale heterogeneity in nutrient sources, especially where very small individual organisms were sampled.

3.4.2.1 *Ocenebra erinacea*

Species of this genus feed principally upon bivalves (such as oysters) by drilling holes through their shells, although some members of the genus have been recorded preying on limpets, barnacles and spirorbid polychaetes (Palmer, 1988). The differences in isotope ratios between sites and years might thus represent predation on different species, perhaps as a result of low abundances of oysters. *Ocenebra erinacea* was not abundant on either reef, and was rather difficult to locate. During the 2008 sampling on the artificial reef, most of the individuals collected were sampled from the underside of one piece of kelp (*Laminaria* spp.).

Indeed, for $\delta^{15}N$ it was these individuals which were found to be different from the other year/site combinations (Table 3.27). These particular individuals might have exploited a different food source; since the kelp does not grow on the artificial reef itself, it is even possible that they attached themselves to the algae somewhere else and were carried to the reef. However, this suggestion is possibly not supported by the results of the analysis for $\delta^{13}C$, which show that the 2008 artificial reef individuals did not differ from the 2007 or 2008 samples from the natural reef and therefore were likely to be exploiting the same basic nutrient sources.

It is thus possible that the reported differences were the result of low sample sizes for some year/site combinations. Only 2 individuals were collected from the artificial reef in 2007 and only one from the natural reef in 2008, making sampling bias a possibility that cannot be ruled out.

3.4.2.2 *Styela clava*

Individuals sampled from artificial and natural reefs appeared to differ substantially, until it was realised that there was a serious size discrepancy between samples from the two reefs. Large individuals were only collected on the natural reef, and only small individuals were collected from the artificial reef. However, one small individual was sampled from the natural reef, and its isotope ratios appeared to match those of the small individuals from the artificial reef (Fig 3.11) suggesting that the differences in isotope ratios between the two sites were in fact a direct result of size-biased sampling, rather than a genuine site difference.

The differences between the small and large individuals are striking, particularly for $\delta^{15}N$ (~5.5‰ for large individuals compared with ~10.5‰ for small individuals). Such a strong change in isotope ratio with size suggests an ontogenetic diet shift. However, it is interesting to note that the shift in $\delta^{15}N$ appears to be 'backwards', with the larger individuals feeding at what appears to be a lower trophic level.

3.4.2.3 *Bispira volutacornis*

This species was sampled from both sites, but not in the same year. Consequently it is not truly possible to determine from the data whether or not there were genuine site or year effects on isotope ratios. However, the fact that no difference was found between the two sets of samples *suggests* the lack of an effect of site or year of sampling. A significant site/year interaction cannot be ruled out, but it is perhaps more parsimonious to assume that no differences were present, rather than that there were real differences between sites that were exactly reversed in 2008 relative to 2007.

3.4.2.4 Amphipoda

The data showed no significant difference in $\delta^{15}N$ of amphipods between sites or years, indicating that they all fed at a common trophic level. However, there was variation in $\delta^{13}C$; amphipods sampled from the natural reef in 2008 had a different mean $\delta^{13}C$ from all other site/year combinations. The magnitude of the mean differences were rather large: mean $\delta^{13}C$ of 2008 natural reef amphipods was 4.03‰ lower than that of 2007 natural reef amphipods, and 3.2‰ lower than that of 2008 artificial reef amphipods.

However, this result needs to be interpreted carefully. While the sample sizes are rather large, making the comparisons statistically sound, a wide range of carbon isotope ratios were recorded for individual amphipods from all sites, indicating that a range of nutrient sources might be being exploited. As a result, the data may be heavily influenced by sampling biases; if (for example) algae-feeding amphipods have different isotope ratios to those feeding on other detritus, then any difference in the mean $\delta^{13}C$ between two sites (or years) might simply represent differences in the number of individuals collected that exploit the various nutrient sources. An alternative (and possibly interacting) problem is that there may be taxonomic divisions between amphipods that affect their isotopic compositions (Jaschinski et al., 2008). If any of these two factors are in operation, then simply comparing the mean isotope ratios of amphipods would not be useful; it was undertaken here only by way of an initial exploration of the data. The problem

might be overcome by detailed quantitative sampling of amphipods from various reef microhabitats, coupled with identification of amphipods to species level.

3.4.2.5 Other taxa

A number of taxa had mean differences which were found to be statistically significant, but which were relatively small (< 1‰). Generally such differences are not considered ecologically interesting because they fall within the range of individual variation (DeNiro and Epstein, 1978). Certainly differences of 0.5‰ or less are not likely to be useful for analysis of food webs, and the site and/or year differences recorded in the mean $\delta^{15}N$ of Paguroidea, *Crepidula fornicata* and *Platynereis* spp. and the mean $\delta^{13}C$ of *Labrus bergylta* were around this magnitude. Consequently, while these differences are acknowledged, they will not be considered further.

3.4.2.6 Size effects

Individual size has been shown to affect $\delta^{15}N$ (Jennings et al., 2002) and $\delta^{13}C$ (Hoeinghaus and Davis, 2007) for some taxa. Therefore, where sample sizes were sufficient, and where appropriate data were collected, possible size-based trends in carbon and nitrogen stable isotope ratios were considered. Patterns were not consistent among taxa; Paguroidea showed size-based trends in $\delta^{15}N$ but not $\delta^{13}C$ (indicating slightly increasing trophic level with increasing individual size, but no shift in ultimate dietary carbon source), whereas the situation was reversed in *Crepidula fornicata*, indicating that small and large individuals may make use of different carbon sources, but at a similar trophic level. *Styela clava* showed sharp distinctions between the isotope ratios (both carbon and nitrogen) of large and small individuals.

Such size-based trends can make a difference to the analysis of data. Where sample sizes were too small, or size data were not consistently collected, including size considerations in the analysis was not feasible. This may have lead to some differences being missed, or being exaggerated by size-biasing in the samples (such

as occurred with *Styela clava*, where only large individuals were sampled on the natural reef).

Size-based isotope effects are not necessarily ubiquitous, and patterns are not identical across taxa, but they have been found in several species, including cephalopods (Parry, 2008), crabs (Hoeinghaus and Davis, 2007) and fish (Jennings et al., 2001; Sweeting et al., 2007), and they should be considered where possible.

3.4.3 Conclusions

Comparisons of taxa found on both the artificial and natural reef sites revealed few differences in stable isotope ratios of carbon and nitrogen between sites or between sampling years. Several differences which were found to be statistically significant were actually very small in magnitude, and are unlikely to be useful indicators of real underlying differences in artificial and natural reef habitats. Furthermore, for those taxa where statistical significance coincided with larger differences in isotope ratios, these effects may have resulted from sampling biases, or high individual variability in some taxa of primary consumers.

Generally, therefore, it is concluded that isotope ratios of consumers did not differ between the two habitats. As mentioned in the introduction to this chapter, differences in the isotope ratios of consumers between sites can result from differences in: trophic baselines; consumer diets; food chain length or food web structure; or relative inputs of basic nutrient sources. Of course, while it is *logically possible* that the artificial and natural reef systems differed in several of the above respects, but in such a way that consumer isotope ratios were not affected, it is more parsimonious to surmise that similar consumer isotope ratios in this case reflected a lack of differences between the two reef systems.

Finally, it appears that isotope ratios largely did not change over the course of the two sampling years, suggesting a degree of stability in the structure of the food webs on the two habitat types.

Chapter 4 considers the trophic structure of the reef food webs in greater detail, taking into consideration some of the possible nutrient sources.

Chapter 4. Nutrient sources and trophic structure on artificial and natural reefs

4.1 Introduction

As described in the previous chapters, the isotope ratios of consumers (particularly the $^{13}C/^{12}C$ ratio) reflect the isotope ratios of their food (DeNiro and Epstein, 1978). Consequently, where there are several potential sources of food, the proportional contributions of these food sources to consumer diets can also be evaluated using stable isotope methods, if the isotope ratios of consumers and their possible food sources are known, and if the isotope ratios of potential food sources are different (Peterson, 1999).

Calculating the diet of a species using isotope ratios is simple if there are few potential food sources; where the number of potential sources is less than or equal to n+1 (where n is the number of stable isotope systems, such as $^{13}C/^{12}C$ and $^{15}N/^{14}N$, used in the analysis), a unique solution is possible (Phillips and Gregg, 2001). However, if there are more than n+1 sources, a range of potential combinations of diets could result in the same consumer isotope ratios, and mixing models, such as IsoSource (http://www.epa.gov/wed/pages/models.htm) must be used to report these ranges of feasible solutions (Phillips and Gregg, 2003).

Multiple nutrient sources are often important in marine ecosystems (Richoux and Froneman, 2007), particularly in coastal areas (Carlier et al., 2007), where production by microphytobenthos (Kang et al., 2007), benthic algae (Kang et al., 2007; Richoux and Froneman, 2007), seagrasses (Richoux and Froneman, 2007), and phytoplankton (Corbisier et al., 2006; Kang et al., 2007), can all be significant, as can riverine nutrient inputs (Chanton and Lewis, 1999), and anthropogenic sources (Corbisier et al., 2006). In these cases it is essential to use mixing models to explore the contributions of potential sources.

Stable isotopes have been used to investigate marine ecosystems ranging from deep sea hydrothermal vents (Bergquist et al., 2007) to intertidal saltmarshes (Kang et al., 2007). However, this approach has not been widely applied to the study of artificial reefs, despite the need to investigate the extent to which these

resemble natural reef habitats. One study (Riera et al., 2004) appeared to show that the food web associated with an artificial habitat was simplified relative to that of natural habitats. However, a subsequent study of another artificial habitat found a complex food web with similar input of nutrient sources, and similar food chain length, to neighbouring natural habitat (Schaal et al., 2008). No other published studies address these issues on artificial reefs.

Therefore, the aim of this chapter is to characterise the structure of the food webs associated with the Poole Bay artificial reef (Section 2.4) and a neighbouring natural reef, by identifying the possible nutrient sources, determining groupings of trophically similar individuals, and using mixing models to assess the possible contributions of each nutrient source to consumer groups on both reefs.

4.2 Materials and methods

4.2.1 Sample collection

The two reef sites (the Poole Bay artificial reef and 'Wrasse Reef') were sampled by SCUBA divers during 2007 and 2008, as described in Section 3.2.1. In addition to the taxa compared in Chapter 3, other species were sampled in order to examine the general structure of reef food webs.

4.2.1.1 Nutrient sources

The construction of the artificial reef (chaotic piles of regular-shaped components) presents a large number of corners, holes, guide ropes and other such snagging points, and consequently drifting pieces of macroalgae are snagged on the reef structure. This includes algal species which do not appear to grow on the artificial reef itself (Table 4.3). A few species of red algae do grow on the artificial and natural reefs, such as *Calliblepharis ciliata* and *Callophyllis* spp., and these were sampled on both habitats. The natural reef, while still jagged and uneven in structure, does not provide such ample opportunities for algae to become entangled, and therefore only small amounts of drifting algae were found caught on this reef. No attempt was made to formally quantify the differences in algal biomass (of snagged algae or of algae growing on the reefs themselves) between

the two reef types. As a result of time constraints during sample processing, full species level identification of algal samples was not always possible.

In order to analyse sedimentary organic matter (SOM), samples of surface sediments were collected, both from very close to each of the reefs and also from sites several meters away from the reefs (see Section 4.2.1.3 below). Finally, plankton and particulate organic matter (POM) were sampled in both years by conducting several vertical tows, from the sea bed to the sea surface, using a 100µm mesh plankton net.

4.2.1.2 Sediment fauna

In order to examine the isotope ratios of consumers from the soft-bottom sediment community, sediment cores were collected by divers during 2007. A plastic gardening trowel was used to excavate an approximately 10cm by 10cm area to a depth of approximately 5cm. Sediment samples were collected in close proximity to both reefs, and also at a distance of approximately 5m from both reefs. Six samples were taken from each site, so that a total of 24 samples were collected. Bagged samples were returned to the surface using inflatable lifting bags, and transferred to labelled buckets for transportation back to the laboratory.

4.2.2 Initial sorting and preservation of samples

Large quantities of sample material were obtained on each sampling date, and because samples to be used for stable isotope analysis can only be preserved by freezing (Bosley and Wainright, 1999), all samples had to be sorted while fresh; little time was available for accurate sample identification. This was somewhat improved in 2008 by the availability of a camera-equipped binocular microscope, which allowed some samples to be photographed for later identification. Nevertheless, identification to species level was not practical for many of the samples, which were thus identified to the greatest taxonomic resolution possible.

In some cases it was clear (based on morphological differences) that samples were of multiple species, but identification to species level was not possible; these were not fully identified, but their presence was recorded (for example, several infaunal

bivalves were not identified to the species level, yet it was clear that multiple species were present). Time constraints also meant that not every individual organism present in the material sampled from the two reefs was recorded and retained – generally only those which were considered likely to provide sufficient material for isotope analysis were processed. Furthermore, sampling effort for reef fauna was not strictly controlled or quantitative, and varied between years, although the two sites were visited a similar number of times.

4.2.2.1 Reef fauna

Sorting and preservation of reef fauna was carried out as described in Section 3.2.2. Where possible, appropriate biometric data (for example length, wet weight, dried weight) were recorded for individual samples. Where no further treatments were necessary, samples were then individually wrapped in tin foil and frozen at -20°C. Samples collected in 2008 were generally photographed for later identification. Prior to sorting and processing, samples were kept in clean seawater in buckets in a cold room at approximately 5°C.

4.2.2.2 Macroalgae

Macroalgal samples were placed in white trays with clean seawater, shaken, and carefully examined in order to extract any hidden fauna, which were processed separately. Representative samples from several species of algae were then used to obtain material for analysis. In the case of branching or bushy algae, small samples were taken and rinsed in Milli-Q water. For algae with flat, broad fronds, a scalpel was used to cut small square sections from the centre of the frond. These sections were carefully scraped with the scalpel in order to remove epiphytic algae and bryozoans, and then rinsed in Milli-Q water. Prepared samples were wrapped in tin foil and frozen at -20°C.

4.2.2.3 Plankton/POM samples

Bottled plankton samples were filtered through Whatman GF/F filters, and the filters were then acidified with 1M HCl to remove carbonates. After acidification,

filters were rinsed through with Milli-Q water to remove excess acid, placed into glass vials and frozen at -20°C.

4.2.2.4 Sediment/SOM samples

At this stage, small surface sediment samples were wrapped in tin foil, individually placed into small zip-loc bags and frozen at -20°C.

4.2.2.5 Sediment fauna

Sediment core samples were sieved using a 1000µm mesh sieve and washed through using fresh water. Any fauna retained by the sieve were identified where possible, and then individually wrapped in tin foil and frozen at -20°C.

4.2.3 Sample preparation and pre-treatment

Table 4.1 Preparation and pre-treatment of samples for stable isotope analysis, detailing which tissues were sampled for analysis (or if whole animals were used), what body parts were removed when whole animals were sampled, and whether or not samples were acidified in order to remove residual carbonates.

Taxon	Sample	Parts removed?	Acidified?
Fish	Dorsal white muscle	n/a	No
Ascidians	Whole animal	None	No
Polychaetes	Whole animal	Feeding tentacles, tubes	No
Gastropods	Foot muscle	n/a	No
Paguroidea	Whole animal	None	Yes
Large crustacea	Leg muscle	n/a	No
Small crustacea	Whole	n/a	Yes
Prawns	Tail muscle	Carapace	No
Bivalves	Adductor muscle	n/a	No
Barnacles	Whole	Feeding appendages, shell	No
Amphipods	Whole	None	No
Bryozoa	Colony sample	None	Yes
Sponges	Sample	None	No

All frozen samples were lyophilised (freeze-dried) for at least 24 hours until completely dry (this was more than sufficient for complete drying of small samples, but larger samples sometimes still contained ice crystals after this time and required longer freeze-drying periods). After freeze-drying, samples were weighed and transferred to sealed glass vials for short-term storage. Samples were then further treated as described in Sections 3.2.3.1 to 3.2.3.7 or as summarised in

Table 4.1. In all cases, including those where no further treatment was necessary, samples were ground to a homogenous powder using a mortar and pestle (thoroughly washed with hot water and cleaning solvents between uses) or a ball mill.

4.2.3.1 Sediment samples

Sediment material was rich in carbonate, derived largely from molluscan shell fragments. Small amounts of each sample were ground to a fine, homogenous powder using a mortar and pestle, and then acidified to remove inorganic carbon (Kennedy et al., 2005). Acid treatment was carried out by placing approximately 1g of sediment from each sample in glass vials, adding 5ml of 1M HCl and shaking vigorously until all effervescence was observed to have ceased. Samples were filtered, rinsed thoroughly with Milli-Q water to remove excess acid, and oven-dried at 60°C. Dried sediments were then placed into sealed glass vials prior to analysis.

4.2.3.2 Plankton/POM samples

Sample material was scraped from the dried glass-fibre filters (see 4.2.2.3), taking care to minimise the amount of filter material retained in the sample (glass fibre should not bias the stable isotope data, since it contains neither carbon nor nitrogen, but it can dilute the sample material), and placed in labelled glass vials.

4.2.4 Stable isotope ratio mass spectrometry procedure

Stable isotope analysis was carried out as described in Section 3.2.4. However, while 600 to 800µg samples were used for animal tissue, other sample types required different quantities (as determined during trial runs). Algal samples contained lower proportions of nitrogen, and so in order to fall within the mass spectrometer's calibration range, larger samples were needed (between 1 and 1.5mg in most cases, but more than 2mg for some samples). Likewise, some of the plankton/POM samples required sample weights in this range. Sediment samples were very low in organic content compared with animal and plant material, and therefore 50 to 60mg samples were required.

4.2.5 Data analysis

Where appropriate, sample data were compared between sites using t-tests/ANOVAs or their non-parametric equivalents where assumptions of normality and homoscedasticity could not be met. Univariate analyses were carried out using SPSS and SigmaStat.

A species-independent approach was adopted in order to identify groups of organisms with similar trophic level and diet source. Hierarchical cluster analyses were computed separately for each site using Bray-Curtis Similarity Indices (Clarke et al., 2006), calculated on $\delta^{15}N$ and $\delta^{13}C$ data for individual invertebrate consumers. $\delta^{15}N$ values were used untransformed, but $\delta^{13}C$ values were transformed by subtraction from zero (Bray-Curtis Similarity indices cannot be calculated on negative values). Groups were defined at the 95% similarity level. This value was selected because it was the lowest level of similarity to produce clear, non-overlapping groups, while higher levels of similarity generated large numbers of small clusters containing only a few individuals. The number, and mean isotope ratios, of clusters were compared between the two reef types. PRIMER (Plymouth Routines In Multivariate Ecological Research) was used for the bivariate data clustering (Clarke and Gorley, 2006).

IsoSource was used to explore the potential contributions of each of the identified nutrient sources to the consumer clusters defined by the cluster analyses above, using carbon isotope ratio data. Carbon and nitrogen stable isotope data were then used in IsoSource models to determine the potential contributions of lower trophic level consumer clusters to the diet of higher trophic level invertebrate clusters, and (on the artificial reef) the fish species *Labrus bergylta*.

4.3 Results

4.3.1 Field sampling observations

In total, approximately 80 taxa were collected from the two reef sites and from the surrounding sediment communities (Table 4.2). This figure is necessarily approximate as a result of the constraints imposed on sample processing time (Section 4.2.2). Statistical comparisons of the numbers of taxa or individuals sampled from the two reef sites, or the four sediment sites, were also not appropriate because of the lack of sampling standardisation.

However, the numbers of individual organisms and taxa sampled from the two reef sites were broadly similar, in both years, with the same taxa generally present. Minor discrepancies cannot be regarded as significant for the reasons outlined previously, but one or two deserve mention. Firstly, a greater number of polychaete taxa were sampled from the natural reef than from the artificial reef. Greater numbers of one species, *Lysidice ninetta*, were sampled on the artificial reef than on the natural reef (during 2008). Other than these small differences, the samples from the two sites appeared similar in terms of taxonomic composition. Both sites were dominated (in terms of the numbers of individuals sampled) by amphipods, polychaete worms (both Errantia and tube-forming Sedentaria), gastropod molluscs, hermit crabs (*Pagurus* spp.), other small crustacea, and barnacles.

Sampling of the sediment community was slightly better standardised, with a pre-determined number of samples being collected, and an approximately defined quantity of material sampled. However, the sediment samples were still subject to all the other constraints mentioned above (such as time available for species identification). All sediment sites appeared to have a similar taxonomic composition, number of taxa present, and number of individuals sampled (Table 4.2), except that samples close to the artificial reef contained fewer individuals (but not taxa). Samples taken from close to both reefs appeared to contain representatives of slightly fewer taxa than those taken further away from the reefs.

Table 4.2 List of taxa collected, with numbers of individuals sampled, on both reef sites, and from neighbouring sediment communities. Colonial taxa are recorded as 'Present' rather than being enumerated. Near = sediment sample taken adjacent to reef, Far = sediment sample taken 5m away from reef. *'Barnacles' refers principally to *Balanus* spp., but small numbers of *Verruca stroemia* and *Acasta spongites* were also sampled. Continues on following page.

			Artificial Reef		Natural Reef		AR Sediment		NR Sediment	
			2007	2008	2007	2008	Near	Far	Near	Far
Fish		*Labrus bergylta*	4	3						
		Spondyliosoma cantharus	1							
		Syngnathus acus	2							
		Gobius paganellus	1							
Crustacea		*Homarus gammarus*	1							
	Brachyura	*Cancer pagurus*	4	3						
		Maja squinado	1			1				1
		Necora puber	3	2	3					
		Inachus dorsettensis		1		1				
		Small Brachyura	2	5		7	1	2	1	1
		Liocarcinus arcuatus				1		1		
	Anomura	*Pagurus* spp.	20	4	16	21	2	10	3	17
		Galathea spp.	1		2					3
		Porcellanidae		1						
	Caridea	*Palaemon serratus*	4	2	1	3				
	Eucaridea	Mysidacea	1							
	Isopoda	Isopoda			1					
	Amphipoda	Gammaridea	11	20	6	23	18	15	19	2
	Cirripedia	Barnacles*	13	20	7	17				
Mollusca	Polyplacophora				1					1
	Gastropoda	*Crepidula fornicata*	10	14		12		15	10	
		Ocenebra erinacea	2	7	8	2			1	
		Buccinum undatum		4		4	6	1	3	4
		Nassarius incrassatus		3						
		Gibbula cineraria	2	4		2	1			3
		Other gastropods		3		1				
	Opisthobranchia	*Polycera quadrilineata*				1			1	
	Bivalvia	*Ostrea edulis*	4		5	1				
		Chlamys varia			2					
		Large sediment bivalve					1	1		
		Infaunal bivalve 1					1	1		
		Infaunal bivalve 2					1			
		Infaunal bivalve 3					4	1	1	3
		Infaunal bivalve 4								5
Tunicata	Ascidiacea	*Ascidiella aspersa*	1							
		Botryllus schlosseri	Present							
		Styela clava		6	4				1	
		Other tunicates	1		1					2

Table 4.2 continued List of taxa collected, with numbers of individuals sampled, on both reef sites, and from neighbouring sediment communities. Colonial taxa are recorded as 'Present' rather than being enumerated. Near = sediment sample taken adjacent to reef, Far = sediment sample taken 5m away from reef.

			Artificial Reef		Natural Reef		AR Sediment		NR Sediment	
			2007	2008	2007	2008	Near	Far	Near	Far
Polychaeta	Errantia	*Nephtys* spp.		2			7	7	19	12
		Hediste diversicolor						2		
		Sthenelais boa						1	1	
		Neanthes virens								1
		Lysidice ninetta		11		2				
		Eteone picta		1						
		Typosyllis prolifera				2				
		Ampharetidae					1	5	9	5
		Glycera spp.					1	1		
		Scolopsis armiger					1	2		
		Sigalionidae				8				
		Harmothoe spp.			2					
		Marphysa spp.				3				
		Eulalia aurea				1				
		Eulalia spp.				1				
		Anaitides mucosa			1			2		
		Platynereis spp.		8		6				
		Other Nereid	9	2	4	5				
		Unidentified Errantia		7						
		Other Errantia	2	3	2	4	4	3	7	13
	Sedentaria	*Eupolymnia nebulosa*				3				
		Terebellidae				6				
		Sabellaria spinulosa		10		10				
		Sabellidae		1		1				
		Bispira volutacornis	3			5				
Cnidaria	Hydroida	*Nemertesia ramosa*	Present							
		Other hydroids	Present		Present					
	Actiniarida	*Anemonia sulcata*			1					
		Other anemone							1	
Bryozoa		*Flustra foliacea*	Present		Present					
		Membranipora mebranacea	Present							
		Other bryozoa	Present		Present					
Porifera		*Suberites* spp.	1							
		Dysidea fragilis	Present		Present					
		Other sponges	2		8					
Echinodermata		Ophiuroid	1					1		1
Sipuncula								1		
TOTAL			107	147	75	154	49	72	77	74
TOTAL NUMBER OF TAXA SAMPLED			34	26	23	29	15	19	14	17

4.3.2 Nutrient sources

Table 4.3 Mean $\delta^{13}C$ and $\delta^{15}N$ (‰) for samples of potential nutrient sources. AR = Artificial Reef; NR = Natural Reef; NAR = Near Artificial Reef; AAR = Away from Artificial Reef; NNR = Near Natural Reef; ANR = Away from Natural Reef; G = algae growing attached to reef; S = algae snagged on reef. Type, colour and taxon columns apply to macroalgal samples only.

Source	Site	Type	Colour	Taxon	Year	n	$\delta^{13}C$	SE	$\delta^{15}N$	SE
POM					2007	3	-19.34	0.33	9.94	0.13
					2008	3	-21.38	0.26	10.03	0.61
SOM	NAR				2007	3	-21.99	0.33	6.73	0.31
	AAR					3	-22.26	0.03	6.21	0.38
	NNR					3	-22.43	0.19	7.50	0.06
	ANR					3	-22.73	0.07	6.45	0.12
Macro-algae	AR	G	Red	*Callophyllis* spp.	2007	1	-23.96		8.72	
					2008	4	-21.69	0.48	9.32	0.18
				Other red	2008	3	-31.75	0.61	8.49	0.50
					2007	1	-32.16		8.02	
						1	-25.71		9.08	
		S	Red	*Callophyllis* - decaying	2008	2	-32.96	0.08	8.62	0.04
				Other red	2007	1	-17.98		11.93	
						1	-21.24		9.57	
					2008	1	-19.23		8.07	
			brown	*Laminaria* spp.	2007	3	-15.81	0.65	8.11	1.65
					2008	4	-16.20	0.76	6.54	1.07
				Laminaria saccharina	2008	1	-19.50		9.17	
				Fucus spiralis	2008	1	-20.15		9.14	
				Other brown	2008	1	-21.58		6.30	
			green	*Ulva lactuca*	2007	3	-13.73	0.78	11.69	0.48
					2008	2	-15.57	1.70	10.97	0.94
				Decayed *Ulva*	2008	1	-19.85		12.30	
				Other green	2008	1	-23.55		8.84	
						1	-22.10		8.42	
						1	-20.38		8.43	
	NR	G	Red	*Callophyllis* spp.	2008	1	-22.18		8.19	
				Other red	2007	1	-23.72		9.48	
						1	-33.47		5.93	
						1	-28.80		7.49	
					2008	1	-22.11		10.05	
						1	-34.31		8.56	
						1	-32.99		8.21	
						3	-30.78	0.60	8.32	0.13
						1	-34.72		7.84	
		S	green	*Ulva lactuca*	2008	2	-15.89	0.14	10.40	0.05

4.3.2.1 Plankton/Particulate Organic Matter samples

POM sampled in 2007 had a mean $\delta^{13}C$ of -19.34‰ compared to -21.38‰ in 2008 (Table 4.3), but this difference was not found to be statistically significant (Mann-Whitney U test; Z = -1.964; n = 6; $p > 0.05$; 2007 data were not normally distributed: Shapiro-Wilk W = 0.750; df = 3; $p < 0.01$).

Mean $\delta^{15}N$ values for the two years were very similar: 9.94‰ in 2007 and 10.03‰ in 2008 (t-test; t = -0.135; d.f. = 4; $p > 0.05$).

4.3.2.2 Sedimentary Organic Matter (SOM) samples

Three replicate sediment samples from each site (Near Artificial Reef: NAR, Near Natural Reef: NNR, Away from Artificial Reef: AAR, Away from Natural Reef: ANR) were analysed to determine the stable isotope ratios of organic carbon (after sample acidification) and nitrogen (Table 4.3).

Neither site nor distance from reef habitat had a significant effect on the $\delta^{13}C$ of SOM (Table 4.4), but sediment samples taken in the vicinity of both reefs were slightly enriched (by 0.784‰) in ^{15}N relative to those collected away from reef habitat (Table 4.5).

Table 4.4 GLM UNIVARIATE ANOVA comparison of $\delta^{13}C$ for sediment samples from near to and away from the natural and artificial reefs. NS = Not Significant ($p > 0.05$)

	df	Mean Square	F
Model	4	1499.391	12956.048
Site	1	0.610	5.273 NS
Reef proximity	1	0.243	2.101 NS
Site * Reef proximity	1	0.000	0.004 NS
Error	8	0.116	

Table 4.5 GLM UNIVARIATE ANOVA comparison of $\delta^{15}N$ for sediment samples from near to and away from the natural and artificial reefs. *$p < 0.05$; NS = Not Significant ($p > 0.05$)

	df	Mean Square	F
Model	4	136.296	693.218
Site	1	0.766	3.896 NS
Reef proximity	1	1.845	9.385 *
Site * Reef proximity	1	0.200	1.017 NS
Error	8	0.197	

4.3.2.3 Macroalgae samples

$\delta^{15}N$ values for macroalgae sampled from the two reef sites covered a relatively small range, with most falling between 8 - 10‰ (Table 4.3). A few samples had lower values, and a similarly small number had values above 10‰. The green alga,

Ulva lactuca, in particular, was ^{15}N enriched relative to the rest of the sampled taxa (10.40‰). One sample of *U. lactuca*, showing signs of decay (discolouration, extreme flaccidity) had the highest $\delta^{15}N$ recorded for any algal sample (12.30‰).

Compared to the $\delta^{15}N$ values, $\delta^{13}C$ values showed a much higher degree of variability, ranging from -34.72 to -12.53‰ for individual samples (Table 4.3). This precluded the possibility of assigning a single mean $\delta^{13}C$ value for 'macroalgae' for further analysis. However, the values for individual samples were arranged into three groups, each covering a range of approximately 5‰ (Table 4.6): one relatively ^{13}C enriched group, one ^{13}C depleted group, and one group with intermediate $\delta^{13}C$ values. Each group contained non-overlapping sets of taxa, representing a potential *a priori* justification in support of the groupings. The mean $\delta^{13}C$ values for each of these three groups were thus considered as three separate potential nutrient sources.

Table 4.6 Summary data for three groups of macroalgae defined by similar $\delta^{13}C$ values (‰). The taxa of growing red algae mentioned in the constituents of the Depleted and Intermediate groups are non-overlapping (different taxa are present in each group).

Group	Range of $\delta^{13}C$	n	Mean $\delta^{13}C$	Constituent samples
Depleted	-34.72 to -28.8	14	-32.14	Several taxa of growing red algae; Decaying *Callophyllis* spp.
Intermediate	-25.71 to -19.23	18	-21.78	Living *Callophyllis* spp.; Several taxa of growing red algae; Several taxa of snagged red algae; Decaying *Ulva lactuca*; Other snagged green algae; Snagged *Fucus spiralis*; Snagged *Laminaria saccharina*; Other snagged brown algae
Enriched	-17.98 to -12.53	15	-15.62	Snagged *Laminaria* spp. Living *Ulva lactuca*

Where a particular species of algae was sampled in a living condition as well as in a partially decayed state (*Ulva lactuca* and *Callophyllis* spp.) the decayed samples appeared strongly depleted in ^{13}C relative to the living samples (Table 4.3); indeed the decayed and living samples of these two taxa were placed into different groups (Table 4.6) as described above.

4.3.2.4 Source $\delta^{13}C$ values

For all further analyses, the following $\delta^{13}C$ values were used as those for the putative nutrient sources for reef organisms. For POM, since no significant effect of year was detected (section 4.3.2.1), the values were averaged across both years (giving a value of -20.36‰). For SOM, since there was no statistically significant effect on $\delta^{13}C$ of site or distance from reef habitat (section 4.3.2.2), the mean $\delta^{13}C$ of all sediment samples was used (-22.35‰). For macroalgae, the mean $\delta^{13}C$ of each of the three macroalgal groups (Table 4.6) were used (-32.14‰, -21.78‰ and -15.62‰).

4.3.2.5 Trophic baseline

Since $\delta^{15}N$ values are typically used to establish trophic position, it is considered worthwhile to calculate a 'trophic baseline' – since the $\delta^{15}N$ value of nutrient sources affects the values that are fed into any food web. While some authors use the mean $\delta^{15}N$ of all nutrient sources as their trophic baseline (Hoeinghaus and Davis, 2007; Jaschinski et al., 2008), this seems to assume that all nutrient sources are fed into the food web in equal proportions. Furthermore, earlier steps in the food chain (from primary producers to primary consumers) have been found to be those most likely to violate assumptions about trophic enrichment (Crawley et al., 2007). As an alternative, some authors suggest the use of the $\delta^{15}N$ values of suitable primary consumers to calibrate the baseline, such as bivalves (Cabana and Rasmussen, 1996; Fukumori et al., 2008). In this project the overall mean $\delta^{15}N$ value of all infaunal bivalves was used: 8.67‰ (standard error = 0.08).

4.3.3 Sediment fauna

Only fauna sampled from the sediment community in the immediate vicinity of the reefs (NAR and NNR) were analysed for stable carbon and nitrogen isotope composition.

Table 4.7 Mean $\delta^{13}C$ and $\delta^{15}N$ (‰) for samples of sediment fauna. NAR = Near Artificial Reef; NNR = Near Natural Reef.

Site		Taxon	n	$\delta^{13}C$	SE	$\delta^{15}N$	SE
NAR	Polychaete	*Nephtys* spp.	7	-17.84	0.37	12.39	0.16
	Crustacea	*Pagurus* spp.	2	-19.31	1.31	10.64	0.24
		Amphipoda	9	-18.95	0.23	9.78	0.17
	Gastropoda	*B. undatum*	6	-17.27	0.16	12.66	0.11
	Bivalvia	Infaunal bivalve 1	1	-20.48	--	8.67	--
		Infaunal bivalve 3	4	-18.46	0.15	8.66	0.13
		Infaunal bivalve 2	1	-16.98	--	8.71	--
NNR	Polychaete	*Nephtys* spp.	9	-18.13	0.18	11.98	0.13
	Crustacea	*Pagurus* spp.	3	-20.64	1.01	10.80	0.10
		Amphipoda	9	-20.18	0.13	10.18	0.09
	Gastropoda	*C. fornicata*	9	-17.76	0.15	8.72	0.16
		B. undatum	3	-17.27	0.16	12.95	0.03
		O. erinacea	1	-14.17	--	11.14	--

4.3.3.1 NAR (Near Artificial Reef) samples

All three (non-indentified but distinct) taxa of infaunal bivalves had extremely similar $\delta^{15}N$ values (Table 4.7; Fig. 4.1) with a mean of 8.67‰, which was adopted as a 'trophic baseline' (Section 4.3.2.5). If an average trophic enrichment of +3.4‰ per trophic level is assumed (Minagawa and Wada, 1984), then secondary consumers would be expected to exhibit $\delta^{15}N$ values of around 12‰. *Nephtys* spp. polychaetes and *B. undatum* had mean values of 12.39 and 12.66‰, respectively, indicating that they were indeed approximately one trophic level higher; they were secondary consumers. Amphipoda and Paguroidea had intermediate $\delta^{15}N$ values (9.78 and 10.64‰ respectively), indicating that they may have occupied intermediate trophic positions (Fig 4.1).

$\delta^{13}C$ values for these taxa showed a smoother, almost linear, progression (Fig. 4.2). This suggests progressive trophic ^{13}C enrichment of food acquired from a single source. Indeed, it appears that the order of increasing $\delta^{13}C$ for some of the species roughly corresponded to that for $\delta^{15}N$: *Nephtys* spp. and *B. undatum* had higher $\delta^{13}C$ and $\delta^{15}N$ than amphipods and hermit crabs. However, the results for infaunal bivalves suggest that this may be an oversimplification.

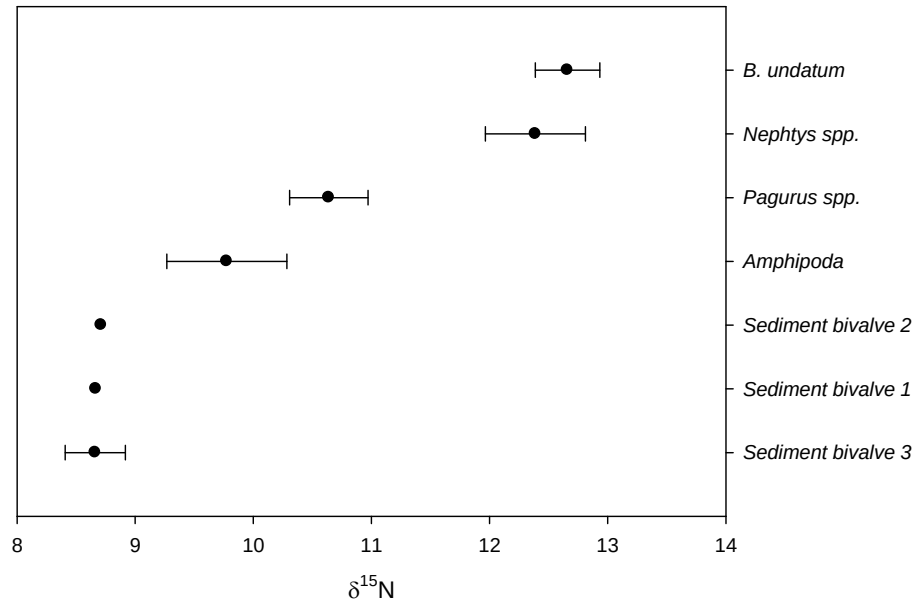


Figure 4.1 Mean $\delta^{15}N$ (‰) of taxa sampled from sediments near to the artificial reef (site NAR). Error bars are ±1 S.D.

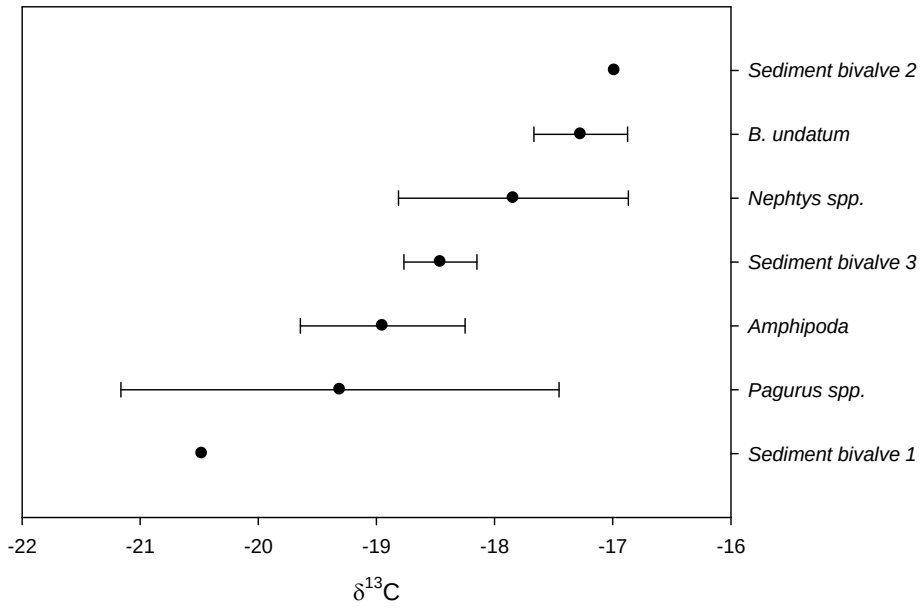


Figure 4.2 Mean $\delta^{13}C$ (‰) of taxa sampled from sediments near to the artificial reef (site NAR). Error bars are ±1 S.D.

4.3.3.2 NNR (Near Natural Reef) Samples

Samples collected from near the natural reef spanned a similar range of $\delta^{15}N$ values (8.72-12.95‰ compared to 8.67-12.66‰ for NAR samples; Table 4.7, Fig.

4.3) as those from near the artificial reef (NAR), with *C. fornicata* representing primary consumers at 8.72‰, and *Nephtys* spp. and *Buccinum undatum* representing secondary consumers at 11.98‰ and 12.95‰ respectively. *Pagurus* spp. and amphipods (similarly to those collected from the NAR site) had intermediate $\delta^{15}N$ values, as did the single individual *Ocenebra erinacea*. For those taxa sampled from both the NAR and NNR sites, the trophic positions were in the same order on both sites (Figs. 4.1 and 4.3) – amphipods had the lowest mean trophic position, with hermit crabs above them, followed by *Nephtys* spp., with *B. undatum* occupying the highest trophic level.

