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Rhodolith Beds in Northern New Zealand: Characterisation of Associated Biodiversity and Vulnerability to Environmental Stressors

New Zealand Aquatic Environment and Biodiversity Report No. 99.

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The ecology of subtidal rhodolith beds has been investigated for the first time in New Zealand, characterising two rhodolith species, *Lithothamnion crispatum* and *Sporolithon durum*, examining the structure and physical characteristics of beds at two locations and documenting their associated biodiversity. The responses of these rhodolith species to environmental stressors have also been investigated for the first time.

Site and characteristics: Field work was conducted in the Bay of Islands, at Kahuwhera Bay and Te Miko Reef, in February and September 2010. The rhodolith beds were mapped using a combination of techniques and the physical characteristics of the habitats were assessed and compared with adjacent areas outside the rhodolith beds.

The rhodolith beds differed significantly in terms of water motion, sediment characteristics and light levels, with Te Miko Reef having characteristics regarded as typical of rhodolith assemblages, and Kahuwhera Bay being atypical with respect to sediments and water clarity. The Te Miko Reef bed was in clear water and rhodoliths were clearly visible sitting on top of the substrate in a more or less single layer over rhodolith- and shell-derived gravel, whereas at Kahuwhera Bay there were fine sediments suspended in the water column and covering rhodoliths and associated biota, and live rhodoliths were in a more or less single layer overlaying grey to blackened rhodoliths in a darkly coloured rhodolith/sediment sublayer. The two rhodolith beds differed in current direction and strength with no clear tidal signal or direction of water motion at Kahuwhera Bay and tidally driven water movement at Te Miko Reef with a dominant northwest flow.

Biodiversity of the rhodolith beds was investigated sampling (1) invertebrates at three levels of association (epifauna, infauna, cryptofauna), (2) macroalgae, (3) fishes, as well as recording the biogenic and non-biogenic substrates:

- a number of undescribed taxa were discovered as well as new records for the New Zealand region, and range extensions of species known elsewhere,
- more than double the number of invertebrate taxa were present in the rhodolith beds than found outside the beds,
- both rhodolith beds harboured high diversity of associated macroalgae and invertebrates but with markedly different species composition.

Invertebrates: A total of 1088 lots of invertebrates (2093 individuals) were collected and 82% of lots and 87% of individuals were identified to species level. Taxa found as cryptofauna on or inside rhodoliths were identified to phylum. Of the 371 taxa considered in this dataset, 147 (40%) were only collected once (39 algae, 4 fish and 104 invertebrate taxa).

The faunal composition differed significantly between sites, with significant differences in infaunal composition of cores taken inside and outside the rhodolith beds. Significant differences were also found in epifaunal species composition between sites within the rhodolith beds as well as significant seasonal variation. More sponges and echinoderms were found inside the rhodolith bed at Kahuwhera Bay than at any of the three sites within the Te Miko Reef location and significantly more molluscs inside the rhodolith beds at Te Miko Reef than at the other sites.

Cryptofaunal abundance (as mean number of invertebrates per rhodolith) was greatest in *Sporolithon durum* collected from Kahuwhera Bay (74 individuals per rhodolith), followed by *Lithothamnion crispatum* from Te Miko Reef B site (49), *S.durum* from the Te Miko Reef B site (44) and *L. crispatum* from Te Miko Reef (34 individuals per rhodolith). The phyla comprising the cryptofauna were dominated by polychaete worms and Ciliophora, accounting for between 68% and 91% of taxa found.

Macroalgae: Collections included both new records for New Zealand, range extensions for Northland, as well as new discoveries of both genera and species. More macroalgae were found inside rhodolith beds than outside beds, and the species composition differed markedly inside and outside the beds. Three hundred and ninety two samples of macroalgae were collected.

Response to stressors: The effects of changes in temperature in combination with the effects of lowered pH, predicted to occur as a consequence of climate change, were investigated in both species of rhodolith examining responses to two pH levels and three temperatures. Both species were found to be vulnerable to the impacts of increasing temperature and decreasing pH. There was a significant difference between the effects of treatments on the two species and further statistical analysis showed significant interaction between temperature and pH level on growth. Overall the greatest effect on growth rate came with the combination of high temperature (25° C) and low pH (7.65) on *Lithothamnion crispatum* which showed negative growth, indicating probable dissolution. In experiments investigating other environmental stressors, temperature was found to be more important for the survival and growth of the rhodolith species examined than the effects of burial, light and fragmentation.

Threats: Changes in water quality and clarity (nutrients, sedimentation), and physical disturbance (dredging, trawling) have been identified internationally as key threats to slow growing rhodoliths. Rhodoliths, as calcified algae, are also recognised as being vulnerable to the impacts of global climate change, in particular the effects of ocean acidification and rising sea temperatures. The rhodolith beds examined in this study do not appear to be threatened by eutrophication or trawling impacts. It is unclear if the increasing sedimentation occurring in the Te Rawhiti Reach is negatively impacting the bed at Kahuwhera Bay, and whether this atypical rhodolith bed (i.e., with abundant fine sediments) is at risk if current sedimentation and mobilisation rates continue.

Management implications: Identification, assessment and mapping of highly biodiverse marine habitats and ecosystems is a priority of the New Zealand Biodiversity Strategy. This study has documented high biodiversity in two subtidal rhodolith beds sited in relatively close proximity in the coastal zone, with significant differences in biotic composition. The extent of rhodolith beds in other parts of the New Zealand region remain to be documented, including those in coastal areas (including intertidal beds) and subtidal beds on the shelf. Information about the locations of rhodolith beds would provide valuable information for resource managers planning for multiple uses of marine areas, for example, indicating sites where aquaculture developments or trawling activities would potentially be damaging to habitats harbouring high biodiversity.

1. INTRODUCTION

1.1 Overview

What are rhodoliths?

Rhodoliths, or maërl, are red algae that are calcified, non-geniculate (i.e., lacking uncalcified joints) and free-living. Maërl, a Breton word for these calcified algae, is used widely in Europe, whereas the term rhodolith is more commonly used in other parts of the world. Individual rhodoliths may grow around a fragment of shell or rock, or they can be composed entirely of coralline algae. An individual may be composed of one or more coralline species, as well as other encrusting organisms such as bryozoans and gastropods (Harvey & Woelkerling 2007). A rhodolith is defined as having more than 50% coralline algal material (Foster 2001). They are not attached to a fixed surface, and so have the potential to be rolled or moved on the seafloor by the action of waves, currents or other disturbances. At least eight non-geniculate coralline genera contain species that form rhodoliths (*Hydrolithon*, *Lithophyllum*, *Lithothamnion*, *Mesophyllum*, *Neogoniolithon*, *Phymatolithon*, *Spongites* and *Sporolithon*) (Harvey & Woelkerling 2007). Rhodoliths can vary widely in their shape and form. Bosence (1983a) proposed a classification scheme for rhodoliths incorporating their taxonomic composition (monospecific or multispecific) and morphology (shape, size and structure). In this scheme rhodoliths are described as spheroidal, ellipsoidal or discoidal, and laminar, branching or columnar, with differing degrees of branching frequency.

Distribution

Rhodoliths occur in localised habitats worldwide, from the tropics to the poles, and from the intertidal zone to depths of over 200 m (Bosence 1983b; Foster 2001). Typically rhodoliths are found subtidally in clear water in areas of coarse sand, gravel or shell debris. A few reports have recorded rhodoliths from muddy embayments and near sea grass beds (e.g., Bosellini & Ginsberg 1971; Bosence 1983b; Perry 2005; Wilson et al. 2011). Rhodoliths are thought to require water motion or bioturbation to maintain them in an unattached and unburied state (Foster et al. 1997; Marrack 1999). Foster (2001) summarised the habitats of living rhodoliths as "…areas where light is high enough for growth and water motion is high enough to inhibit burial by sediment but not so high or unidirectional as to cause mechanical destruction or rapid transport out of favourable growing conditions".

Rhodolith beds can cover extensive areas. The largest beds that have been reported are on the Brazilian Shelf spanning the equator from 2° N to 27° S (Kempf 1970; Milliman 1977; Testa & Bosence 1999; Foster 2001; Amado-Filho et al. 2007, 2012; Figueiredo et al. 2007; Pereira-Filho et al. 2012). Peña & Bárbara (2008a & b, 2009) reported on extensive beds in Galicia (NW Spain) occurring from the low intertidal to depths of 41 m. Major beds of rhodoliths occur in Mexico's Gulf of California in a transitional setting between tropical and temperate climatic zones. Extensive studies have been carried out on this area (e.g., Steller & Foster 1995; Foster et al. 1997; Steller et al. 2003; Yabur-Pacheco & Riosmena-Rodríguez 2006; Riosmena-Rodríguez et al. 2012). Rhodoliths are also found in the Gulf of Chiriquí, Panama (Littler & Littler 2008); in the North Pacific in Japan in deep fore-reef to shelf areas at water depths of 50 to 135 m (Matsuda & Iryu 2011); in British Columbia (Foster 2001) and Alaska (Konar et al. 2006); in the North Atlantic along the coast of arctic Norway (Freiwald 1998); in Ireland and Scotland (e.g., De Grave & Whitaker 1999; Hall-Spencer & Bamber 2007; Hall-Spencer et al. 2008a); in Newfoundland and Labrador (Canada) (Gagnon et al. 2012); and in the South Pacific they have been recorded from French Polynesia (Payri 1997). In Australia, beds are reported from Western Australia, Queensland and Victoria (summarised in Harvey & Bird 2008). Rhodoliths are also known to occur extensively in the fossil record from a wide range of latitudes including reports from New Zealand (e.g., Burgess & Anderson 1983; Freiwald et al. 1991; Foster et al. 1997; Cintra-Buenrostro et al. 2002; Nalin et al. 2008).