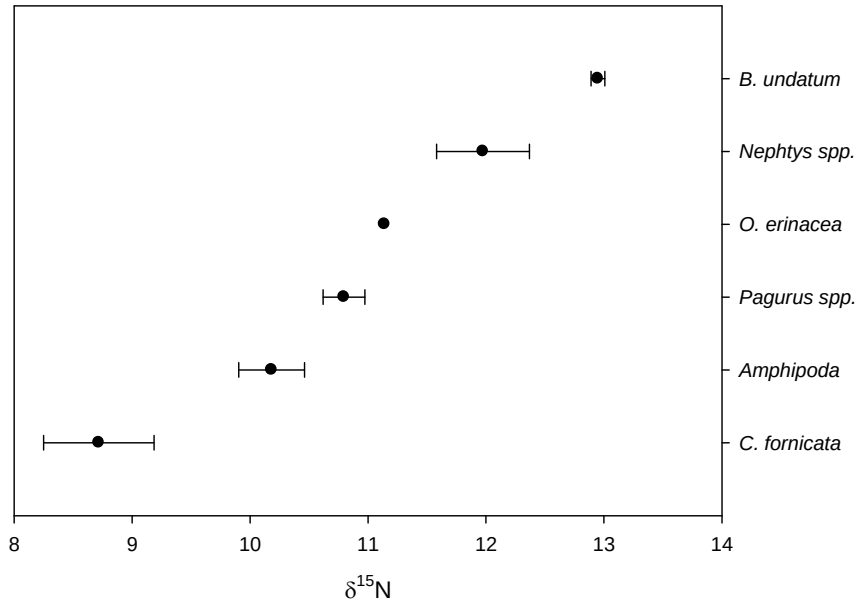


Figure 4.3 Mean $\delta^{15}N$ (‰) of taxa sampled from sediments near to the natural reef (site NNR). Error bars are ±1 S.D.

$\delta^{13}C$ values, similarly to those from the NAR site, seemed to show a pattern of enrichment with increasing trophic level (Fig. 4.4), however in this case closer inspection reveals that several of the secondary consumer species were ^{13}C depleted relative to the single representative primary consumer (*C. fornicata*). This suggests the influence of more than one nutrient source.

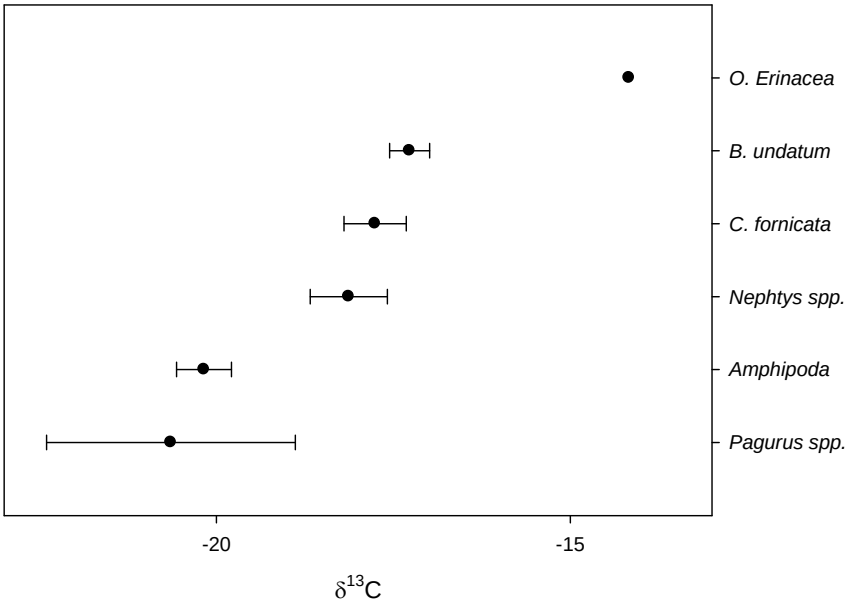


Figure 4.4 Mean $\delta^{13}C$ (‰) of taxa sampled from sediments near to the natural reef (site NNR). Error bars are ±1 S.D.

4.3.3.3 Comparing taxa sampled on both sites

With one exception, none of the four taxa which were common to both sediment sites showed significantly different stable isotope ratios between the two sediment sites (Table 4.8). Amphipods had a significantly higher (more ^{13}C enriched) $\delta^{13}C$ value in the sediment close to the artificial reef (-18.95‰, compared to -20.18‰ for NNR samples, a difference of 1.23‰).

Table 4.8 Statistical comparisons of mean $\delta^{13}C$ and $\delta^{15}N$ values between the two sediment sites. All data normally distributed, except for $\delta^{13}C$ values for *Nephtys* spp.; this comparison was made using the non-parametric Mann-Whitney U test. All other comparisons made using t-test. ***p < 0.001, NS = Not Significant (p > 0.05)

	Comparison	d.f.	t-statistic
Nephtys spp.	$\delta^{13}C$	n/a (n: 7,9)	n/a (Z = 71.00 NS)
	$\delta^{15}N$	14	-2.012 NS
Pagurus spp.	$\delta^{13}C$	3	-0.817 NS
	$\delta^{15}N$	3	0.718 NS
Amphipoda	$\delta^{13}C$	16	-4.622***
	$\delta^{15}N$	16	2.105 NS
B. undatum	$\delta^{13}C$	7	-0.00 NS
	$\delta^{15}N$	7	1.771 NS

4.3.4 Artificial Reef fauna

Table 4.9 Mean $\delta^{13}C$ and $\delta^{15}N$ (‰) for fauna sampled from the artificial reef. *O. erinacea* were found to differ in both $\delta^{13}C$ and $\delta^{15}N$ between the 2007 and 2008 samples (see Chapter 3) so data for both years are presented separately.

	Taxon	n	$\delta^{13}C$	SE	$\delta^{15}N$	SE
Fish	*Labrus bergylta*	7	-18.04	0.12	15.29	0.18
	Spondyliosoma cantharus	1	-15.65	--	17.53	--
	Syngnathus acus	2	-17.97	0.02	13.56	0.16
	Gobius paganellus	1	-18.16	--	13.41	--
Crustacea	*Homarus gammarus*	1	-16.90	--	14.28	--
	Cancer pagurus	7	-16.37	0.22	13.42	0.30
	Maja squinado	1	-16.73	--	14.01	--
	Necora puber	5	-17.44	0.21	12.85	0.28
	Pagurus spp.	13	-20.47	0.24	10.28	0.11
	Palaemon serratus	4	-15.89	1.14	13.93	0.62
	Amphipoda	21	-21.40	0.63	9.95	0.16
	Cirripedia	19	-17.28	0.21	11.00	0.11
Mollusca	*Crepidula fornicata*	19	-17.97	0.13	8.22	0.08
	Ocenebra erinacea 2007	2	-16.53	0.04	11.14	0.20
	Ocenebra erinacea 2008	7	-15.35	0.15	12.57	0.18
	Buccinum undatum	3	-16.52	0.20	12.76	0.66
	Gibbula cineraria	4	-20.08	0.20	10.48	0.30
	Ostrea edulis	4	-19.78	0.09	10.44	0.23
Tunicata	*Styela clava*	4	-21.61	0.25	10.47	0.11
Polychaeta	*Nephtys* spp.	2	-18.92	0.99	12.26	0.89
	Lysidice ninetta	10	-19.77	0.31	11.66	0.30
	Eteone picta	1	-24.85	--	12.06	--
	Platynereis spp.	7	-27.08	0.89	10.22	0.14
	Nereis spp.	6	-27.66	0.60	10.53	0.13
	Other Nereid	11	-20.38	0.94	10.93	0.19
	Sabellaria spinulosa	10	-17.66	0.13	10.42	0.14
	Bispira volutacornis	3	-19.12	0.41	8.92	0.32
Bryozoa	*Flustra foliacea*	1	-22.42	--	8.28	--
Porifera		3	-20.53	0.73	9.84	0.34

4.3.4.1 $\delta^{15}N$ and trophic level of consumers

Invertebrate fauna sampled on the artificial reef had a range of mean $\delta^{15}N$ values (Table 4.9) from 8.22‰ (*Crepidula fornicata*) to 14.28‰ (*Homarus gammarus*, the common lobster), corresponding to a range of 1.76 trophic levels (Fig.4.5). Most invertebrate taxa fell between trophic levels 1 (primary consumers, 8.67‰) and 2 (secondary consumers, 12.17‰). Large crustacea (*H. gammarus, Cancer pagurus*,

Necora puber and *Maja squinado*) had the highest mean $\delta^{15}N$ (14.28‰, 13.42‰, 12.85‰, and 14.01‰ respectively); these were among the highest trophic level invertebrate consumers sampled. Those species of fish that were successfully sampled also had among the highest $\delta^{15}N$ values, with *Labrus bergylta* (15.29‰) and *Spondyliosoma cantharus* (17.53‰) displaying the highest values of all taxa sampled. The taxa with the lowest $\delta^{15}N$ values (those closest to the theoretical trophic position of primary consumers) were *F. foliacea* (8.28‰), *Bispira volutacornis* (8.92‰), and *C. fornicata* (8.22).

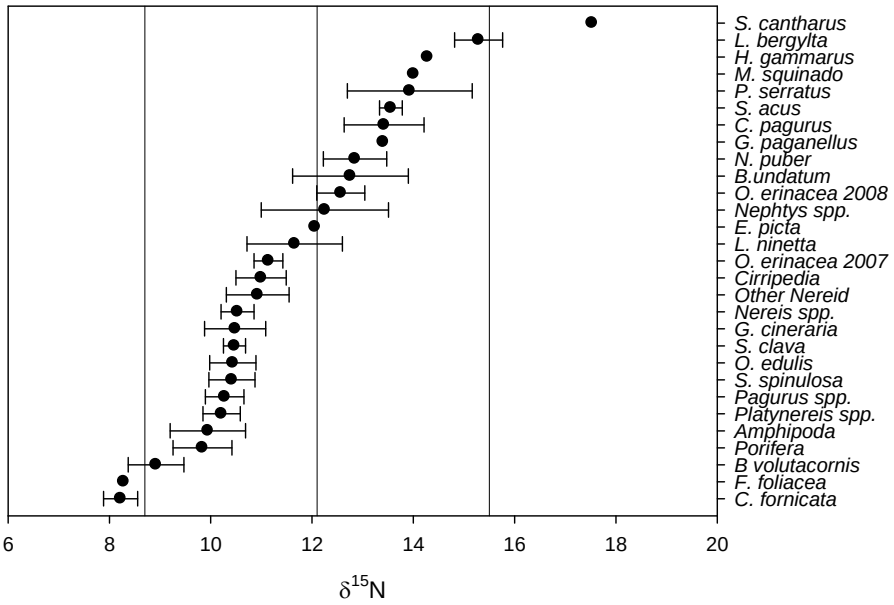


Figure 4.5 Mean $\delta^{15}N$ (‰) of taxa sampled from artificial reef habitat. Error bars are ±1 S.D. Vertical lines indicate the position of theoretical trophic levels of consumers, assuming a $\delta^{15}N$ of 8.7‰ for primary consumers (trophic level 1) and a trophic ^{15}N enrichment of 3.4‰ per trophic level.

4.3.4.2 $\delta^{13}C$ values of consumers

Artificial reef taxa (including fish) showed a wide range of mean $\delta^{13}C$ values, from -27.66‰ to -15.35‰ (Table 4.9, Fig. 4.6). However, most of the taxa sampled had mean $\delta^{13}C$ values in a relatively narrow range between -21‰ and -15‰. Broader taxonomic groupings such as 'Amphipoda' and 'Other Nereids' (a category which included more than one species, but not all sampled members of the Nereidae) showed greater individual variation in $\delta^{13}C$ than other taxa. Unlike for $\delta^{15}N$, reef fish were not the most enriched in $\delta^{13}C$, mostly showing mean $\delta^{13}C$ values around -18‰. However, compared to the more reef-bound fish (*L. bergylta*, *Syngnathus*

acus and *Gobius paganellus*) the single specimen of *Spondyliosoma canthurus* appeared to have a more enriched $\delta^{13}C$ value (-15.65‰).

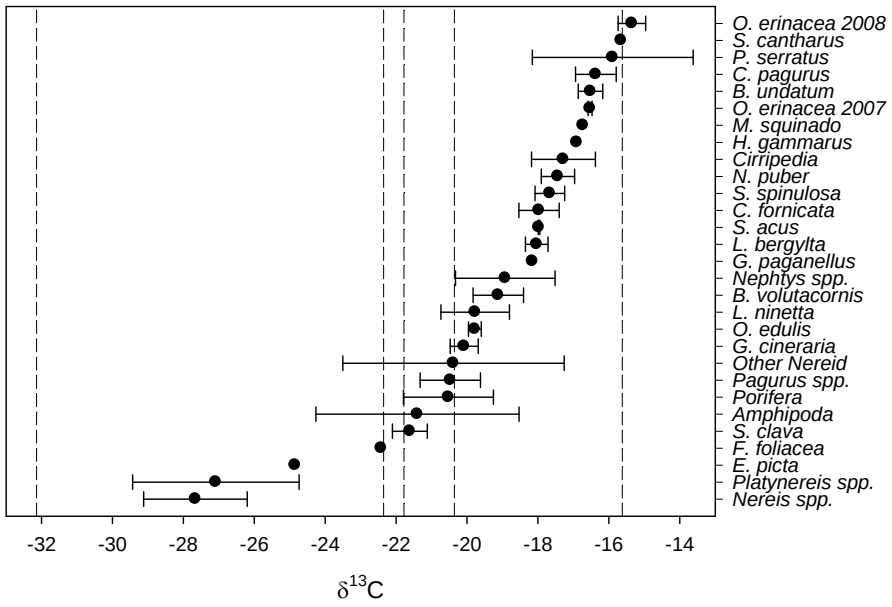


Figure 4.6 Mean $\delta^{13}C$ (‰) of taxa sampled from artificial reef habitat. Error bars are ±1 S.D. Dashed lines mark mean $\delta^{13}C$ values of putative nutrient sources, and are for reference only (-32.14 = Depleted algae group; -22.35 = SOM; -21.78 = Intermediate algae group; -20.36 = POM; -15.62 = Enriched algae group).

4.3.4.3 Cluster analysis

The cluster analysis identified seven non-overlapping clusters at 95% similarity (Table 4.10). The $\delta^{15}N$ and $\delta^{13}C$ values for individuals in each cluster are shown in Fig. 4.7. The clusters do not correspond exactly to any obvious taxonomic divisions, but they may correspond to trophic categories (Table 4.10). Some clusters, however, do contain individuals from taxa assumed to be in different trophic guilds. For example, Cluster 4A contains suspension feeders such as oysters and barnacles, but also grazers such as *G. cineraria*, and hermit crabs (Paguroidea).

Table 4.10 Summary data for artificial reef sample groups determined by hierarchical cluster analysis (95% similarity), including mean stable isotope ratios (with standard deviation in brackets) and taxonomic composition for each cluster.

Cluster	n	$\delta^{13}C$	$\delta^{15}N$		n	Description
1A	12	-28.11	10.40	*Platynereis* spp.	5	Polychaete
		(1.27)	(0.41)	*Nereis* spp.	5	consumers and
				Other Nereid	1	Amphipoda
				Amphipoda	1	(^{13}C depleted)
2A	18	-23.48	9.80	Amphipoda	9	Amphipods and
		(1.25)	(0.88)	*Platynereis* spp.	2	polychaete
				Styela clava	2	consumers
				Pagurus spp.	1	
				Eteone picta	1	
				Nereis spp.	1	
				Other Nereid	1	
				Flustra foliacea	1	
3A	23	-18.01	8.38	*Crepidula fornicata*	19	Suspension
		(0.61)	(0.51)	*Bispira volutacornis*	2	feeders
				Sabellaria spinulosa	2	
4A	50	-19.95	10.63	*Pagurus* spp.	12	Suspension
		(0.80)	(0.77)	Other Nereid	8	feeders and
				Lysidice ninetta	7	secondary
				Amphipoda	6	consumers
				Gibbula cineraria	4	
				Ostrea edulis	4	
				Cirripedia	3	
				Porifera	3	
				Styela clava	2	
				Bispira volutacornis	1	
5A	33	-17.36	10.82	Cirripedia	15	Suspension
		(0.55)	(0.35)	*Sabellaria spinulosa*	8	feeders and
				Amphipoda	5	secondary
				Ocenebra erinacea	2	consumers
				Lysidice ninetta	1	
				Nephtys spp.	1	
				Other Nereid	1	
6A	3	-20.69	12.92	*Lysidice ninetta*	2	Errant
		(0.70)	(0.21)	*Nephtys* spp.	1	polychaetes
7A	29	-16.26	13.10	*Ocenebra erinacea*	7	Higher level
		(1.10)	(0.94)	*Buccinum undatum*	3	consumers:
				Homarus gammarus	1	gastropods/
				Cancer pagurus	7	crustacea
				Necora puber	5	
				Maja squinado	1	
				Palaemon serratus	4	
				Cirripedia	1	

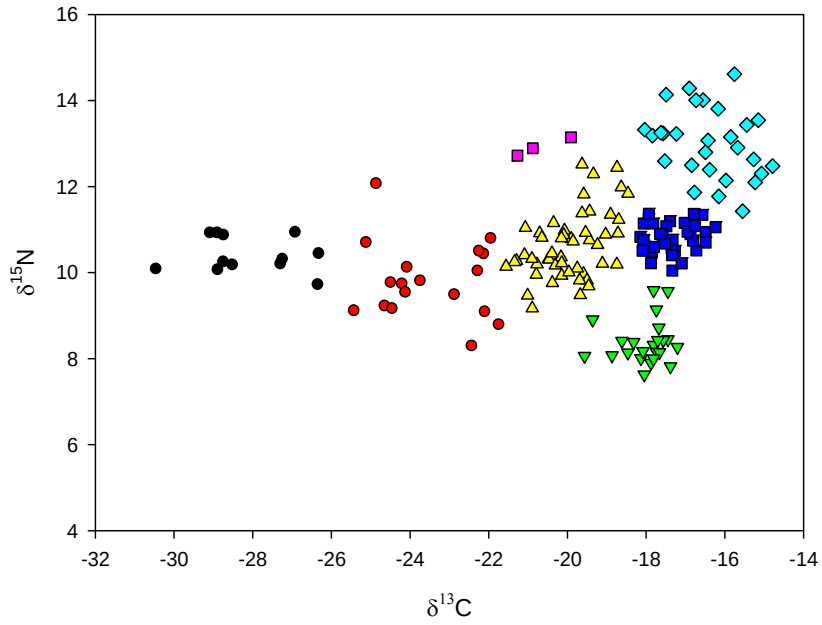


Figure 4.7 Artificial reef invertebrate consumer stable isotope ratios (‰), organised into groups of >95% similarity (determined by hierarchical cluster analysis, see Table 4.10). Black circles: Cluster 1A, Red circles: Cluster 2A, Green triangles: Cluster 3A, Yellow triangles: Cluster 4A, Blue squares: Cluster 5A, Pink squares: Cluster 6A, Cyan diamonds: Cluster 7A.

4.3.4.4 IsoSource modelling

IsoSource models were computed for Clusters 1A to 5A, using the $\delta^{13}C$ values of each of the potential nutrient sources (Tables 4.3 and 4.6). $\delta^{13}C$ values of the nutrient sources were adjusted by +1‰ to compensate for trophic fractionation. Models were run using a 1% increment, and a tolerance of 0.1 (Phillips and Gregg, 2003).

Figure 4.8 displays the range of possible contributions of the five nutrient sources to Clusters 1A to 5A. SOM, POM, and Intermediate Macroalgae were all potentially significant contributors to the diets of Clusters 2A, 3A and 4A, which included a variety of fauna exploiting several feeding modes: suspension feeders, detritivores and algal grazers (SOM, POM and Intermediate Macroalgae had very similar $\delta^{13}C$ values, making it impossible to disentangle their individual contributions). POM was also a potentially significant contributor to 5A, which contained filter feeding organisms. Depleted Macroalgae were a dominant contributor to the diet of Cluster 1A (with other nutrient sources making potentially very small or zero contributions), and may have made some small contribution to Cluster 2A. Finally,

Enriched Macroalgae made potentially very strong contributions to the diet of clusters 3A (*Crepidula fornicata*) and 5A (suspension feeders).

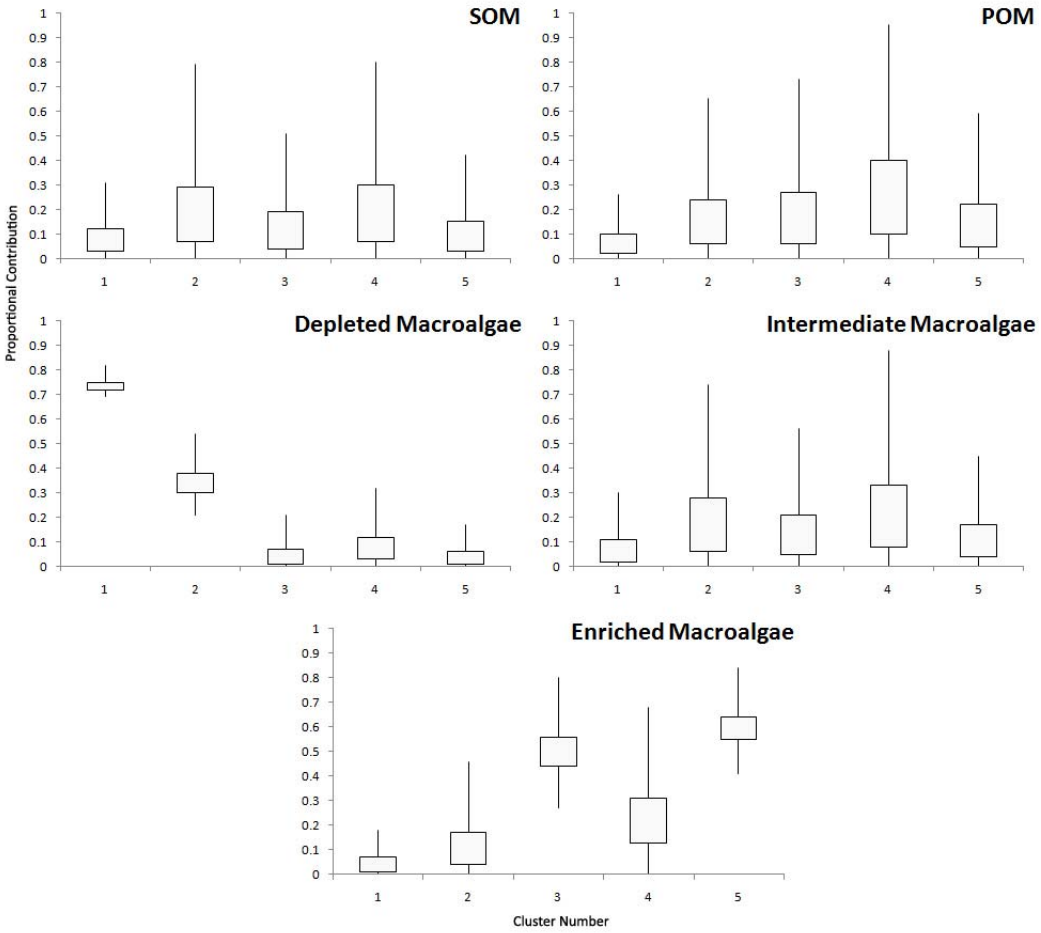


Figure 4.8 Proportional contributions of each of the putative nutrient sources to artificial reef trophic clusters 1A to 5A. Plots indicate full range of feasible contributions, as well as 25th and 75th percentiles.

IsoSource models were also computed for higher level invertebrate consumers (Clusters 6A and 7A), and for *Labrus bergylta*, considering the other consumer clusters as potential dietary sources (Table 4.10). $\delta^{13}C$ values of potential food sources were adjusted by +1‰, and $\delta^{15}N$ values by +3.4‰ to account for trophic fractionation. A 1% increment and tolerance of 0.1 were used for the modelling the diet of Clusters 6A and 7A, and a 2% increment/0.5 tolerance were used for *L. bergylta*.

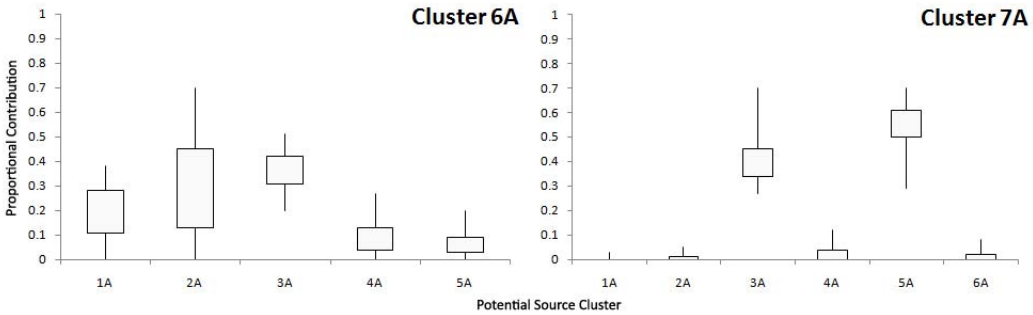


Figure 4.9 Proportional contributions of trophic clusters to the diet of higher level invertebrate consumers (Clusters 6A and 7A) on the artificial reef. Plots indicate full range of feasible contributions, as well as 25th and 75th percentiles.

Members of Cluster 6A (polychaete secondary consumers) appeared to derive some of their diet from the more ^{13}C depleted clusters (1A and 2A). Cluster 3A was also a potentially significant contributor, with 4A and 5A making little, if any contribution (Fig 4.9).

The diet of higher invertebrate consumers in Cluster 7A (predatory gastropods and large crustacea) was dominated by clusters 3A and 5A, which were composed mainly of suspension feeders (polychaetes, barnacles, and *C. fornicata*). Other clusters made little, if any, contribution to the diet of species in Cluster 7A.

Fish (*L. bergylta*) appeared to derive much of their diet from Clusters 4A to 7A (with wide margins of uncertainty about the exact contributions of each of these clusters), with little (if any) contribution of Clusters 1A, 2A and 3A (Fig 4.10)

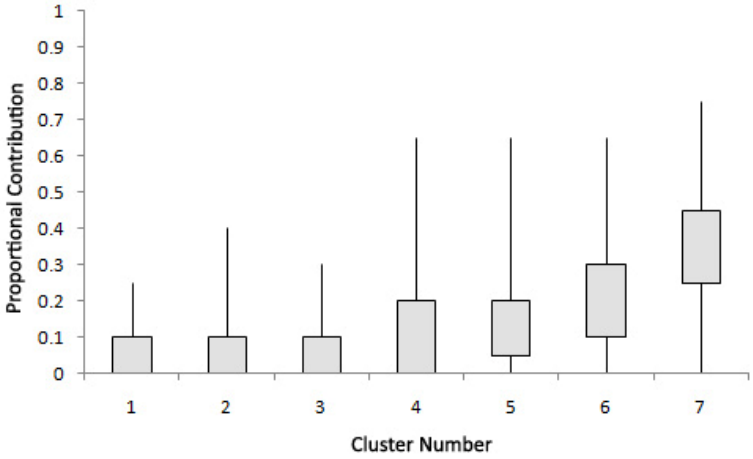


Figure 4.10 Proportional contributions of Clusters 1A to 7A to the diet of reef fish (*L. bergylta*). Plots indicate full range of feasible contributions, as well as 25th and 75th percentiles.

4.3.5 Natural reef fauna

Table 4.11 Mean isotope ratio ($\delta^{13}C$ and $\delta^{15}N$) data expressed in permil (‰) for fauna sampled from the natural reef system. *O. edulis* and *S. clava* are both divided into two size classes.

	Taxon	n	$\delta^{13}C$	SE	$\delta^{15}N$	SE
Crustacea	*Maja squinado*	1	-17.55	--	12.32	--
	Necora puber	3	-17.16	0.14	12.70	0.58
	Pagurus spp.	29	-20.42	0.19	10.41	0.10
	Amphipoda	24	-22.95	0.78	10.09	0.16
	Cirripedia	15	-16.98	0.47	11.01	0.18
Mollusca	*Crepidula fornicata*	10	-17.48	0.13	8.44	0.06
	Ocenebra erinacea	8	-15.68	0.12	11.69	0.22
	Buccinum undatum	3	-16.83	0.10	12.31	0.19
	Gibbula cineraria	2	-20.04	0.28	9.59	0.04
	Ostrea edulis (large)	5	-19.52	0.15	10.16	0.11
	Ostrea edulis (small)	1	-19.80	--	8.72	--
Tunicata	*Styela clava* (large)	3	-19.53	0.49	5.49	0.39
	Styela clava (small)	1	-22.19	--	10.89	--
Polychaeta	*Lysidice ninetta*	2	-19.77	0.37	12.92	0.18
	Typosyllis prolifera	1	-17.94	--	13.08	--
	Sigalionidae	6	-19.40	0.28	12.29	0.16
	Marphysa spp.	3	-21.72	1.45	11.53	0.33
	Eulalia aurea	1	-16.37	--	12.79	--
	Platynereis spp.	5	-29.58	0.53	10.68	0.10
	Other Nereid	5	-27.11	2.01	11.95	0.20
	Eupolymnia nebulosa	3	-19.97	0.64	10.46	0.23
	Terebellidae	4	-20.81	0.87	10.89	0.25
	Sabellaria spinulosa	10	-18.09	0.19	9.99	0.18
	Sabellidae	1	-20.52	--	8.90	--
	Bispira volutacornis	5	-18.99	0.20	8.92	0.08
Porifera		3	-20.20	0.71	10.57	0.34

4.3.5.1 $\delta^{15}N$ and trophic level of consumers

No fish were sampled from the natural reef, but the range of isotope values for invertebrate consumers was similar to that on the artificial reef (Table 4.11; Fig. 4.11): from 8.44‰ (*C. fornicata*) to 13.08‰ (*Typosyllis prolifera*), a spread of 1.36 trophic levels. Large crustacea (*M. squinado* and *N. puber*) had similar $\delta^{15}N$ values to those collected from the artificial reef ($\delta^{15}N$ values for *N. puber* were not found to differ significantly between the two sites; see Section 3.3.1.2). Species with $\delta^{15}N$ values very close to those of theoretical primary consumers were *B. volutacornis*,

C. fornicata (similar to conspecifics sampled from the artificial reef) *Ostrea edulis* (small size class, one individual), and a single individual of the family Sabellidae. The large size class of the tunicate *Styela clava* had an unusually low mean $\delta^{15}N$ (5.49‰) while the smaller size class was at a higher trophic level (see Section 3.4.2.2). Again, similar to the artificial reef, the bulk of consumer taxa fell somewhere between the isotope ratios of theoretical primary and secondary consumers (Fig4.11).

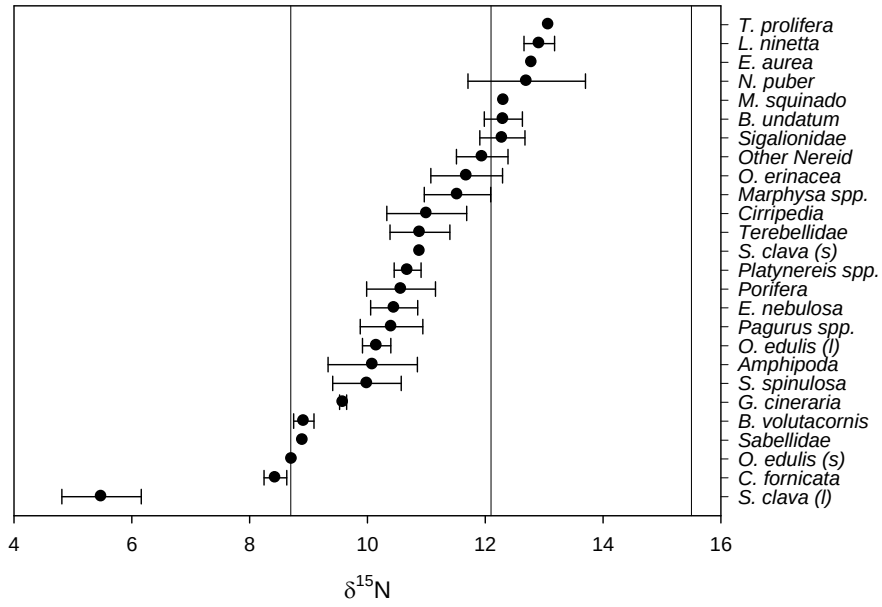


Figure 4.11 Nitrogen stable isotope ratios (expressed in ‰) of taxa sampled from natural reef habitat. Error bars are ±1 S.D. Vertical lines indicate the position of theoretical trophic levels of consumers, assuming a $\delta^{15}N$ of 8.7‰ for primary consumers (trophic level 1) and a trophic ^{15}N enrichment of +3.4‰ per trophic level.

4.3.5.2 $\delta^{13}C$ values of consumers

Natural reef taxa also showed a wide range of mean $\delta^{13}C$ values, from -29.58‰ to -15.68‰ (Fig. 4.12), similar to those on the artificial reef, despite the absence of fish sampling on this site. Again, the bulk of consumer taxa had mean $\delta^{13}C$ values between -21‰ and -15‰. The taxonomic categories 'Amphipoda' and 'Other Nereid' showed much greater individual variation in $\delta^{13}C$ than other taxa, just as they did on the artificial reef.

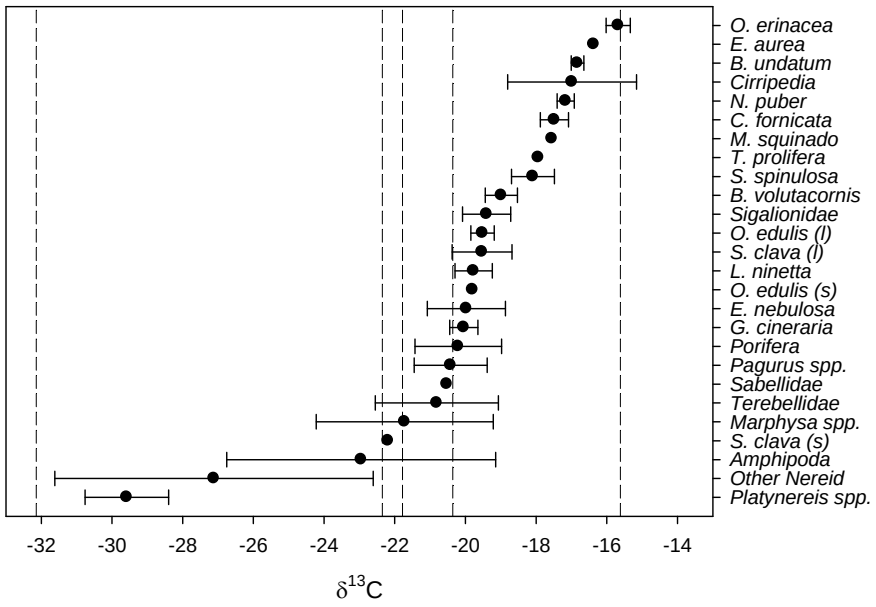


Figure 4.12 Carbon stable isotope ratios (expressed in ‰) of taxa sampled from natural reef habitat. Error bars are ±1 S.D. Dashed lines mark mean $\delta^{13}C$ values of putative nutrient sources, and are for reference only (-32.14 = Depleted algae group; -22.35 = SOM; -21.78 = Intermediate algae group; -20.36 = POM; -15.62 = Enriched algae group).

4.3.5.3 Cluster analysis

In total, 10 clusters were identified at 95% similarity (Table 4.12; Fig. 4.13). Similarly to the artificial reef faunal clusters, there was some correspondence with taxonomic divisions, but the groups mainly appeared to match trophic divisions.

Table 4.12 Summary data for natural reef sample groups determined by hierarchical cluster analysis (95% similarity), including mean stable isotope ratios (with standard deviation in brackets) and taxonomic composition for each cluster.

Cluster	n	$\delta^{13}C$	$\delta^{15}N$		n	Description
1N	14	-28.98	10.67	*Platynereis* spp.	5	Amphipods and
		(1.97)	(0.98)	Other Nereid	4	polychaetes
				Amphipoda	5	(^{13}C depleted)
2N	9	-24.05	10.06	Amphipoda	8	Amphipods
		(0.58)	(0.71)	*Marphysa* spp.	1	
3N	13	-21.99	10.79	*Pagurus* spp.	7	Hermit crabs
		(0.35)	(0.73)	Amphipoda	2	and other
				Terebellidae	2	consumers
				Marphysa spp.	1	
				Styela clava (small)	1	
4N	8	-19.51	12.62	Sigalionidae	4	Errant
		(0.40)	(0.27)	*Lysidice ninetta*	2	polychaetes
				Other Nereid	1	
				Cirripedia	1	
5N	7	-16.75	12.70	*Buccinum undatum*	3	Higher level
		(0.32)	(0.51)	*Necora puber*	2	consumers:
				Eulalia aurea	1	gastropods
				Ocenebra erinacea	1	and crustacea
6N	8	-15.69	11.40	*Ocenebra erinacea*	7	Predatory
		(0.36)	(0.60)	Cirripedia	1	gastropods
7N	22	-17.54	11.21	Cirripedia	12	Suspension
		(0.48)	(0.70)	*Sabellaria spinulosa*	3	feeders and
				Amphipoda	2	secondary
				Maja squinado	1	consumers
				Necora puber	1	
				Pagurus spp.	1	
				Typosyllis prolifera	1	
				Sigalionidae	1	
8N	58	-19.64	10.11	*Pagurus* spp.	21	Suspension
		(0.87)	(0.69)	*Sabellaria spinulosa*	7	feeders and
				Amphipoda	7	secondary
				Ostrea edulis	6	consumers
				Bispira volutacornis	4	
				Eupolymnia nebulosa	3	
				Porifera	3	
				Gibbula cineraria	2	
				Terebellidae	2	
				Sabellidae	1	
				Sigalionidae	1	
				Marphysa spp.	1	
9N	11	-17.56	8.46	*Crepidula fornicata*	10	*C. fornicata*
		(0.45)	(0.19)	*Bispira volutacornis*	1	
10N	3	-19.53	5.49	*Styela clava* (large)	3	
		(0.84)	(0.67)			

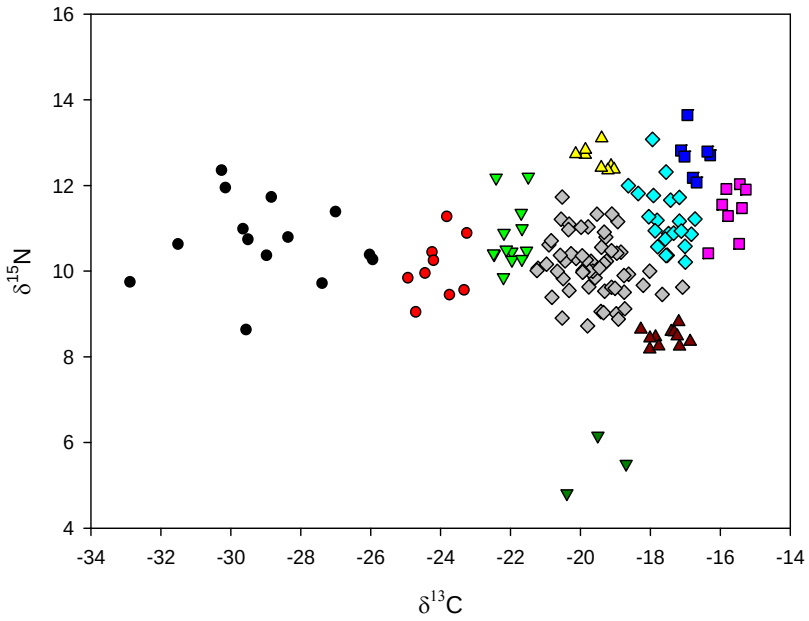


Figure 4.13 Natural reef invertebrate consumer stable isotope ratios (‰), organised into groups of >95% similarity. Black circles: Cluster 1N, Red circles: Cluster 2N, Bright green triangles: Cluster 3N, Yellow triangles: Cluster 4N, Blue squares: Cluster 5N, Pink squares: Cluster 6N, Cyan diamonds: Cluster 7N, Grey Diamonds: Cluster 8N, Brown triangles: Cluster 9N, Dark Green triangles: Cluster 10N.

A number of the clusters identified by the cluster analysis appeared to correspond to those from the artificial reef: both in terms of their taxonomic composition and in terms of their mean $\delta^{13}C$ and $\delta^{15}N$ values. These pairs of corresponding clusters were compared, and it was found that in all but one case, the artificial and natural reef clusters did not have significantly different mean $\delta^{15}N$ and $\delta^{13}C$ values (Table 4.13). The one difference was between the $\delta^{15}N$ of clusters 4A and 8N (secondary consumers, detritivores, and filter feeders): the artificial reef cluster had a higher value, though only by 0.52‰.

Three clusters had no obvious counterpart from the artificial reef. Cluster 10N was composed solely of the larger size class of *S. clava*, (only small individuals of this species were collected on the artificial reef). 6N was composed mainly of the predatory gastropod *O. erinacea*, which was placed largely within cluster 7A on the artificial reef, rather than being placed in its own cluster. Finally, Cluster 3N was composed largely of *Pagurus* spp. and a few polychaetes.

Table 4.13 Comparisons between artificial and natural reef consumer groups. Data correspondence to normal distribution tested using Shapiro-Wilk test. Test statistics: t (where t-test used for normally distributed data) or U (where Mann-Whitney U test used for non-normally distributed data[1]). *$p < 0.05$; NS = Not Significant ($p > 0.05$) following sequential Bonferroni correction (Rice, 1989).

Comparison		Data normal?	Mean difference	d.f. (n)	Test statistic
1A vs. 1N	$\delta^{13}C$	Yes	0.874	24	1.317 NS
	$\delta^{15}N$	Yes	0.274		-0.902 NS
2A vs. 2N	$\delta^{13}C$	Yes	0.573	25	1.296 NS
	$\delta^{15}N$	Yes	0.254		-0.750 NS
3A vs. 9N	$\delta^{13}C$	No [1]	0.407 (median)	(11, 23)	72.000 NS
	$\delta^{15}N$	No [1]	0.191 (median)		81.000 NS
4A vs. 8N	$\delta^{13}C$	Yes	0.311	106	-1.931 NS
	$\delta^{15}N$	Yes	0.518		3.698 *
5A vs. 7N	$\delta^{13}C$	Yes	0.183	53	1.261 NS
	$\delta^{15}N$	No [1]	0.224 (median)	(22, 33)	248.000 NS
6A vs. 4N	$\delta^{13}C$	Yes	1.184	9	-3.616 NS
	$\delta^{15}N$	Yes	0.293		-1.688 NS
7A vs. 5N	$\delta^{13}C$	Yes	0.488	34	1.145 NS
	$\delta^{15}N$	Yes	0.404		-1.087 NS

4.3.5.4 IsoSource Modelling

IsoSource models were computed for Clusters 1N, 2N, 9N, 8N and 7N (the natural reef counterparts of clusters 1A to 5A – see above and Table 4.13), using the $\delta^{13}C$ values of each of the potential nutrient sources (Tables 4.3 and 4.6). $\delta^{13}C$ values of the nutrient sources were adjusted by +1‰ to compensate for trophic fractionation. Models were run using a 1% increment, and with a tolerance of 0.1.