Structure and ecosystem functions of rhodolith beds

The rounded, layered or branching thalli of rhodoliths provide very heterogeneous habitats. Collectively the thalli within a rhodolith bed form a fragile, structured biogenic matrix (Foster 2001; Konar et al. 2006). Rhodoliths are considered to act as ecosystem engineers modifying the physical characteristics of their environment, producing a habitat that can support a high diversity and abundance of marine animals and algae in comparison with surrounding habitats (e.g., Littler et al. 1991; Steller & Foster 1995; Foster 2001;Steller et al. 2003; Barbera et al. 2003; Kamenos et al. 2004a, b; Foster et al.

2007; Peña & Bárbara 2008a, b; Nelson 2009; Hernández-Kantún et al. 2010; Meihoub Berlandi et al. 2012).

Over 300 species of algae and invertebrates were associated with one rhodolith bed in the Gulf of California (Steller et al. 2003; Hinojosa-Arango & Riosmena-Rodríguez 2004) and more than 450 species on beds in the Iberian Peninsula (Bordehore et al. 2003). A rhodolith bed in Maltese Islands (Mediterranean) proved to have high species diversity with 244 animal and 87 algal taxa recorded (Sciberras et al. 2009). Many species found in rhodolith beds appear to be rhodolith-specific, including some cnidarians, echinoderms and chitons (Clark 2000; James 2000; Steller et al. 2003). Meihoub Berlandi et al. (2012) found twenty-six families of polychaete worms over two rhodolith beds on the southern Brazilian coast. Rhodolith beds in southern Espirito Santo State provide an important habitat for epibenthic communities, supporting 25% of the known macroalgal species richness along the Brazilian coast (Amado-Filho et al. 2010). The abundance of cryptofauna (invertebrates within the natural cavities between rhodolith branches and also inside branches) that rhodoliths support has been found to increase with increasing size and three-dimensional complexity of the individual rhodoliths and the beds (e.g., Steller et al. 2003; Grall et al. 2006; Figueiredo et al. 2007; Foster et al. 2007).

Live rhodolith beds appear to provide a critically important service as nursery areas among a patchwork of shallow coastal habitats in the north-east Atlantic.The complex habitat structure of rhodolith beds has been shown to provide refugia for juvenile fish and settlement habitat for shellfish larvae, some of which are commercially important (e.g., scallops, crabs, cod) (e.g. Hall-Spencer et al. 2003; Kamenos et al. 2004a, b; Steller & Cáceres-Martínez 2009). Howarth et al. (2011) investigated the effects of a fully protected marine reserve on commercially valuable scallops and benthic habitats in Isle of Arran, United Kingdom. A greater abundance of juvenile scallops was related to the greater presence of macroalgae and rhodoliths within the reserve boundaries, with the rhodolith habitat apparently positively influencing spat settlement. The high levels of functional diversity typically supported by rhodolith beds may indirectly contribute to maintaining productivity of commercial fisheries (e.g., Kamenos et al. 2004a; Hall-Spencer et al. 2003; 2008b; Ordines & Massuti 2009; Steller & Caceres-Martinez 2009).

Foster (2001) considered that in terms of area covered rhodoliths beds may be comparable in extent with kelp beds, seagrass meadows, and non-geniculate coralline reefs. Despite evidence of their importance for biodiversity and for juvenile stages of commercial species, the functional ecology of such peculiar and complex habitats has received very little attention in contrast to other marine communities such as kelp forests or seagrass beds. Grall et al. (2006) analysed the benthic food web within rhodolith beds in the Bay of Brest, investigating the community structure parameters (species richness, abundance, biomass and dominant species) along with the carbon and nitrogen isotopic composition of the main benthic species (macrofauna and megafauna). This assessment of trophic levels and differences in the potential food sources of maërl inhabitants using stable isotopes, revealed a complex system including a high diversity of feeding strategies. Epiphytic macroalgae and microphytobenthos both growing on maërl thalli together with sedimenting particulate organic matter originating from the water column provided the sources of carbon for this system. Grall et al. (2006) concluded that the physical complexity of the rhodoliths, combined with the diversity of carbon sources, would provide a wide variety of microhabitats that 'would explain, at least partly, the high number of species observed in this exceptional habitat'.

Pereiro-Filho et al. (2012) calculated that the summits of several seamounts covered by extensive rhodolith beds within the tropical southern western Atlantic are responsible for 0.3% of the world's carbonate production, and Amado-Filho et al. (2012) recorded the production from Brazilian rhodolith beds to be comparable to the world's largest $CaCO_3$ deposits, describing these beds as "major $CaCO_3$ biofactories".