Again, unique solutions to mixing equations were not possible, and therefore ranges of possible contributions are presented (Fig. 4.14). The contributions of each of the five nutrient sources to each of the consumer clusters were substantially similar to those on the artificial reef (compare Fig. 4.14 with Fig. 4.8). SOM, POM, and Intermediate Macroalgae were potentially significant contributors to the diets of Clusters 2N, 9N, 8N and 7N (although SOM and Intermediate Macroalgae may have been less important to Clusters 9N and 7N). These clusters included a variety of fauna exploiting several feeding modes: suspension feeders, detritivores and algal grazers (Table 4.12). SOM, POM and Intermediate Macroalgae had very similar $\delta^{13}C$ values, making it impossible to disentangle their

individual contributions. Depleted Macroalgae were a dominant contributor to the diet of Cluster 1N (with other nutrient sources making potentially very small or zero contributions), and may also have contributed significantly to the nutrient intake of Cluster 2N. Finally, Enriched Macroalgae made potentially very strong contributions to the diet of clusters 9N and 7N, which were largely composed of suspension feeders.

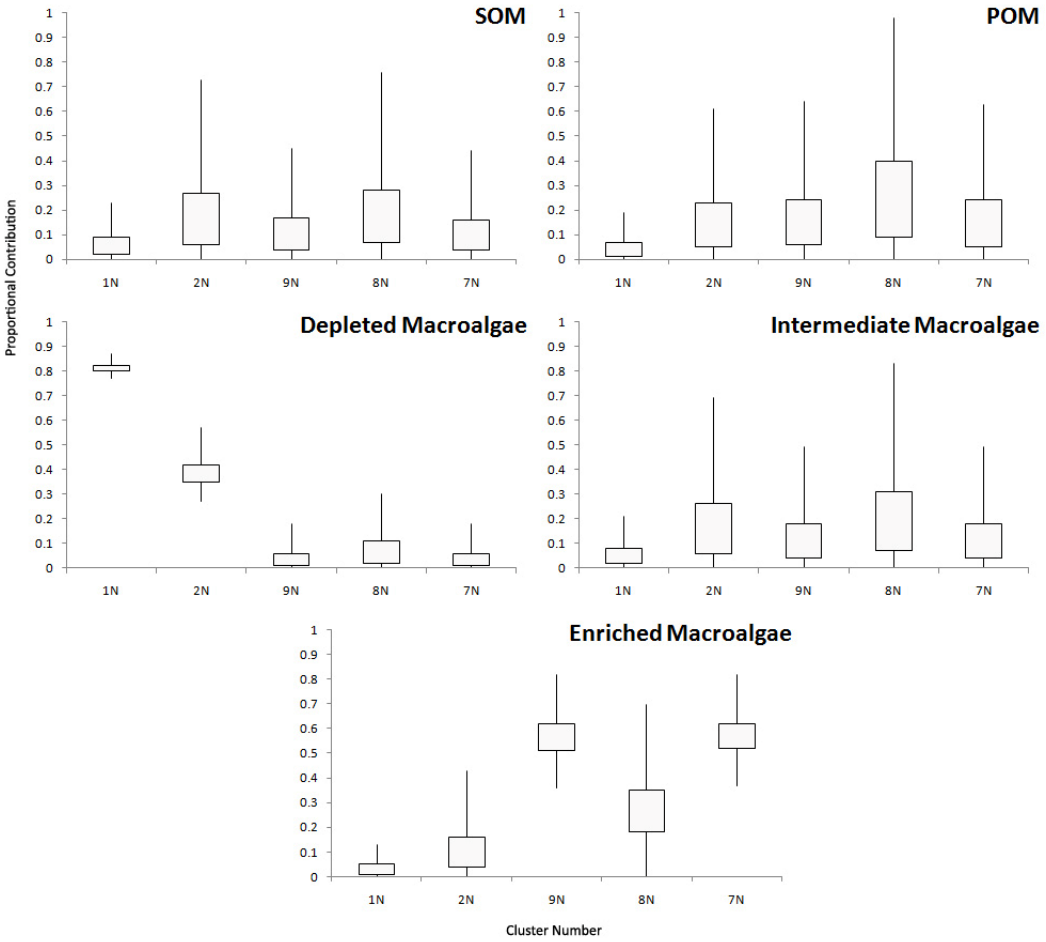


Figure 4.14 Proportional contributions of each of the putative nutrient sources to natural reef trophic clusters. Plots indicate full range of feasible contributions, as well as 25th and 75th percentiles.

4.4 Discussion

4.4.1 Reef nutrient sources

Given the wide ranges of mean $\delta^{13}C$ values of consumer taxa, it is clear that multiple nutrient sources were important on both sites. Furthermore, these ranges were similar on both reef systems (-27.66‰ to -15.35‰ on the artificial reef and -29.58‰ to -15.68‰ on the natural reef) with the majority of invertebrate taxa having values between -21‰ and -15‰. This is a similar range to that of invertebrate consumers reported in other studies of artificial (Schaal et al., 2008) and natural (Carlier et al., 2007) shallow-water coastal ecosystems.

The presence of consumers with more negative $\delta^{13}C$ values (those in Clusters 1A, 2A, 1N and 2N) means that a similarly ^{13}C depleted nutrient source must be available. The 'Depleted Macroalgae' was the only identified nutrient source to meet this criterion. This source consisted of several taxa of red algae which were found growing on both the natural and artificial reef systems. It is clear that algae growing on these reefs contributed to the diet of some consumers. Other isotope studies have identified macroalgae as an important nutrient source where present (Richoux and Froneman, 2007; Sara et al., 2007).

The more enriched $\delta^{13}C$ of some primary/secondary consumer clusters, particularly 3A (-18.01‰), 5A (-17.35‰), 9N (-17.56‰) and 7N (-17.54‰) also indicated a potentially important contribution of a more enriched nutrient source, confirmed by the potentially large contribution of the 'Enriched Macroalgae' source to the diets of these four clusters (Figs. 4.8 and 4.14). This nutrient source (Table 4.6) consisted of snagged algae (laminarians which do not grow on the reefs themselves, and were only found on the artificial reef as a result of its tendency to trap drifting algae) and the green alga, *Ulva lactuca*, which may be present as a result of growth on the reef as well as trapping by the artificial reef structure. However, this group of algae seem unlikely candidates for the diets of Clusters 3A, 5A, 9N and 7N, which are composed mainly of suspension feeders. Furthermore, despite the fact that snagged algae were sparse on the natural reef, natural reef

clusters 9N and 7N did not differ isotopically from their artificial reef counterparts (Table 4.13).

There are two potential explanations. The first is that there is another source of primary production on both reefs, not sampled during this project, with a similar $\delta^{13}C$ value to that of the Enriched Macroalgae group. Diatoms, for example, have been found to have relatively enriched $\delta^{13}C$ values (Fry and Wainright, 1991) making them a potential candidate, along with other microphytobenthos (Kang et al., 2007). The second possibility is that the assumed trophic fractionation for carbon (+1‰) was not correct. Further field sampling would be required to investigate the first possibility, whereas the second would require detailed species-by-species experimental work to address.

Discrimination of the importance of the three other nutrient sources (SOM, POM and Intermediate Macroalgae) was not possible, because of their similar mean $\delta^{13}C$ values (-22.35‰, -21.78‰, and -20.36‰ respectively), which led to large uncertainties in the IsoSource model outputs (see Figs. 4.8 and 4.14). Source $\delta^{15}N$ values might have been included in the analysis to reduce these uncertainties, but this was not appropriate, because the $\delta^{15}N$ of most consumers was clearly not 3.4‰ greater than that of the SOM, POM or any macroalgae. This initial trophic step, from primary producers to primary consumers, is the one most likely to violate the standard trophic fractionation assumptions (Crawley et al., 2007). In any case, some combination of these three sources was certainly of importance to reef fauna, since most consumer taxa had mean $\delta^{13}C$ values that were enriched by 1-7‰ relative to these sources (Figs 4.6 and 4.12). This 'group' of sources was also a potentially significant contributor to almost every cluster of primary consumers (Fig. 4.8). Macroalgae, as previously stated, can certainly be an important source of nutrient input to some food webs, while SOM and POM are also commonly important sources in isotope studies of marine ecosystems (Carlier et al., 2007; Sara et al., 2007; Schaal et al., 2008).

4.4.2 Trophic levels

Consumer taxa on both reefs showed a smooth progression in $\delta^{15}N$ values (Figs. 4.5 and 4.11), with wide overlaps in the values for some taxa. Both of these properties are indicative of relatively complex food webs, with numerous trophic linkages and dietary overlap between taxa (Richoux and Froneman, 2007).

Another relatively simple way of assessing the relative complexity of food webs is to compare the total length of their food chains (Cabana and Rasmussen, 1996; Schaal et al., 2008). This is particularly simple to estimate using isotope methods; since $\delta^{15}N$ can be used to estimate trophic level, the range of $\delta^{15}N$ values of consumers can be used as a proxy measure of food chain length. Considering only invertebrate taxa (because fish tended to have higher $\delta^{15}N$ and were only sampled on the artificial reef), the range of mean $\delta^{15}N$ values for the artificial reef was 8.22-14.28‰ (6.06‰; 1.76 trophic levels), compared to 8.44-13.08‰ on the natural reef (4.64‰; 1.36 trophic levels). This does appear to indicate a longer food chain on the artificial reef. However, some of the taxa with the highest mean $\delta^{15}N$ values were also not sampled from the natural reef (*H. gammarus*, *P. serratus*, and *Cancer pagurus*) and furthermore, the values for some of those taxa were obtained from very low sample sizes or even single individuals. The range of $\delta^{15}N$ values for another study of consumers on a marine artificial habitat was similar in magnitude to that on the artificial reef sampled here (6.10‰, 1.79 trophic levels), although the actual $\delta^{15}N$ values were lower (Schaal et al., 2008), perhaps resulting from differences in the trophic baselines (Cabana and Rasmussen, 1996) between Poole Bay and the site studied by those authors.

4.4.3 Trophic structure and diet of higher level consumers

Figure 4.15 shows a schematic representation of a possible structure of the artificial reef food web. Each nutrient source contributed to one or more of the trophic clusters, and several of the clusters may have exploited nutrients derived from more than one source.

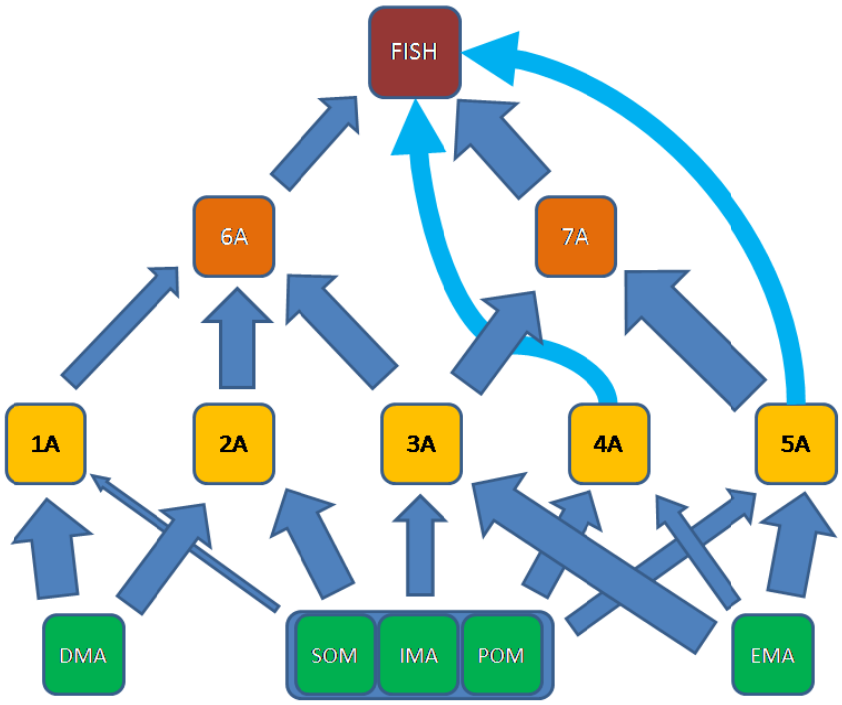


Figure 4.15 Possible configuration of artificial reef food web, based on results of IsoSource mixing models. Green boxes represent potential nutrient sources; DMA = Depleted Macroalgae, SOM = Sedimentary Organic Matter, IMA = Intermediate Macroalgae, POM = Particulate organic Matter, EMA = Enriched Macroalgae. SOM, IMA and POM are grouped together: the similarity of their isotope ratios makes it impossible to separate their contributions. Numbered boxes represent clusters identified by a hierarchical cluster analysis. Thicknesses of arrows indicate likely strength of contributions; however these are only qualitatively judged and are not intended to accurately represent the available data.

Analysis of the nutrient sources for the lowest trophic level consumer clusters was complicated by the similarity of $\delta^{13}C$ values for POM, SOM, and Intermediate Macroalgae. Higher level consumers on the artificial reef were assumed to derive their diet from one or more of the lower trophic level clusters (1A to 5A). For this trophic step, a $\delta^{15}N$ fractionation around +3.4‰ seemed more reasonable, as the $\delta^{15}N$ values for Clusters 6A (predatory polychaetes) and 7A (large crustaceans and predatory gastropods) were 2.1-4.63‰ enriched compared to those of the lower trophic level clusters (1A to 5A). Therefore, both $\delta^{15}N$ and $\delta^{13}C$ were used in the IsoSource model, reducing the uncertainties in the estimates of consumer diets (Fig. 4.9).

Consumers in Cluster 6A (predatory polychaetes) clearly derived a proportion of their diet from the more $\delta^{13}C$ depleted clusters (1A and 2A, consisting mainly of amphipods and polychaetes); potentially incorporating some of the production from red algae growing on the reefs ('Depleted Macroalgae', an important nutrient source for clusters 1A and 2A) into higher trophic levels. Consumers in Cluster 7A (crustaceans and predatory gastropods) appeared to derive much of their diet from suspension feeding taxa in Clusters 3A and 5A (Fig. 4.9).

Finally, the highest level consumers on the reef, fish (here represented by *L. bergylta*) clearly derived some of their diet from Clusters 6A and 7A, but also from 4A and 5A: this includes hermit crabs, other polychaetes, barnacles, and gastropods. This is certainly consistent with the expected diet of fish on artificial reefs (Relini et al., 2002a; Sanchez-Jerez et al., 2002).

The pattern of clustering was broadly similar between the two reefs, with all clusters on the artificial reef having a natural reef 'counterpart' (Table 4.13). The outputs from IsoSource models of the contributions of nutrient sources to consumer clusters on both reefs also appeared very similar (Figs. 4.8 and 4.14). Clearly the same nutrient sources were important on both reef systems, and made similar contributions to both reef food webs. There were a few minor differences in the pattern of clustering between the two sites, namely that the cluster analysis of the natural reef fauna identified three groups which had no counterparts on the artificial reef. One of these was solely composed of the large size class of *S. clava* (only small *S. clava* were sampled on the artificial reef, and these have different isotope ratios to large individuals; Section 3.4.2.2). The other two clusters which had no artificial reef equivalents were made up largely of taxa which were present on both reefs. Cluster 3N contained amphipods and hermit crabs (most *Pagurus* spp. were, however, placed in Cluster 8N), but also representatives of polychaete taxa not sampled from the artificial reef. The inclusion of these polychaetes, perhaps along with individual hermit crabs with more negative $\delta^{13}C$, may have been enough to result in the identification of this additional cluster by the software. Finally, Cluster 6N was almost solely composed of *O. erinacea*. This species was one of the only taxa for which a statistically significant (and observably

large) difference in isotope ratios between the two sites was detected. These data do not suggest an obvious cause of this difference.

4.4.4 Species-independent analysis

A species-by-species analysis of dietary sources using IsoSource would have been extremely time-consuming, and of dubious worth. For higher trophic level consumers in particular, the range of potential prey organisms would have meant the inclusion of a very large number of potential food sources in the IsoSource mixing models. The description of food webs involving numerous taxa is sometimes simplified by clustering taxa on the basis of their mean stable isotope ratios (Carlier et al., 2007; Richoux and Froneman, 2007). However, this may be problematic because of two effects: individual diet variation, and size-based isotope ratio shifts. Consumer diets can vary substantially, and individuals within a species can have isotope values as much as 2‰ different, even on the same diet (DeNiro and Epstein, 1978). Amphipods and some polychaetes in this project showed large variation around their mean $\delta^{13}C$ values (Figs. 4.6 and 4.12). If there is spatially-driven variation in the diets of consumers, then the mean isotope ratios for those taxa could suffer from severe sampling biases. There is also evidence that isotope ratios are in some cases affected by size (Jennings et al., 2001; Hoeinghaus and Davis, 2007). For the two taxa in this analysis where sample sizes were large enough to explore possible size effects (*C. fornicata* and *Pagurus* sp.), effects of size on isotope ratios were found (Sections 3.3.1.1 and 3.3.2.1). Size effects were also observed for *S. clava* (Section 3.3.5). If sampling is size-biased, this can therefore impact the reported mean isotope ratios. Where there is a large isotope shift between size classes (such as for *S. clava*) large individuals may be more trophically similar to members of other species than to smaller conspecifics. A clustering strategy based on mean isotope ratios for taxa may thus generate a misleading picture of the food web.

Clustering on the basis of individual isotope data avoids problems resulting from size-biased sampling and spatial variation in diet, while producing a manageable number of groups of trophically similar individuals, which can then be compared

among habitats or used in IsoSource modelling. For example, Jennings (2001) found that using data from individual fish revealed strong size-based trophic structuring of fish communities, which was not evident in a taxonomically defined analysis.

4.4.5 Sediment community results

While no significant differences were detected between taxa adjacent to either reef, it is of interest that a difference was found between the $\delta^{15}N$ of sediments close to the reef and distant from the reef (Section 4.3.2.2). Those closest to the reefs were ^{15}N enriched, which is consistent with studies showing ^{15}N enrichment as a result of nutrient inputs, such as human urban waste (Riera et al., 2000) or fish farm waste (Gao et al., 2006; Dolenec et al., 2007). This might provide a way of investigating 'reef effects' on surrounding sediment communities.

4.4.6. A 'reef signature' for fish?

Only one individual fish was collected that might be considered a member of an off-reef, schooling species, *Spondyliosoma cantharus* (black bream). This individual had the highest $\delta^{15}N$ and least negative $\delta^{13}C$ of any fish sampled (Table 4.9; Figs. 4.7 and 4.8), indicating a trophic position higher than, and dietary composition distinct from, any taxa of reef fish. If the stable isotope ratios (particularly for carbon) of reef and off-reef fish are distinct (as this result suggests), then this raises the possibility of using isotope ratios to determine what contribution (if any) artificial reef food resources make to the diets of fish which are attracted by these structures. If aggregated fish opportunistically feed on artificial reefs, then their isotope ratios might be expected to be intermediate between those of fully reef-based and fully pelagic or demersal individuals. Of course, this speculation is based on one individual, but further investigation is warranted into the stable isotope ratios of off-reef fish and their potential food sources.

4.4.7 Limitations of IsoSource models

4.4.7.1 Dealing with multiple sources

Where there are larger numbers of food sources, mixing models can only reliably estimate the ranges of potential contributions, as reported here (Sections 4.3.5.4 and 4.3.4.4). There are two means of reducing the uncertainty and constraining the model outputs.

Using another isotope system: If stable isotope ratio data concerning other elements are available for the same samples, then these can be incorporated into the model, potentially allowing unique solutions to be found (Phillips and Gregg, 2001), but only if the number of sources is less than or equal to *n*+1 (where *n* is the number of isotope ratios measured). In this project, $\delta^{15}N$ was measured for each sample, and where appropriate, this was used in IsoSource modelling, alongside $\delta^{13}C$. However, in the case of higher consumers in this investigation, there were five potential groups of prey, and wide bounds of uncertainty remained. Of course, further isotope systems might be added (such as $^{34}S/^{32}S$ or $^{18}O/^{16}O$) to reduce uncertainty, but this would entail additional analytical expense and, again, would only be useful if there was a significant difference in the ratios of these isotopes between potential sources (Peterson, 1999).

Applying non-isotopic constraints: Another means of constraining the potential ranges of outcomes from mixing models is to use related scientific knowledge to adjust the analysis (Phillips and Gregg, 2003). Data from other types of dietary study (such as stomach content analysis) can be used in this manner, if for example, they categorically rule out one potential food source.

4.4.7.2 Trophic fractionation

The key assumption which underpins the use of models such as IsoSource is that trophic fractionation values (the difference in isotope ratios between diet and consumer) conform to standard estimates. In order to run the models, fractionation values of +1‰ (for carbon) and +3.4‰ (for nitrogen) were assumed, and the isotope ratios of sources were adjusted accordingly, prior to running the

models. Unfortunately, despite the widespread use of such estimates in the literature, there is plenty of evidence to indicate that the use of these standard values is flawed. Studies have repeatedly demonstrated that not only does the fractionation vary among species (Minagawa and Wada, 1984; Dubois et al., 2007), but it can also vary between tissues in a single individual (Pinnegar and Polunin, 1999; Yokoyama et al., 2008), as well as between conspecific individuals on different diets (Crawley et al., 2007). A large amount of work would be required to determine specific fractionation values for all possible consumer-diet combinations, so some degree of assumption is essential and is likely to remain so.

4.4.8 Chapter Conclusions

The results outlined in Chapter 3 indicated that, in terms of the stable isotope ratios of consumer taxa, there were few differences between the natural and artificial reef sites investigated here. This suggested common nutrient sources, and common trophic positions for consumers present on both sites.

The results of this chapter are broadly supportive of this conclusion. A similar range of species were collected from both sites, and similar numbers of individual animals were also collected. Fauna sampled from both reefs also displayed a similar range of $\delta^{15}N$ and $\delta^{13}C$ values. Cluster analyses carried out on faunal isotope ratios from both reef systems identified similar 'trophic clusters' on both sites, and statistical comparison of clusters from the two sites failed to detect large significant differences. Sedimentary organic matter, plankton, and various groups of macroalgae all contributed to reef food webs, though the uncertainties involved in the analyses make their relative importance unclear. In agreement with Schaal et al. (2008), no justification was found for declaring the artificial reef food web to be significantly simplified relative to that associated with a natural habitat.

Finally, although based on a single data point, it was observed that off-reef fish had notably different isotope ratios to obligate reef dwelling fish. This raises the intriguing possibility of using isotope analysis to provide new data for the long standing attraction-production debate.

Chapter 5. Acid treatment of carbonate-rich samples for stable isotope analysis

5.1 Introduction

5.1.1 Preservation of samples

Samples collected in the field are often preserved in formalin, alcohol, or $HgCl_2$ (for example), in order to prevent bacterial decay. However, chemical preservation techniques have been repeatedly shown to alter the stable isotope ratios of sample material (Gloutney and Hobson, 1998; Bosley and Wainright, 1999; Edwards et al., 2002; Sweeting et al., 2004; Kelly et al., 2006), either as a result of compounds leaching out of the samples or because they become contaminated with carbon and nitrogen from the preservatives themselves (Bosley and Wainright, 1999). Furthermore, the effect of such treatments is often inconsistent between samples (Sweeting et al., 2004), or at the very least is often taxon-specific, meaning that it may not be appropriate to apply a generic arithmetic correction to obtain the 'real' isotope ratio from that recorded. In any case, to derive taxon-specific correction factors would require substantial pilot work, involving measurement of the exact deviation resulting from preservation for each taxon of interest (Kelly et al., 2006). This may be feasible for single-species studies, but it is likely to be too sample-intensive for food web studies involving numerous taxa.

To avoid these problems, samples intended for isotopic analysis are not usually preserved chemically, but are instead frozen as soon as possible after collection; this does not affect isotopic ratios relative to fresh-dried samples (Gloutney and Hobson, 1998; Bosley and Wainright, 1999; Sweeting et al., 2004). Samples must be sorted prior to freezing, however, since bulk freezing of samples followed by later defrosting for sorting has itself been shown to affect stable isotope ratios (Dannheim et al., 2007). The need to sort the samples prior to freezing greatly increases the work required at the point of sampling, and necessitates access to freezers, which can be difficult while working in the field. Nevertheless, these requirements need to be met if reliable isotopic measurements are to be obtained. Alternatively, if the facilities exist, immediate oven drying of samples is suitable

(Gloutney and Hobson, 1998; Bosley and Wainright, 1999), but this can be impractical for large numbers of samples.

5.1.2 Treatment of samples for stable isotope analysis

5.1.2.1 Lipid removal

Lipids are ^{13}C depleted compared to protein and carbohydrate (DeNiro and Epstein, 1978), and since the amount of lipids in tissues varies in response to numerous factors (species, condition, age, season) they are often removed prior to analysis. However, lipid extraction can also affect the sample C:N ratio and $\delta^{15}N$ value (Soreide et al., 2006; Mintenbeck et al., 2008), so separate analyses may need to be run on treated and untreated samples. Alternatively, various 'lipid normalisation' models exist which are intended to mathematically correct for the lipid effect (Sweeting et al., 2006). However, exact relationships between initial lipid content and changes in C:N/$\delta^{13}C$ are often species specific, and thus these models need to be verified on a species by species basis (Mintenbeck et al., 2008). These problems can be avoided by using muscle tissue or other lipid-poor tissues for analysis (Sweeting et al., 2006).

5.1.2.2 Carbonates

Ecological studies using stable isotope analysis are often concerned with diet. Carbon and nitrogen stable isotope ratios are used to identify the trophic position and ultimate nutrient source for consumers (Peterson, 1999), as prey-derived compounds are incorporated into their tissues. However, when dealing with whole organisms, not every part of the body is necessarily derived from the diet. Marine organisms, especially many invertebrates, have hard components built from a combination of proteins and inorganic carbonates, such as crustacean carapaces, or mollusc shells. The carbonates used in these structures are not obtained from the diet, but are absorbed from the surrounding water (Fritz and Poplawski, 1974). Carbon obtained in this way is very isotopically 'heavy' (highly enriched in ^{13}C) relative to organically derived carbon (Haines and Montague, 1979), and the

isotopic signature of a whole animal can be substantially biased if this carbonate is included in the sample.

In the case of larger animals, it is easy to obtain carbonate-free samples by dissecting out pieces of muscle tissue, or by removing all traces of shell material, but this is not always practical for smaller organisms. When carbonates cannot be physically avoided in this manner, a simple chemical treatment is often recommended: the use of dilute acid (usually HCl) to dissolve away the carbonates (Haines and Montague, 1979). Carbonates react with the acid, and inorganic carbon is given off as carbon dioxide. The carbon isotope ratio of the remaining material, once dried, is then assumed to represent only what has been taken up in the diet. Acid treatment of carbonate-free tissue samples (usually muscle) has been repeatedly shown to have no significant effect on $\delta^{13}C$, and therefore acid treatment of such samples is considered unnecessary (Bunn et al., 1995; Bosley and Wainright, 1999; Chanton and Lewis, 1999; Serrano et al., 2008).

However, despite several decades of ecological isotope studies, there is still wide variation in the methods used to deal with carbonates in samples (Serrano et al., 2008). Different authors have used different strengths of acid, times of exposure to acid and even different methods of acid exposure (Serrano et al., 2008), which may lead to different results for carbonate-rich samples (Soreide et al., 2006). Once acidified, the sample will contain excess acid and chloride salts. Generally it is preferable to keep the gases entering a mass spectrometer as 'clean' as possible, to prevent erroneous readings and to extend the working life of the mass spectrometer components, such as the filament. This is achieved using a chemical column which removes contaminants, in addition to ensuring complete reduction of the nitrogen and carbon dioxide gas entering the mass spectrometer. Halogens are removed using silver (in the form of silvered cobaltous cobaltic oxide in this study). If numerous samples containing large amounts of chloride are analysed, it is possible that the relevant reagents in the filter column will be consumed, allowing chloride ions to enter the mass spectrometer. This could be addressed by increasing the amount of silver-containing compound in the filter column (Yamamuro and Kayanne, 1995), but these columns are of fixed length and this

would require reducing the quantities of other chemicals in the column. Another option would be to change the filter column more regularly, but this will slow down the analysis of samples and increase consumable costs. A simple process of rinsing the samples with distilled water after acidification should remove acid and chlorides, avoiding the problem. However, there is a risk that this rinsing process itself may affect the resulting isotope ratios. Rinsing of acidified samples certainly causes additional loss of organic carbon and nitrogen from samples (Yamamuro and Kayanne, 1995), and may alter the isotope ratio (Carabel et al., 2006; Mateo et al., 2008), although some authors claim that rinsing has no significant further effect (Soreide et al., 2006). Only one study appears to consider (briefly) the effect of rinsing without prior acidification (Carabel et al., 2006), and the results of washing alone are not actually discussed in detail.

Any potential effect on the nitrogen stable isotope ratio should also be considered. Acid treatment is intended to remove only inorganic carbon, and it might be reasonable to assume that it would not affect $\delta^{15}N$, given that dilute hydrochloric acid seems unlikely to attack nitrogen-containing organic compounds during a brief exposure. However, some evidence suggests that the stable nitrogen isotope ratio can be impacted significantly (Bunn et al., 1995; Carabel et al., 2006), while other studies disagree (Bosley and Wainright, 1999; Chanton and Lewis, 1999; Serrano et al., 2008). These disagreements may stem from variation in effects across different taxa. Of course, if acidification is followed by rinsing with distilled water, this might further affect the nitrogen isotope ratio (Carabel et al., 2006). Therefore, as a precaution, it is considered advisable to run separate analyses (Corbisier et al., 2006; Mateo et al., 2008) on treated samples (for the carbon isotope ratio) and untreated samples (for the nitrogen isotope ratio), although this increases the number of individual samples which need to be analysed. However, it appears that it is quite common practice to acidify all samples as a matter of course (whether or not there is an *a priori* reason to suspect the presence of significant amounts of carbonate), and to use the treated samples for both carbon and nitrogen results (Rautio and Vincent, 2007; Yokoyama and Ishihi, 2007; Pasquaud et al., 2008; Schaal et al., 2008; Yokoyama et al., 2008).

5.1.3 Purpose of this chapter

The purpose of this chapter is to use samples collected during the main study to explore the effects of two acid treatment regimes on the carbon and nitrogen stable isotope ratios of individual carbonate-rich samples, as well as the effect of simply rinsing the samples without acidification.

5.2 Materials and Methods

5.2.1 Sample collection and preparation

Twenty hermit crabs (family Paguridae) were collected from the natural reef site during dives conducted in 2008, as a part of the general sampling effort. Individual animals were returned to the laboratory for processing, where they were removed from their acquired gastropod shells and individually frozen. Samples were subsequently freeze-dried for 24 hours prior to further preparation. Dried individuals were weighed whole and ground into homogenous powder using a mortar and pestle. Each individual animal provided sufficient material for both acidification treatments to be carried out on sub-samples, but only ten individuals provided enough for a third treatment, involving only distilled water rinsing.

5.2.2 Sample treatment

Prior to acidification, 600 to 800µg amounts of dried untreated sample material from each individual were weighed into tin capsules. The remaining material from each crab was divided up in order to be subjected to further treatments. The first sub-sample from each individual was subjected to an 'Acidification' treatment. Samples in this group were placed into labelled 5ml centrifuge tubes. 1ml of 1M HCl was added to each tube, and tubes were agitated vigorously until all effervescence ceased, indicating complete dissolution of carbonates. Tubes were then spun in a centrifuge at 5000 rpm for 5 minutes, after which the supernatant was discarded. The samples were then dried for 24 hours at 60°C. Small amounts of each dried sample (600 to 800µg) were weighed into tin capsules for analysis.

The second subsample from each individual was subjected to an 'Acidification with Rinsing' treatment. Samples in this group were similarly placed into labelled 5ml centrifuge tubes. 1ml of 1M HCl was added to each tube and all tubes were agitated vigorously until effervescence ceased. Tubes were then spun in a centrifuge at 5000rpm for 5min, after which the supernatant was discarded. A 2ml aliquot of Milli-Q water was then pipetted into the tube, which was vigorously agitated for 30 seconds before being spun in the centrifuge for another 5 minutes at 5000rpm, after which the supernatant was discarded. This rinsing process was repeated twice more (giving a total of three rinses), checking the pH of the discarded supernatant at each step with indicator paper in order to verify that the sample was free of acid (three rinses were always sufficient, whereas some acid often remained after only two rinses). The rinsed sample material was then dried for 24 hours at 60°C, after which 600 to 800µg amounts of each sample were weighed out into tin capsules.

Where sufficient material remained (10 out of the original 20 samples met this condition), this was subjected to a third treatment: 'Rinsing Only'. Samples in this group were placed into labelled 5ml Eppendorf tubes, and 2ml aliquots of Milli-Q water were added to each tube. The tubes were then sealed and vigorously agitated for 30 seconds, before being spun in a centrifuge for 5 minutes at 5000 rpm. The supernatant was discarded. A total of 3 rinse/spin cycles were carried out, followed by oven drying, such that samples in this group were treated identically to those in the 'Acidification with Rinsing' group, apart from the omission of the acidification step. Small amounts of each dried sample (600 to 800µg) were weighed into tin capsules for analysis.

5.2.3 Sample analysis

Weighed-out samples in tin capsules were simultaneously analysed for $\delta^{13}C$ and $\delta^{15}N$ by stable isotope ratio mass spectrometry (as described in Section 3.2.4). A total of 70 analyses were run: 20 Untreated, 20 Acidification, 20 Acidification with Rinsing, and 10 Rinsing Only.

5.2.4 Data analysis

Mean $\delta^{13}C$ and $\delta^{15}N$ values for samples subjected to the various treatments were compared using repeated measures ANOVA and paired sample t-tests (after tests for normality and homoscedasticity) computed with SPSS and SigmaStat.

5.3 Results

5.3.1 Effect of acid treatments on mean $\delta^{13}C$

Table 5.1 Stable carbon isotope ratio data for hermit crab samples subjected to two acidification regimes, compared with untreated samples from the same individuals. Correspondence with normal distribution tested using the Kolmogorov-Smirnov statistic.

	Untreated	Acidification	Acidification with Rinsing
Sample number	20	20	20
Data normally distributed?	Yes ($p > 0.05$)	Yes ($p > 0.05$)	Yes ($p > 0.05$)
Mean $\delta^{13}C$	-17.271	-19.210	-20.417
Standard Error (mean)	0.242	0.156	0.226
Difference from Untreated	-	-1.939	-3.146

$\delta^{13}C$ data for all treatments were normally distributed (Kolmogorov-Smirnov test, $p > 0.05$; Table 5.1) and homoscedastic (Levene's statistic = 0.559; d.f. = 2, 57; $p = 0.575$). Both acidification regimes ('Acidification' and 'Acidification with Rinsing') resulted in significantly depleted mean $\delta^{13}C$ values (Fig. 5.1; Table 5.2b); 'Acidification with Rinsing' also resulted in significantly depleted $\delta^{13}C$ relative to acidification alone (Tukey test, $p < 0.001$; Table 5.2b).

Table 5.2 (a) Repeated Measures ANOVA for the effect of two acidification regimes on the $\delta^{13}C$ of hermit crabs; ***$p < 0.001$.

Source of Variation	DF	SS	MS	F
Between Subjects	19	39.792	2.094	
Between Treatments	2	100.753	50.376	171.750***
Residual	38	11.146	0.293	
Total	59	151.690		

(b) Pairwise comparisons, Tukey test; ***$p < 0.001$.

Comparison	Difference of means	Test Statistics (p,q)
Untreated vs. Acidification with Rinsing	3.146	3, 25.978***
Untreated vs. Acidification	1.939	3, 16.007***
Acidification vs. Acidification with Rinsing	1.207	3, 9.971***

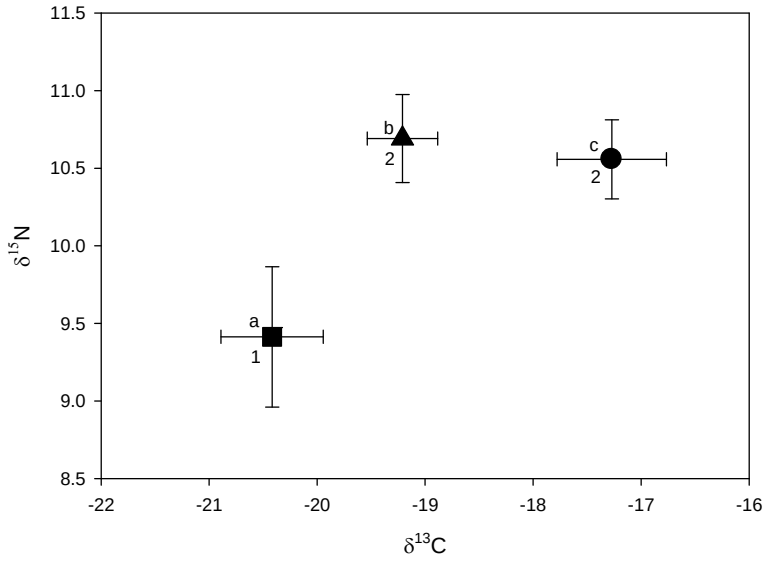


Figure 5.1 Mean nitrogen and carbon stable isotope ratios (expressed as ‰ relative to international standards) for hermit crabs. Circle: Untreated; Triangle: Acidification; Square: Acidification with Rinsing (n=20 for all treatments). Error bars are ±95% confidence intervals of the mean. Treatments resulting in significantly different $\delta^{13}C$ ($p < 0.001$) are marked with different letters, and treatments resulting in different $\delta^{15}N$ ($p < 0.001$) are marked with different numbers.

5.3.2 Effect of acid treatments on mean $\delta^{15}N$

$\delta^{15}N$ data for all treatments were normally distributed (Kolmogorov-Smirnov test; Table 5.3) and homoscedastic (Levene's statistic = 1.766; d.f. = 2, 57; $p = 0.180$). Acid treatment significantly affected the mean $\delta^{15}N$ of samples ($F = 25.344$, $p < 0.001$; Table 5.4a, Fig. 5.1). However, while acidification regimes had a significantly different effect from each other, only Acidification with Rinsing resulted in a significantly different mean $\delta^{15}N$ from Untreated samples ($p < 0.05$; Tukey's test, Table 5.4b).

Table 5.3 Stable nitrogen isotope ratio data for hermit crab samples subjected to two acidification regimes, compared with untreated samples from the same individuals. Correspondence with normal distribution was tested using the Kolmogorov-Smirnov statistic.

	Untreated	Acidification	Acidification with Rinsing
Sample number	20	20	20
Data normally distributed?	Yes ($p > 0.05$)	Yes ($p > 0.05$)	Yes ($p > 0.05$)
Mean $\delta^{15}N$	10.557	10.691	9.413
Standard Error (mean)	0.122	0.135	0.216
Difference from Untreated	-	+0.134	-1.144

Table 5.4 (a) Repeated Measures ANOVA for the effect of two acidification regimes on $\delta^{15}N$; ***p < 0.001, ns = Not Significant (p > 0.05).

Source of Variation	DF	SS	MS	F
Between Subjects	19	15.573	0.820	
Between Treatments	2	19.745	9.872	25.344***
Residual	38	14.802	0.390	
Total	59	50.120		

(b) Pairwise comparisons

Comparison	Difference of means	Test Statistics (p,q)
Untreated vs. Acidification with Rinsing	1.144	3, 8.200***
Untreated vs. Acidification	0.134	3, 0.959 ns
Acidification vs. Acidification with Rinsing	1.278	3, 9.160***

5.3.3 Effect of rinsing (without acidification) on mean $\delta^{13}C$ and $\delta^{15}N$

The 'Rinsing Only' treatment was only carried out on samples from 10 individuals, so 'Rinsing Only' data were only compared to the 'Untreated' data for those specific individuals. Data for all treatments were normally distributed (Table 5.5). Rinsing had a significant effect (Fig. 5.2) on both $\delta^{13}C$ (paired t-test; t = -2.687; d.f. = 9; p = 0.025) and $\delta^{15}N$ (paired t-test; t = 2.272; d.f. = 9; p = 0.049).

Table 5.5 Summary data for comparison of samples subjected to a 'Rinsing Only' treatment with untreated samples. Carbon and nitrogen stable isotope data presented. Correspondence with normal distribution tested using Kolmogorov-Smirnov statistic.

		Untreated	Rinsing Only
	Sample number	10	10
$\delta^{13}C$	Mean	-17.579	-16.401
	Standard Error (mean)	0.323	0.493
	Data normal?	Yes (p > 0.05)	Yes (p > 0.05)
	Difference from Untreated	-	+1.177
$\delta^{15}N$	Mean	10.790	9.917
	Standard Error (mean)	0.192	0.364
	Data normal?	Yes (p > 0.05)	Yes (p > 0.05)
	Difference from Untreated	-	-0.873

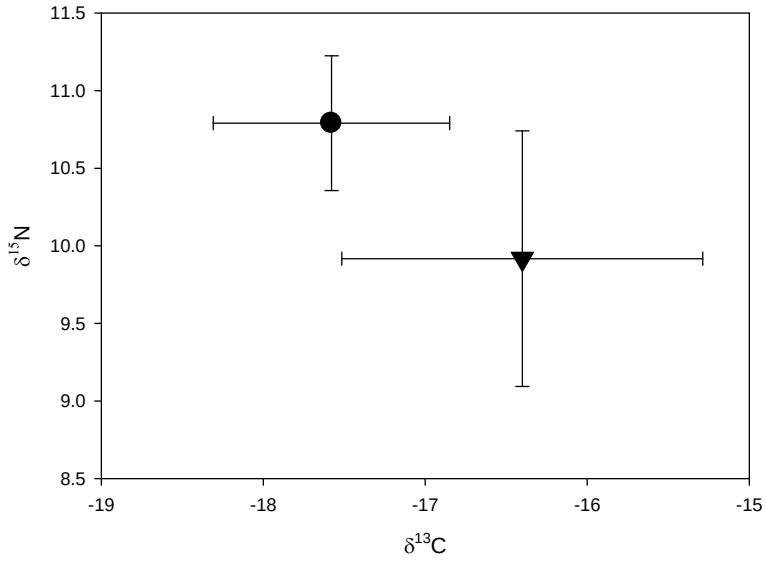


Figure 5.2 Mean nitrogen and carbon stable isotope ratios (expressed as ‰ relative to international standards) for 'Untreated' (Circle) samples and 'Rinsing Only' (Triangle) samples (n=10). Error bars are ±95% confidence intervals of the mean.

5.3.4 Consistency of treatment effects on individuals

Since samples from the same individuals were subjected to multiple treatments, the extent to which the overall trends in mean values were reflected in the effects of the treatments on individual organisms can be examined (Figs 5.3 and 5.4).

5.3.4.1 $\delta^{13}C$

Acidification resulted in a consistent reduction in $\delta^{13}C$ for all individuals except one, which showed a very small increase. Acidification followed by rinsing resulted in a larger decrease in $\delta^{13}C$, which was consistent across all samples (Fig. 5.3). The effects of rinsing alone were less consistent, with some samples showing an increase in $\delta^{13}C$ and others showing a decrease. The effect of rinsing on acidified samples (inferred from the difference between Acidification and Acidification with Rinsing; Fig. 5.4) was quite consistent, in all cases but two resulting in a further decrease in $\delta^{13}C$.

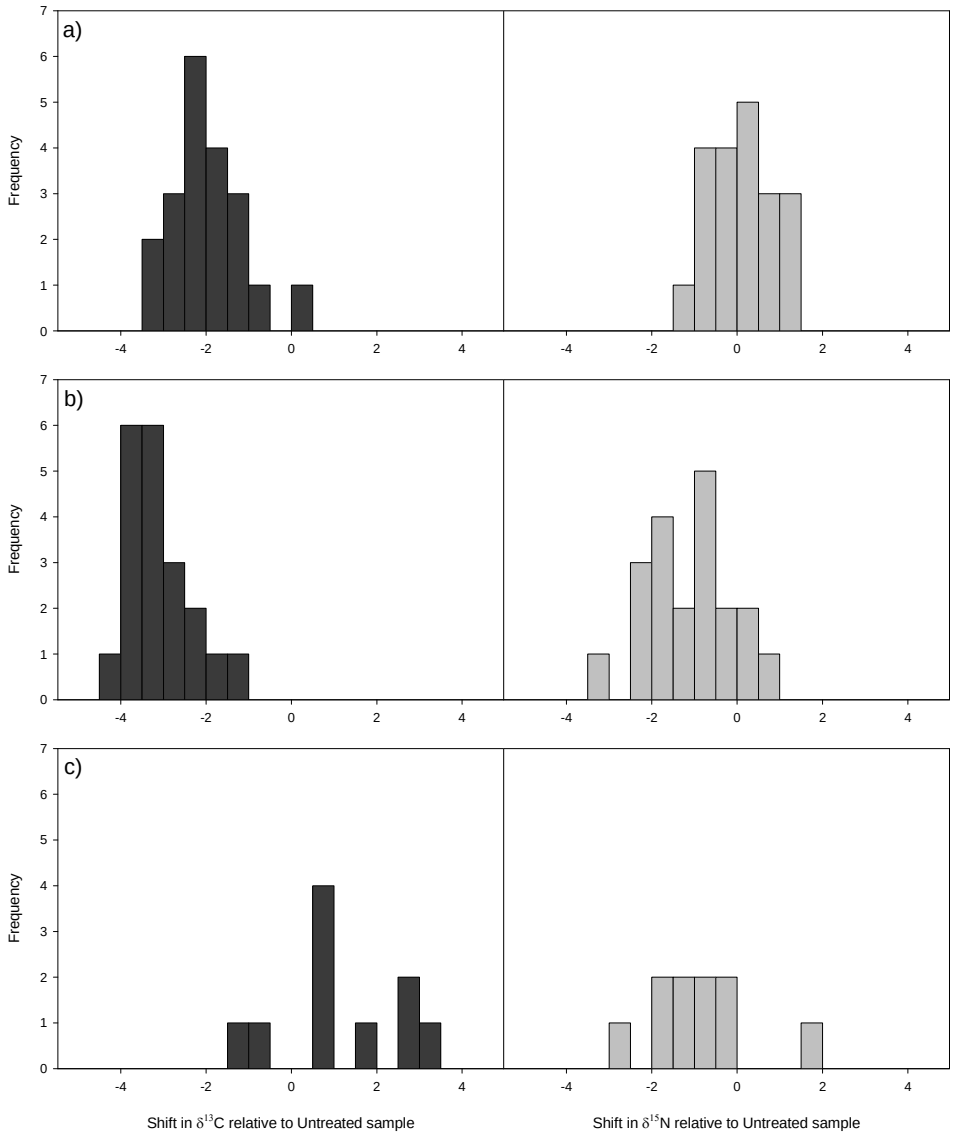


Figure 5.3 Frequency distribution of post-treatment shifts in $\delta^{13}C$ (left, dark grey) and $\delta^{15}N$ (right, light grey) relative to Untreated values for individual crabs, following a) Acidification, b) Acidification with Rinsing, and c) Rinsing only.

5.3.4.2 $\delta^{15}N$

Effects of treatments on $\delta^{15}N$ were less consistent across individuals. Acidification alone had no clear effect (Section 5.3.2; Fig. 5.1), and the decrease in $\delta^{15}N$ after Acidification with Rinsing (Section 5.3.2; Fig. 5.1) was not entirely consistent across all individuals, with some showing an increase (Fig. 5.3). The effect of rinsing on acidified samples (inferred from the difference between Acidification and Acidification with Rinsing; Fig. 5.4) was quite consistent, in all cases but three resulting in a decrease in $\delta^{15}N$. The effect of rinsing alone was also relatively

consistent (a decrease in $\delta^{15}N$; Fig. 5.3), with only one sample showing the opposite effect to the majority.

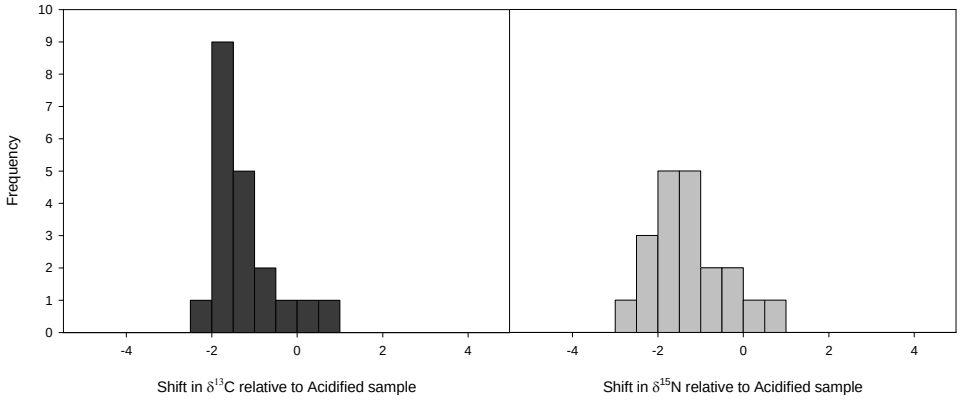


Figure 5.4 Frequency distribution of shifts in $\delta^{13}C$ (left, dark grey) and $\delta^{15}N$ (right, light grey) after Acidification with Rinsing, relative to those after Acidification; representing the effects of post-acidification rinsing on acidified samples.

5.4 Discussion

5.4.1 Effect of acidification on isotope ratios

It is clear that acidification affects the stable isotope ratios of biological samples which contain carbonates (Bunn et al., 1995; Soreide et al., 2006). The results here verify that acidification is necessary in the case of carbonate-rich samples. Hermit crabs use abandoned gastropod shells for protection, and their abdomen is not encased in a hard carapace. Therefore it is to be expected that there may be a greater proportion of inorganic carbon in the whole bodies of other crustacean families where the entire body is encased in thicker hard carapace; acidification is even more important in samples with greater amounts of carbonate (Serrano et al., 2008).

However, the differences between the isotope ratios measured after acidification alone, and after acidification followed by rinsing, raise a further question. Which are the 'correct' values? In order to understand this, we should first consider the effects of the treatments on the $\delta^{15}N$ of the samples, on which acidification was not expected to have an effect.

5.4.1.1 Effect of treatments on $\delta^{15}N$

The data show, in line with expectation, that acidification alone had no significant effect on the mean $\delta^{15}N$ of samples (Fig. 5.1; Table 5.4). This is supported by the lack of any consistent directionality in the response of individual samples to treatment (Figs. 5.1 and 5.3), which is compatible with results from a recent meta-analysis (Mateo et al., 2008) showing that effects of acid treatment on sample $\delta^{15}N$ are not consistent. However, when acidification was followed by rinsing, a significant reduction in $\delta^{15}N$ occurred. This is not consistent with some other results: in one study acidification with rinsing resulted in ^{15}N *enrichment* (Bunn et al., 1995), and there is a general pattern of little or no effect of this type of treatment in the literature (Mateo et al., 2008). The results of the 'rinsing only' treatment suggest this effect of rinsing is independent of acidification; rinsing without prior acidification also resulted in a reduction in $\delta^{15}N$ (Fig. 5.2), and the mean reduction in $\delta^{15}N$ after simple rinsing was of similar magnitude to that after acidification with rinsing (0.873 versus 1.144 respectively). The acidification itself may thus be irrelevant, and it may simply be the case that there are easily leached nitrogen-containing compounds which can be lost as a result of any rinsing whatsoever.

It therefore seems reasonable to conclude that the mean $\delta^{15}N$ for untreated samples is the 'correct' value, and therefore the one that should be used in analyses. Further support is provided by examining the variability of sample $\delta^{15}N$ after treatments. The data were checked for homoscedasticity prior to statistical analysis, and there were no significant differences among the variances of samples subjected to different treatments (Section 5.3.2). However, it is interesting to note that every treatment resulted in a slight increase in the standard error of the mean relative to the untreated samples (Table 5.3). Other studies corroborate this observation of increased $\delta^{15}N$ variability after acid treatments (Mateo et al., 2008) supporting the presumption that the untreated values are the 'correct' ones. Nevertheless, there was no difference between the *mean* $\delta^{15}N$ of untreated versus acidified (but not rinsed) samples, therefore $\delta^{15}N$ data from acidified (but not

rinsed) samples could still be used to compare groups of individuals without adversely affecting the results.

5.4.1.2 Effect of treatments on $\delta^{13}C$

As expected, acidification affects the $\delta^{13}C$ of samples. Acid treatment significantly reduced $\delta^{13}C$, and subsequent rinsing resulted in a further reduction (Tables 5.1 and 5.2; Figs. 5.1, 5.3, 5.4). However, rinsing alone without prior acidification actually resulted in an *increase* in the mean $\delta^{13}C$. Clearly, unlike for $\delta^{15}N$, the effect of rinsing was modified by prior acidification. Rinsing otherwise untreated samples may have resulted in the loss of some carbon-containing compounds, with a bias towards isotopically lighter carbon, causing the sample $\delta^{13}C$ to rise. Acidification removed inorganic carbon (which is isotopically heavy), reducing the $\delta^{13}C$ of samples as expected. Rinsing of acidified samples then resulted in further loss of 'heavy' carbon; acidification may have liberated some compounds from the samples, which were then lost during rinsing (but which would not have been lost if the samples had not been first acidified). Acidification with rinsing may thus remove some organic (dietary) carbon from samples in addition to the inorganic carbon lost during the acidification itself (Yamamuro and Kayanne, 1995; Serrano et al., 2008).

Determining the 'correct' value for use in further analysis is not as simple as for $\delta^{15}N$. Acidification aims to remove inorganic carbonates, and it might be assumed that this 'unwanted' carbon was all lost as carbon dioxide during the acidification, and therefore that further changes in $\delta^{13}C$ following subsequent rinsing were undesirable deviations away from the 'correct' values. If we examine the variability of the sample $\delta^{13}C$ values before and after the various treatments, it is interesting to note that of all the groups, the acidified (but un-rinsed) samples had the lowest standard error (Table 5.1) compared to the other treatments. Tests for homoscedasticity (Section 5.3.1) suggest that these differences were not significant, but it is worth noting, since it has been observed previously that acidification can reduce sample variability (Mateo et al., 2008). Acidification without rinsing might thus be the best treatment. However, the most appropriate

acidification method remains unproven. Other taxa should be studied in this manner to determine if the effects of post-acidification rinsing are consistent, although very detailed chemical work would be required in order to understand which compounds are being lost as a result of sample rinsing.

5.4.2 Conclusions and Recommendations

Where it is likely that carbonates are present in significant amounts, acidification is necessary in order to obtain useful measurement of carbon stable isotope ratios. It is not possible to be certain from the results outlined above, but it seems possible that post-acidification rinsing may shift samples away from their true $\delta^{13}C$ values (Mateo et al., 2008). Simple acidification without rinsing results in the lowest variability between samples, and may be the optimal approach for comparing groups of organisms. However, there may be practical reasons why it is desirable to remove unreacted acid from the sample by rinsing; the effects this additional step has on the carbon isotope ratio should be considered.

When no rinsing is carried out, acidification alone has no significant effect on the mean $\delta^{15}N$ of samples. Given this result, it appears that it is not necessary to run duplicate untreated samples to obtain results for nitrogen isotope ratios when simply acidifying samples without rinsing. However, post-acidification rinsing can strongly influence the results (Bunn et al., 1995; Carabel et al., 2006; Mateo et al., 2008). Some authors routinely acidify all samples, without running duplicate untreated samples. As long as samples are not rinsed (post-acidification) to remove excess acid, this approach may not invalidate the resulting nitrogen data. However, if rinsing is carried out, then duplicate untreated samples *must be run* in order to obtain valid nitrogen data. The best approach would seem to be to avoid sample pre-treatments where possible, by using samples from tissues which are low in both lipids and carbonates (Soreide et al., 2006).

Chapter 6. Summary of Part I: Artificial reefs and stable isotopes

6.1 Summary of results of Part I

6.1.1 Chapter 3: Diets of artificial and natural reef consumers

Stable carbon and nitrogen isotope ratios of consumers were generally found to be similar on an artificial reef and a nearby natural reef system. While not conclusive, this indicated that both reefs were likely to support similar trophic structures.

6.1.2 Chapter 4: Trophic complexity on artificial and natural reefs

A more detailed exploration of the food webs on an artificial and nearby natural reef revealed broad similarities. Invertebrate fauna from both reefs showed a similarly wide range of $\delta^{13}C$ values, indicating that multiple nutrient sources were important, and a similar range of $\delta^{15}N$ values, indicating that there were approximately equivalent numbers of trophic levels. Further analysis demonstrated that the same nutrient sources were similarly important on both reefs. Many reef fauna seemed to be supported by a nutrient source made up of an indeterminate mixture of sedimentary organic matter, particulate organic matter/plankton and certain macroalgae with similar isotope ratios. Several taxa of red algae which were growing on the reefs (with depleted $\delta^{13}C$ values) also contributed to the food webs. Reef fauna were divided up into clusters of trophically similar individuals, and the pattern of clustering was largely the same on both reefs.

6.1.3 Chapter 5: Acid treatment of samples for stable isotope analysis

An analysis of the effect of sample acidification on the $\delta^{13}C$ and $\delta^{15}N$ of carbonate-rich samples confirmed the need to acidify such samples in order to obtain accurate carbon isotope ratio results. Acidification alone did not significantly impact the $\delta^{15}N$ of samples; however, acidification followed by distilled water rinsing had a significant effect on this isotope ratio, as did rinsing without acidification. Where samples need to be acidified to remove carbonate and are subsequently rinsed with distilled water, duplicate (untreated) samples should be analysed to ensure that valid $\delta^{15}N$ results are obtained.

6.2 Applicability of stable isotope methods

Isotope methods are ideal for exploring food webs, and have been used in many marine systems (Peterson, 1999). They avoid some of the problems and biases inherent in other diet assessment methods such as stomach content analysis (Hyslop, 1980), and can be used to explore the diet and trophic position of small invertebrate species for which gut content analysis would not be possible.

Isotope methods do have their own problems and biases. Interpretation of isotope data, and modelling of dietary contributions, is usually based on assumed trophic fractionation values of +1‰ for carbon (DeNiro and Epstein, 1978) and +3.4‰ for nitrogen (Minagawa and Wada, 1984). These values are commonly used in isotope studies, although other estimates are also available and are sometimes used (for example, see Vanderklift and Ponsard, 2003). However, there is increasing evidence that these assumed values are not valid for many species/diet combinations (Gannes et al., 1997; Vanderklift and Ponsard, 2003; Crawley et al., 2007; Dubois et al., 2007). Choice of trophic fractionation value in an analysis can thus affect the interpretation of the results, particularly for nitrogen, where the trophic fractionation is larger and more variable (Vanderklift and Ponsard, 2003).

The presence of lipids within consumer tissues is also a concern; lipid compounds within biological samples are known to bias the results of carbon stable isotope ratios (Pinnegar and Polunin, 1999), because lipids are isotopically 'light' and exist in variable quantities in individual organisms or tissues (Sweeting et al., 2006). Therefore it is recommended that lipids are removed from samples prior to analysis. This is usually accomplished by treating samples with polar solvents following the Soxhlet (7% methanol in dichloromethane; Soreide et al., 2006; Bodin et al., 2007) or Folch (2:1 chloroform:methanol; Soreide et al., 2006; Sweeting et al., 2006) methods. While the presence of lipids is not thought to significantly bias the results of any nitrogen stable isotope analysis, it is often recommended that separate samples are run on lipid-extracted and untreated sub-samples because of the possibility that the extraction method may alter the sample $\delta^{15}N$, possibly to an inconsistent degree (Sweeting et al., 2006; Bodin et al., 2007; Mintenbeck et al.,

2008). This, of course, requires double the number of samples to be analysed, with consequent expense, and adds another step of laboratory processing. Another option is the use of lipid-correction factors, calculated based on assumed $\delta^{13}C$ values for lipids and measurements of the lipid content of samples. However, while some studies have used such methods (Sweeting et al., 2006), controversy still exists over whether or not the adjustments made using them are valid (Mintenbeck et al., 2008).

As an alternative to these more complex procedures, where possible, samples of lipid-poor tissues can be used, as it has been demonstrated that lipid extraction often has no effect on their carbon stable isotope ratios (Soreide et al., 2006). Usually, muscle tissue is regarded as being lipid-poor, including fish white muscle (Mintenbeck et al., 2008), crustacean tail muscle (Bodin et al., 2007), and bivalve adductor muscle (Yokoyama et al., 2008). Where possible, muscle tissue samples were used for the analyses carried out for this project. However, in some cases this was not possible, including in the case of some invertebrates such as *Pagurus* spp. and most of the polychaetes and amphipods analysed.

6.3 Isotope methods as a part of integrated research efforts

The limitations of stable isotope methods can be overcome by using them in conjunction with other methods. Isotope methods can be used in conjunction with more traditional methods such as stomach content analysis, to alleviate some of the biases in the latter method, such as the presence in the stomach contents of items that are not actually assimilated by consumers (Ho et al., 2007). Other modern techniques, such as lipid biomarker analysis, are available (Sargent et al., 1987). Different primary producers can have unique combinations of fatty acids (this can even be used in species identification), which allows their importance in consumer diets to be assessed (Sargent et al., 1987). This can produce results which corroborate and enhance those from stable isotope analysis (Gao et al., 2006; Guest et al., 2008; Tucker et al., 2008). Lipid analysis can prove particularly useful where potential food sources have similar stable isotope ratios (Gao et al.,

2006), as was the case for sedimentary organic matter, particulate organic matter, and some species of macroalgae, in this investigation (as described in Chapter 4).

Ideally, lipid biomarker analysis, stable isotope analysis, and stomach content analysis can all be used together (Alfaro, 2008) to produce a much more accurate and detailed account of consumer diets, with each technique potentially compensating for the biases and inherent assumptions of the others. Conducted alongside spatially and temporally replicated quantitative sampling of communities, this could represent a powerful means of exploring food webs, both on natural and artificial reefs.

6.4 Concluding remarks

In agreement with one previous study on a sea wall in France (Schaal et al., 2008), no evidence was found that food webs on artificial reefs are 'simplified' relative to those in natural habitats. Consumers on the Poole Bay artificial reef exploit a variety of nutrient sources, and occupy a range of trophic positions.

However, this project was only one of an extremely small number of studies using stable isotopes on artificial reefs (only two published studies were found: Riera et al., 2004; Schaal et al., 2008), and was based on a relatively restricted amount of sampling. Stable isotope ratio analysis should be more widely incorporated into research projects on artificial reefs, alongside traditional methods and other contemporary techniques.

PART II

MARINE COMMUNITIES OF NORTH SEA OFFSHORE PLATFORMS

Chapter 7. Introduction to Part II: Offshore structures as marine habitats

7.1 Offshore production platforms: *de facto* artificial reefs

There are over 7000 offshore platforms world-wide (Hamzah, 2003), the negative impacts of which have been widely studied (Gray et al., 1999). However, these structures are also *de facto* artificial reefs, and since some are now more than 30 years old, they may constitute stable and mature reef systems.

The most common type of offshore structure consists of a lattice of tubular steel (the 'jacket') extending from the surface to the sea floor, on which may sit various types of deck section, providing drilling facilities, quarters or other required infrastructure (the 'topsides'). Other elements (such as the 'conductors' which bring the oil/gas to the surface) are located within the jacket, and there may also be significant seabed infrastructure in the vicinity of a platform, such as pipelines and manifolds.

These structures present extensive surface area for fouling organisms: 8,173 m^2 for a typical platform in 30m water depth (Gallaway and Lewbel, 1982) and approximately 12,000-16,000m^2 for a structure in 45m water depth (Bohnsack et al., 1991). Structures located in deeper water (over 200m in some cases) obviously provide much greater surface area. Settlement opportunities are presented over a wide depth range, allowing colonisation by a variety of taxa, ranging from intertidal algae to deep water corals (Lopez-Bautista et al., 2003; Gass and Roberts, 2006). Their size, very high profile, and open lattice structure, also make platforms potent fish aggregators, attracting fish at all depths (Aabel et al., 1997a).

However, active platforms are sites of hydrocarbon extraction, and disruption of natural communities is also to be expected. Platforms can introduce contaminants into the water column (Hylland et al., 2008) and sediments (Gray et al., 1999) in their vicinity. The 'drill cuttings' piles (accumulations of drilling wastes at the base of some platforms), for example, can contain large amounts of toxic materials (Hartley and Watson, 1993).

7.2 Worldwide research on platform ecology

7.2.1 USA

The Gulf of Mexico hosts over 4000 platforms (Sammarco et al., 2004); the largest concentration of offshore structures anywhere in the world. Most of the available literature on platform ecology comes from studies carried out in the USA, where research on platforms in the Gulf of Mexico and off the coast of California has been ongoing since the 1960s (Wolfson et al., 1979).

7.2.1.1 Fouling communities

Platforms in the USA host diverse and extensive fouling assemblages (Wolfson et al., 1979; Lewbel et al., 1987), differing markedly in some respects from those on natural rocky reefs (Sammarco et al., 2004; Bram et al., 2005; Page et al., 2007). The differences can be positive, such as greater abundances of important prey species (Wolfson et al., 1979; Page et al., 2007), or negative, such as the presence of higher abundances of invasive or exotic species (Page et al., 2006). Fouling composition shows vertical zonation (Lewbel et al., 1987; McGinnis et al., 2001), and can vary between structures, depending on factors such as distance from shore (Lewbel et al., 1987).

7.2.1.2 Fish populations

Very high densities of fish have been observed at American platforms (Stanley and Wilson, 1996; McGinnis et al., 2001). Some natural reef species are absent from platform assemblages, usually those that rely on resources that are absent or reduced on platforms, or those with limited dispersal potential (Carr et al., 2003). Fish assemblages also vary among platforms (Love et al., 2000; McGinnis et al., 2001), with those further offshore supporting a lower diversity of shallow water fishes (Schroeder and Love, 2004). At deeper platforms, fish assemblages can show both taxonomic (Love et al., 2000; Carr et al., 2003) and ontogenetic (Love et al., 2000; 2006) depth zonation, and biomass tends to increase towards the sea floor (Love et al., 2000).

There is some evidence of enhanced fish production at American platforms. High abundances of certain prey species are exploited by fish at platforms, leading to better fish condition (Page et al., 2007), faster growth (Schroeder and Love, 2004), or greater size (Carr et al., 2003) for certain fish species on platforms. Some species also recruit to platforms at higher densities than seen on natural reefs (Carr et al., 2003; Love et al., 2006) and may even be exported from platforms to natural habitats (Love et al., 2005). Bocaccio (*Sebastes paucispinis*) in Californian waters, for example, appear to benefit from the presence of offshore structures, as they may allow the recruitment of larvae that would not have otherwise survived (Emery et al., 2006).

7.2.1.3 Effects on surrounding environment

Large quantities of bivalves grow on some platforms (Wolfson et al., 1979). The death and dislodgement of these animals leads to the formation of a 'shell mound' at the base of platforms, which can be extensive (Schroeder and Love, 2004), and provides hard substrate, altering bottom topography and adding to the structural complexity of the system. This encourages greater biological diversity and supports different invertebrate and fish assemblages to neighbouring soft-bottoms (Wolfson et al., 1979; Love et al., 2000; Bomkamp et al., 2004). The organic input from dead mussels also represents a benthic food subsidy, supporting extremely high densities of some consumers (Wolfson et al., 1979; Bomkamp et al., 2004).

Infauna outside of the area occupied by a platform and its shell mound are affected (Wolfson et al., 1979), however, effects on surrounding communities result from a complex combination of factors: contamination from the platform, physical modification of the seabed by bivalve shells, changes in the hydrodynamic regime, organic input from the shell mound, and potential ecological effects of interactions with the fauna attracted by the platform. The physical and ecological effects are generally thought to be more important than any sediment contamination effects, at least for some shallow Gulf of Mexico platforms with small contamination radii (Montagna et al., 2002). Studies of fauna from Californian platforms have also found no significant accumulation of petroleum hydrocarbons; mussels harvested

from these structures have been deemed fit for human consumption and are reputed to be of very high quality (Schroeder and Love, 2004).

7.2.2 Asia

Very little published research is available on the ecology of platforms in Asian waters, despite the presence of large numbers of structures in some regions (Stachowitsch et al., 2002). However, studies of offshore platforms in China (Yan and Yan, 2003), Brunei Darussalam (Holyoak et al., 2005), and Abu Dhabi (Stachowitsch et al., 2002) generally describe similar patterns to those observed elsewhere: well developed fouling communities which differ from those on natural reefs, marked vertical zonation, attraction of fish, and variations in platform assemblages driven by factors such as distance from shore.

7.2.3 Continental Europe

Research on Mediterranean platforms has mainly centred on a few shallow water structures in the Adriatic, which are heavily fouled by bivalves (Relini et al., 1998), and attract diverse fish assemblages which show significant seasonality (Fabi et al., 2002b)

7.2.4 The North Sea

There are around 500 large offshore installations in the North-Eastern Atlantic, most of which are fixed steel structures in the North Sea (OSPAR, 2009). However, in comparison with platforms in the USA, very little research has been published; less than ten peer-reviewed papers on the biology of North Sea platforms were available (Forteath et al., 1982; Jorgensen et al., 2002; Lokkeborg et al., 2002; Soldal et al., 2002; Whomersley and Picken, 2003; Todd et al., 2009)

7.2.4.1 Fouling assemblages

North Sea platforms are well fouled, and show vertical zonation patterns (Houghton, 1978; Forteath et al., 1982). The fouling communities continue to change even after very long periods of time (Whomersley and Picken, 2003), and there are some differences between platforms; for example, bivalve fouling is

extensive on some platforms, but not others (Forteath et al., 1982). Differences may result from the location of platforms, and the time since their installation (Whomersley and Picken, 2003). Platforms in the Celtic Sea appear similar in terms of fouling composition to those in the southern parts of the North Sea (Southgate and Myers, 1985).

7.2.4.2 Fish

North Sea platforms aggregate substantial numbers of fish at all depths (Lokkeborg et al., 2002; Soldal et al., 2002), including commercially important species (such as the Atlantic cod *Gadus morhua*); as many as 80,000 fish have been estimated to be present around a typical structure (Aabel et al., 1997a). Acoustic and fishing studies around platforms have also found evidence of strong diel and seasonal patterns of abundance around platforms (Lokkeborg et al., 2002; Soldal et al., 2002). Cetaceans may also be attracted; there is some initial evidence that porpoises feed in the vicinity of platforms (Todd et al., 2009).

7.2.4.3 Effects on surrounding environment

Disturbance and contaminant effects around North Sea platforms have altered benthic assemblages up to 6km away from some platforms (Gray et al., 1990; Olsgard and Gray, 1995), reducing biodiversity and leading to dominance of typical tolerant species (Hartley and Watson, 1993). Organisms feeding in the sediment might accumulate toxins; but while some flatfish show a tendency to be 'tainted' by oil from drill cuttings piles (Picken, 1994), semi-pelagic fish such as cod and saithe have been found to be taint-free (Aabel et al., 1997a). No detailed research has explored any 'reef effect' of these platforms on seabed communities.

7.3 Rigs-to-reefs

7.3.1 Rationale

It is generally assumed that all platforms will be removed once they reach the end of their working lives. However, there are reasons to reconsider this assumption. These structures are used as habitat, and their removal will result in the death of

all attached organisms, and loss of habitat for any surviving motile fauna. This may have negative impacts on species of conservation importance. For example, Cowcod (*Sebastes levis*) in the USA is overfished and subject to a "rebuilding plan" (Schroeder and Love, 2004). Oil platforms in the Southern California Bight represent important habitat for significant numbers of this species and removal of these platforms may be equivalent to the removal of large areas of natural habitat (Love et al., 2005). Some species of hard coral which have been found growing on platforms in the Gulf of Mexico are protected by national and international law (Sammarco et al., 2004).

Decommissioning activities are not free of wider environmental impacts. Underwater explosives can cause significant local mortality of fish (Schroeder and Love, 2004), and can harm marine mammals and sea turtles. Removal of structures and clearance of debris will result in seabed disturbance, and drill cuttings piles may be disrupted, potentially dispersing contaminants over a wider area. Even if this is avoided, drill cuttings would no longer be protected by the structure, exposing them to future disturbance by fishing gears (Picken, 1994). The whole process of decommissioning will also require considerable energy use (Watson, 1996), and consequent emissions.

There are also financial implications. Some structures are heavily used by recreational anglers and SCUBA divers (Hiett and Milon, 2002), and their loss could have a significant economic impact on local communities. The cost of decommissioning is also likely to be very high; globally, at least US$30 billion (Watson, 1996), and possibly considerably more.

As a result of these factors, decommissioned platforms are sometimes used as material to construct permanent artificial reefs. This is called 'rigs-to-reefs'.

7.3.2 Rigs-to-reefs approaches

7.3.2.1 Leave in Place

The simplest and (initially) cheapest option consists of plugging the oil wells, removing all sources of contamination, and then leaving the installation in place.

This might facilitate various alternative uses (Abraham, 2001; Schroeder and Love, 2004); suggestions have included use as aquaculture sites (Relini et al., 1998; McGinnis et al., 2001), power stations (Talisman, 2004), research facilities, luxury hotels, military training facilities (Talisman, 2004), prisons, or industrial plants.

Ecologically, leave-in-place has some advantages. Platform assemblages will be undisturbed, and the upper part of the jacket is not removed; this retains the most biologically productive part of the system, maintaining the supply of material to the shell mound (McGinnis et al., 2001; Bomkamp et al., 2004) and supporting greater numbers of fish (Carr et al., 2003). Any drill cuttings would be protected by the jacket.

However, the ongoing maintenance costs and liability concerns mean that only in a very few cases will leaving a structure in place be viable, and even in those cases it may not be a permanent solution.

7.3.2.2 Topple in Situ

Following the removal of all sources of contamination, the jacket can be cut at (or near) the base, pulled over and allowed to settle on the sea floor. Short-term effects will be negative as the fouling assemblages and local benthos will be disturbed. The shallowest assemblages (fouling and fish) will be lost, and the shell mound (where present) will gradually degrade owing to cessation of input from shallow bivalve populations (Carr et al., 2003; Bomkamp et al., 2004). Depending on how close to the seabed the jacket is cut, cuttings piles may or may not continue to be protected. Toppling can be undertaken by smaller vessels than are needed for total removal activities; costs and carbon emissions will be reduced (Schroeder and Love, 2004).

7.3.2.3 Move and Topple

Instead of allowing the platform to fall where it stands, the jacket can be lifted from the seabed and relocated to a designated site, allowing the creation of large artificial reef systems using multiple jackets. The impacts on fouling organisms and

fish will be the same as for topple-in-place, and the original site will need to be fully cleared.

While this may not seem different from total removal (from a practicality and cost point of view), the jacket will not need to be lifted entirely out of the water, and therefore smaller lifting vessels can be used, reducing costs and emissions (Schroeder and Love, 2004).

7.3.2.4 Partial removal

Alternatively, the jacket and conductors can be severed at a depth adequate to leave the required overhead clearance (typically 55m). The upper part of the jacket may then be removed to shore, or placed on the seafloor to increase the amount of habitat (Schroeder and Love, 2004). Advantages of this method include a potentially higher reef profile and a reduction in the trauma to the biological assemblages, especially since non-explosive methods are more feasible in this context (Dauterive, 2000). Again, the shallowest assemblages would be lost, and any shell mound would no longer be replenished, but the retention of the platform base would ensure the protection of any drill cuttings pile.

7.3.2.5 Increasing the value of 'reefed' rigs

Reefs built using decommissioned platforms could be enhanced by the addition of materials such as quarry rock (Schroeder and Love, 2004), which would contribute complexity and additionally microhabitat space, compensating for the loss of shell mound (McGinnis et al., 2001). Reefed rigs could be also protected in order to prevent overfishing (Love et al., 2005).

7.3.3 Opposition to Rigs-to-Reefs

Some environmentalists object to the use of decommissioned platforms as artificial reefs. Decommissioned platforms might be considered to be sources of contamination in the marine environment, although cleaned reef components would consist of little other than steel. Assemblages on artificial reefs can also be considered 'unnatural', particularly if they are placed in an area with little or no

existing hard-bottom habitat, and some would prefer that the sea bed be restored to its 'natural' state once platforms are decommissioned. Finally, some oppose rigs-to-reefs because they consider that it is simply a way for oil companies to avoid their responsibility to remove these structures (Baine, 2002).

Opposition to rigs-to-reefs among commercial fishermen is generally strong, as they perceive the areas as lost fishing grounds, and any structures on the seabed present a snagging hazard for trawlers. Strong evidence of benefits to fish stocks would be required before they became supportive. However, fishermen using certain gear types (lobster fishermen in California, for example) are sometimes in favour of rigs-to-reefs (McGinnis et al., 2001).

7.3.4 Rigs-to-reefs in the Gulf of Mexico

The US Gulf of Mexico hosts the world's largest rigs-to-reefs program. All five states with coasts on the Gulf have active artificial reef programs, and those of Texas and Louisiana are based almost entirely on decommissioned platforms (Kaiser and Pulsipher, 2005). Nearly 200 retired platforms have been converted to reefs in the Gulf (Kaiser and Pulsipher, 2005), an arrangement which is supported and encouraged at a federal level, albeit without any federal funding (McGinnis et al., 2001).This arrangement first came about as a result of pressure from the recreational fishing sector, which was concerned about the removal of structures. Platforms in the Gulf of Mexico are estimated to provide over 1600 ha of hard habitat (Lewbel et al., 1987); these structures have been heavily utilised for recreational fishing, which is economically important in the Gulf states (Hiett and Milon, 2002).

Platforms that have been approved for 'reefing' are usually toppled *in situ* or relocated prior to toppling (Kaiser and Pulsipher, 2005), although 'partial removal' is becoming more common as larger, deeper platforms are decommissioned (Schroeder and Love, 2004). Companies donating platforms to a state rigs-to-reef program also donate half the cost savings (relative to complete removal) to the state artificial reef program. Liability for the platform and responsibility for

maintaining safety of navigation are transferred to the state (Kaiser and Pulsipher, 2005).

7.3.5 Could rigs-to-reefs work elsewhere?

Rigs-to-reefs has been successful in the Gulf of Mexico, but this has resulted from a rather unique combination of factors (Schroeder and Love, 2004). Firstly, the paucity of natural hard-bottom habitat in the Gulf of Mexico means that platforms make up a significant amount of the total hard habitat in these waters. The rigs-to-reefs program has also benefited from the considerable support of the recreational fishing and diving tourism sectors. Where these elements are missing or reduced, support for rigs-to-reefs is low.

In California, for example, there is significant opposition to rigs-to-reefs from environmental groups and the commercial fishing industry, despite the support of many politicians, and some other conservation groups. Additionally, Californian waters already contain large natural hard-bottom areas; unlike in the Gulf of Mexico, platforms may not make a significant contribution to regional hard bottom habitat (McGinnis et al., 2001). Consequently, although a proposal for a rigs-to-reefs program was approved by the California legislature, it was vetoed by the state Governor in 2001.

Ongoing research into the ecosystems associated with offshore platforms in other locations may help to facilitate a reconsideration of rigs-to-reefs outside of the Gulf of Mexico.

7.4 Aims and Objectives of Part II

The following chapters are concerned with the marine communities associated with platforms in the North Sea. The main aims are:

- To describe the marine communities of offshore structures in the North Sea
- To determine how fouling assemblages in particular vary between platforms

- To evaluate the use of 'footage-of-opportunity' in studies of biological communities

These aims are discussed in the context of the (admittedly sparse) literature concerning North Sea platform communities, and some consideration is given to the feasibility of using these structures as permanent artificial reefs.

Chapter 8. Marine communities of North Sea offshore platforms

8.1 Introduction

8.1.1 Studies of North Sea platforms

Very little peer-reviewed work has been published on the marine life associated with offshore platforms in the North Sea. A few studies have focused on aggregated fish at platforms (Jorgensen et al., 2002; Lokkeborg et al., 2002; Soldal et al., 2002), a handful have documented the fouling communities (Forteath et al., 1982; Whomersley and Picken, 2003), one has described the occurrence of the cold water coral, *Lophelia pertusa*, on North Sea platforms (Gass and Roberts, 2006), and one has recently been published on the possible use of these structures by foraging porpoises (Todd et al., 2009). Any other knowledge about the biology of these vast artificial reefs is contained in a variety of 'grey literature', much of which is not generally available.

This is partly a consequence of the nature of oil and gas platforms in the North Sea. They are located many kilometres offshore, making it difficult and expensive for researchers to visit them. Biological sampling can be difficult, especially in the often challenging conditions of the North Sea. Above all, these are working structures, and normal operations take precedence over other activities. Nonetheless, it is sometimes possible to gain useful data from offshore platforms, especially where scientific work can be carried out during normal operational activities, such as structural surveys.

8.1.2 The SERPENT project

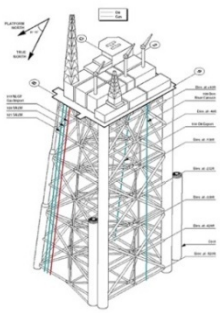
The SERPENT project (Scientific and Environmental ROV Partnership utilising Existing iNdustrial Technology) is a collaborative venture involving the oil and gas industry and scientists based at numerous institutions throughout the world. It allows researchers access to offshore locations where the industry is engaged in exploration and extraction activities, and the use of equipment such as ROVs (Remotely Operated Vehicles), some of which are capable of working in depths of up to 6000m (Jones, 2009). The scientific community generally has access to only a

very small number of such vehicles and they are expensive to operate and maintain. The SERPENT collaboration allows use of such equipment at no cost, albeit with the restrictions that scientific work must fit around existing operational schedules, and that work can only take place in locations where the industry has assets in place. However, this situation is ideal for the study of the marine life associated with oil and gas infrastructure itself.

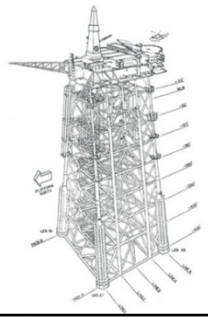
8.2 Methods

8.2.1 Study sites

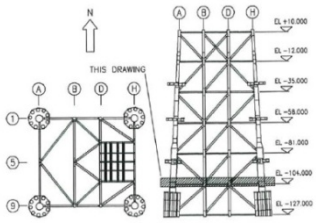
Several offshore platform operators and contractors involved in SERPENT were contacted with a request for footage and images of steel-jacketed offshore structures in the North Sea. Three responded favourably, providing extensive footage and images of a total of eight structures (Fig 8.1), covering a range of locations in the North Sea (Fig 8.2), and a selection of platform ages. Offshore structures are regularly inspected for corrosion, damage, or other deformation, and this is usually carried out using ROVs. All parts of a platform are inspected visually, and footage of the inspection is recorded and retained; it was this footage which was made available for the project. Underwater inspection of offshore structures is hampered by marine growth: it can restrict access to certain parts of the structure and it obscures the surface, hiding corrosion or damage (Zvyagintsev and Ivin, 1995). However, this makes structural survey footage ideal for assessing the biological community.