Knowledge about rhodoliths in New Zealand

Taxonomy: The taxonomy of rhodoliths has been regarded as difficult because of their morphological variability and because they are reported to be infrequently fertile, and thus critical distinguishing characters are unable to be evaluated. In New Zealand the common non-geniculate coralline algae of both central and northern areas of New Zealand have been studied (Harvey et al. 2005; Farr et al. 2009). This research has enabled us to identify appropriate molecular markers for the identification of sterile material thereby overcoming a significant barrier to work on corallines (Broom et al. 2008), as

well as to develop a database of information on the distribution and species composition of different areas. Farr et al. (2009) have identified at least four species of rhodolith-forming non-geniculate coralline algae in New Zealand – *Sporolithon durum* (Foslie) R.A.Towns. & Woelk., *Lithothamnion crispatum* Hauck (as *L. indicum* Foslie) (Basso et al. 2011), *Lithothamnion proliferum* Foslie, and *Lithophyllum* sp. The latter two are known from very few specimens and further work is required before their distribution in the New Zealand region is fully understood. In New Zealand fertile rhodoliths of both *S. durum* and *L. crispatum* are commonly found, contrary to experience internationally.

Distribution & ecology: There is little published information about the location, extent or ecosystem functioning of rhodolith beds in New Zealand. Foster (2001) reviewed the global distribution of rhodolith beds and reported on one within the New Zealand region at Kapiti Island (based on Battershill et al. (1993). This bed was reported on the eastern side of Kapiti Island, although Battershill et al. (1993) were unable to define its full extent.

Rhodoliths have also been reported from sites in north eastern North Island. For example, in a study of the macrobenthos of the Cavalli Islands, rhodoliths were recorded as "an important component of the sediments" of the Cavalli Passage (Grace & Hayward 1980). The rhodoliths were in depths of 5-10 m and associated with the bivalve *Tawera spissa*. Grace & Hayward (1980) observed that the rhodoliths provided attachment surfaces for bryozoa, serpulid polychaetes and small algae, as well as grazers such as chitons, limpets and a variety of epifauna such as amphipods, crabs, isopods, ophiuroids and gastropods. The position of the rhodolith beds coincided with the highest diversity recorded in the survey area. Hayward et al. (1981) reported both living and dead rhodoliths from an area of coarse sediment in the Bay of Islands south of Urupukapuka Island. In a study of sub-tidal associations at Tutukaka, Brook et al. (1981) reported on the occurrence of non-geniculate coralline algae encrusting dead cockle shells in areas of fine muddy sand. In addition they found a *Corallina-Maoricolpus-Notomithrax*- association, with *Corallina* turf, encrusting non-geniculate corallines and the presence of poorly developed rhodoliths in some areas at depths of 1–7 m in gravelly muddy sand, associated with a diverse epi- and infauna. Hayward et al. (1982) recorded a gravelly substrate-association at Rakitu Island (east of Great Barrier Island) that included various algae (*Caulerpa*, *Codium*, '*Lithothamnion*', *Zonaria*), chitons, polychaetes and bryozoa associated with pebbles and large shells, in pebbly to coarse sandy pebble gravel at 12–18 m depth. They also recorded rhodoliths from the "gravelly substrate" association and a *Selenaria squamosal*-association, although made no specific comment on their association with other taxa.

In a study west of Great Barrier Island, Hayward et al. (1986) reported a very distinctive rhodolith-holothurian *Cucumaria-Glycymeris laticostata*- (now *Tucetona laticostata*) association, in depths of 10–15 m in high energy situations in coarse sediment that was characterised by a rich subsidiary epifauna. They reported that a rich and diverse fauna indicated that the rhodoliths provided a favourable habitat for epifauna within the coarse substrate as well as for infauna. Pink finely branched live rhodoliths were present in all stations and dead white specimens comprised a large proportion of the sediment. Rhodoliths were also found in smaller numbers associated with a *Corbula zelandica* and *Venericardia purpurata* sub-association. Around Great Mercury Island, Grace & Grace (1976) reported that beds of rhodoliths were found to be associated with the abundant *Tawera spissa* and *Venericardia purpurata* community, and they mapped the presence of rhodoliths occurring in coarse sand to shell gravel at 4–15 m depth, in a channel with strong currents. They commented on the problems of obtaining representative samples with dredge sampling in this kind of habitat and the likelihood of recording a higher proportion of epifauna to infauna than actually occurs on the sea bed. The species forming the rhodoliths were not identified in any of these papers.