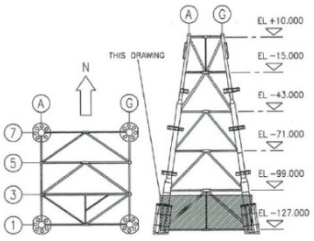
THISTLE ALPHA
Water Depth: 160m
Subsea structure mass: 31,000 tonnes
Installation Date: 1977-1978
Operator: BP
Data provided: Selected digital stills of organisms on structure
Date surveyed: Images captured in 2004 and 2005



HEATHER ALPHA
Water Depth: 144m
Subsea structure mass: 18,700 tonnes
Installation Date 1977-1978
Operator: Lundin Oil
Data provided: Structural survey footage (legs) and digital stills of points of interest
Date surveyed: July 2002, digital stills obtained in 2005

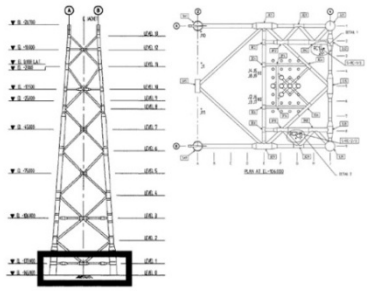


NORTH ALWYN A (NAA)
Water Depth: 125m
Subsea structure mass: 18,500 tonnes
Installation Date: 1985
Operator: Total
Data provided: Complete structure survey (video) including conductors
Date surveyed: June 2006

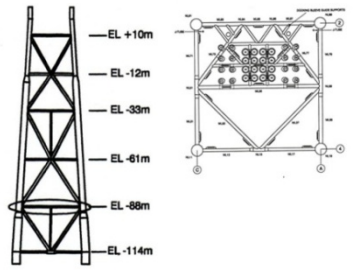


NORTH ALWYN B (NAB)
Water Depth: 125m
Subsea structure mass: 14,500 tonnes
Installation Date: 1986
Operator: Total
Data provided: Complete structure survey (video)
Date surveyed: July 2006

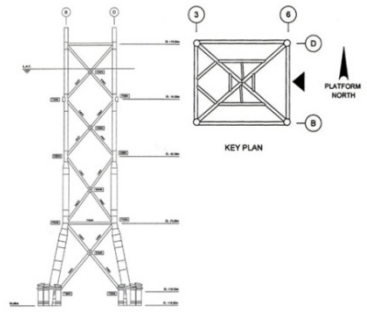
Figure 8.1 Summary information for platforms investigated during this study. Schematics were provided by platform operators or survey contractors, other information was obtained from OPSAR database (OSPAR, 2009). Figure continues overleaf.



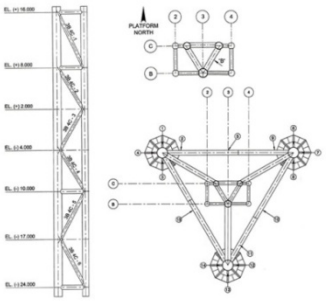
DUNBAR
Water Depth: 127m
Subsea structure mass: 9500 tonnes
Installation Date: 1993-1994
Operator: Total
Data provided: Complete structure survey (video)
Date Surveyed: August 2006



BRUCE (DRILLING)
Water Depth: 118m
Subsea structure mass: 7700 tonnes
Installation Date: 1993
Operator: BP
Data provided: Survey footage (legs, conductors)
Date surveyed: June 2006



ANDREW
Water Depth: 116m
Subsea structure mass: 6500 tonnes
Installation Date: 1996
Operator: BP
Data provided: Survey footage (legs, conductors)
Date surveyed: June 2006



HOTON
Water Depth: 32m
Subsea Structure mass: 428 tonnes
Installation date: 2001
Operator: BP
Data provided: Complete structure survey (video)
Date surveyed: August 2006

Figure 8.1 continued

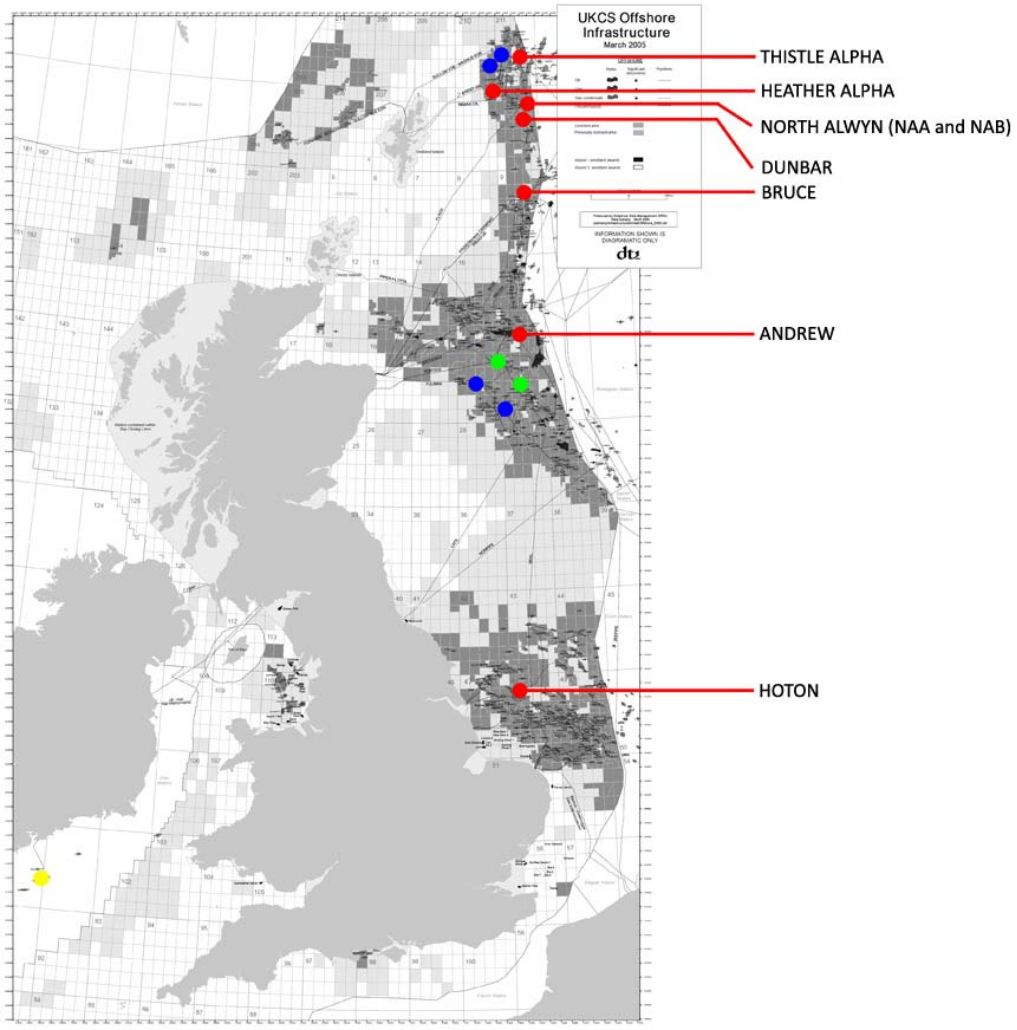


Figure 8.2 UK Sector Oil and Gas infrastructure map, showing positions of the platforms examined for this study. For reference, positions of platforms examined in previous studies also marked in different colours: Yellow (Southgate and Myers, 1985), Green (Forteath et al., 1982) and Blue (Whomersley and Picken, 2003). Source: DTI (http://www.og.dti.gov.uk/)

8.2.2 Terminology

Terms used to refer to features on steel offshore platforms (Fig 8.3) are explained below.

- **Jacket**: overall term for the underwater structure of the platform, which extends from the seabed to above the sea surface and supports the weight of the topsides; specifically refers to structures composed of a lattice of tubular steel members.
- **Topsides**: those parts of the platform structure supported atop the jacket: accommodations, drilling and production decks, etc.
- **Member**: any tubular steel element of the platform jacket; these can be vertical, horizontal, or any other orientation.

- **Node**: any point where multiple members meet, at which they are attached together by welds
- **Elevation**: platform jackets are divided up into a number of levels, to assist with locating elements of structure in the schematics. Elevations are numbered from the seabed upwards, with major horizontals at various intervals as the jacket is ascended. For example, the NAA platform has 7 elevations at which major horizontal members are found; 1 (seabed), 3 (104m), 7 (81m), 11 (58m), 15 (35m), 17 (12m) and 19 (which is above sea level). The layout of a typical major elevation on NAA is shown in Fig 8.3.
- **Leg**: largest vertical members, usually referring only to those on the four corners of the jacket
- **Conductors**: vertical elements with a relatively small diameter, extending from the seabed to the surface, which carry oil/gas. Often numerous, housed within the jacket.
- **Conductor Guide Frames**: horizontal elements; these are the frameworks which hold the conductors in place within the jacket.
- **Caissons, J-Tubes, Risers**: other vertical elements, responsible for carrying various substances to or from the surface. Caissons do not always extend all the way from the surface to the seabed.
- **Pile guides**: steel jacket structures are typically held in place by a series of tubular steel piles which are driven into the seabed. These piles pass through 'pile guides', which are present at the base of the structure as well as at various depths. The largest pile guide assemblies (at the base of a structure) are also sometimes referred to as **pile sleeves**.
- **Footings**: general term referring to the very bottom parts of the structure: the bases of legs, conductors, etc.

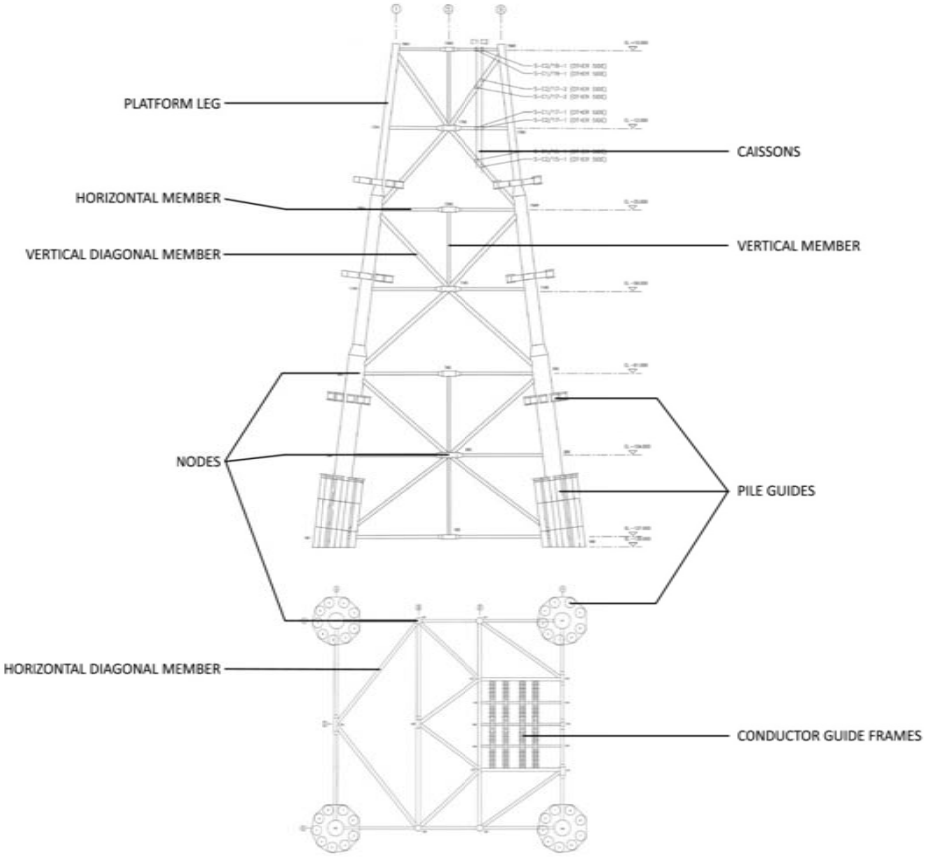


Figure 8.3 Layout of typical steel jacket offshore structure, with representative features marked. Schematic is of platform North Alwyn A, provided by Total E&P.

8.2.2 Habitat availability on offshore platforms

Offshore structures present a very large surface area for attached organisms to settle on. A steel jacket structure in 30m depth (for example) provides over 8,000m^2 of surface area for colonisation (Gallaway and Lewbel, 1982). Given that some of the platforms examined here were installed in over 140m depth, the area provided is considerably greater. These structures extend throughout the entire water column, providing habitat for a variety of sessile organisms, ranging from intertidal species to deep water corals. Furthermore, habitat diversity is increased by the availability of surfaces with a variety of orientations, including vertical surfaces, horizontal surfaces facing both upwards and downwards, and diagonally inclined surfaces at various angles; surface orientation is known to affect fouling assemblage composition (Glasby, 2000; Glasby and Connell, 2001; Perkol-Finkel et al., 2006). Additional surfaces are presented at various depths by conductor guides and pile guides. Caissons, risers and conductors are often arranged in groups,

creating small spaces between them which some species can occupy; grout pipelines, reference electrodes, redundant stubs and cut-offs, towing eyes, control panels and other such components (left over from the installation phase) further increase structural complexity and microhabitat diversity. Finally, hard fouling species themselves provide surfaces for colonisation by other epibiota and increase structural complexity yet further.

Debris from platforms can also provide additional habitat structure on the seafloor, which is readily used by fouling species and fish (Caselle et al., 2002). Hard remains of fouling organisms that have become dislodged from the structure further modify the seabed, altering habitats (Bomkamp et al., 2004).

8.2.3 Preliminary species list for North Sea structures

Published studies were examined in order to generate a list of species which might be expected on North Sea platforms (Table 8.1). Only two published studies were located which dealt specifically with fouling species on structures in the North Sea (Forteath et al., 1982; Whomersley and Picken, 2003). An additional study, concerning fouling on two structures in the Celtic Sea, was included (Southgate and Myers, 1985). Each study had a slightly different focus: Forteath et al (1982) focused on one particular platform, using biological samples from platform legs and images of growth on platform legs to explore depth zonation; Southgate and Myers (1985) sampled fouling assemblages on two platforms down to a depth of 30m; while Whomersley and Picken (2003) utilised video surveys of the clamps on oil export risers from four platforms to explore coarse-resolution changes in the fouling assemblages over an 11-year period. Differences in the taxa observed between these studies may thus derive from differences in focus and methodologies, rather than any other factors.

8.2.4 Observations of reef fauna based on survey footage

An assessment of the organisms (fouling organisms, motile invertebrates, and fish) associated with all eight structures was carried out by viewing of the survey footage, and careful examination of any digital stills that were provided. For each

platform where footage was provided, survey footage of all legs was viewed, recording all species that could be identified. It should be noted that the video footage used, even at its best, did not allow confident identification of some of the species encountered. In these cases, higher taxonomic classifications were used. The species list derived from previous studies on North Sea platforms (Table 8.1) was used to identify likely candidate species, alongside identification guides (Hayward and Ryland, 1995; Moen and Svensen, 2004; Picton and Morrow, 2009).

Different amounts of footage were made available for each structure (complete structure surveys were provided for some platforms, while only leg and conductor guide frame surveys were provided for others), and footage from some platforms was processed in greater detail than others. Furthermore, certain specific types of footage were only provided for some platforms. During structure surveys, ROVs were sometimes required to undertake measurements on platform members; this usually entailed actually coming into contact with a member and holding still for a few seconds or longer. This still did not provide enough detail to identify all species encountered, but occasionally it showed some smaller taxa which would otherwise not have been seen at all. Digital stills from Heather Alpha and Thistle Alpha also allowed some smaller taxa to be identified, but the limited number of these images meant that few species were spotted in this fashion.

Consequently, a semi-quantitative approach was adopted for the assessment of species presence at each structure. Taxa were assigned to one of several abundance categories based on observations made on still images or video footage. Precise definitions of each of the categories varied depending on the type of organism concerned, and are explained in table legends accompanying the data. Briefly, species were listed as 'Present' (P) where they were sighted at a structure, perhaps based on a single observation, but no quantification was possible. 'Uncommon' (U) taxa were those observed infrequently, in small numbers (<20) of individuals, or small colonies of fouling species. Taxa were listed as 'Common' (C) if they occurred in larger numbers (up to 100 colonies or individuals), while 'Abundant' (A) taxa were observed in numbers greater than 100, and 'Very Abundant' (VA) taxa were those for which more than 1000 individuals were

observed. Taxa which were not recorded on any particular platform were referred to as 'Not Observed' (rather than absent). In some cases, however, it was possible to be confident that failure to observe a species corresponded to an absence of that species, and these were recorded as 'Not Present' (NP). For example, *L. pertusa* was not observed on the Hoton platform, and since this platform is located in a water depth of only 30m (*L. pertusa* was not observed shallower than approximately 40m depth) and is located south of the expected distribution for this species on platforms in the North Sea (Gass and Roberts, 2006), it can be confidently asserted that this species was indeed absent from Hoton. Given the limitations imposed by availability of footage, these semi-quantitative abundance estimates should in all cases be regarded as minimum estimates of the true abundances.

8.2.5 Fish survey methodology

To further explore fish occupation of North Sea platforms, a fish survey of NAA was undertaken using the structural survey footage. Two types of footage were used for the fish survey. At each of the major elevations (1 (seabed), 3 (104m), 7 (81m), 11 (58m), 15 (35m), 17 (12m)) all horizontal members were surveyed by the ROV, which made two slow passes of each member, one above and one below. Since the ROV faced forward along the member during this type of survey, the footage was suitable for fish counts, and all fish observed by the ROV were recorded, with one restriction: only fish within a short distance of the member (approximately three times the diameter of the member) were counted, since very large numbers of *P. virens* were often visible on the footage, but were too distant to be counted. This meant that only fish directly associated with platform structure were surveyed. At elevation 1 (seabed), many of the members were buried, however the ROV followed the route of each member, so fish among the platform footings were similarly counted.

In addition to the footage of the major elevations, footage of the pile guide assemblies at the base of each of the four legs was also analysed. Pile guide surveys included two passes of each pile sleeve, and one circuit of the interior of

the assembly (between the piles and the base of the platform leg). All fish encountered during this part of the platform survey were counted. Each elevation was generally surveyed over the course of a few hours on one particular occasion, however, different elevations were surveyed at different times (Table 8.5). All pile guides were surveyed between 15:00 and 17:00 on one particular day.

Footage of platform legs, and other vertical or diagonal members above the level of the pile guides was not used. Preliminary viewing of this footage revealed that few fish were directly associated with such elements, except at nodes (which were included in the surveys of the horizontals) and footage of these members generally involved the ROV looking directly at the member; this was not useful for counting fish.

8.3 Results and Discussion

Table 8.1 Invertebrate species list for offshore platforms in UK waters, derived from three published studies of fouling communities. [1]Whomersley and Picken (2003) focuses on fouling patterns of a few fouling categories, and does not provide a species list. [2]The category described as 'hydroids' in this paper is likely to include all of these species, and possibly others. [3]The category described as 'tubeworms' in this paper is likely to include these species.

		Forteath et al., 1982	Southgate and Myers, 1985	[1]Whomersley and Picken, 2003
		North Sea	Celtic Sea	North Sea
Macroalgae	*Polysiphonia brodiaei*	Present	Present	
	Polysiphonia urceolata	Present		
	Ulva lactuca		Present	
	Ulva intestinalis	Present		
	Laminaria hyperborea	Present		
	Laminaria digitata	Present		
	Alaria esculenta	Present		
Bryozoans	*Bicellariella ciliata*	Present		
	Bugula avicularia	Present		
	Electra pilosa	Present		
	Omalosecosa ramulosa	Present		
	Tubulipora liliacea	Present		
	Alcyonidium hirsutum	Present		
Hydroids	*Tubularia larynx*	Present	Present	[2]Present
	Obelia dichotoma	Present		[2]Present
	Bougainvillia ramosa	Present		[2]Present
	Laomedea flexuosa	Present		[2]Present
Anemones	*Metridium senile*	Present	Present	Present
	Sagartia troglodytes		Present	
Soft corals	*Alcyonium digitatum*	Present	Present	Present
Sponges	*Leucosolenia complicata*	Present		
Polychaetes	*Protula tubularia*		Present	[3]Present
	Pomatoceros triqueter	Present	Present	[3]Present
	Hydroides norvegica	Present	Present	[3]Present
	Filograna implexa	Present		
	Perinereis cultrifera		Present	
	Sabella pavonina		Present	
	Nereis pelagica	Present		
Crustaceans	*Caprella aequilibra*		Present	
	Jassa falcata	Present	Present	
	Parajassa pelagica		Present	
	Stenothoe valida		Present	
	Cancer pagurus		Present	
	Hyas araneus		Present	
	Inachus spp.		Present	
	Idotea pelagica		Present	
	Balanus (=*Chirona*) *hameri*		Present	
Pycnogonids	*Nymphon gracile*		Present	
Molluscs	*Aeolidia papillosa*		Present	
	Dendronotus frondosus	Present		
	Anomia ephippium		Present	
	Chlamys distorta		Present	
	Hiatella arctica		Present	
	Modiolus barbatus		Present	
	Musculus discors		Present	
	Mytilus edulis		Present	Present
	Ostrea edulis		Present	
Echinoderms	*Echinus esculentus*		Present	
	Psammechinus miliaris		Present	
	Asterias rubens	Present	Present	Present
	Marthasterias glacialis		Present	
	Ophiothrix fragilis		Present	

8.3.1 Fouling organisms on North Sea structures

Table 8.2 List of fouling organisms observed on 8 North Sea platforms, with estimated abundances. **P** = Present: based on a single observation, (or a few observations) however sightings were affected by restricted sampling or other constraints, and therefore no statements can be made about abundance; **U** = Uncommon: present in small numbers (less than 20 individuals for solitary species, or less than 20 small colonies for colonial species); **C** = Common: observed more frequently, but never accounting for large areas of coverage (up to 100 individuals or colonies); **A** = Abundant: more than 100 individuals, many large colonies, or dominance over some areas; **VA** = Very Abundant: more than 1000 individuals, or dominance over substantial areas; **Blank cells** = Not Observed: taxon not recorded, but presence cannot be ruled out; **NP** = Not Present: similarly to Not Observed, the taxon was not recorded, but in this case other factors (such as platform depth or location) mean that its presence can be more confidently ruled out.

		Heather Alpha	Thistle Alpha	NAA	NAB	Dunbar	Bruce	Andrew	Hoton
Macroalgae	*Polysiphonia* spp.	P		P	P	P	P	P	P
	Alaria esculenta	P		P		P			
	Other red algae	P		P	P	P	P	P	P
	Other green algae	P		P	P	P	P	P	P
Anemones	*Metridium senile*	A	A	A	VA	VA	VA	VA	VA
	Urticina spp.	P		A	A	A	C	C	P
	Sagartia spp.	P	P	A	P	A	C	U	
	Protanthea simplex	C	C	C	A	P	C	P	
	Corynactis viridis			P		P	P	U	
Soft coral	*Alcyonium digitatum*	A	A	A	A	A	C	A	
Hydroids	*Tubularia* spp.	VA	VA	A	A	A	C	A	
	Other hydroids	VA	VA	VA	VA	VA	VA	VA	A
Hard corals	*Lophelia pertusa*	VA	A	VA	C	C	U		NP
	Caryophyllia smithii	A	A	A	A	A		P	
Tunicates	*Ciona intestinalis*			U		P		P	
Bryozoans	*Omalosecosa ramulosa*			P					
	Membranipora membranacea	P							
Polychaetes	Serpulidae	A	A	A	A	A	C	A	A
Barnacles	*Chirona hameri*			U	C	C	C	U	
Sponges	*Mycale lingua*	P	P	P		P	P	P	
Bivalves	*Mytilus edulis*	P	P	P	P	P	P	P	

8.3.1.1 Shallow fouling assemblages

Detailed examination and accurate identification of species in the shallowest parts of the platforms was not possible. Proximity to the sea surface meant that water movement here was greatest, making it hard for the ROV pilot to maintain position, so footage was often blurred and less useful. Furthermore, high light levels from the surface meant that the legs could often only be seen in silhouette. However, some observations were possible.

The assemblages of organisms found at the shallowest parts of the platforms (legs and other vertical members down to a depth of approximately 20m, and any

horizontal and diagonal members within that depth range) resembled the communities which occur on a typical rocky shore (Fig 8.4). Sessile fauna were dominated by hydroids (including *Tubularia* spp.) and mussels (Fig 8.4); these were assumed to be mostly *Mytilus edulis*, but other mussel species have been indentified on platforms in waters around the British Isles (Southgate and Myers, 1985), so a mixture cannot be ruled out. Uniform beds of mussels were not observed on all platforms, although the upper surfaces of shallow horizontal members presented suitable habitat, often being covered in mussel growth (Fig 8.4).

Figure 8.4 Typical shallow-water fouling assemblages on North Sea platforms. **A**: Mussel (*Mytilus edulis*) dominated fouling on the upper face of a horizontal member (Elevation 17, Platform NAA, 10m depth). **B**: Algae (*Alaria esculenta*) dominated assemblage on a platform leg (Leg H9, Platform NAA, 10m depth). **C**: Algae (*Polysiphonia* spp.) dominated assemblage on a platform leg (Leg B6, Platform Andrew, 8m depth).

However, these fauna were largely overgrown by macroalgae, which were prevalent in these depths; platform legs were often completely shrouded in algae at depths less than 10m (Fig 8.4). *Polysiphonia* spp. were the most common algae, and appeared to be present on every platform surveyed here, while other algae, both red and green, were important but could not be readily identified. *Alaria esculenta*, a species typical of highly exposed rocky shores (Little and Kitching, 1996), formed extensive beds on some structures (Heather Alpha, North Alwyn A), and was present in lesser quantities on others (Dunbar), but was not observed on all platforms. At the air-sea interface on a few platform components, thin layers of red and green algae were seen, which may have included *Ulva intestinalis* and *Ulva lactuca*.

Macroalgae generally appeared most prevalent on the outward facing surfaces of platform legs (see Chapter 9), but also on the upper surfaces of horizontal members (particularly those around the sides of platforms (horizontal members

within the interior of the jacket were more shaded by the topsides above). Undersides of horizontal members were more dominated by hydroids (such as *Tubularia* spp.) and some species of anemone. Shading and surface orientation are known to affect fouling composition (Glasby, 1999a, b).

8.3.1.2 Actinaria (anemones)

The plumose anemone, *Metridium senile*, was present on every structure, albeit with some large differences between platforms in the extent of coverage (see Chapter 9). In places, the fouling assemblage was totally dominated by this species (Fig 8.5), which was generally greatest in abundance between 30m and 90m. However, *M. senile* did not appear to be depth-limited over the depth range occurring on these platforms; it was present at every depth excluding the very shallow, algae-dominated regions. It was also not restricted to growing on surfaces with particular orientations; it was found on legs and other vertical members, and also on both upper and lower surfaces of horizontal and diagonal members.

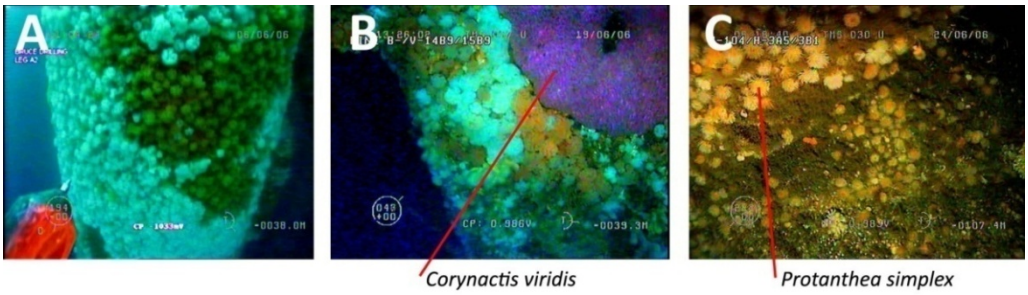


Figure 8.5 Anemone fouling on North Sea platforms. **A**: 100% coverage of *Metridium senile* on a platform leg (Leg A2, Platform Bruce, 38m depth). **B**: *Corynactis viridis* (individual *C. viridis* are too small to be resolved in this frame) among *M. senile* (Leg B9, Platform NAA, 39m depth). **C**: *Protanthea simplex* anemones growing among hydroids on the underside of a horizontal member (Elevation 3, Platform NAA, 107m depth).

Urticina spp. and *Sagartia* spp. anemones were observed on most platforms, and were likely to be ubiquitous on North Sea platforms. *Sagartia* anemones were seen individually or in small clusters of less than 10 individuals, while *Urticina* anemones were usually solitary. *Corynactis viridis* and *Protanthea simplex* (Fig 8.5) were observed in small patches, with the former usually on legs and other vertical surfaces, while the latter also occurred on the undersides of horizontal members.

8.3.1.3 Soft coral (*Alcyonium digitatum*)

Alcyonium digitatum (Fig 8.6D) was observed on every platform except for Hoton, in varying abundances (see Chapter 9). It was observed at all depths, although it was generally most abundant between 30m and 50m. It occurred on legs and other verticals, the sides of horizontal members, and the undersides of horizontal and diagonal members, but generally not on the upper faces of horizontals or diagonals. In a few areas on platform Andrew, coverage of *A. digitatum* approached 100%, but mostly it was found among *M. senile* or hydroid-dominated fouling.

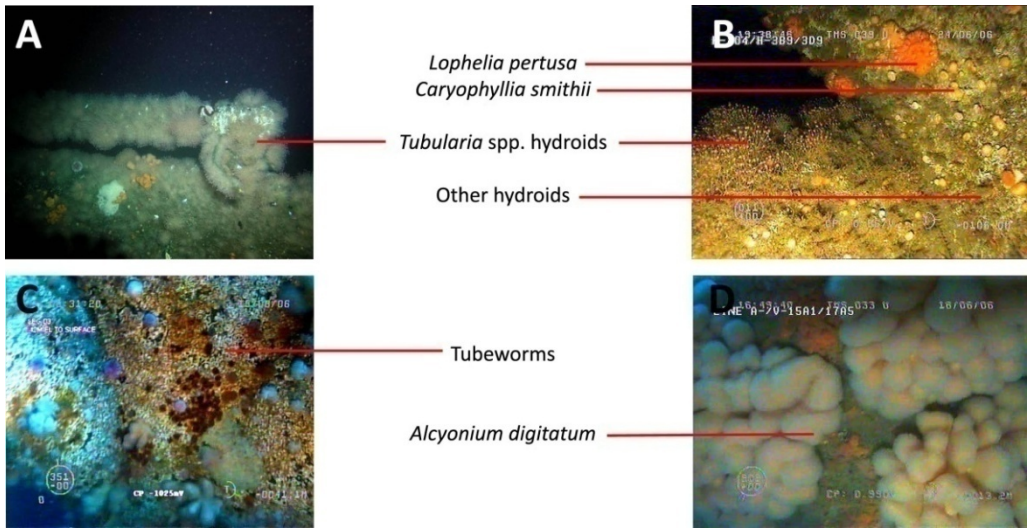


Figure 8.6 Other typical fouling assemblages on North Sea platforms. **A:** Heavy fouling of a horizontal member by *Tubularia* spp. hydroids (Platform Heather Alpha, 37m depth). **B**: Lighter, mixed hydroid fouling assemblage featuring *Tubularia* spp., other hydroids, as well as several *Caryophyllia smithii* polyps and small colonies of *Lophelia pertusa* (Elevation 3, Platform NAA, 106m depth). **C**: Fouling by tubeworms, approaching 100% coverage with living polychaetes (Family Serpulidae) and abandoned worm casts, on the interior face of a platform leg (Leg D3, Platform Andrew, 40m depth). **D**: Heavy fouling by *Alcyonium digitatum* on the underside of a vertical-diagonal member (Platform NAA, 15m depth).

8.3.1.4 Hydroids

Areas of hydroid dominated fouling occurred on all platforms, although there were variations in the amount of coverage and in the density of hydroid growth (Fig 8.6B). Bryozoans may also have been present in large quantities (Forteath et al., 1982), but were not generally identifiable from the video footage or still images. Hydroid-dominated assemblages varied in composition: shallower assemblages (in depths from 20m to 90m) were generally thicker and incorporated more anemones

(*M. senile*, *C. viridis*, *P. simplex*), soft corals (*Alcyonium digitatum*) and sometimes mussels. At greater depths, hydroid cover was generally thinner, with less inclusion of other soft-fouling species (anemones and soft corals) but with hard corals sometimes present (Fig. 8.6B).

8.3.1.5 Hard fouling species (Tubeworms, barnacles, and bryozoans)

Tubeworms (Family Serpulidae) were present on all platforms. Living individuals were observed, and abandoned worm casts were also present, covering substantial areas of platform members in places (Fig 8.6C). Species identification was not possible, but it is likely that they are the same species that were identified during previous studies: *Protula tubularia, Pomatoceros triqueter, Hydroides norvegica* and *Filograna implexa* (Forteath et al., 1982; Whomersley and Picken, 2003).

Large barnacles (*Chirona hameri*) were observed on the deeper parts of some platforms; however, they never appeared to cover more than a few percent of any areas viewed by the ROVs. They may have been obscured by other types of growth in places.

Bryozoans were almost certainly undersampled by the methods used here; they were identified very infrequently (*Membranipora membranacea* was observed on *Alaria esculenta* in images from Heather Alpha, and one colony of *Omalosecosa ramulosa* was observed on a platform member on NAA). Several species of bryozoan have been sampled from North Sea platforms (Table 8.1); indeed they were found to be dominant fouling taxa on Montrose Alpha (Forteath et al., 1982). Arborescent bryozoans observed on video may have been assumed to be hydroids. Direct biological sampling would be required to explore the extent of their presence on these structures.

8.3.1.6 Scleractinia (hard corals)

Lophelia pertusa colonies were also present on some platforms among the hydroid-dominated assemblages, although in some places their growth was so great that *L. pertusa* itself was the dominant fouling organism (Fig 8.7). This species was not observed on Andrew or Hoton, but was present on all other platforms to

some extent. A range of colony sizes and densities were observed (Fig 8.7). Large colonies (colony diameters estimated to be greater than 0.5m) were observed on legs, large diagonal members, and other structural elements of Heather Alpha, Thistle Alpha, NAA and (to a lesser extent) NAB. Less than 50 small colonies (colony diameters estimated to be less than 0.5m, see Figs. 8.6B and 8.7A) were observed on legs and conductor guide frames on the Bruce platform, while on the Dunbar platform many such small colonies (>100) were present, but appeared to be restricted to the conductors. It has been estimated that colonies smaller than approximately 150mm are not reliably detected on survey footage (Gass and Roberts, 2006).

The distribution of colony sizes on North Sea platforms may be a result of their temporal pattern of colonisation. Gass and Roberts (2006) recorded several hundred small colonies (colony diameter less than 0.5m) on the conductors of Heather and NAA, along with a smaller number (less than 50) of larger colonies, and suggested that these large colonies were primary colonists (larvae from natural *L. pertusa* reefs), while the smaller colonies were founded by larvae produced by sexually mature colonies on the platforms themselves. The size of some larger (up to 132cm in diameter) colonies on North Sea platforms, combined with data on colony growth rates, suggests that some platforms were colonised by *L. pertusa* almost immediately after installation (Gass and Roberts, 2006).

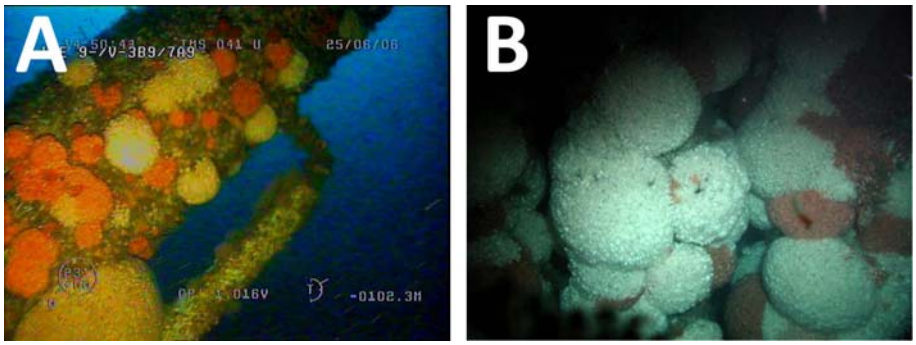


Figure 8.7 Variations in the extent of fouling by *Lophelia pertusa*. **A**: Growth of multiple colonies on the underside of a vertical-diagonal member. Various sizes of colony are visible, indicating that colonisation has occurred repeatedly over a number of years (Platform NAA, 102m depth). **B**: Complete overgrowth of conductors with *L. pertusa* (Platform Heather Alpha, 120m depth).

Cup corals (*Caryophyllia smithii*) were present in great numbers (often many more than 100 individuals) on some platforms (Table 8.2; Fig 8.6). They were generally

present on vertical surfaces and on the undersides of diagonal and vertical members, and were observed over a wide depth range, from around 30m to over 100m, but not on Bruce or Hoton.

8.3.2 Motile invertebrates on North Sea structures

Twenty taxa of motile invertebrates were observed in the structural survey videos and still images (Table 8.3). Several of these taxa were small and not frequently observed on video footage; however they were identified from close examination of still images, or on occasions when the ROV moved very close to a member being inspected.

Table 8.3 List of motile invertebrate taxa observed on 8 North Sea platforms, with estimated abundances. **P** = Present: based on a single observation, (or a few observations) however sightings were affected by restricted sampling or other constraints, and therefore no statements can be made about abundance; **U** = Uncommon: present in small numbers (less than 20 individuals); **C** = Common: observed more frequently (up to 100 individuals); **A** = Abundant: more than 100 individuals; **VA** = Very Abundant: more than 1000 individuals; **Blank cells** = Not Observed: taxon not recorded, but presence cannot be ruled out.

		Heather Alpha	Thistle Alpha	NAA	NAB	Dunbar	Bruce	Andrew	Hoton
Echinoderms	*Echinus esculentus*	C	C	C	C	C	P	U	P
	Psammechinus miliaris						P		C
	Asterias rubens	P	P	A	A	A	C	C	O
	Henricia spp.						P	U	
	Marthasterias glacialis	P	P						
	Ophiothrix fragilis	P	P	VA	P	P		P	
	Ophiocomina nigra			A		P		P	
Crustaceans	Paguroidea							P	U
	Necora puber			P					U
	Cancer pagurus			U		U		U	C
	Hyas spp.			P					
	Inachus spp.			P					
	Lithodes maja							P	
	Galathea spp.	P	P	VA	A				
	Caridea			P					
Chelicerates	Pycnogonid		P						
Molluscs	*Neptunea antiqua*			P					
Ctenophora				C	C			C	
Scyphozoa	*Aurelia aurita*			C				C	
	Cyanea capillata	U		U	U	U		U	P

8.3.2.1 Echinoderms

Echinoderms were present on all platforms, at all depths, including the shallowest parts of some structures. Fouling organisms on platforms may provide substantial quantities of food for asteroids such as *Marthasterias glacialis* and *Asterias rubens* (more than 100 individuals of this species were observed around the footings of

some of the platforms). These will feed on live *Mytilus edulis* present on platforms, but may also make use of any organic matter which falls to the seafloor. The fall of organic material from platforms can provide a substantial food subsidy to seafloor communities, which Asteroidea (amongst other taxa) will readily exploit (Bomkamp et al., 2004). However, abundances around these North Sea platforms were not as high as they are reported to be in the vicinity of some platforms in the USA, where densities as high as $29m^{-2}$ have been reported (Wolfson et al., 1979).

On some platforms, the presence of extensive anemone coverage is thought to prevent echinoderms from climbing the vertical members (Wolfson et al., 1979); some echinoderms are known to avoid anemones, even those which do not normally prey on them (Riedel et al., 2008). However, urchins and starfish were observed at most depths, and were even seen among *M. senile*. This filter feeding anemone may present no threat to these organisms. It is not known if all echinoids and asteroids migrate to structures as adults (high abundances observed at some platforms suggest that many do; Wolfson et al., 1979) or if some might recruit directly to structures as larvae.

Very high densities of ophiuroids (mainly *Ophiothrix fragilis*) were present on North Alwyn A within the hydroid dominated fouling assemblages. They were not observed to occur in such high abundances on other platforms, but footage from NAA was generally examined in greater detail. Ophiuroids, particularly in such high abundances, can provide a substantial food resource for foraging fish. Cod, for example, have been shown to take advantage of high ophiuroid densities, despite the fact that they are not normally an important component of their diet (Rae, 1967).

8.3.2.2 Crustaceans

Crustaceans were not observed in large numbers. Adult *Necora puber* and *Cancer pagurus* were recorded, mostly around platform footings, although several *C. pagurus* were also observed on horizontal members (and even occasionally on vertical surfaces), particularly among *M. senile* on Hoton. *Cancer pagurus* were also occasionally observed in association with *L. pertusa* colonies on NAA.

Smaller crustaceans were also observed. *Hyas* spp. and *Inachus* spp. were recorded during occasional close views of members, *Galathea* spp. were frequently observed, and Caridea (prawns) were also sighted. Hydroid dominated assemblages in particular appeared to host many small crustacea. Destructive sampling of fouling assemblages would be required to accurately identify and quantify these small fauna, which may represent a substantial food base for exploitation by fish and larger invertebrates. Large numbers of amphipods are also very likely to be associated with platform fouling communities, and on platforms in California these are an important food resource which is utilised by some fish species (Page et al., 2007).

8.3.2.3 Other invertebrates

Apart from two individual *Neptunea antiqua* observed on North Alwyn A, no motile molluscs were recorded. However, during occasional close up views on the platforms (particularly NAA), distinctive spirals of nudibranch eggs were observed. The presence of nudibranchs is consistent with the very high abundances of anemones and hydroids, both of which are consumed by these molluscs (Moen and Svensen, 2004).