Basso et al. (2009) presented research on a shallow rhodolith bed on the Whangaparaoa Peninsula, a site dominated by the species *Sporolithon durum* although with rare *Lithophyllum* sp. rhodoliths also present. They considered this bed to be of particular interest because of the large size (10–97 mm, mean 38.7 mm) and density of the rhodoliths, and their occurrence in such a shallow habitat (low intertidal and upper subtidal zones). They considered that in this very shallow environment the tidal and wave currents act on a relatively wide gently sloping platform, preventing sedimentation of fine particles on the rhodoliths. Dewas & O'Shea (2012) reported on bivalve/rhodolith patches in the Hauraki Gulf and the associated benthic invertebrate assemblages which were found to have higher

taxon richness and abundance when compared to gravel substrates. Unfortunately they did not identify which rhodolith-forming species were present, and did not characterise the size or form of the individual rhodoliths. They were not able to differentiate "any effects of rhodoliths on seabed taxon richness and abundance from those of *T. laticostata* shell". Rhodoliths were collected from the southern Manukau Harbour in 1976 from a subtidal bank (NIWA Invertebrate Collection) and there are some historical accounts of rhodoliths from Pouto in the Kaipara Harbour (L. Makey, AUT - pers. comm.) but no rhodoliths have been collected recently in either harbour.

Rhodoliths are also known to occur at sites in the South Island. Davidson (1992) recorded rhodolith beds from the Totaranui area in areas of strong tidal currents although was not able to document the extent of these algae. He considered that these rhodoliths were the "only significant beds recorded in the Nelson/Marlborough region". However, Davidson et al. (2011) reported on rhodoliths occurring "in a small number of distinct locations in the Sounds" including Picnic Bay in Pelorus Sound (dense cover over 1.9 ha of seafloor), and Ponganui Bay and Catherine Cove (dense beds between 6 and 26 m), D'Urville Island. A dense bed of rhodoliths was found at approximately 13 m depth in Okiwa Bay, Croisilles Harbour, with rhodoliths forming a layer 15–25 cm deep (R. Murdoch – pers. comm.). Reid et al. (2011) reported on macroalgal communities on the Kaikoura coast, including the presence of rhodoliths, and Fleming (1950) reported on branching calcareous algae from Fiordland. In addition through field work, personal communications and liaison with other research teams we are aware of rhodolith beds in North Otago and Foveaux Strait.

Threats and vulnerability

Coralline algae are long-lived, slow growing marine organisms, with growth rates ranging from 0.015–2.5 mm per year) (e.g., Adey & McKibbin 1970; Frantz et al. 2000; Blake & Maggs 2003; Bosence & Wilson 2003; Rivera et al. 2004). Frantz et al. (2000) determined the growth rate for an individual rhodolith, *Lithothamnium crassiusculum*, from the southern Gulf of California through ^{14}C analysis using accelerator mass spectrometry (AMS) to be 0.6 mm/year. This growth rate suggests large *L. crassiusculum*, which have been found with radii in excess of 6 cm, may be over 100 years old. It is postulated that these slow growth rates give rhodoliths a limited ability to respond to or recover from damage or burial (Grall & Hall-Spencer 2003; Hall-Spencer et al. 2010).

Recent international studies indicate that rhodoliths are at risk from the impacts of a range of human activities e.g., physical disruption (trawling, dredging, anchoring) (Hall-Spencer & Moore 2000), reduction in water quality (siltation and coastal runoff, anoxia, eutrophication, effluent discharges, offshore dumping) (e.g., Wilson et al. 2004; Riul et al. 2008), alterations to water movement (breakwaters, quays, sea-walls, causeways, marinas, bridges), and aquaculture installations (shellfish rafts and lines, fish cages) (Hall-Spencer et al. 2003, 2006). Impacts of fragmentation on individuals and on beds may be critical in terms of biodiversity and abundance associated with rhodolith beds because the diversity and abundance of organisms supported by a rhodolith significantly increase with complexity (branching density) and the space available (thallus volume) (Steller et al. 2003).

Although rhodolith beds are dependent upon some level of motion or disturbance for their maintenance, extreme levels of disturbance can result in a reduction of thallus density, size and structure, loss of living thalli and ultimately transition into sand flats. Effects of towed and static demersal gear (e.g. scallop dredges, otter trawls, hydraulic gear) have been investigated by several authors. Demersal gear deposits sediment over a wide area around fished tracks and suspension feeders suffer from clogged gills, while algae are smothered. Kamenos et al. (2003) looked at the heterogeneity of substrates in dredged versus undredged rhodolith beds and found that unimpacted beds had higher structural heterogeneity, and that much of the rhodolith bed was killed post-burial by a lack of light. Similar results have been reported by Bordehore et al. (2003) examining the impacts of otter trawling on rhodolith beds in Spain, and by Riul et al. (2008) who observed decreases in primary production of up to 70% when rhodoliths were buried by a thin sediment layer. Jackson (2008) summarised the habitat destruction that occurs as a consequence of trawling and dredging, namely the reduction of the three-dimensional structure and complexity of sea floor habitats to bare sediment; reduction in the size, biological diversity and turnover time of dominant species; and the consequent new associations of species that may persist for decades even if trawling is halted. While it is clear that in general the effects of trawling reduce epifaunal and infaunal diversity through large scale homogenising of the benthos (e.g., Engel & Kvitek 1998), this localised benthic disturbance also has a potentially large negative effect. Long-term effects are difficult

to estimate, but it is clear from the extremely low coralline growth rates reported worldwide that recovery of the substrate after disturbance is likely to be very slow. For example, Hall-Spencer & Moore (2000) compared the effects of scallop dredges used on a previously unfished rhodolith bed in Scotland with similar beds that had been fished. A single tow of three dredges was found to have physical effects that remained clearly discernible four years after the event.

A number of studies examining the impacts of various types of aquaculture (e.g., mussel farms, salmon sea cages) on benthic habitats have found rhodoliths to be very sensitive and negatively affected when compared with other unvegetated benthic habitats, particularly as deposits of detritus and fine sediment can result in the burial of the rhodoliths (e.g., Wilson et al. 2004; Riul et al. 2008; Peña & Bárbara 2008a; Sanz-Lázaro et al. 2011; Wilding 2011; Aguado-Giménez & Ruiz-Fernández 2012).

In Britain and France, calcareous marine sediments have been used for centuries to enrich soils and rhodolith beds are currently actively exploited for a variety of industrial processes including agriculture, water purification, mineralisation and the manufacture of cosmetics (Grall & Hall-Spencer 2003). In Brazil 96 000 to 120 000 metric tonnes of rhodoliths are extracted per year (Riul et al. 2008). Harvesting activities and disturbance of the beds releases sediment in the water column. Within the European Union "Habitats Directive" rhodolith beds have been recognised as key habitats warranting protection and moves to limit extraction in European waters have been underway for some time (e.g., Birkett et al. 1998). To date there has been no commercial interest in extraction of rhodoliths in New Zealand.

As calcified organisms, rhodoliths will be affected by acidification of the oceans resulting from global climate change. Although the potential impacts are not yet fully understood, they are likely to be complex and variable between species (Hall-Spencer et al. 2008a; Kuffner et al. 2008; Doney et al. 2009; Martin & Gattuso 2009). It is thought that sensitive reef-building species such as coralline algae may be pushed beyond their thresholds for growth and survival within the next few decades (Anthony et al. 2008). Jokiel et al. (2008) have shown that rhodoliths are profoundly adversely affected by acidification, and show a much greater impact than exhibited by other coralline algae or corals. The impact of acidification may be more pronounced in colder seas where carbonate saturation states will be lower. Büdenbender et al. (2011) have shown strong negative response of an Arctic species of *Lithothamnion* to increased CO_2 levels. Hepburn et al. (2011) examined carbon use strategies in a kelp forest community and observed that coralline algae could potentially be less physiologically competitive as calcification and maintenance of calcified structures become more difficult but could also be overwhelmed by fleshy algae which respond positively to elevated carbon dioxide levels.

In a review of the contributions of calcareous algae to global carbonate production Basso (2012) summarised the response of coralline red algae to marine acidification and rising temperature – these algae show decreased net calcification, decreased growth and reproduction, as well as reduced abundance and diversity, leading to death and an ecological shift to dominant non-calcifying algae. Basso (2012) concluded that current knowledge of the distribution of coralline-dominated habitats and the quantification of their carbonate production is not adequate to allow proper environmental management and confident modelling of a global carbon budget, and considered that the priorities for future research are locating the algal carbonate factories around the world and evaluating their extent and their production.

1.2 Objectives

This research focused on both field and laboratory investigations of rhodoliths in New Zealand. Rhodoliths at two locations have been studied, employing a range of approaches to characterise the beds and their associated biodiversity. In the laboratory rhodoliths have been grown under a range of conditions to examine their response to a range of environmental parameters, specifically examining the effects of light, burial, fragmentation, temperature and acidification. We present a qualitative assessment of agents of change affecting biodiversity and ecosystems in coastal marine areas of New Zealand, as identified by the Millennium Ecosystem Assessment - http://www.millenniumassessment.org/.

1.2.1 Overall Objectives

1.To evaluate the vulnerability of New Zealand rhodolith species to environmental stressors and to characterise diversity of rhodolith beds.

1.2.2 Specific Objectives

1. To characterise the distribution and physical characteristics of two New Zealand rhodolith beds and characterise the associated biodiversity.

2. To measure the growth rates and evaluate the vulnerability of New Zealand species of rhodoliths to environmental stressors.

2. METHODS

Species investigated

The rhodolith-forming species investigated in this study were *Sporolithon durum* (Foslie) R.A.Towns. & Woelk. (Sporolithaceae) and *Lithothamnion crispatum* Hauck (Hapalidiaceae) (Basso et al. 2011; previously as *L. indicum*, Farr et al. 2009) (Figure 1). They were identified both by morphological and anatomical features as well as molecular sequence data. Rhodoliths of *S. durum* are generally larger than *L. crispatum* and have thicker branches and a more open branching pattern. *Sporolithon durum* produces cruciately divided tetrasporangia in calcified compartments in sori. *Lithothamnion crispatum* rhodoliths are tightly branched, and produce zonately divided tetrasporangia in multiporate conceptacles that have flat tops and are flush or raised above the level of the thallus (Farr et al. 2009). Both species have uniporate gametangial conceptacles.