8.3.3 Fish associated with North Sea structures

Sixteen taxa of fish were identified on the platforms studied during this project (Table 8.4). With the exception of fish associated with North Alwyn A (Section 8.3.4), a semi-quantitative approach was taken, with abundances classified using broad categories (similar to the approach taken for taxa in previous sections).

8.3.3.1 *Brosme brosme*

A reef-associated fish, which generally feeds on benthic invertebrates (Moen and Svensen, 2004), *B. brosme* was present on every platform except Hoton, always in direct association with elements of platform structure. It was often encountered at nodes (indeed on some platforms it was found at almost every intersection between members at suitable depths), where structural complexity was greatest (such as around conductor guide frames) and where infrastructure (or debris)

provided convenient crevices in which to rest. This species was not observed any shallower than approximately 80m, and was present from this depth down to the seabed, where individuals were usually associated with the bases of platform legs or items of debris.

Table 8.4 List of fish taxa observed on 8 North Sea platforms, with estimated abundances. **P** = Present: based on a single observation, (or a few observations) however sightings were affected by restricted sampling or other constraints, and therefore no statements can be made about abundance; **U** = Uncommon: present in small numbers (less than 20 individuals); **C** = Common: observed more frequently (up to 100 individuals); **A** = Abundant: more than 100 individuals; **VA** = Very Abundant: more than 1000 individuals; **Blank cells** = Not Observed: taxon not recorded on a structure; **NP** = Not Present: similarly to Not Observed, the taxon was not recorded, but in this case other factors (such as platform depth or location) mean that its presence can be more confidently ruled out.

	Heather Alpha	Thistle Alpha	NAA	NAB	Dunbar	Bruce	Andrew	Hoton
Brosme brosme	P	P	C	C	U	U	U	NP
Gadus morhua	P	P	C		P		P	NP
Molva molva	P	P	U					
Pollachius pollachius	P	P	A	C	P	P	P	
Pollachius virens	VA	P	VA	VA	VA	VA	VA	
Trisopterus luscus			P					A
Trisopterus spp.			VA	A	P	P	P	A
Melanogrammus aeglefinus			U				P	
Lophius piscatorius			U					
Entelurus aequoreus			U				P	
Sebastes spp.	A	A	A	A	C	C	U	NP
Myoxocephalus scorpius								P
Trachurus trachurus								P
Labrus bergylta			U					
Anarhichas lupus	P	P	U	P		P		
Pleuronectiformes			U				U	U

8.3.3.2 Atlantic cod (*Gadus morhua*)

Cod were observed in relatively small numbers on some of the platforms. Several were observed during the survey of NAA (see Section 8.5.2), and at Heather Alpha, Thistle Alpha, Dunbar, and Andrew. These fish were generally found near the seabed around the footings of structures, and not aggregated around shallower members.

8.3.3.3 Redfish (*Sebastes* spp.)

These were commonly observed around the footings of structures, occasionally in groups of greater than 50 individuals (particularly evident in some of the stills collected on Heather Alpha and Thistle Alpha). Redfish were usually found in association with elements of platform structure (rather than in open water or on the seabed), and were often observed at nodes, or among the pile guides at the bases of platform legs. They were generally not observed any shallower than a depth of around 75m on any of the platforms nor were they present on Hoton, which was presumably too shallow and too far south.

8.3.3.4 Saithe (*Pollachius virens*) and Pollock (*Pollachius pollachius*)

Saithe were the most commonly observed fish around the platforms studied here (except for the Hoton platform, from which they were absent, presumably because it was not in sufficiently deep water), and were seen in groups consisting of greater than 100 fish during some platform surveys, as well as being photographed on Heather Alpha and Thistle Alpha. They were sighted at all depths; from just below the surface, to just above the sea floor at depths greater than 100m. While individuals and small groups (5 to 20 fish) were often observed passing close to platform members, or swimming among conductors, this species was most abundant in the open spaces within platform jackets and in the open waters outside of the jacket, too distant from the ROV for counting to be possible.

Pollock were also observed, usually as single large individuals rather than in groups. Some were present among groups of saithe, making it hard to be sure of accurate discrimination between the two species. The larger groups of fish present within the void spaces of the platform jacket were assumed to be mostly saithe.

8.3.3.5 Wolffish (*Anarhichas lupus*)

Platforms were often inhabited by several individuals of this species, typically located on the seabed, next to platform legs or other vertical members, or occasionally resting on horizontal and diagonal members at slightly higher

elevations. None were observed on the more southerly platforms (Hoton and Andrew), and only one was seen in footage from the Bruce platform.

8.3.3.6 *Trisopterus* spp.

Three species of this genus were present in the study region: pouting (*Trisopterus luscus*), poor cod (*Trisopterus minutus*), and Norway pout (*Trisopterus esmarkii*). These can be difficult to distinguish, particularly when moving rapidly on low resolution video footage. *Trisopterus luscus* was certainly distinguished on Hoton, where it was seen close to the ROV, in the vicinity of the platform footings. However, identification was less certain around the footings of the more northerly platforms, where large numbers of small gadoid fish were observed at a distance where positive identification was difficult. However, these were more likely to have been *T. minutus* or *T. esmarkii*, given occasional closer observations of some individuals. Generally, where individuals could be clearly identified as *T. luscus*, they were recorded as such. Where there was greater uncertainty, fish were recorded as *Trisopterus* spp.; obviously this may have included some *T. luscus*. As a further complication, these groups of fish around the northern platforms may have been mixed groups including whiting (*Merlangius merlangus*). These fish were generally observed in the vicinity of platform footings, on the seabed or a short distance above it (but not more than 10m).

8.3.3.7 Flatfish (Pleuronectiformes)

A small number of flatfish were seen in association with the platforms. However, the quality of the video often precluded positive identification. One plaice (*Pleuronectes platessa*) was observed on the sea floor adjacent to the Hoton platform, and flatfish were occasionally observed on the horizontal and diagonal members of NAA. These fish were generally small and cryptic, and were only sighted on occasions when the ROV was very close to the structure, and/or where an individual fish moved conspicuously against its background; flatfish on the structures may have been much more numerous than was apparent.

8.3.3.8 Wrasse

A small number of ballan wrasse, (*Labrus bergylta*) were observed in the shallower sections of North Alwyn A. These fish were only seen in direct association with platform structure, including vertical, horizontal or diagonal members, and one was seen at the shallowest pile guides at around 30m depth. None were sighted deeper than this. Wrasse have not previously been reported at North Sea platforms.

8.3.3.9 Other fish

Small numbers of ling (*Molva molva*) and haddock (*Melanogrammus aeglefinus*) were observed around the footings of NAA and other deeper platforms. One monkfish (*Lophius piscatorius*) was recorded on the seabed at the base of NAA. Pipefish (*Entelurus aequoreus*) were occasionally observed moving among the fouling assemblages on NAA and Andrew. On platform Hoton, scorpionfish (*Myoxocephalus scorpius*) were observed among the footings, large groups of schooling fish (> 100 fish, but not seen clearly, perhaps the black bream, *Spondyliosoma cantharus*) were seen swimming past structural members and a single individual horse mackerel (*Trachurus trachurus*) was observed.

8.3.4 Quantitative fish survey (North Alwyn A)

8.3.4.1 Results

Fish counts (Table 8.5) provide an estimate of the total number of fish associated with the jacket of the North Alwyn A platform, with the exception of saithe (*P. virens*). Individuals of this species were counted in the survey, but only those that passed close to the member being surveyed. Hundreds (and in some cases thousands) of saithe were, however, often visible in the survey footage, both in the large open spaces within the jacket, and in the water column around the outside of the structure. The presence of other fish species within these groups cannot be ruled out; mackerel (*Scomber scombrus*) have been observed to aggregate around some North Sea platforms (Soldal et al., 2002).

Excluding *P. virens*, most fish were found around the footings of the platform; over 1000 *Trisopterus* spp., over 100 *P. pollachius* and over 100 *Sebastes* spp. were associated with the members at elevation 1 and the pile guide assemblies. Fish numbers declined sharply with decreasing depth and, excluding *P. virens*, only around 10 individual fish from those taxa found around the footings were recorded shallower than Elevation 3. A small number of wrasse (*Labrus bergylta*) and pipefish (*Entelurus aequoreus*) were counted around the shallower areas of the platform.

Table 8.5 Results of fish survey on the pile guides and major horizontals of the North Alwyn A platform. Survey dates are all days in June 2006. Survey times: **D** = Day (0600 to 1800); **N** = Night (1800 to 0600).

	Horizontals						Piles/Pile guide assemblies				**TOTAL**
Elevation	1	3	7	11	15	17	1 to 3				
Depth	127m	104m	81m	58m	35m	12m	104 to 127m				
Leg							A1	A9	H1	H9	
Date of survey	22nd	24th	21st	18th	16th	17th	24th	24th	24th	24th	
Time of survey	D	N	N	N	N	D	D	D	D	D	
Brosme brosme	14	14	3	2			14	12	10	8	**77**
Sebastes spp.	20	10					35	46	34	20	**165**
Gadus morhua	25		1						1		**27**
Pollachius pollachius	68	11	3				10	8	12	3	**115**
Pollachius virens		19	37	5	30	75					**166**
Trisopterus spp.	1262						35	24	33	37	**1391**
Labrus bergylta						2					**2**
Melanogrammus aeglefinus	2	1									**3**
Anarhichas lupus	10							1	1	1	**13**
Lophius piscatorius	1										**1**
Molva molva	2							1			**3**
Pleuronectiformes	1			1	1						**3**
Entelurus aequoreus				1	1	1					**3**
TOTALS	**1405**	**55**	**44**	**9**	**32**	**78**	**94**	**92**	**91**	**69**	**1969**
NUMBER OF TAXA	**10**	**5**	**4**	**4**	**3**	**3**	**4**	**6**	**6**	**5**	

8.3.4.2 Evaluation of fish survey results

Video footage from ROV inspections of horizontal members and pile guides was successfully used to provide data on the fish around the North Alwyn A platform. However, this was only useful for assessing numbers of fish which were directly associated with platform structure (such as *B. brosme* and *Sebastes* spp.). These were found to be most abundant around the footings of the structure, with far fewer fish recorded shallower than 100m. Previous attempts to use inspection

videos to assess fish around North Sea offshore platforms (Aabel et al., 1997b; Cripps et al., 1998a) have not been able to systematically explore the horizontal elevations of platforms in this way; they have been restricted to the use of footage of vertical elements (such as risers), which is not well suited to fish surveys. However, results have been qualitatively in agreement with those here: most fish species (*G. morhua*, *Trisopterus* spp., *Sebastes* spp.) found in greatest numbers close to the seabed, with *P. virens* observed at shallower depths in and around the jacket.

Fish surveys using video footage can suffer from biases resulting from fish behaviour (Trenkel et al., 2004). Fish can be repelled or attracted by ROV lights and sounds, leading to incorrect counts. If fish are highly mobile they may be counted more than once. For most of the species encountered here, these biases are unlikely to have been particularly important, since individual fish appeared largely indifferent to the ROV until it almost came into contact with them. *Pollachius virens* and *P. pollachius* often swam past the camera at very close range. *Anarhichas lupus* were usually encountered resting on the seabed and seldom reacted to the ROV. *Brosme brosme* displayed little aversion to the ROV, although they occasionally showed apparent curiosity, and in one case even aggression. However, *Trisopterus* spp. did show some tendency to move away from the ROV. They were mostly encountered on the seafloor within the jacket footprint, and the entire area under the jacket was not surveyed (Fig 8.3 shows the extent of the horizontal members surveyed, which gives an approximation of the area covered by the surveys, which cannot be strictly quantified given the lack of a reliable means of measuring distances on the ROV footage) which means that underestimation of *Trisopterus* spp. and other species not directly associated with platform members is likely. A previous survey using ROV footage (Aabel et al., 1997b) recorded approximately 5000 *Trisopterus* spp. around a typical northern North Sea structure, which is within an order of magnitude of the numbers observed in this study (~1400), and similar numbers of cod were also observed (30 compared to 27 in this investigation).

Another problem concerns the potential for diurnal variation in fish numbers, since the time of day at which survey footage was collected was not controlled, and ROV operations were continuous throughout the 24-hour cycle. Fish might have different depth distributions at different times (Soldal et al., 2002), and may leave the vicinity of the platform during the night, as has been observed in some artificial reef studies (Fowler et al., 1999; Santos et al., 2002). Diurnal variations in fish occupancy of a structure could be explored using static fish counts (holding the ROV static for a period of time and counting all fish observed), repeated at various times of day, or by acoustic monitoring of the water column around a structure (as conducted by Soldal et al. 2002).

Fish in the water column around the jacket or in the open spaces within the jacket structure were not adequately sampled. Static fish counts might be suitable, but the very large numbers of fish spread over distances as great as 100m away from the ROV, the variable light and visibility levels, and the lack of a reliable means of estimating size and distance from the footage, mean that acoustic survey methods are more appropriate. Acoustic surveys have been undertaken on North Sea platforms (Soldal et al., 2002), but these types of measurement are usually carried out only outside of the jacket (Stanley and Wilson, 1996; Soldal et al., 2002), and fail to account for fish located in the open spaces within the jacket or simply assume similar densities inside the structure.

8.3.5 Seabed effects

Evidence of shell fall was observed at the base of all platforms. However, the amount of shell on the seabed did seem to vary between platforms. NAA appeared to have surprisingly little shell accumulation at its base; this was perhaps mostly covered by (or incorporated into) the extensive drill cuttings pile at the base of the platform. It is also possible that there was insufficient mussel growth to generate a substantial shell mound. Some areas of seabed at the base of other platforms (such as Andrew and Bruce) were covered in bivalve shell material. However, the available footage was not suitable for a systematic exploration of the extent of seabed modification.

8.4 Conclusions

Offshore structures in the North Sea provide habitat for a variety of species. Many fish are attracted, utilising both structural members and the open spaces between them, and a large (unquantified) biomass of motile invertebrates is present, representing a potentially valuable food resource. The fouling community itself appears dominated by a small number of cnidarian species: hydroids, anemones, and corals. How the coverage of these organisms varies with depth, and between platforms, is the subject of Chapter 9.

Chapter 9. Comparing fouling assemblages on North Sea platforms

9.1 Introduction

Several key taxa dominate the fouling communities on offshore structures in the North Sea (Forteath et al., 1982; Southgate and Myers, 1985; Picken, 1986; Whomersley and Picken, 2003). Perhaps the most prevalent are the cnidarians: hydroids such as *Tubularia larynx*, anemones (most notably *Metridium senile*), and both soft and hard corals. However, other taxa are commonly present, such as tube building polychaetes (Serpulidae), bryozoans, and (in the shallower parts of platforms) macroalgae and bivalves (*Mytilus edulis*). Assemblages of these organisms on offshore structures, just like those on natural substrates, exhibit patterns of vertical zonation, which may vary between platforms (Forteath et al., 1982; Southgate and Myers, 1985).

However, while anecdotal accounts of variations in fouling between structures in different locations in the North Sea have been reported (Picken, 1986), only one systematic study of how fouling assemblages on North Sea platforms vary spatially and temporally has been published (Whomersley and Picken, 2003).

The aim of this chapter is therefore to compare, in detail, the fouling on several North Sea platforms, using structural survey footage provided by the oil and gas industry.

9.2 Materials and Methods

9.2.1 Video footage

In order to explore platform fouling assemblages, the structural survey footage introduced in Chapter 8 was used. For all platforms except Heather Alpha, footage was provided as digital video files; footage of Heather Alpha was provided on S-VHS tapes and subsequently digitised using Microsoft Windows Movie Maker. No survey footage was provided from Thistle Alpha, and this platform was not considered further.

Survey footage of platform legs proved most suitable for assessing the fouling community. Leg surveys consisted of several vertical passes of each leg; each survey pass viewed either the interior or exterior face of a leg, and the orientation of each pass is referred to here as the 'Aspect'. One leg was selected at random from each platform, using footage from one exterior and one interior pass for analysis of marine growth.

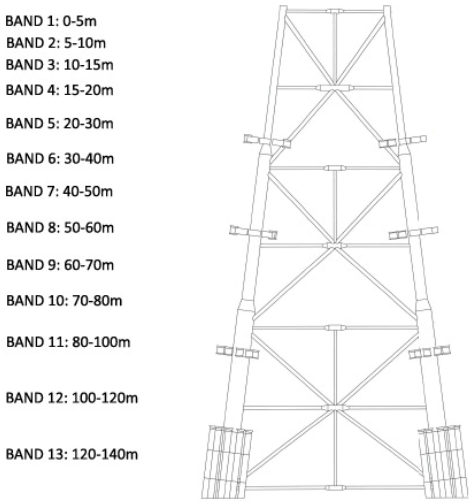


Figure 9.1 Platform depth bands.

9.2.2 Video sampling

Suitable individual frames were extracted from the digitised video footage using VLC media player (http://www.videolan.org/vlc) and saved as JPEG image files. Platform legs were divided up into a number of depth bands, in order to determine if variation between platforms was similar at different depths. Initially, platforms were divided up into 10m depth bands (0-10m, 10-20m, 20-30m, etc.), as obtaining replicate, non-overlapping frame grabs over shorter distances was more difficult. However, physical conditions are expected to change most rapidly in the shallowest depths (Little and Kitching, 1996), and therefore 5m depth bands were used for the top 20m of each platform (Fig. 9.1). At depths greater than 80m, broader depth bands were used, as change was expected to be more gradual at these depths.

9.2.3 Data extraction

For each individual frame, percentage cover of each of the most dominant fouling organisms was recorded. Only a limited number of organisms could be reliably identified and measured on all footage, and therefore only the following six 'principal fouling types' were recorded: Macroalgae; *Metridium senile*; Other Actinaria, *Alcyonium digitatum*; *Lophelia pertusa*; and *Tubularia larynx*. In addition, for any framegrab where these taxa did not completely cover the whole surface area, one of three 'background fouling types' was assigned (Table 9.1).

Table 9.1 Background fouling types used to describe general fouling on areas of structure not covered by one of the principal fouling types.

Background Fouling Type	Description
1 - Very light fouling	Typically sparse hydroids, small amounts of other taxa. Some hard fouling. Unfouled metal visible, sometimes large areas
2 – Hard fouling	Structure covered with hard fouling – barnacles, calcareous bryozoans, tubeworm casts (active and abandoned) and other reef-building species. While this fouling type was thin (< 20mm) in places, no unfouled structure was generally visible
3 – Hydroid dominated	Complex mixture of fouling species, typically dominated by hydroids, but containing other taxa such as arborescent bryozoans, anemones, mussels, and soft/hard corals. Variable thickness, but often >40mm, with no unfouled metal visible

For each principal fouling type, percentage cover in each extracted frame was measured using an image segmentation approach. Images were imported into Adobe Photoshop (http://www.adobe.com), and after adjustments to contrast and brightness (to improve image clarity), a paint brush tool was used to manually demarcate the areas covered by each of the principal fouling types. Video footage was considered alongside the images, in order to assist with correct marking of areas where image quality was low. Any areas which were not to be considered (for example any open water visible to either side of the platform leg) were masked off using a neutral colour (brown). An 'image mask' for each principal

fouling type was thus created, and the proportional coverage of each fouling type could then be calculated (Fig 9.2). Percentage cover data were rounded (up) to the nearest 2%.

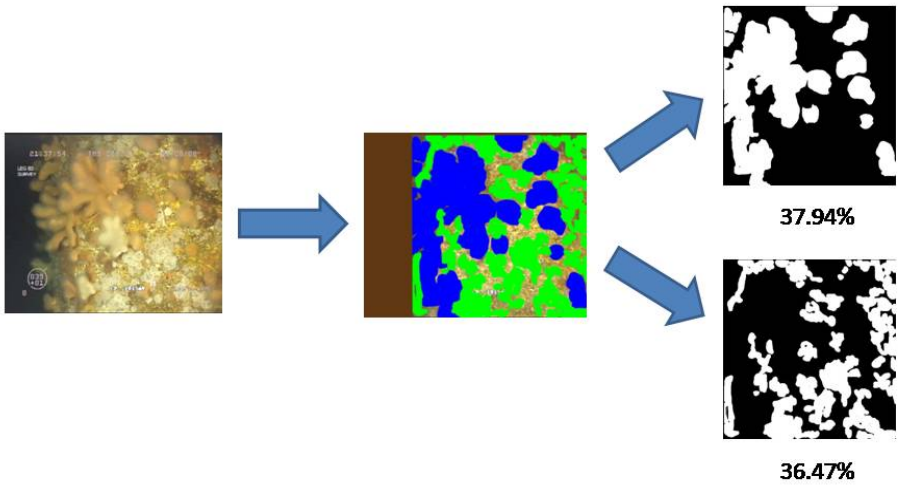


Figure 9.2 Illustration of the steps in the segmentation of a typical image, taken on the Andrew Platform at a depth of 40-50m. The area to the left of the leg is not of interest and is first 'masked off' in a neutral colour. Areas occupied by the principal fouling types are then marked using different colours (green areas are those occupied by *Metridium senile*, and blue represents *Alcyonium digitatum*). An image mask is generated for each taxon, from which percent cover is measured. The residual area is assigned to one of three 'background fouling types'.

9.2.4 Data analysis

Two separate sets of multivariate analyses were carried out to explore the effects of platform location and year of installation on the fouling assemblages within each depth band, using PERMANOVA (Anderson, 2001). These analyses were carried out in all cases using Bray-Curtis dissimilarities (Clarke et al., 2006) calculated on fourth-root transformed percentage cover data. This transform down-weights more abundant species, preventing them from overriding the contributions of other, less abundant taxa (Clarke and Gorley, 2006). This was felt to be appropriate given that the abundances of *Metridium senile* were often found to be much higher than those of other taxa.

Heather Alpha, North Alwyn A, North Alwyn B and Dunbar are all located in an area to the East of the Shetland Islands (Fig. 8.2) but were installed in different years (1978, 1985, 1986 and 1993 respectively; see Fig. 8.1). Fouling on these platforms was compared to determine whether or not the year in which they were installed

(and therefore also the length of time for which they had been in place) had a lasting effect on the platform assemblages, independent of any effect of location within the North Sea. For each depth band, a two-way crossed PERMANOVA analysis was carried out with Platform and Aspect as the two factors (Platform was treated as a random factor, while Aspect was a fixed factor). Where a significant ($p < 0.05$) Platform*Aspect interaction was found, separate pairwise comparisons were carried out between platforms for both levels of Aspect (Interior/Exterior), and pairwise comparisons between Aspects were carried out for all platforms. Where there was no significant Platform*Aspect interaction ($p > 0.05$) pairwise tests were conducted for between-platform comparisons using data from both Aspects combined. Between-aspect pairwise tests were in such cases not necessary because there were only two levels of Aspect (Interior/Exterior). Separate sequential Bonferroni corrections were applied to *p* values for each set of pairwise comparisons (Rice, 1989).

Dunbar, Bruce, and Andrew were all installed in the mid-1990s; however, they are located some distance apart (Fig. 8.2). Dunbar is located furthest north, to the east of the Shetland Islands, while Bruce is at a latitude further south than the Shetland Islands, and Andrew is located to the east of mainland Scotland. The fouling assemblages of these three platforms were compared using a series of two-way crossed PERMANOVA analyses (one for each depth band), with Platform and Aspect as factors, to explore the effect of location on the fouling assemblages of these structures. Pairwise tests were carried out as appropriate, following the approach detailed in the previous paragraph.

For the two sets of analyses described above, one leg was selected from each platform at random. In order to determine whether or not choice of leg may have influenced the between-platform comparisons, a further set of comparisons were carried out, on data from all four legs of Dunbar. Between-leg comparisons were carried out in the same manner as the between-platform comparisons above (two-way crossed PERMANOVA for each depth band, with Leg and Aspect as factors), except that Leg was treated as a fixed factor, since all legs on Dunbar were sampled.

9.3 Results

9.3.1 Effect of platform installation year

A summary of the results of tests for the influence of Aspect and Platform (and any Platform*Aspect interaction) on the fouling assemblages of Dunbar, Heather Alpha, NAA, and NAB in all 13 depth bands, is presented in Table 9.2 (full PERMANOVA statistics and details of pairwise comparisons can be found in Appendix 1).

Table 9.2 Significance of factors in two-way, crossed PERMANOVA comparisons between four platforms of different ages but all located in the most northerly region, with Aspect and Platform as factors, for each depth band. Full results presented in Appendix 1. *p < 0.05; **p < 0.01; ns = not significant, p > 0.05; p values evaluated following sequential Bonferroni corrections.

Depth	Platform	Aspect	Platform*Aspect
0-5m	**	ns	**
5-10m	**	**	**
10-15m	**	**	**
15-20m	**	ns	**
20-30m	**	ns	ns
30-40m	**	ns	ns
40-50m	**	ns	ns
50-60m	**	ns	ns
60-70m	**	ns	ns
70-80m	**	ns	ns
80-100m	**	ns	ns
100-120m	**	ns	ns
120-140m	*	ns	ns

9.3.1.1 Depth band 1: 0-5m

There was a significant Platform*Aspect interaction at this depth (p < 0.01; Table 9.2). Pairwise comparisons (Appendix 1) identified no differences between platforms on the Exterior aspect of platform legs (no test statistic or p value was calculated, as all replicates for all platforms were extremely similar: close to 100% cover of macroalgae on the exterior of all platform legs). Comparing the Interior aspect of platform legs (Figs. 9.3 to 9.6), Dunbar differed significantly from all other platforms (p < 0.01) as it was the only platform to have no macroalgae recorded on the interior face of the analysed leg (Fig. 9.6) NAA, NAB, and Heather Alpha were

not significantly different from each other ($p > 0.05$). Dunbar and Heather both had significant variation between their Interior and Exterior faces ($p < 0.05$), because of large differences in the coverage of macroalgae (Figs. 9.5, 9.6).

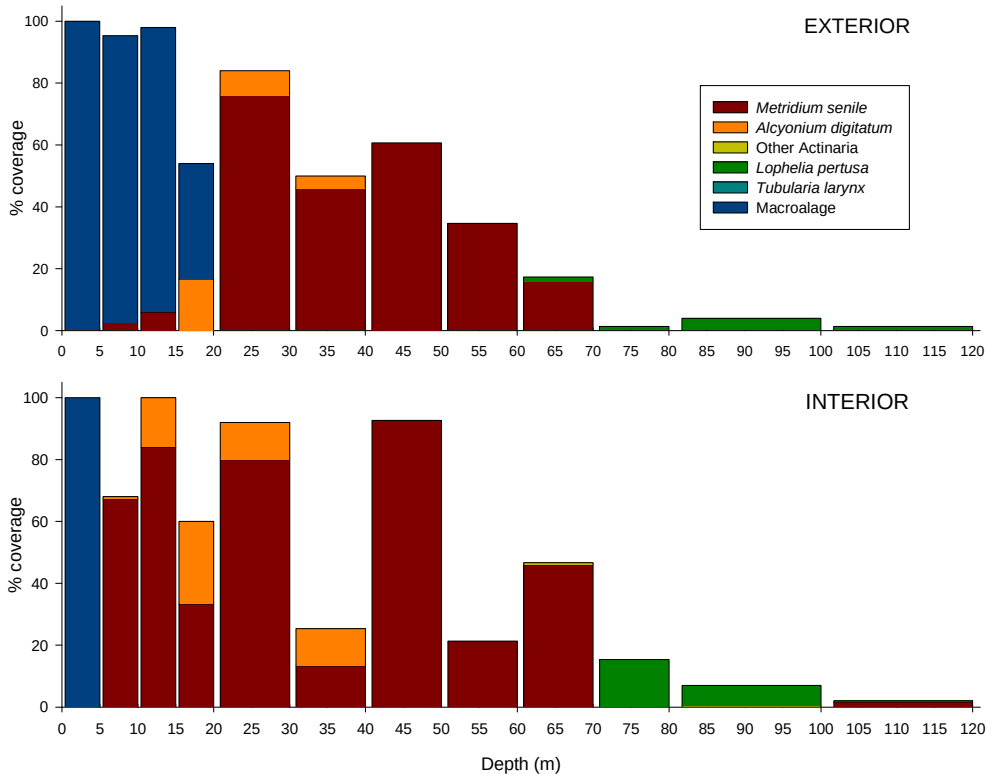


Figure 9.3 Distribution of principal fouling types on the Interior and Exterior faces of a platform leg on North Alwyn A, from the surface down to the seabed at 120m

9.3.1.2 Depth band 2: 5-10m

Assemblages varied significantly between Platforms and Aspects at this depth ($p < 0.01$) and there was also a significant Platform*Aspect interaction ($p < 0.01$). Pairwise testing revealed that the only variation among platforms was that Heather Alpha differed significantly from both NAA and NAB ($p < 0.01$), although these latter two platforms did not differ from each other ($p > 0.05$). Heather Alpha (Fig. 9.5) lacked both *M. senile* and *A. digitatum* at this depth, which were present on the interior of legs on NAA and NAB (Figs. 9.3 and 9.4). All platforms showed significant differentiation between the fouling on the Interior and Exterior aspects ($p < 0.05$ for Dunbar and NAB; $p < 0.01$ for Heather and NAA); the exterior faces of legs had greater coverage of macroalgae and less (or no) cover of other fouling types.

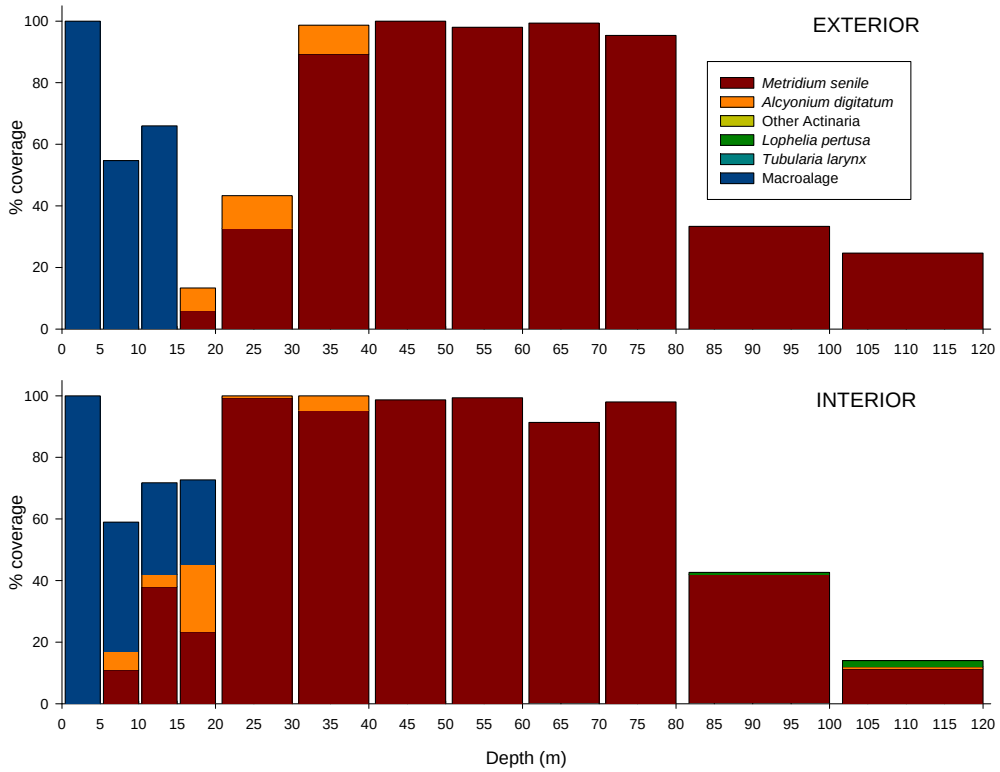


Figure 9.4 Distribution of principal fouling types on the Interior and Exterior faces of a platform leg on North Alwyn B, from the surface down to the seabed at 120m

9.3.1.3 Depth band 3: 10-15m

Platform and Aspect were again highly significant factors ($p < 0.01$), and there was a significant ($p < 0.01$) interaction (Table 9.2). Pairwise testing (Appendix 1) showed that no platforms significantly differed on their Exterior aspect ($p > 0.05$ in all cases), but all differed significantly from all others in terms of the fouling on their Interior aspect ($p < 0.05$ for all comparisons, and $p < 0.01$ for Dunbar/Heather Alpha and Dunbar/NAA comparisons). Heather Alpha (Fig. 9.5) had substantial coverage (~75%) of *T. larynx*, which was not noticeably present on the other platforms, while NAB (Fig. 9.4) had over 25% cover of macroalgae, which was absent from the interior faces of all other platforms in this comparison. NAA (Fig. 9.3) had 15-20% cover of *A. digitatum,* which was not present on Dunbar or Heather Alpha, while Dunbar (Fig. 9.6) was dominated by effectively complete cover of *M. senile* on the interior. All platforms showed significant differences ($p < 0.05$) between their Interior and Exterior aspects, again because of the dominance of macroalgae on their exterior faces.

9.3.1.4 Depth band 4: 15-20m

There was again a significant Platform*Aspect interaction at this depth, however, only NAB showed significant ($p < 0.05$) differentiation between exterior and interior faces (Appendix 1). Pairwise test for between-platform comparisons were still carried out separately for Interior and Exterior aspects (Appendix 1). Comparing the interior faces of platform legs, all platforms differed significantly from all other platforms ($p < 0.05$), apart from NAA and NAB, which were not significantly different from each other ($p > 0.05$). Comparing fouling on the exterior aspect of platforms, all platforms were similar ($p > 0.05$) except for Heather Alpha, which differed from all other platforms ($p < 0.05$, and $p < 0.01$ for comparisons with NAA and NAB; Appendix 1), by having a very high (> 80%) cover of *T. larynx* (Fig. 9.5).

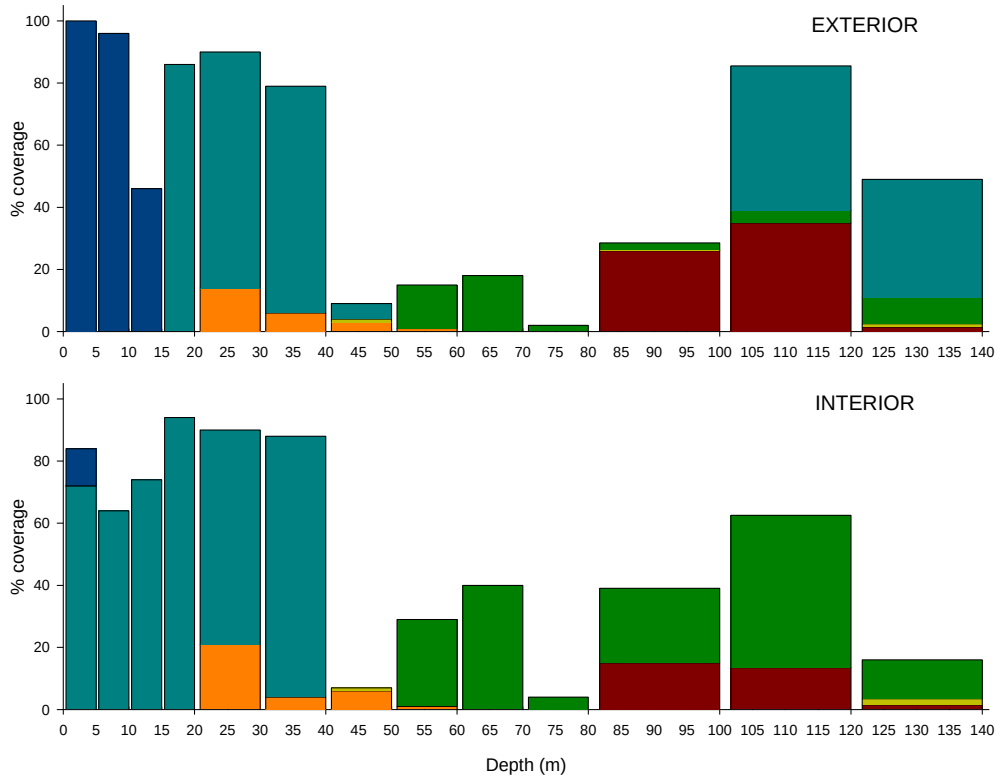


Figure 9.5 Distribution of principal fouling types on the Interior and Exterior faces of a platform leg on Heather Alpha, from the surface down to the seabed at 140m

9.3.1.5 Depth band 5: 20-30m

There was no significant effect of Aspect ($p > 0.05$), and no significant Platform*Aspect interaction ($p > 0.05$) at this depth, or at any depth band below it (Table 9.2). However, there was a significant effect of Platform ($p < 0.01$), and pairwise tests (Appendix 1) showed that Heather Alpha differed from all other platforms at this depth ($p < 0.05$ for all pairwise comparisons including Heather Alpha); it had over 70% cover of *T. larynx*, which was absent from other platforms at this depth (Figs. 9.3 to 9.6). Dunbar also differed from NAA ($p < 0.05$).

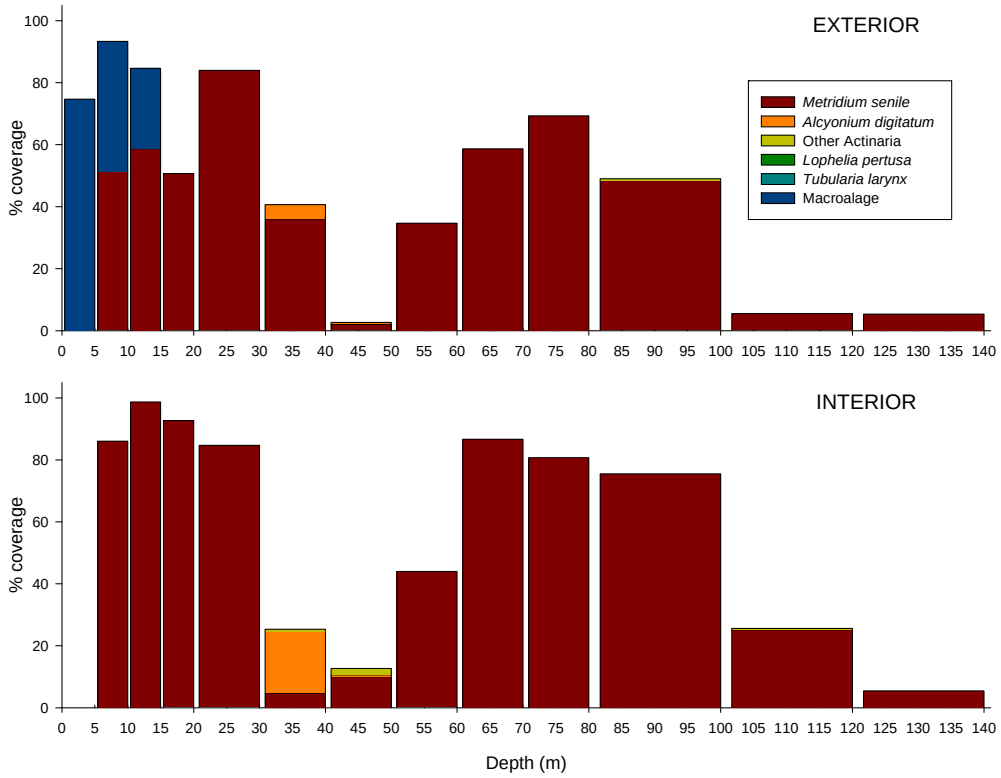


Figure 9.6 Distribution of principal fouling types on the Interior and Exterior faces of a platform leg on Dunbar (Leg A4), from the surface down to the seabed at 140m

9.3.1.6 Depth band 6: 30-40m

There was significant variation among platforms at this depth ($p < 0.01$). Heather Alpha differed significantly from all other platforms ($p < 0.01$; Appendix 1); again there was substantial coverage of *T. larynx* on this platform and no others (Fig. 9.5). The only other difference detected by the pairwise tests (Appendix 1) was a significant ($p < 0.05$) difference between Dunbar and NAB, which may have been a

result of the greater abundance of *M. senile* on NAB (>90% cover) compared to Dunbar (<40% cover).

9.3.1.7 Depth band 7: 40-50m

There was significant variation among platforms at this depth ($p < 0.01$; Table 9.2), with all platforms differing from all other platforms ($p < 0.01$ for all between-platform comparisons except NAA/NAB, for which $p < 0.05$; Appendix 1). Heather Alpha was distinguished by the presence of *T. larynx* (Fig. 9.5), although at much lower abundance (<10% cover) compared to shallower depths, and the absence of *M. senile,* which was abundant on the other platforms. The other platforms were differentiated by having different amounts of *M. senile*, which was most abundant on NAB (close to 100% cover; Fig. 9.4), with less (60-90% cover) on NAA (Fig. 9.3), and least (<10% cover) on Dunbar (Fig. 9.6).

9.3.1.8 Depth band 8: 50-60m

Significant variation among platforms was detected at this depth ($p < 0.01$; Table 9.2), mainly because Heather Alpha (with up to 25% cover of *L. pertusa*, which was absent from other platform legs at this depth) was again distinct from all other platforms ($p < 0.01$ in all cases; Appendix 1). NAA and NAB also differed significantly from each other ($p < 0.01$); NAB had substantially more *M. senile* (~100% cover) compared to NAA (less than 30% cover).

9.3.1.9 Depth band 9: 60-70m

All platforms differed from all other platforms ($p < 0.05$ for all pairwise comparisons; Appendix 1, and $p < 0.01$ for overall effect of 'Platform' factor in PERMANOVA test; Table 9.2). Heather Alpha (Fig. 9.5) had up to 40% cover of *L. pertusa*, distinguishing it from other platforms, while NAA (Fig. 9.3) had only small (<5% cover) amounts of *L. pertusa,* which nonetheless distinguished it from NAB and Dunbar (Figs. 9.4 and 9.6), from which this species was absent at this depth. NAB had almost total cover of *M. senile* (close to 100%), while cover of this species varied from 60-90% on Dunbar.