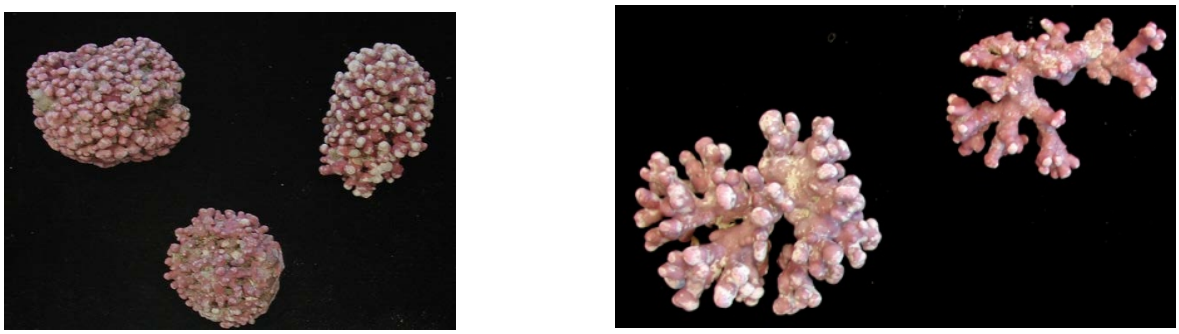

Figure 1: *Lithothamnion crispatum*, maximum size approximately 4 cm (left), and *Sporolithon durum*, maximum size approximately 7 cm (right).

Locations and sites

Two rhodolith beds within the Bay of Islands, northern New Zealand, were selected as the locations for the majority of the field work in this study. These beds were identified as potential study locations during earlier field work (ZBD2004-07; Bay of Islands OS20/20 project). Kahuwhera Bay, (35^{o} 15' 40.00" S, 174^{o} 10' 55.00" E) is situated on the southern side of the larger Manawaora Bay on the mainland, and Te Miko Reef (35° 13' 43.80" S, 174° 10' 55.00" E) lies 4 km to the north in the 0.7–1.2 km wide channel between Moturua Island and Motuarohia Island (Figure 2). Refer to Appendix 1 for details of the locations and sites in the Bay of Islands. Field work was carried out in February and September 2010, allowing late summer and early spring sampling of biological and physical data (temporal sampling referred to in analyses as seasonal).

Figure 2: Map of the Bay of Islands indicating the position of the two study locations, Kahuwhera Bay and Te Miko Reef.

2.1 Objective 1

2.1.1 Characterisation of the beds: qualitative and quantitative site characteristics

2.1.1.1 Mapping the beds

The method of Steller et al. (2003) was used to distinguish between sandy benthos versus rhodolith bed habitat: if there was more than 10% cover of living coralline thalli the area was considered to be inside a rhodolith bed. A combination of techniques – drop-down digital video with ground truthing by SCUBA diving and dredge samples – was used to map the extent of the beds and to evaluate the presence/absence of rhodoliths, species composition at each location, and percentage cover of the two rhodolith species.

Survey grids with points 250 m apart were apportioned to the locations at Kahuwhera Bay and Te Miko Reef, each covering 5 to 7 km^2 with 90 and 95 targeted points respectively. At Kahuwhera Bay the survey points were allocated in a grid ranged in depths from 15 m in the northwest and to 4 m in the south and east of the area and extended across the outer reaches of the entire Manawaora Bay, an area of gently sloping seafloor of approximately 3.5 by 2 km. At Te Miko Reef, in the channel between Moturua Island and Motuarohia Island, the saddle between the islands shallows to a depth of 6 m. The video sample points extended to depths of 20 m to the south east and 25 m depth to the north east of the reef, a distance of approximately 2 km.

The drop-down digital video, consisting of a light-weight pyramid shaped steel frame with a stable base measuring 66 cm by 41 cm and a height of 44 cm, was deployed by hand-hauled cable. The vertically downward facing video camera, a 'Splashcam Deep Blue unit' manufactured by Ocean Systems inc. USA, was mounted 40 cm above the base and gave a field of view in water of 33 cm by

21 cm or approximately 0.07 m^2. Power was supplied to the camera via a pair of conductors in the cable from a 12 V DC rechargeable battery at the surface. Live video travelled up the coaxial cable for viewing in real-time and recording to mini DV tapes on a Sony video Walkman.