9.3.1.10 Depth band 10: 70-80m

Platforms again varied significantly ($p < 0.01$; Table 9.2), but Heather Alpha and NAA did not differ significantly from each other ($p > 0.05$; Appendix 1); both these platforms had small (<10% cover) amounts of *L. pertusa*, but none of the other principal fouling types were present (NAA and Heather Alpha both differed from the other two platforms; $p < 0.05$). While both NAB and Dunbar had no observable fouling by any of the principal fouling types except for *M. senile*, NAB had greater coverage (~100%, compared to <80% on Dunbar); NAB and Dunbar differed significantly ($p < 0.05$).

9.3.1.11 Depth band 11: 80-100m

Platform fouling varied significantly ($p < 0.01$; Table 9.2) in this depth band. NAA (Fig. 9.3) differed from all other platforms ($p < 0.05$ for all pairwise comparisons involving NAA; Appendix 1); lack of *M.* senile distinguished it from Dunbar, NAB and Heather Alpha, on all of which this species was present at this depth (Figs. 9.6, 9.4 and 9.5).

9.3.1.12 Depth band 12: 100-120m

There was significant variation at this depth ($p < 0.01$), but pairwise comparisons (Appendix 1) only identified significant variation ($p < 0.05$) between two platforms: Dunbar and Heather Alpha (all other pairwise comparisons were non-significant; $p > 0.05$). Heather Alpha had up to 40% cover of *T. larynx*, and up to 40% cover of *L. pertusa,* while Dunbar lacked both of these fouling taxa.

9.3.1.13 Depth band 13: 120-140m

Data were only available for Heather Alpha and Dunbar below 120m, and these platforms differed significantly ($p < 0.05$; Table 9.2). Similarly to Depth band 12, Heather Alpha had *T. larynx* (up to 30% cover) and *L. pertusa* (up to 10% cover), together with less than 5% cover of *M. senile* and Other Actinaria, while Dunbar was only very lightly fouled (Fig 9.6), with less than 5% cover of *M. senile* and no other principal fouling types present.

9.3.1.14 Background fouling cover.

The dominant background fouling types for each of the four platforms compared above are presented in Table 9.3. Down to a depth of approximately 50m, any surfaces not covered by the principal fouling types (macroalgae, *M. senile*, *A. digitatum,* Other Actinaria, *L. pertusa*, and *T. larynx*) were covered by background fouling type 3 (hydroid and arborescent bryozoan dominated fouling), with no unfouled structure generally visible. Below 50m, platforms North Alwyn A and Heather Alpha were dominated by hard fouling (background fouling type 2: tubeworms, calcareous bryozoans), while background fouling on North Alwyn B and Dunbar was much lighter, with less hard fouling, and areas of unfouled structure visible.

Table 9.3 Dominant background fouling types for the four most northerly platforms (to the east of the Shetland Islands). Gold cells: fouling type 1 (very light fouling); Green cells: fouling type 2 (hard fouling); Blue cells: fouling type 3 (Hydroid dominated fouling); Unshaded cells (0): total fouling cover by principal fouling organisms; Black cells: platform did not extend to that depth.

Platform	NAA		NAB		Heather Alpha		Dunbar	
Pass	EXT	INT	EXT	INT	EXT	INT	EXT	INT
0-5m	0	0	0	0	0	3	0	3
5-10m	3	3	3	3	3	3	3	3
10-15m	3	3	3	3	3	3	3	3
15-20m	3	3	3	3	3	3	3	3
20-30m	3	2	3	0	3	3	3	3
30-40m	3	2	3	0	3	3	3	3
40-50m	3	2	0	3	3	2	3	3
50-60m	2	2	3	2	2	2	2	1
60-70m	2	2	2	2	2	2	2	3
70-80m	2	2	2	2	2	2	1	2
80-100m	2	2	1	1	2	2	1	1
100-120m	2	2	1	1	2	2	1	1
120-140m					2	3	1	1

9.3.2 Effect of platform location

Summarised results of comparisons of the fouling assemblages of Andrew, Bruce and Dunbar in all 13 depth bands, are presented in Table 9.4 (full PERMANOVA statistics and details of pairwise comparisons can be found in Appendix 2).

9.3.2.1 Depth band 1: 0-5m

Significant variation was detected between platforms at this depth ($p < 0.01$; Table 9.4), but there was also a significant Platform*Aspect interaction ($p < 0.01$). Pairwise tests (Appendix 2) indicate that this variation involved the Dunbar platform; Dunbar was the only platform for which there was a significant difference between the fouling assemblages on interior and exterior faces of platform legs ($p < 0.01$), as this platform had no macroalgae on the Interior aspect (Fig. 9.6). This also accounts for the significant differences ($p < 0.01$) between the interior faces of Dunbar and the other two platforms (Andrew and Bruce), while there was no significant variation among exterior faces of the three platforms ($p > 0.05$; Appendix 2).

Table 9.4 Significance of factors in two-way, crossed PERMANOVA comparisons between three similarly aged platforms in different locations, with Aspect and Platform as factors, for each depth band. Full results presented in Appendix 2. *$p < 0.05$; **$p < 0.01$; ns = not significant, $p > 0.05$; p values evaluated following sequential Bonferroni corrections.

Depth	Platform	Aspect	Platform*Aspect
0-5m	**	ns	**
5-10m	**	*	ns
10-15m	*	*	**
15-20m	*	ns	*
20-30m	**	ns	ns
30-40m	**	ns	ns
40-50m	**	ns	ns
50-60m	**	ns	ns
60-70m	**	ns	ns
70-80m	**	ns	**
80-100m	ns	ns	ns
100-120m	ns	ns	ns

9.3.2.2 Depth band 2: 5-10m

There was significant variation among platforms ($p < 0.01$) and aspects ($p < 0.05$) at this depth, but no Platform*Aspect interaction ($p > 0.05$). Macroalgae were abundant (>40% cover) on the exterior aspect of all platforms, but absent from the interior aspect. However, despite the significant variation among platforms apparently detected by the global PERMANOVA test, no significant differences

between individual platforms were found ($p > 0.05$ for all between-platform pairwise comparisons; Appendix 2).

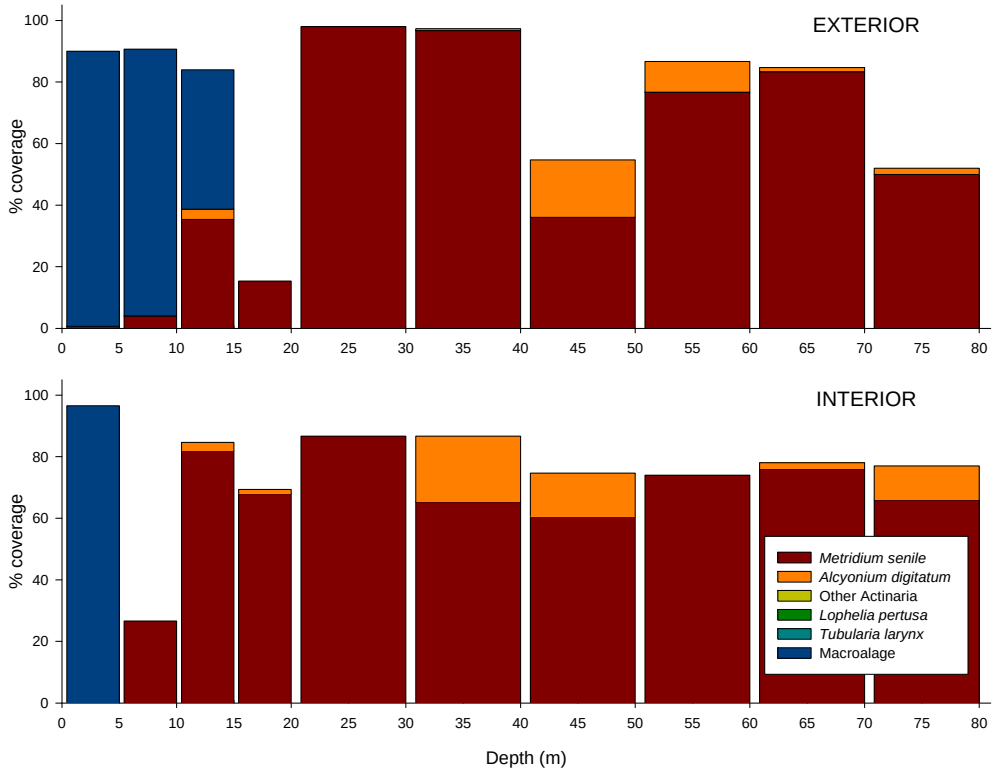


Figure 9.7 Distribution of principal fouling types on the Interior and Exterior faces of a platform leg on Andrew, from the surface down to 80m (deeper footage not available)

9.3.2.3 Depth band 3: 10-15m

There was a significant Platform*Aspect interaction for this depth band ($p < 0.05$; Table 9.4). All platforms showed significant variation between their Interior and Exterior aspects ($p < 0.05$ for Andrew, $p < 0.01$ for Bruce and Dunbar; Appendix 2), with all three platforms having more than 20% cover of macroalgae on their exterior faces, but none on their interiors (Figs. 9.6-9.8). The only between-platforms comparison (Appendix 2) to find a significant difference was that between Dunbar and Bruce for their Interior aspect ($p < 0.05$). Bruce had less than 35% combined cover of *M. senile* and *A. digitatum* at this depth (Fig. 9.8), while Dunbar had close to 100% cover of *M. senile* alone (Fig. 9.6).

9.3.2.4 Depth band 4: 15-20m

There was a significant Platform*Aspect interaction at this depth ($p < 0.05$; Table 9.4). The only platform to have a significant difference between the fouling assemblages on Interior and Exterior aspects was Bruce ($p < 0.01$; Appendix 2), as it had 100% macroalgal cover on the exterior and none on the interior (Fig 9.8). The interior aspect on platform Bruce also differed from that on Dunbar ($p < 0.01$) as the latter platform had much higher coverage of *M. senile* (>90% compared to <20%) and lacked *A. digitatum*, which was present on Bruce (Figs. 9.6 and 9.8).

9.3.2.5 Depth band 5: 20-30m

There was significant variation among platforms at this depth ($p < 0.01$); Bruce was significantly different from Andrew and Dunbar ($p < 0.05$ in both cases), as it was the only platform with recorded coverage of *A. digitatum* (~5% cover) at this depth, and had less *M. senile* (Andrew and Dunbar both had >80% cover of this species on both aspects), particularly on the interior aspect where cover was less than 40% (Fig. 9.8).

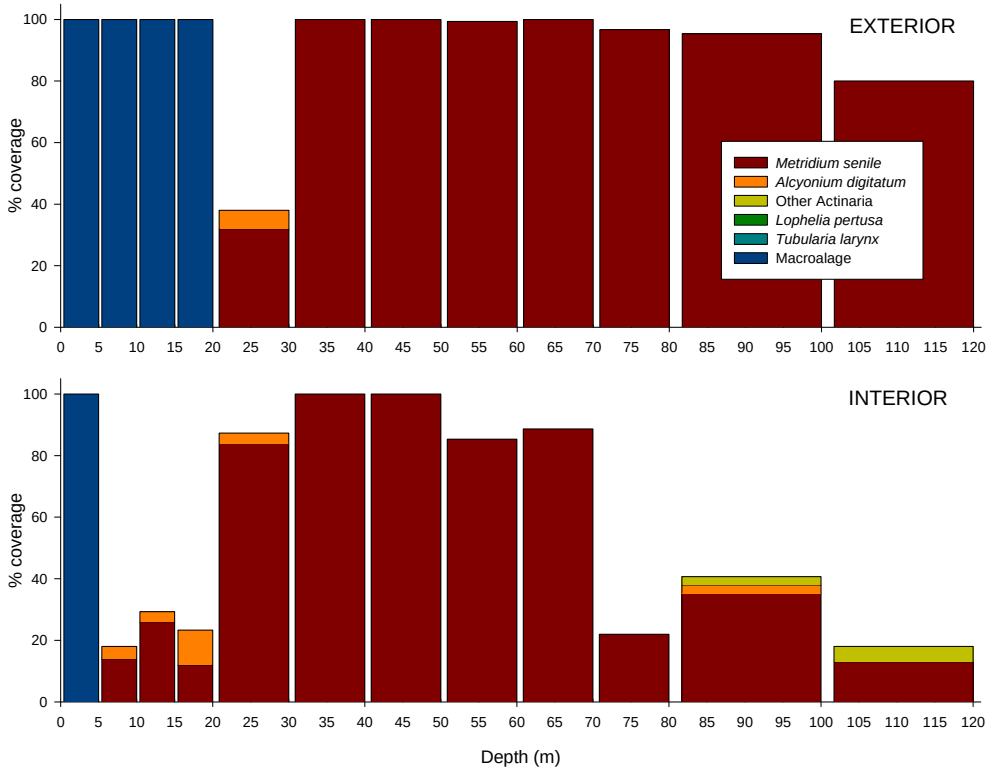


Figure 9.8 Distribution of principal fouling types on the Interior and Exterior faces of a platform leg on Bruce, from the surface down to the seabed at 120m

9.3.2.6 Depth bands 6 to 9: 30-70m

Across all four of these depth bands, there was significant variation among platforms ($p < 0.01$ for depth bands 6-9; Table 9.4), and all three platforms differed from each other ($p < 0.05$ for most pairwise comparisons in this depth range; Appendix 2). Bruce was almost 100% fouled by *M. senile* between 30 and 70m, with none of the other principal fouling types present (Fig. 9.8), while Andrew had generally greater than 60% cover of *M. senile*, but also up to 25% cover of *A. digitatum* in places (Fig. 9.7), and Dunbar had much less *M. senile* (mostly <60% cover from 30-70m, except for on the interior aspect in depth band 9), modest amounts (<20% cover) of *A. digitatum* in depth band 6, and very small amounts (<5% cover) of Other Actinaria (Fig. 9.6). There were no significant effects of Aspect on fouling composition, and no Platform*Aspect interactions for any of depth bands 6-9 (all $p > 0.05$; Table 9.4).

9.3.2.7 Depth band 10: 70-80m

There was a significant Platform*Aspect interaction at this depth ($p < 0.01$; Table 9.4). Bruce was the only platform for which there was a significant effect of Aspect ($p < 0.05$; Appendix 2); abundance of *M. senile* was much lower on the interior (<20% cover) compared with the exterior (>80% cover). Considering only the Interior aspect of platforms (Appendix 2), all three platforms differed significantly ($p < 0.05$ for Dunbar/Bruce; $p < 0.01$ for Dunbar/Andrew and Andrew/Bruce comparisons). The interior of Bruce had much less *M. senile* (<20% cover) than Andrew and Dunbar (both >75% cover), while Andrew also had *A. digitatum* present (Fig. 9.7).

9.3.2.8 Depth bands 11 and 12: 80-120m

Data were only available for Bruce and Dunbar at depths greater than 100m. No significant variation was detected between these two platforms, or between aspects, at these depths (all $p > 0.05$; Table 9.4).

9.3.2.9 Background fouling types

The dominant background fouling types for each of the three platforms compared above are presented in Table 9.5. Most background fouling down to 50m on Bruce and Dunbar was type 3 (hydroid dominated), although extensive *M. senile* cover on Bruce (up to 100% in places, see Fig. 9.8) meant that in several places no background fouling could be recorded on this platform. Unlike Bruce and Dunbar, Andrew (the most southerly platform) had areas of much lighter fouling between 5 and 50m, with unfouled surfaces visible in places. Below 50m, however, Andrew and Dunbar both had areas where the background fouling was predominantly of type 2 (hard fouling: tubeworms, calcareous bryozoans), while Bruce had more light background fouling, with area of unfouled surface visible. Below 80m all areas not occupied by the principal fouling types were only lightly fouled, or unfouled.

Table 9.5 Dominant background fouling types for three platforms installed at similar times (mid-1990s) but spread across three locations in the North Sea. Gold cells: fouling type 1 (very light fouling); Green cells: fouling type 2 (hard fouling); Blue cells: fouling type 3 (Hydroid dominated fouling); Unshaded cells (0): total fouling cover by principal fouling organisms; Black cells: platform did not extend to that depth.

Platform	Andrew		Bruce		Dunbar	
Pass	EXT	INT	EXT	INT	EXT	INT
0-5m	3	3	0	0	0	3
5-10m	3	1	0	3	3	3
10-15m	3	1	0	3	3	3
15-20m	1	1	0	3	3	3
20-30m	1	1	3	3	3	3
30-40m	1	1	0	0	3	3
40-50m	2	2	0	0	3	3
50-60m	2	1	1	1	2	1
60-70m	2	2	0	1	2	3
70-80m	1	1	1	1	1	2
80-100m			1	1	1	1
100-120m			1	1	1	1

9.3.3 Between-legs comparison, Dunbar platform

Summarised results of comparisons of the fouling assemblages of all four legs of Dunbar in all 13 depth bands, are presented in Table 9.6 (full PERMANOVA statistics and details of pairwise comparisons can be found in Appendix 3). The leg used for previous comparisons involving Dunbar was leg A4 (Fig. 9.6).

Table 9.6 Significance of factors in two-way, crossed PERMANOVA comparisons between the four legs of the Dunbar platform, with Aspect and Leg as factors, for each depth band. Full results presented in Appendix 3. *p < 0.05; **p < 0.01; ns = not significant, p > 0.05; p values evaluated following sequential Bonferroni corrections.

Depth	Leg	Aspect	Leg*Aspect
0-5m	ns	**	ns
5-10m	**	**	**
10-15m	**	**	**
15-20m	ns	**	ns
20-30m	**	*	*
30-40m	**	ns	ns
40-50m	**	ns	ns
50-60m	*	ns	ns
60-70m	**	*	*
70-80m	*	ns	**
80-100m	ns	ns	ns
100-120m	ns	ns	ns
120-140m	ns	ns	ns

9.3.3.1 Depth bands 1 to 4: 0-20m

Aspect had a significant effect on fouling composition in all four of the shallowest depth bands (p < 0.01) with significant Leg*Aspect interactions in depth bands 2 and 3 (p < 0.01). Between 5 and 10m (depth band 2), only Leg B2 showed significant variation between aspects (p < 0.01; Appendix 3), while between 10 and 15m (depth band 3) all legs except A2 showed significant variation between aspects (p < 0.01; Appendix 3). Interior faces of legs had much lower abundances of macroalgae than Exterior faces (which had up to 100% macroalgal cover), or even no macroalgae at all (Figs. 9.6 and 9.9-9.11).

There was no significant variation among legs from 0-5m and 15-20m (depth bands 1 and 4, Table 9.6; p > 0.05), and although there were significant variations among

legs ($p < 0.01$) and significant Leg*Aspect interactions ($p < 0.01$) in depth bands 2 and 3 (5-15m; Table 9.6), pairwise tests (Appendix 3) revealed very few significant differences between legs. For depth band 2 (5-10m) the only significant pairwise difference was between the Interior aspects of A2 and B2 ($p < 0.01$); A2 had macroalgae and *M. senile* fouling at this depth, while B2 lacked both of these fouling types (Figs. 9.9 and 9.10). In depth band 3, the Interior aspect of B2 differed from that of A4 and B4 ($p < 0.01$ in both cases; Appendix 3); both A4 (Fig. 9.6) and B4 (Fig. 9.11) had over 60% cover of *M. senile* on their interior faces at this depth, while this fouling type was absent from the interior of leg B2 (Fig. 9.10). No other between-leg comparisons (for either aspect) found significant differences at this depth ($p > 0.05$; Appendix 3).

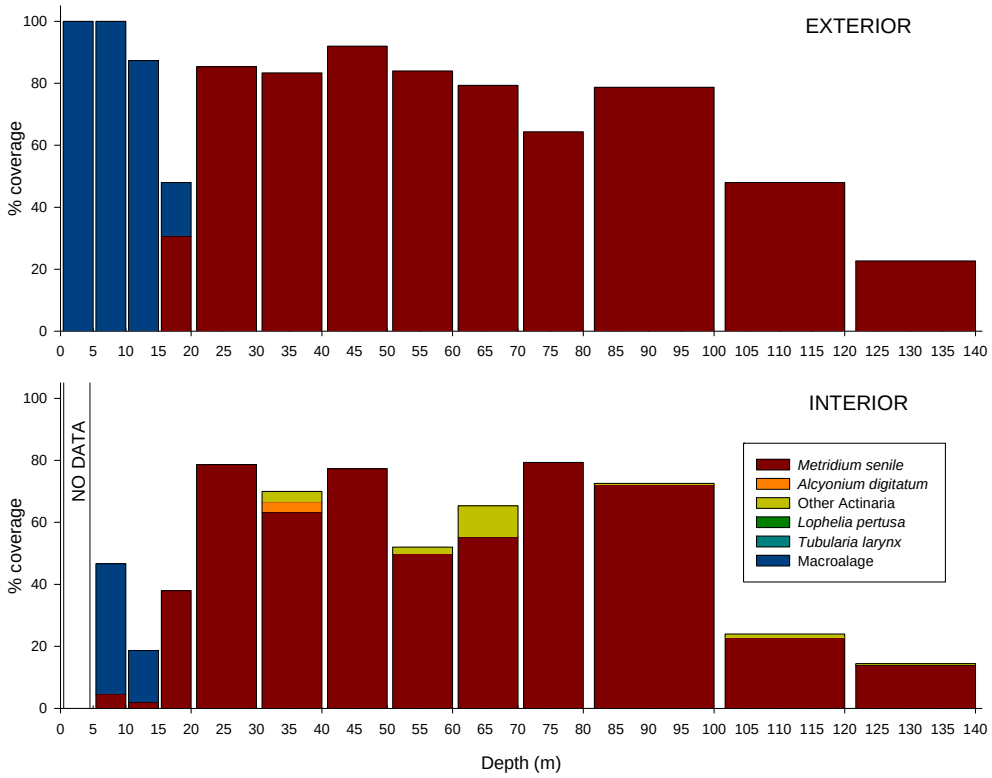


Figure 9.9 Distribution of principal fouling types on the Interior and Exterior faces of Leg A2 on Dunbar, from the surface down to the seabed at 140m

9.3.3.2 Depth band 5: 20-30m

There was a significant Leg*Aspect interaction at this depth ($p < 0.05$; Table 9.6). No individual legs showed significant variation between Exterior and Interior aspects at this depth ($p > 0.05$; Appendix 3). The only between-leg variations

detected by pairwise testing (Appendix 3) were significant differences between the Interior aspect of Leg B4 and the Interior aspects of all other legs ($p < 0.05$ in all cases). Leg B4 (Fig. 9.11) was the only leg to have a large recorded abundance of *A. digitatum* on its interior face (~40% cover), with very little (<5% cover) *M. senile*; *Alcyonium digitatum* was absent from the interiors of A2, A4, and B2 (Figs. 9.6, 9.9 and 9.10), which all had more than 75% cover of *M. senile*.

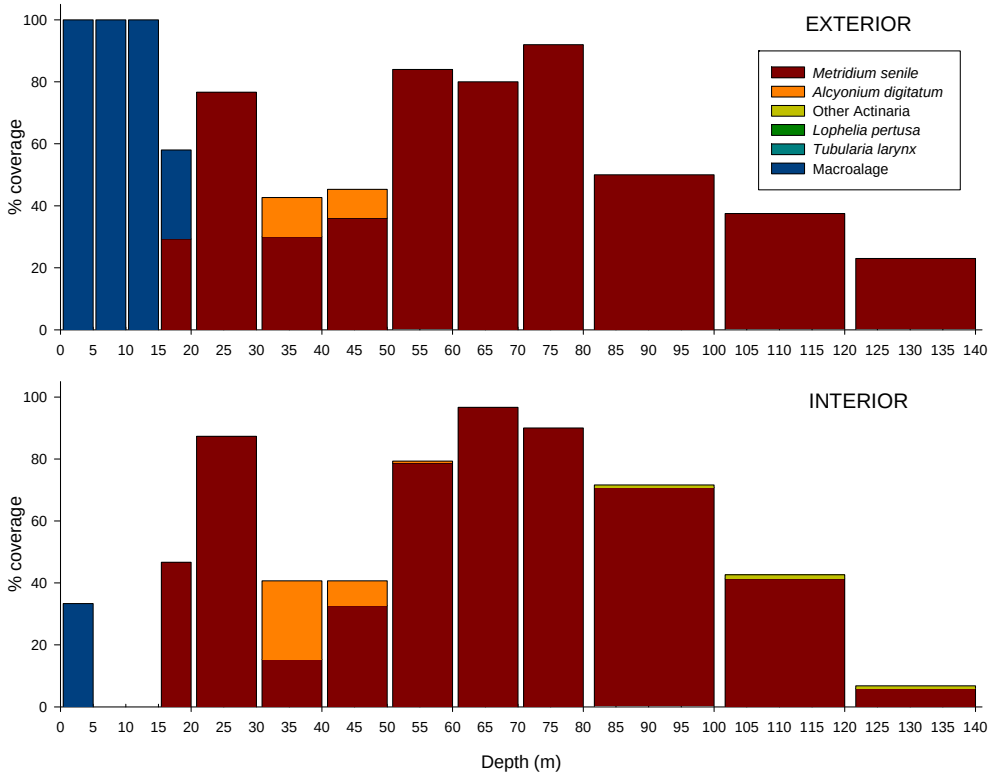


Figure 9.10 Distribution of principal fouling types on the Interior and Exterior faces of Leg B2 on Dunbar, from the surface down to the seabed at 140m

9.3.3.3 Depth band 6: 30-40m

There was significant variation among legs at this depth ($p < 0.01$; Table 9.6). Leg B4 was significantly different from all other legs ($p < 0.05$ for all pairwise comparisons involving B4; Appendix 3); B4 lacked *M. senile* (Fig. 9.11), which was present on all other legs at this depth (Figs. 9.6, 9.9 and 9.10). There was no significant variation between Aspects ($p > 0.05$) and no significant Leg*Aspect interaction ($p > 0.05$).

9.3.3.4 Deeper fouling comparisons (40-120m)

There was little variation among legs below 40m depth (Appendix 3). Between 40 and 50m Leg A2 differed significantly from A4 and B4 ($p < 0.05$ in both cases); the latter two legs had less than 10% cover of *M. senile* (Figs. 9.6 and 9.11), which was abundant (>75% cover) on A2 (Fig. 9.9). Between 50 and 60m B4 differed from A2 and A4 ($p < 0.05$ in both cases); B4 had less than 10% combined cover of *M. senile* and *A. digitatum* (Fig. 9.11), while A2 and A4 lacked *A. digitatum* and had at least 40% cover of *M. senile* (Figs. 9.9 and 9.6).

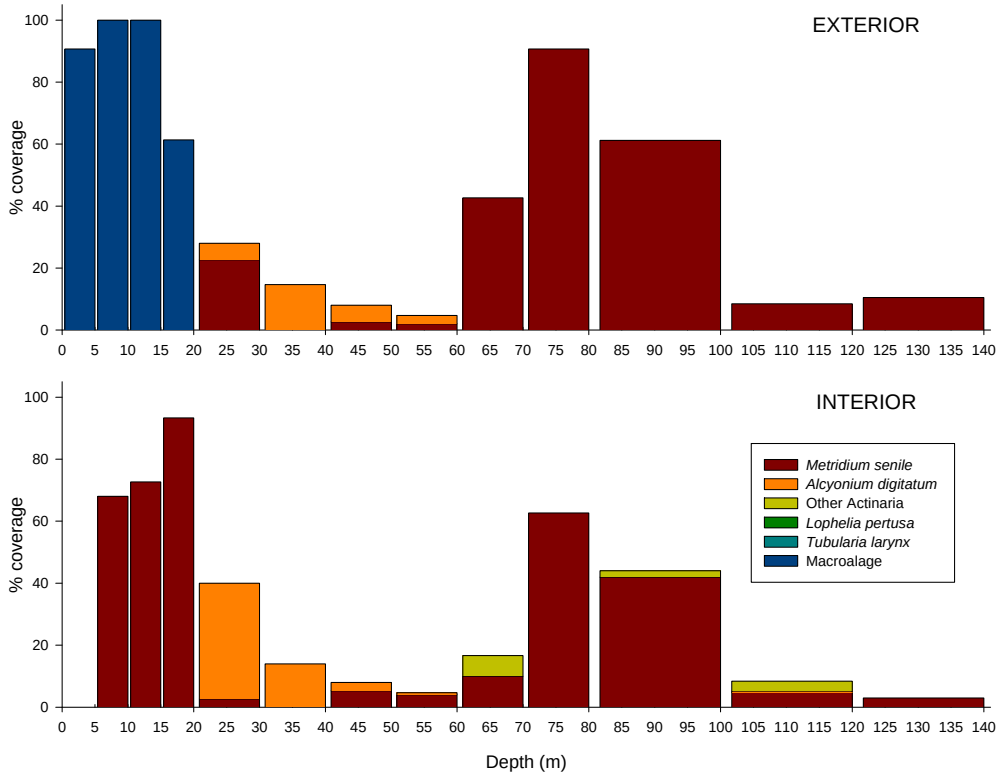


Figure 9.11 Distribution of principal fouling types on the Interior and Exterior faces of Leg B4 on Dunbar, from the surface down to the seabed at 140m

Between 60 and 70m there was a significant Leg*Aspect interaction ($p < 0.05$) and Leg B4 differed from legs A4 and B2, but only on the Interior face ($p < 0.01$ in both cases; Appendix 3). The Interior aspect of B4 had around 5% cover of Other Actinaria, but this fouling type was not recorded on the Interior aspects of A4 or B2 (Figs. 9.6 and 9.10), which also had much greater coverage of *M. senile* (more than 80% on A4 and B2 compared to less than 15% on B4).

The only variation among Legs or Aspects in depth band 10 (70-80m) was that the Exterior of A2 differed from those of B2 and B4 ($p < 0.05$; Appendix 3). The only obvious difference was that A2 had only approximately 65% exterior cover of *M. senile* at this depth; this figure was closer to 90% on B2 and B4 (Figs 9.9 to 9.11). There was no significant variation among Legs or Aspects at depths greater than 80m (Table 9.6).

9.3.4 Hoton platform

Hoton was not included in the multivariate comparisons, because it was located much further south and much more recently installed (during 2001) than the other platforms (Figs. 8.1 and 8.2). It is also located in much shallower water (approximately 30m maximum depth). However, data on the fouling of this structure were collected (Fig. 9.12). While macroalgae were present (up to 30% cover) from 0-5m, most of the structure was fouled almost entirely by *M. senile*, with none of the other principal fouling types recorded.

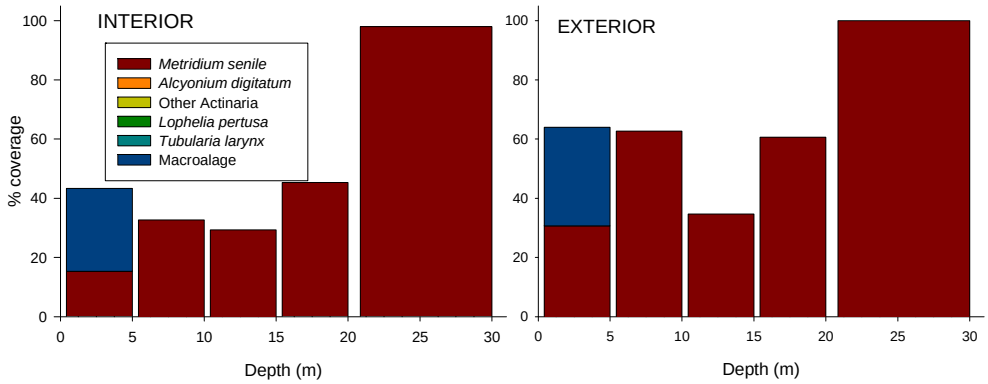


Figure 9.12 Distribution of principal fouling types on the Interior and Exterior faces of a platform leg on Hoton, from the surface down to the seabed at 30m

9.4 Discussion

9.4.1 Effect of aspect on fouling assemblages

On the shallowest parts of the platforms examined here (0-20m depth), there were significant differences between the assemblages on the inner and outer faces of platform legs. The outer faces of legs were generally dominated by macroalgal growth, which was less true of assemblages on the inner faces. This difference may

be the result of two effects: outer faces may be exposed to greater wave action, and inner faces may receive less sunlight as a result of shading by the topsides and horizontal members. Wave exposure has long been known to be a factor structuring rocky shore communities (Lewis, 1976), however, increasing wave exposure is often thought to result in greater dominance by sessile fauna (Little and Kitching, 1996), and not, as was seen here, by algae. Degree of shading is well established as a factor structuring fouling assemblages, including on artificial substrates (Glasby, 1999a, b); lack of light limits algal growth, allowing sessile fauna to dominate. It seems likely that differences in the level of shading between interior and exterior faces were the cause of the differences observed here. However, it would be necessary to make field measurements in order to confirm any possible differences in the shading and wave exposure of different faces of legs, and no such data were available.

9.4.2 Variation in fouling between platforms

While the principal fouling organisms were common to most of the platforms sampled here, it is clearly not true that all North Sea offshore platforms share an identical pattern of fouling distribution with depth. However, it is also not true that each platform had a unique and distinctive pattern of fouling. There was significant variation among platforms that appeared to be driven by factors such as their age and location.

9.4.2.1 Effect of year of installation

Four platforms for which data were provided (Heather Alpha, North Alwyn A, North Alwyn B and Dunbar) were located to the east of the Shetland Islands, in the most northerly area of the UK sector of the North Sea to have significant oil and gas infrastructure (Fig. 8.2). North Alwyn A and North Alwyn B are actually located immediately adjacent to one another, while Dunbar is approximately 20km south. Heather Alpha is approximately 50km away from NAA and NAB, however it is located around 45km to the east, and is in fact less than 20km further north than NAA, meaning that in terms of their latitudes, these platforms are all within 40km of each other (by contrast, Bruce is 120km south of the Alwyn platforms, and

Andrew is a further 190km south of Bruce; distances measured by analysis of Fig. 8.2). While these four platforms are thus relatively close together, they were all installed in different years. The comparisons undertaken in Section 9.3.1 were thus intended to explore any variation in the fouling assemblages resulting from this age difference.

The oldest platform, Heather Alpha, was distinguished from the other platforms over much of the sampled depth range. It differed significantly from Dunbar in all but one depth band, and differed from NAA and NAB in 9 of the 12 depth bands in which they were compared (Appendix 1). Since Dunbar was the youngest platform, with NAA and NAB being of intermediate age, it is not surprising that Heather Alpha appeared more different from Dunbar than these latter two platforms. Dunbar, similarly, differed from Heather Alpha in more depth bands than it did from NAA and NAB (8 and 7 respectively). NAA and NAB were the least dissimilar, being significantly different from each other in only 6 out of 12 depth bands over which they were compared. NAA and NAB mostly appeared to differ as a result of the greater coverage of *M. senile* on NAB (almost 100% cover between 20 and 80m) and the noticeably greater growth of *L. pertusa* on the sampled leg of NAA (Figs. 9.3 and 9.4), which is consistent with greater age.

Greater growth of *L. pertusa* also distinguished Heather Alpha, and it was present at higher abundances than on NAA (up to 40% between 100 and 120m on the interior of the sampled leg; Fig. 9.5). Cover of *M. senile* was also lower on this platform (and on NAA) than on the younger platforms, NAB and Dunbar. However, Heather Alpha was differentiated over depths ranging from the surface down to 50m by the presence of substantial (up to 100% cover in some depth bands) growth of *T. larynx*, a feature unique to Heather Alpha in this study. However, during the early years of the colonisation of the Montrose platform (Forteath et al., 1982), *T. larynx* was also dominant over a similar depth range, but was gradually replaced by other species. It thus seems unlikely to represent a climax community, so such high abundances on an older structure such as Heather Alpha are unusual. It might be possible that the structure was cleaned of fouling at these depths at some stage prior to the survey, such that clean surfaces were available for

colonisation. Information about previous platform maintenance was not made available. However, the presence of some substantial amounts of *A. digitatum* on the legs within this zone (in similar quantities to some of the other platforms where no clearing had obviously taken place) suggests that this had not occurred. Another possibility is that the high abundances observed during the survey represent a seasonal presence; *Tubularia* spp. can be seasonally important in some habitats (Zintzen et al., 2008b). Footage from earlier or more recent inspections might help to determine if this is a stable or transient feature of the fouling on this platform.

In reality, the factor being examined here (date of installation) was a composite of two factors. Firstly, it included the time since installation. Fouling assemblages continue to change even several years after initial colonisation (Butler and Connolly, 1999), particularly where slow growing species such as hard corals are present (Perkol-Finkel et al., 2005). *Lophelia pertusa,* for example, can be expected to continue growing, and thus potentially altering the composition of fouling assemblages, until such time as these structures are removed, and other hard fouling species such as tube-building polychaetes may also be expected to continue to accumulate.

However, the actual year in which these platforms were installed may exert an effect independent of the length of time that has elapsed since. Seasonal and inter-annual differences in the availability of colonists can certainly influence the composition of fouling assemblages over the short term (Underwood and Anderson, 1994; Brown, 2005), but the extent to which these differences might persist over decadal time-scales is less certain (Butler and Connolly, 1999; Brown, 2005). It is interesting that North Alwyn A and North Alwyn B showed some differences in their fouling assemblages, despite having an age difference of only one year. These platforms have been in place for twenty years, so it seems unlikely that such differences resulted solely from this one year age difference. Initial variation in the colonisation and early succession on NAB may thus account for the differences. However, a further confounding factor is proximity to NAA itself. To what extent might the presence of an already colonised structure immediately

adjacent to NAB have influenced its colonisation, perhaps by acting as a source of larvae?

9.4.2.2 Platform location

Platforms Dunbar, Bruce, and Andrew were installed at a similar time, but are spread across different latitudes within the North Sea. Section 9.3.2 details the comparisons among these platforms, which were intended to explore the influence of location (within the UK sector of the North Sea) on fouling composition. These platforms did vary significantly, at most depths, indicating that over this spatial scale (hundreds of km; Andrew was about 300km south of Dunbar and 190km south of Bruce), platform location has an effect on fouling composition. All three platforms showed some differentiation from the others. Dunbar differed from Bruce in 9 of 12 sampled depth bands, while Andrew differed from Dunbar and Bruce in 6 and 5 depth bands respectively (Appendix 2). Generally, it appeared that the more southerly platforms had greater abundances of *M. senile* and *A. digitatum* (Figs. 9.6 to 9.8), while *L. pertusa* was restricted to platforms further north (it was not recorded on the legs of Bruce or Andrew in this part of the analysis, although it was present on Bruce; see Chapter 8),

Location may affect these fouling communities via the influence of water quality factors such as temperature and salinity (Thiyagarajan et al., 2003). No physical data were provided alongside the footage, so no useful speculation can be made concerning the importance of such factors in this situation. Location, particularly in relation to local current and tidal regimes, can also affect supply of colonists (Sammarco et al., 2004; Zintzen et al., 2008a). The distribution of *L. pertusa* on North Sea platforms, for example, is thought to be determined by a combination of water temperature and larval supply factors (Gass and Roberts, 2006); waters around southerly platforms may be too warm and/or currents may not carry larvae far enough south.

9.4.3 Variation among platform legs

Platforms were compared using data from one randomly selected leg from each structure. This approach fails to account for possible variation between legs, meaning that the between-platform comparisons described above may have been affected by choice of leg. However, during the course of making general observations of marine organisms on each platform (Chapter 8), footage from all legs was viewed, and gave little reason to suspect substantial differences between the fouling on legs within platforms. Nevertheless, a comparison of legs within Dunbar was carried out to test for potential differences. Significant variation between legs was detected in 8 out of 13 depth bands (Table 9.6). However, most of these differences seem to have involved one particular leg, Leg B4, which differed significantly from the other legs over depths between 20-40m and 50-70m, mainly because of much lower coverage of *M. senile*, and the presence of more *A. digitatum*. Differentiation between other legs was infrequent: A2 only differed from A4 in two depth bands (from 30-50m) and from B2 in one depth band (5-10m), while A4 was only significantly different from B2 in one depth band (10-15m). At most depths where there was significant variation, only one leg differed from the others. Each leg, therefore, provided a generally representative sample of fouling on the Dunbar platform, and therefore choice of a different leg (A4 was used here) may not have altered the overall results of the between-platform comparisons. Ideally, however, future analyses should use data from all legs (Leg would be incorporated as a fixed factor, nested within Platform), so that between-leg variation can be fully accounted for and analysed within a statistical framework.

9.4.4 Relative influence of factors on fouling composition

Ideally, the two key factors under investigation here ('year of installation' and 'location') would be explored in a single set of analyses, so that the magnitude of their influence on fouling assemblages could be directly compared. However, the availability of platform surveys was at the discretion of the oil and gas industry, and it was only fortuitous that the surveys provided included not only a cluster of

platforms of different ages in a similar location, but also three platforms of similar age in different locations. However, since different aged platforms were only included for one of the 'locations' examined, this meant that both factors could not be included in a single analysis (two-way crossed analysis was not appropriate, as several 'cells' in the analytical structure were empty, and nested analysis was not appropriate as the design would have been extremely unbalanced). Therefore the two factors were considered using separate analyses, and the magnitude of their effects could not be compared directly.

However, if we consider the comparisons undertaken for each factor, we can make some remarks about possible differences in the influences of the factors. If year of installation (for example) is a more influential factor than location, it might be expected that there would be a greater number of significant ($p < 0.05$) differences among the pairwise comparisons of platforms of different ages (Section 9.3.1) than among pairwise comparisons of platforms of similar age in different locations (Section 9.3.2). Indeed, this appears to be the case. In the comparisons of platforms installed in different years, a total of 59 significant ($p < 0.05$) pairwise differences were found over all depth bands (Appendix 1). In comparisons of platforms of similar age in different locations (Appendix 2), a total of 21 significant ($p < 0.05$) pairwise differences were found. Finally, a total of 19 significant ($p < 0.05$) pairwise differences were found between legs on the Dunbar platform (Appendix 3). However, in each of these cases, a different number of pairwise comparisons were carried out (102 for the between-ages comparison, 48 for the between-locations comparisons, and 108 for the comparisons between legs on Dunbar). More differences would be expected where more comparisons are made, and therefore the number of differences must be standardised by dividing by the total number of comparisons. This gives a figure of 0.58 for the between-ages comparison, 0.44 for the between-locations comparison, and 0.18 for the between-legs comparison. This (rather crude) index, which is simply the proportion of pairwise comparisons which found a significant difference, suggests that of the primary factors under consideration here, age/time of installation is more influential than location, as there was more variation among platforms of different

ages than between platforms in different locations. Finally, the comparatively low value (0.18) for the between-legs comparison on Dunbar confirms that variation between legs was relatively minor compared with that between platforms. However, this in no substitute for a rigorous, balanced, multi-factor analysis including age, location, and leg (which was not possible using the data provided); for example, it permits no consideration of the *magnitude* of any of the detected differences. It is only included here in order to make some initial estimate of the relative importance of these factors.

9.4.5 Development of fouling assemblages on North Sea platforms.

Clearly, the amount of time for which a platform has been in place is a significant predictive factor for the nature of the overall fouling community, and so (possibly to a slightly lesser extent), is location within the North Sea. Based on the data from this project, alongside conclusions from other studies, it is possible to propose a general scheme for the development of platform fouling communities over time.

At the shallowest parts of a platform, corresponding to the intertidal and shallow sublittoral zones of a typical rocky shore, colonisation by algae, barnacles, mussels and other such species is likely to be rapid (even the youngest platform, Hoton, installed only 5 years prior to the survey, showed this pattern). This typical shallow fouling assemblage, as well as forming relatively quickly (Forteath et al., 1982), also appears to be stable (it was universal to all platforms studied here, regardless of age).

Below this depth, platforms are first colonised by hydroids, tubeworms and bryozoans, (Forteath et al., 1982; Whomersley and Picken, 2003) after which heavy fouling by *M. senile* occurs, leading to almost total dominance of this species by the time a structure has been in place for five to 10 years. The development of this 'anemone zone' may take longer on more northerly platforms (Whomersley and Picken, 2003). Beneath and around *M. senile*, a background fouling community of soft and hard fouling species develops, including tubeworms, hydroids, anemones, etc. Over time, *M. senile* appears to decline in abundance, particularly at the base of platforms, as the background fouling community, including a greater proportion

of hard fouling organisms, increases in coverage. The decline of *M. senile* is more pronounced for more northerly platforms. *Alcyonium digitatum* begins to appear after 5 years, particularly within the 30-50m depth range, and then increases over the following 5-10 years, displacing some *M. senile*.

By the time a structure has been in place for 20 years, *M. senile* has declined markedly, with hard fouling species dominant over much of the platform (although coverage remains thinnest near the base of the platform), particularly if it is located further north. If far north enough, after 10 years in place large *L. pertusa* colonies become evident (although this species may colonise platforms almost immediately after installation, it takes several years before colonies become large enough to be resolved on survey footage; Gass and Roberts, 2006), and these may continue to grow indefinitely, or until they become too heavy to remain in place. For the largest, most northerly platforms, this species may become dominant over deeper parts of the structure.

9.4.6 Estimating species abundances from structural survey footage

Evaluating benthic communities using digital stills or video recordings is an established approach with several advantages (as well as some disadvantages) compared to *in-situ* field methods (Meese and Tomich, 1992; Whorff and Griffing, 1992; Tkachenko, 2005). Collecting footage or images requires considerably less time spent in the field, and can allow much greater areas to be surveyed. It also produces a permanent record of the survey, which can be returned to for further data extraction or to confirm questionable results. Furthermore, if a site is visited periodically, changes in assemblages can be monitored, and in some cases even individual organisms or colonies can be revisited in order to assess growth (Gass and Roberts, 2006). Importantly, methods involving the assessment of percentage coverage in digital images have been found to be more precise, to have greater sensitivity (ability to detect low abundance species), and show less inter-observer variability than rival methods (Meese and Tomich, 1992; Whorff and Griffing, 1992).

There are disadvantages to any methods involving video and photographic sampling. Firstly, images or video can have various quality problems. Still images can be underexposed, blurred by camera movement, poorly lit, or badly focussed; this can be minimised with the use of digital cameras which allow images to be immediately reviewed in the field, and can often store very high numbers of images, allowing multiple shots of each sample to be taken. However, there is still the possibility that poor images will not be noticed until the analysis stage. Video recording suffers from similar problems. Bad lighting, lack of focus or rapid camera movements can render footage useless, and while some adjustments can be made to correct problems, this cannot rescue extremely poor footage. These problems can go unnoticed until the footage is returned to the laboratory for analysis, by which time it is too late to undertake corrective action. All of these problems were in evidence in the video footage analysed for this project, given that it was not originally recorded for this purpose.

Another problem with image-based sampling methods is the potential for difficulties in species identification (Meese and Tomich, 1992). Fortunately, the main fouling taxa recorded here were easy to distinguish. However, the limited video resolution meant that identification of smaller or less dominant taxa was impossible, hence the use of broad 'background fouling types'. There may therefore have been inter-platform variation that was not detected as a result of this relatively coarse scale approach to the data analysis.

The principle disadvantage of photographic and video sampling of biological communities is usually thought to be the cost of the equipment (Whorff and Griffing, 1992). This factor was irrelevant here, since no equipment purchase was necessary and no costs were incurred for deployment or usage of the equipment, as all footage was recorded during standard ROV survey operations. This is a key advantage of the use of such 'footage of opportunity'. The main disadvantage is, of course, the lack of control over video recording. However, ROV crews have shown a willingness to work with scientists to provide more usable footage in future, at the very least by trying to undertake sections of their structural surveys more slowly and orient the ROV perpendicular to the platform.

Several means are available to extract percentage cover data from video footage. In this case, a manual segmentation approach was used, as this has been found to be more precise, sensitive, and repeatable than other techniques such as point sampling methods (Whorff and Griffing, 1992; Tkachenko, 2005). It is also superior to automatic colour segmentation (Tkachenko, 2005), whereby areas of a certain colour are selected automatically. The latter approach was not suitable for two reasons: firstly because low video quality made it unfeasible for many images, and secondly because several of the main fouling taxa not only occurred in multiple colour morphs, but were also often not distinct in colour from other taxa. The disadvantage of manual segmentation is that it is far more time consuming than other methods (Meese and Tomich, 1992; Tkachenko, 2005); individual images can take anywhere from <1 minute (in areas with very little fouling or almost total fouling by one species) to >30 minutes to process.

9.4.7 Conclusions

Structural inspection footage from seven North Sea offshore platforms was used to compare the fouling assemblages present on these structures. Platforms were not identically fouled; there were differences in how the fouling community changed with depth, which were influenced both by the age and location of the structures. More recently built and more southerly platforms had a tendency to approach total coverage of *M. senile*, while more mature platforms had somewhat less *M. senile* and significant establishment of *L. pertusa* if located in more northerly parts of the North Sea. However, location and age do not totally explain the differences and similarities between platforms; events governing the identities of the initial colonists may have long term effects on the fouling assemblage, and other factors, such as proximity to other platforms, were not explored.

Chapter 10. Summary of Part II

10.1 Summary of results from Part II

Offshore platforms, including those in the North Sea, are *de facto* artificial reefs. They represent unique features in the North Sea environment – no natural formations provide vertical surfaces that stretch from the seabed to the sea surface in these remote offshore locations, with water depths of up to 200m. Sessile marine invertebrates and algae utilise the entire surface area of these platforms, and a variety of different organisms occupy different depths, leading to a pattern of vertical zonation. This means that these structures host, in a single location, species ranging from typical intertidal organisms to deep water corals.

The pattern of fouling was compared among seven North Sea platforms. Both age and location, as expected given previous studies on platforms and other artificial habitats (Forteath et al., 1982; Butler and Connolly, 1999; Whomersley and Picken, 2003; Zintzen et al., 2008a, b), exerted an influence over the fouling communities; younger and more southerly platforms had greater coverage of *Metridium senile* than older and more northerly platforms, which had more hard fouling generally, as well as growth of *Lophelia pertusa*. However, age and location did not completely explain the patterns of similarities between platforms.

Fish were also associated with these structures. Over 1000 small gadoids (*Trisopterus* spp. and *Merlangius merlangus*) were observed at the seabed around the footings of one structure (North Alwyn A), along with smaller numbers (< 50 fish) of larger gadoids such as *Gadus morhua* and *Melanogrammus aeglefinus*. Potentially very large numbers of saithe (*Pollachius virens*) were also observed among the jackets of most of the platforms studied here.

10.2 Use of structural survey footage for scientific research

10.2.1 Epifaunal studies

Limited as our knowledge of North Sea fouling communities is, it is almost entirely based on structural survey footage (Forteath et al., 1982; Whomersley and Picken,

2003; Gass and Roberts, 2006). Structural survey footage (particularly footage of platform legs) proved suitable in this study for assessing variation among platform fouling assemblages, revealing patterns of variation between platforms that were easily resolved using percentage cover data for only a small number of reliably identifiable and measurable fouling types.

However, this may miss other patterns within the fouling communities. There may have been unseen variation within each of the principal fouling types, such as differences in the species composition of macroalgae on different platforms. The amount of mussel growth has been found to differ between some platforms (Forteath et al., 1982), and this could not be assessed using this footage. Furthermore, video footage allows no assessment of the biomass of fouling organisms, nor measurement of the abundance, species composition and biomass of associated small motile fauna such as amphipods, which can vary between artificial reefs even where the fouling assemblages appear superficially similar (People, 2006). Physical sampling of platform assemblages would have to be carried out in order to explore any such variation.

10.2.2 Fish studies

Structural survey footage is less suitable for fish assessment. Video surveys of fish abundance are subject to numerous biases resulting from fish behaviour (Trenkel et al., 2004), and in order to produce useful density measurements, ancillary data such as observer speed and field of view need to be provided (Trenkel, 2003). Technical options such as stereo-video (Harvey et al., 2002), structured illumination or laser grids (Tusting and Davis, 1993) can allow size and distance measurements to be made, but these are not used by inspection ROVs and are unlikely to be available in most circumstances. However, complete structure surveys such as those provided here can allow the enumeration of those fish species which are directly associated with platform structure, as was carried out for North Alwyn A in this study. Assessment of other fish (such as the saithe observed in the vicinity of most platforms here) is perhaps best carried out using acoustic methods (Stanley and Wilson, 1996; Soldal et al., 2002)

10.3 Further research on North Sea offshore structures

North Sea platforms are under-studied marine ecosystems. This is understandable given the difficulties involved in conducting research offshore in challenging conditions. However, there are many avenues of potential research which would add to our knowledge of the ecology of these platforms, artificial reefs, and general ecological processes such as dispersal, settlement and succession on hard substrata.

The analysis of fouling assemblages using survey footage could be extended. It may be possible to use survey video to compare fouling on surfaces with different orientations, but footage of the upper and lower faces of horizontal members was usually unsuitable for percent cover measurement. There are also numerous other platform features (for example risers and conductors) which could be compared to legs. It would certainly be beneficial to study equal numbers of platforms in each location, so that balanced statistical analyses could be conducted. Assuming the availability of archival footage or the acquisition of future survey video, multiple surveys of individual platforms can also be analysed to investigate the development of fouling communities over time, as was carried out for a few structures by Whomersley and Picken (2003).

However, it is perhaps more important to extend the analysis to the associated assemblages of small invertebrates, in order to see how these vary with depth, and between platforms. This will also establish whether or not the patterns in the 'principal fouling types' recorded for this study are a good proxy for patterns in the complete fouling assemblages. Most importantly, however, studies of these organisms are needed to establish the size of the potential food base provided by such structures for fish and large invertebrates (Page et al., 2007).

Nothing is known about the origins of the larvae which colonise these platforms, the extent to which populations on platforms are self-sustaining, and whether or not they can act as sources of colonists for other platforms or even natural reef habitats. These 'offshore islands' may represent a fascinating opportunity to undertake studies of population genetics, especially if coupled with oceanographic

data on water movements, as has been carried out for fish larvae on some platforms in California (Emery et al., 2006; Love et al., 2006)

While acoustic and fishing studies have been carried out to establish the abundance (Lokkeborg et al., 2002; Soldal et al., 2002) and occupancy patterns (Jorgensen et al., 2002) of some fish at some North Sea platforms, and some studies have investigated the possibility of fish around platforms being 'tainted' by hydrocarbons (Picken, 1994; Aabel et al., 1997b), very little other work has been done on fish. In contrast with platforms in Californian waters, where work has been carried out on the diet of fish at platforms (Page et al., 2007), nothing is known about the diet of North Sea fish around platforms.

Similarly, nothing is known about the role of food subsidies provided by fouling organisms to the benthic community. Fouling communities on platforms in the USA have been shown to contribute considerable organic input to the benthos (Wolfson et al., 1979; Lewbel et al., 1987; Bomkamp et al., 2004), but no studies have examined this question in the North Sea.

10.4 Rigs-to-reefs in the North Sea

Many North Sea oil and gas platforms are reaching the end of their productive lives, and decommissioning decisions need to be made for these structures. Obviously any decommissioning option which entails removal of platforms will result in a loss of habitat for the species found in association with them. One possible alternative would be to use the jackets as permanent artificial reefs (Aabel et al., 1997a), either by toppling them in place, moving them to particular sites and then placing them on the seabed, or by removing the top parts of the structures and leaving the rest in place (partial removal). Eventually, the steel would corrode away, but it has been estimated that a structure could remain intact for over 150 years, even without maintenance (Hovda et al., 1998), continuing to function as an artificial reef for at least that length of time.

Such reefs could fulfil one of two roles, as 'fishing reefs' or as 'protection reefs' (Cripps et al., 1998b). Fishing reefs would take advantage of the aggregation of fish

by artificial reefs to increase the Catch Per Unit Effort of fishing vessels, and the predictability of fishing catches, reducing running costs (Whitmarsh et al., 2008) and potentially relieving fishing pressure on other areas. However, in the absence of substantial production of fish biomass at such reefs, this could very easily lead to overfishing if not carefully managed (Polovina, 1991). Alternatively 'protection reefs' could be used to create or supplement 'no fishing zones', using the physical structure to prevent trawling. Even if it is not established that artificial reefs result in production of fish biomass, 'protection reefs' can still be effective (Pitcher and Seaman, 2000). Large artificial reefs in protected areas could act as refugia for overfished populations of important fish such as cod, but only relatively small numbers of cod have been recorded around platforms. Less than 30 cod were recorded at the base of North Alwyn A in this study and similar numbers were observed during another study using ROV footage (Aabel et al., 1997b), and it has not been demonstrated that platforms provide any substantial benefit to fish populations over the scale of entire North Sea fisheries. However, recent observations of the hard coral *Lophelia pertusa* on many of these platforms (Gass and Roberts, 2006) suggest the possibility that these structures could be used to establish permanent *L. pertusa* reefs in the North Sea. Naturally occurring *L. pertusa* reefs are threatened by deep water fishing, and offshore platforms may provide refugia for this species.

However, unlike in the Gulf of Mexico, these structures are too far offshore to be useful as recreational angling or SCUBA diving sites, so support for rigs-to-reefs is lacking. The fishing industry opposes the use of platform materials to construct artificial reefs, as this is perceived as preventing access to fishing grounds (Baine, 2002). Environmental groups oppose rigs-to-reefs in the North Sea largely because it is perceived as a way for the oil and gas industry to dump waste materials, regardless of any actual environmental benefits which might be accrued as a result of avoiding the disruption and energy use inherent in platform decommissioning (Wilkinson and Bellamy, 2000). As a result of the 'Brent Spar incident', in which the approved option of deep-water disposal for an offshore oil storage buoy (the Brent Spar) was made untenable by environmental activists, opposition to offshore

disposal of platform materials (even if used as artificial reefs) has solidified (Side, 1997), with the OSPAR Commission (responsible for regulations regarding environmental protection in the North Sea) effectively banning offshore disposal in 1998 (OSPAR, 1998).

The prospects for rigs-to-reefs in the North Sea therefore seem bleak. More evidence of the efficacy of these structures as reefs may help to change this situation, and as the difficulties involved in removing some of the larger structures become apparent, it may become acceptable to leave some parts of some structures in place. Derogations from the requirement for complete removal can be sought, for example the footings of structures weighing over 10,000 tonnes and installed before 1999 may be considered for abandoning in place (OSPAR, 1998). Unfortunately, as described in Section 10.3 above, and as should be clear from this thesis, decommissioning decisions in the North Sea are currently taking place despite a paucity of rigorous published scientific knowledge about these structures, and the potential effects of their removal on the environment.

Appendix 1. PERMANOVA results for investigation of age-related variation

Comparison of platforms of different ages/time of installation in similar locations.
TWO-WAY CROSSED PERMANOVA
Platform: Random factor, 4 levels (Dunbar, Heather, NAA, NAB)
Aspect: Fixed factor, 2 levels (Interior, Exterior)
Sequential Bonferroni corrections applied to *p*-values
ND = No Differentiation - no within-platform or between-platform variation
TD = Total Differentiation - no within-platform variation, but platforms differ
**p* < 0.05; ***p* < 0.01; ns = not significant (*p* > 0.05).

Depth band 1: 0-5m

Source	df	SS	MS	F
Platform	3	15088.1697	5029.3899	144.1691**
Aspect	1	7727.7111	7727.7111	1.5365 ns
Platform*Aspect	3	15088.1697	5029.3899	144.1691**
Residual	16	558.1656	34.8854	
Total	23	38462.2162		

Pairwise comparisons (t-statistic)

Between Platform, Interior Aspect		Between Platforms, Exterior Aspect		Between Aspects, by Platform	
Dunbar, Heather	10.2711**	Dunbar, Heather	ND	Dunbar	TD
Dunbar, NAA	TD	Dunbar, NAA	ND	Heather	7.4841*
Dunbar, NAB	TD	Dunbar, NAB	ND	NAA	ND
Heather, NAA	7.4841 ns	Heather, NAA	ND	NAB	ND
Heather, NAB	7.4841 ns	Heather, NAB	ND		
NAA, NAB	ND	NAA, NAB	ND		

Depth band 2: 5-10m

Source	df	SS	MS	F
Platform	3	17690.444	5896.8147	12.2927**
Aspect	1	26671.9813	26671.9813	6.0927**
Platform*Aspect	3	13133.1172	4377.7057	9.1259**
Residual	16	7675.2081	479.7005	
Total	23	65170.7507		

Pairwise comparisons (t-statistic)

Between Platform, Interior Aspect		Between Platforms, Exterior Aspect		Between Aspects, by Platform	
Dunbar, Heather	2.6457 ns	Dunbar, Heather	1.5503 ns	Dunbar	2.6295*
Dunbar, NAA	1.9491 ns	Dunbar, NAA	1.3786 ns	Heather	779.941**
Dunbar, NAB	2.1941 ns	Dunbar, NAB	0.5954 ns	NAA	8.625**
Heather, NAA	14.3546**	Heather, NAA	0.9945 ns	NAB	4.6481*
Heather, NAB	13.5815**	Heather, NAB	5.2331 ns		
NAA, NAB	5.5049 ns	NAA, NAB	1.7714 ns		

Depth band 3: 10-15m

Source	df	SS	MS	F
Platform	3	20320.8465	6773.6155	45.9983**
Aspect	1	31109.3105	31109.3105	23.0908**
Platform*Aspect	3	4041.7824	1347.2608	9.149**
Residual	16	2356.1279	147.258	
Total	23	57828.0674		

Pairwise comparisons (t-statistic)

Between Platform, Interior Aspect		Between Platforms, Exterior Aspect		Between Aspects, by Platform	
Dunbar, Heather	15.9274**	Dunbar, Heather	2.0994 ns	Dunbar	41.747**
Dunbar, NAA	12.9661**	Dunbar, NAA	1.0627 ns	Heather	4.0988*
Dunbar, NAB	5.0224*	Dunbar, NAB	1.5161 ns	NAA	9.2386**
Heather, NAA	12.7032*	Heather, NAA	2.0515 ns	NAB	5.41**
Heather, NAB	9.6553*	Heather, NAB	1.9199 ns		
NAA, NAB	4.8762*	NAA, NAB	1.5794 ns		

Depth band 4: 15-20m

Source	df	SS	MS	F
Platform	3	44655.2103	14885.0701	45.5782**
Aspect	1	1947.3049	1947.3049	0.6427 ns
Platform*Aspect	3	9089.5661	3029.8554	9.2774**
Residual	16	5225.3284	326.583	
Total	23	60917.4096		

Pairwise comparisons (t-statistic)

Between Platform, Interior Aspect		Between Platforms, Exterior Aspect		Between Aspects, by Platform	
Dunbar, Heather	18.012**	Dunbar, Heather	4.671*	Dunbar	2.5977 ns
Dunbar, NAA	6.7121*	Dunbar, NAA	2.445 ns	Heather	2.0627 ns
Dunbar, NAB	7.791*	Dunbar, NAB	3.289 ns	NAA	2.8519 ns
Heather, NAA	10.1914*	Heather, NAA	5.8937**	NAB	4.0908*
Heather, NAB	8.0084**	Heather, NAB	17.2951**		
NAA, NAB	3.3307 ns	NAA, NAB	2.8652 ns		

Depth band 5: 20-30m

Source	df	SS	MS	F
Platform	3	33686.1521	11228.7174	158.6214**
Aspect	1	852.0958	852.0958	3.7082 ns
Platform*Aspect	3	689.3531	229.7844	3.246 ns
Residual	16	1132.631	70.7894	
Total	23	36360.232		

Pairwise comparisons	
Dunbar, Heather	20.284*
Dunbar, NAA	14.563*
Dunbar, NAB	2.6596 ns
Heather, NAA	14.1304*
Heather, NAB	8.624*
NAA, NAB	-0.6359 ns

Depth band 6: 30-40m

Source	df	SS	MS	F
Platform	3	27191.7033	9063.9011	32.149**
Aspect	1	-182.4513	-182.4513	-0.5375 ns
Platform*Aspect	3	1018.4188	339.4729	1.2041 ns
Residual	16	4510.9443	281.934	
Total	23	32538.6151		

Pairwise comparisons	
Dunbar, Heather	6.3287**
Dunbar, NAA	1.5877 ns
Dunbar, NAB	3.8044*
Heather, NAA	8.8353**
Heather, NAB	14.237**
NAA, NAB	2.5986 ns

Depth band 7: 40-50m

Source	df	SS	MS	F
Platform	3	32887.112	10962.3707	10.9186**
Aspect	1	522.3751	522.3751	0.9253 ns
Platform*Aspect	3	1693.611	564.537	0.5623 ns
Residual	16	16064.1668	1004.0104	
Total	23	51167.2649		

Pairwise comparisons	
Dunbar, Heather	2.5224**
Dunbar, NAA	2.9676**
Dunbar, NAB	3.1658**
Heather, NAA	4.0534**
Heather, NAB	4.0643**
NAA, NAB	2.586*

Depth band 8: 50-60m

Source	df	SS	MS	F
Platform	3	36052.963	12017.6543	18.8241**
Aspect	1	1008.7209	1008.7209	1.0826 ns
Platform*Aspect	3	2795.2841	931.7614	1.4595 ns
Residual	16	10214.7038	638.419	
Total	23	50071.6718		

Pairwise comparisons	
Dunbar, Heather	4.1136**
Dunbar, NAA	0.7332 ns
Dunbar, NAB	1.8189 ns
Heather, NAA	4.3246**
Heather, NAB	4.3981**
NAA, NAB	5.1862**

Depth band 9: 60-70m

Source	df	SS	MS	F
Platform	3	41228.2317	13742.7439	20.2499**
Aspect	1	1305.1045	1305.1045	1.028 ns
Platform*Aspect	3	3808.6682	1269.5561	1.8707 ns
Residual	16	10858.4956	678.656	
Total	23	57200.4999		

Pairwise comparisons	
Dunbar, Heather	23.5299*
Dunbar, NAA	1.3077*
Dunbar, NAB	2.6942*
Heather, NAA	3.3078*
Heather, NAB	26.0245*
NAA, NAB	1.36*

Depth band 10: 70-80m

Source	df	SS	MS	F
Platform	3	45121.3943	15040.4648	11.5482**
Aspect	1	619.4348	619.4348	0.9852 ns
Platform*Aspect	3	1886.1788	628.7263	0.4827 ns
Residual	16	20838.5297	1302.4081	
Total	23	68465.5376		

Pairwise comparisons	
Dunbar, Heather	3.634*
Dunbar, NAA	5.3174*
Dunbar, NAB	3.5621*
Heather, NAA	0.6707 ns
Heather, NAB	3.6531*
NAA, NAB	5.3361*

Depth band 11: 80-100m

Source	df	SS	MS	F
Platform	3	28936.4403	9645.4801	6.3675**
Aspect	1	2081.0856	2081.0856	2.1151 ns
Platform*Aspect	3	2951.8076	983.9359	0.6496 ns
Residual	16	24236.6668	1514.7917	
Total	23	58206.0003		

Pairwise comparisons	
Dunbar, Heather	1.9153*
Dunbar, NAA	5.0399*
Dunbar, NAB	1.3422 ns
Heather, NAA	2.4084*
Heather, NAB	0.9231 ns
NAA, NAB	2.7268*

Depth band 12: 100-120m

Source	df	SS	MS	F
Platform	3	20621.8445	6873.9482	4.3424**
Aspect	1	1513.1242	1513.1242	0.5073 ns
Platform*Aspect	3	8947.2513	2982.4171	1.884 ns
Residual	16	25328.0082	1583.0005	
Total	23	56410.2282		

Pairwise comparisons	
Dunbar, Heather	2.8181*
Dunbar, NAA	2.3339 ns
Dunbar, NAB	1.0969 ns
Heather, NAA	1.2178 ns
Heather, NAB	2.1761 ns
NAA, NAB	1.7462 ns

Depth band 13: 120-140m (only Heather Alpha and Dunbar compared)

Source	df	SS	MS	F
Platform	1	12266.4624	12266.4624	7.2154 *
Aspect	1	4505.2722	4505.2722	1.0336 ns
Platform*Aspect	1	4359.0052	4359.0052	2.5641 ns
Residual	8	13600.2224	1700.0278	
Total	11	34730.9623		

Appendix 2. PERMANOVA results for investigation of between-location variation

Comparison of platforms of similar ages/time of installation in different locations.
TWO-WAY CROSSED PERMANOVA.
Platform: Random factor, 3 levels (Bruce, Andrew, Dunbar).
Aspect: Fixed factor, 2 levels (Interior, Exterior).
Sequential Bonferroni corrections applied to *p*-values.
ND = No Differentiation - no within-platform or between-platform variation.
TD = Total Differentiation - no within-platform variation, but platforms differ
**p* < 0.05; ***p* < 0.01; ns = not significant (*p* > 0.05).

Depth band 1: 0-5m

Source	df	SS	MS	F
Platform	2	9990.0982	4995.0491	253.891**
Aspect	1	4957.7861	4957.7861	0.9819 ns
Platform*Aspect	2	10098.1319	5049.066	256.6366**
Residual	12	236.0879	19.674	
Total	17	25282.1041		

Pairwise comparisons (t-statistic)

Between Platform, Interior Aspect		Between Platforms, Exterior Aspect		Between Aspects, by Platform	
Dunbar, Bruce	TD	Dunbar, Bruce	ND	Dunbar	TD**
Dunbar, Andrew	321.5571**	Dunbar, Andrew	1.058 ns	Bruce	ND
Andrew, Bruce	2.3695 ns	Andrew, Bruce	1.058 ns	Andrew	0.9734 ns

Depth band 2: 5-10m

Source	df	SS	MS	F
Platform	2	8684.099	4342.0495	6.9437**
Aspect	1	28010.3634	28010.3634	19.3977*
Platform*Aspect	2	2888.002	1444.001	2.3092 ns
Residual	12	7503.9053	625.3254	
Total	17	47086.3696		

Pairwise comparisons	
Dunbar, Bruce	1.0587 ns
Dunbar, Andrew	1.7776 ns
Andrew, Bruce	0.9735 ns

Depth band 3: 10-15m

Source	df	SS	MS	F
Platform	2	2348.9387	1174.4694	10.6259*
Aspect	1	32015.331	32015.331	21.1275*
Platform*Aspect	2	3030.6852	1515.3426	13.7099**
Residual	12	1326.353	110.5294	
Total	17	38721.3079		

Pairwise comparisons (t-statistic)

Between Platform, Interior Aspect		Between Platforms, Exterior Aspect		Between Aspects, by Platform	
Dunbar, Bruce	5.6678*	Dunbar, Bruce	1.8444 ns	Dunbar	41.747**
Dunbar, Andrew	1.149 ns	Dunbar, Andrew	3.5473 ns	Bruce	15.044**
Andrew, Bruce	2.828 ns	Andrew, Bruce	3.7784 ns	Andrew	4.4933*

Depth band 4: 15-20m

Source	df	SS	MS	F
Platform	2	9068.3354	4534.1677	5.6505*
Aspect	1	14495.0346	14495.0346	4.2454 ns
Platform*Aspect	2	6828.5248	3414.2624	4.2549*
Residual	12	9629.1564	802.4297	
Total	17	40021.0511		

Pairwise comparisons (t-statistic)

Between Platform, Interior Aspect		Between Platforms, Exterior Aspect		Between Aspects, by Platform	
Dunbar, Bruce	16.6981**	Dunbar, Bruce	2.0028 ns	Dunbar	2.5977 ns
Dunbar, Andrew	0.9671 ns	Dunbar, Andrew	1.4072 ns	Bruce	39.4794**
Andrew, Bruce	4.634 ns	Andrew, Bruce	2.6245 ns	Andrew	1.0864 ns

Depth band 5: 20-30m

Source	df	SS	MS	F
Platform	2	2467.7467	1233.8733	27.8769**
Aspect	1	159.7679	159.7679	0.762 ns
Platform*Aspect	2	419.311	209.6555	4.7367 ns
Residual	12	531.1385	44.2615	
Total	17	3577.9641		

Pairwise comparisons	
Dunbar, Bruce	4.0534*
Dunbar, Andrew	1.3552 ns
Andrew, Bruce	4.1418*

Depth band 6: 30-40m

Source	df	SS	MS	F
Platform	2	9727.276	4863.638	21.05**
Aspect	1	263.3466	263.3466	0.758 ns
Platform*Aspect	2	694.8104	347.4052	1.5036 ns
Residual	12	2772.6189	231.0516	
Total	17	13458.0519		

Pairwise comparisons	
Dunbar, Bruce	6.2368**
Dunbar, Andrew	3.1798*
Andrew, Bruce	2.753*

Depth band 7: 40-50m

Source	df	SS	MS	F
Platform	2	6337.6336	3168.8168	16.591**
Aspect	1	760.4493	760.4493	1.0647 ns
Platform*Aspect	2	1428.5183	714.2592	3.7396 ns
Residual	12	2291.9576	190.9965	
Total	17	10818.5588		

Pairwise comparisons	
Dunbar, Bruce	3.17**
Dunbar, Andrew	2.1018*
Andrew, Bruce	12.6929**

Depth band 8: 50-60m

Source	df	SS	MS	F
Platform	2	851.9794	425.9897	2.972**
Aspect	1	351.6337	351.6337	2.4653 ns
Platform*Aspect	2	285.2679	142.634	0.9951 ns
Residual	12	1720.0069	143.3339	
Total	17	3208.8879		

Pairwise comparisons	
Dunbar, Bruce	1.6691*
Dunbar, Andrew	1.6462*
Andrew, Bruce	1.6154 ns

Depth band 9: 60-70m

Source	df	SS	MS	F
Platform	2	835.5684	417.7842	11.4107**
Aspect	1	9.4171	9.4171	0.1751 ns
Platform*Aspect	2	107.5545	53.7772	1.4688 ns
Residual	12	439.3624	36.6135	
Total	17	1391.9024		

Pairwise comparisons	
Dunbar, Bruce	2.705*
Dunbar, Andrew	3.5125*
Andrew, Bruce	3.3044*

Depth band 10: 70-80m

Source	df	SS	MS	F
Platform	2	2239.0284	1119.5142	24.6146**
Aspect	1	28.4052	28.4052	0.0751 ns
Platform*Aspect	2	756.1441	378.072	8.3126**
Residual	12	545.781	45.4818	
Total	17	3569.3587		

Pairwise comparisons (t-statistic)

Between Platform, Interior Aspect		Between Platforms, Exterior Aspect		Between Aspects, by Platform	
Dunbar, Bruce	3.8887*	Dunbar, Bruce	4.8371*	Dunbar	3.3201 ns
Dunbar, Andrew	10.5317**	Dunbar, Andrew	1.9745 ns	Bruce	4.3822*
Andrew, Bruce	7.3676**	Andrew, Bruce	2.4402 ns	Andrew	1.2896 ns

Depth band 11: 80-100m

Source	df	SS	MS	F
Platform	1	1424.4799	1424.4799	2.2642 ns
Aspect	1	1459.1505	1459.1505	0.9665 ns
Platform*Aspect	1	1509.6589	1509.6589	2.3995 ns
Residual	8	5033.1416	629.1427	
Total	11	9426.4308		

Depth band 12: 100-120m

Source	df	SS	MS	F
Platform	1	1424.4799	1424.4799	2.2642 ns
Aspect	1	1459.1505	1459.1505	0.9665 ns
Platform*Aspect	1	1509.6589	1509.6589	2.3995 ns
Residual	8	5033.1416	629.1427	
Total	11	9426.4308		

Appendix 3. PERMANOVA results for investigation of between-leg variation

Comparison of legs on the Dunbar platform.
TWO-WAY CROSSED PERMANOVA
Leg: Fixed factor, 4 levels (A2, A4, B2, B4)
Aspect: Fixed factor, 2 levels (Interior, Exterior)
Sequential Bonferroni corrections applied to *p*-values
ND = No Differentiation - no within-platform or between-platform variation
TD = Total Differentiation - no within-platform variation, but platforms differ
**p* < 0.05; ***p* < 0.01; ns = not significant (*p* > 0.05).

Depth band 1: 0-5m

Source	df	SS	MS	F
Platform	3	1252.1307	417.3769	1.0012 ns
Aspect	1	50413.6533	50413.6533	120.9369**
Platform*Aspect	3	1251.4735	417.1578	1.0007 ns
Residual	16	6669.7464	416.8592	
Total	23	59587.0039		

Depth band 2: 5-10m

Source	df	SS	MS	F
Platform	3	12273.4128	4091.1376	4.7391**
Aspect	1	26712.4856	26712.4856	30.9433**
Platform*Aspect	3	12364.1431	4121.381	4.7741**
Residual	16	13812.3304	863.2706	
Total	23	65162.3717		

Pairwise comparisons (t-statistic)

Between Platform, Interior Aspect		Between Platforms, Exterior Aspect		Between Aspects, by Platform	
A2, A4	2.2719 ns	A2, A4	1.7052 ns	A2	2.7337*
A2, B2	11.9408**	A2, B2	ND	A4	2.6295*
A2, B4	1.9699 ns	A2, B4	ND	B2	TD
A4, B2	1.00 ns	A4, B2	1.7052 ns	B4	2.6458*
A4, B4	1.2249 ns	A4, B4	1.7052 ns		
B2, B4	2.6458 ns	B2, B4	ND		

Depth band 3: 10-15m

Source	df	SS	MS	F
Platform	3	14011.7437	4670.5812	10.986**
Aspect	1	32896.1968	32896.1968	77.3777**
Platform*Aspect	3	13989.7758	4663.2586	10.9688**
Residual	16	6802.2099	425.1381	
Total	23	67699.926		

Pairwise comparisons (t-statistic)

Between Platform, Interior Aspect		Between Platforms, Exterior Aspect		Between Aspects, by Platform	
A2, A4	2.0764 ns	A2, A4	1.0946 ns	A2	1.0758 ns
A2, B2	2.6383 ns	A2, B2	2.9395 ns	A4	41.747**
A2, B4	2.0427 ns	A2, B4	2.9395 ns	B2	TD
A4, B2	587.9218**	A4, B2	1.8444 ns	B4	29.3093**
A4, B4	1.3663 ns	A4, B4	1.8444 ns		
B2, B4	29.3093**	B2, B4	ND		

Depth band 4: 15-20m

Source	df	SS	MS	F
Platform	3	3355.0752	1118.3584	2.8357 ns
Aspect	1	23087.1853	23087.1853	58.5394**
Platform*Aspect	3	2609.9158	869.9719	2.2059 ns
Residual	16	6310.1979	394.3874	
Total	23	35362.3743		

Depth band 5: 20-30m

Source	df	SS	MS	F
Platform	3	12559.0162	4186.3387	31.462**
Aspect	1	1683.0176	1683.0176	12.6485*
Platform*Aspect	3	5042.0851	1680.695	12.6311*
Residual	16	2128.964	133.0602	
Total	23	21413.0828		

Pairwise comparisons (t-statistic)

Between Platform, Interior Aspect		Between Platforms, Exterior Aspect		Between Aspects, by Platform	
A2, A4	0.7041 ns	A2, A4	0.0735 ns	A2	0.6981 ns
A2, B2	1.8293 ns	A2, B2	0.8155 ns	A4	0.1292 ns
A2, B4	7.5204*	A2, B4	1.5967 ns	B2	1.4751 ns
A4, B2	0.4226 ns	A4, B2	0.9356 ns	B4	3.5805 ns
A4, B4	7.5064*	A4, B4	1.6035 ns		
B2, B4	7.5396*	B2, B4	1.5344 ns		

Depth band 6: 30-40m

Source	df	SS	MS	F
Platform	3	23616.0441	7872.0147	15.4855**
Aspect	1	937.6299	937.6299	1.8445 ns
Platform*Aspect	3	1082.7278	360.9093	0.71 ns
Residual	16	8133.5576	508.3473	
Total	23	33769.9594		

Pairwise comparisons	
A2, A4	3.6447*
A2, B2	2.7169 ns
A2, B4	8.2506*
A4, B2	0.6443 ns
A4, B4	3.3221*
B2, B4	3.646*

Depth band 7: 40-50m

Source	df	SS	MS	F
Platform	3	13239.1779	4413.0593	7.9133**
Aspect	1	1608.831	1608.831	2.8849 ns
Platform*Aspect	3	999.035	333.0117	0.5971 ns
Residual	16	8922.8089	557.6756	
Total	23	24769.8527		

Pairwise comparisons	
A2, A4	3.0212*
A2, B2	2.7726 ns
A2, B4	8.0667*
A4, B2	1.3377 ns
A4, B4	2.5021 ns
B2, B4	1.6995 ns

Depth band 8: 50-60m

Source	df	SS	MS	F
Platform	3	13217.7755	4405.9252	4.432*
Aspect	1	442.2768	442.2768	0.4449 ns
Platform*Aspect	3	2287.5618	762.5206	0.767 ns
Residual	16	15905.9565	994.1223	
Total	23	31853.5705		

Pairwise comparisons	
A2, A4	1.4351 ns
A2, B2	1.6187 ns
A2, B4	2.3275*
A4, B2	1.5699 ns
A4, B4	2.1508*
B2, B4	2.2053 ns

Depth band 9: 60-70m

Source	df	SS	MS	F
Platform	3	3058.3191	1019.4397	7.6999**
Aspect	1	950.9954	950.9954	7.1829*
Platform*Aspect	3	1723.9454	574.6485	4.3403*
Residual	16	2118.3523	132.397	
Total	23	7851.6122		

Pairwise comparisons (t-statistic)

Between Platform, Interior Aspect		Between Platforms, Exterior Aspect		Between Aspects, by Platform	
A2, A4	1.0795 ns	A2, A4	7.1878*	A2	1.0379 ns
A2, B2	1.1408 ns	A2, B2	0.0216 ns	A4	8.2307*
A2, B4	2.1376 ns	A2, B4	1.2891 ns	B2	1.6426 ns
A4, B2	1.3937 ns	A4, B2	4.6732 ns	B4	3.1007 ns
A4, B4	7.7104**	A4, B4	0.724 ns		
B2, B4	7.9342**	B2, B4	1.2815 ns		

Depth band 10: 70-80m

Source	df	SS	MS	F
Platform	3	52.0039	17.3346	4.4062*
Aspect	1	2.109	2.109	0.5361 ns
Platform*Aspect	3	85.0154	28.3385	7.2032**
Residual	16	62.9465	3.9342	
Total	23	202.0749		

Pairwise comparisons (t-statistic)

Between Platform, Interior Aspect		Between Platforms, Exterior Aspect		Between Aspects, by Platform	
A2, A4	0.285 ns	A2, A4	1.4921 ns	A2	3.0123 ns
A2, B2	0.8768 ns	A2, B2	8.4405*	A4	3.3201 ns
A2, B4	1.4227 ns	A2, B4	5.9328*	B2	0.2921 ns
A4, B2	0.7883 ns	A4, B2	4.4628 ns	B4	2.2012 ns
A4, B4	1.5821 ns	A4, B4	4.1252 ns		
B2, B4	1.7862 ns	B2, B4	0.3259 ns		

Depth band 11: 80-100m

Source	df	SS	MS	F
Platform	3	378.0109	126.0036	1.321 ns
Aspect	1	278.743	278.743	2.9222 ns
Platform*Aspect	3	276.3657	92.1219	0.9658 ns
Residual	16	1526.2082	95.388	
Total	23	2459.3279		

Depth band 12: 100-120m

Source	df	SS	MS	F
Platform	3	3677.9006	1225.9669	1.7109 ns
Aspect	1	2265.2851	2265.2851	3.1612 ns
Platform*Aspect	3	2266.386	755.462	1.0543 ns
Residual	16	11465.2709	716.5794	
Total	23	19674.8427		

Depth band 13: 120-140m

Source	df	SS	MS	F
Platform	3	1892.688	630.896	0.628 ns
Aspect	1	2169.7592	2169.7592	2.1598 ns
Platform*Aspect	3	2020.9149	673.6383	0.6705 ns
Residual	16	16073.8654	1004.6166	
Total	23	22157.2274		

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