Lighting was enhanced by a pair of custom-built LED lights consisting of two 5 W LED MR16 downlight bulbs mounted in a block of clear acrylic resin. Power was provided from the surface by a separate PVC cable and a surface switch to enable the seafloor to be examined in differing lighting regimes. At each drop-down video sample point the vessel was anchored and the camera frame was lowered to the seafloor and footage recorded for approximately 10 seconds of contact with lights on and off, this was repeated on two other occasions to give three non-overlapping replicates at each sample point. A small dredge with a mouth area of 36 cm by 15.5 cm and 45 cm deep was then deployed at about 20% of the sample points to ground-truth species mix and associations with other flora and fauna. Observations from divers' logs served as a further method for cross checking rhodolith bed characteristics at the grid points. Post processing of video adopted the protocols outlined in Hall-Spencer et al. (2008a).

All replicate footage was scored for:
a) Rhodolith species present i.e., *Lithothamnion crispatum, Sporolithon durum,*
b) Percentage cover of rhodoliths
c) Percentage alive/dead (based on pigmentation of rhodoliths),

2.1.1.2 Physical characteristics of the beds: *in situ* characterisation of the habitat

a. Sediment characteristics

Sediment samples were taken during the biological sampling of the infauna in both February and September (refer below) and later analysed for particle size. Sediments were characterised using the Laser Sizer Protocol. A Beckman Coulter LS13320 Laser Diffraction Particle Size Analyser was used to determine the percent by volume of particles ranging in size from 0.04 μm to 2000 μm. This was done by taking a small (0.25–6 g) subsample of sediment and soaking it in 50 ml of washing solution (containing NaH_2CO_3 and $NaHCO_3$). The sample was agitated in an ultrasonic bath (Cole-Parmer 8891) for up to 15 seconds to disaggregate the fine particles prior to being washed through a 1.6 mm sieve and into the laser sizer. The results were uploaded into Gradistat version 6 for statistical analysis. The results were grouped into the categories gravel (larger than 2 mm), sand (63 μm – 2 mm) and mud (less than 63 μm).

b. Light, temperature, and water motion

Spot measurements of *in situ* light levels were made using a scalar PAR (photosynthetically active radiation) probe (Biospherical® Instruments QSP-2100), to which we added an underwater digital display interface for recording values underwater. The scalar probe measures light from all directions and therefore gave more appropriate measures of light availability to marine macroalgae. Measurements were made directly on the seafloor within rhodolith beds as well as approximately 1 m above the bed at both locations. Mean values were obtained from sets of 10–22 measurements. In addition short-term water current characteristics were obtained from two high resolution current meters (Aanderaa RCM Recording Current Meters) which were deployed for five days in September to record current speed and direction at the locations.

To obtain longer term environmental measurements of temperature and light six Onset HOBO Pendant® Temperature/Light data loggers (UA-002-64, 64K) were deployed at the field locations in February, and retrieved in September. All light level values derived from the HOBO loggers (in lux) were converted to give approximate estimates of PAR as described by Herrera et al. (2009).

2.1.2 Characterisation of biodiversity of the rhodolith beds

2.1.2.1 Sampling protocols

Sampling was designed to characterise the rhodolith beds at the two locations and to assess biodiversity inside the rhodolith beds and compare this with biodiversity found in sites outside rhodolith beds. Sampling was carried out at both locations in February and September, enabling temporal comparisons.

At each location, sites inside and outside the rhodolith beds were assessed (two sites at each location, i.e., Kahuwhera Bay - KWB and KWB_OUT, Te Miko Reef - TMR and TMR_OUT). At Te Miko Reef an additional site containing mixed rhodolith species was also assessed (TMR_B). As the beds were more or less at single depth (approximately 8 m), it was not necessary to stratify sampling by depth.

Adapting the methods used in published studies (especially Foster et al. 2007, Steller et al. 2003, Steller et al. 2007a, b, and Harvey & Bird 2008) to the conditions at our study sites, we developed a protocol which allowed us to sample:

1) invertebrates - at three levels of association with the rhodoliths (i.e., epifauna (on the surface of the substrate, either rhodolith or sand), infauna (within the sediment), and cryptofauna (invertebrates within the natural cavities between rhodolith branches, and also inside branches) (definitions adapted from Steller et al. 2003), as well as macrofauna on the surface of the beds/substrate,
2) macroalgae,
3) fish,

as well as record the presence of biogenic or non-biogenic substrates. A summary of the locations, sites and collection methods used is presented in Table 1.

Table 1: A summary of the locations and sites and the collection methods used at each site. The same methods were used in February and September. (LC = *Lithothamnion crispatum*, SD = *Sporolithon durum*; RPQ = random point quadrat).

Location	Site	In or out of beds	Rhodo-liths	In-fauna	Epi-fauna	Macro-fauna	Fish	Epi-flora	Crypto-fauna	Biogenic Substrate
				Cores	Transect	Swath	Rot-enone	Algal search		RPQ
Kahu-whera Bay	KWB	In	SD	Y	Y	Y	Y	Y	Y	Y
	KWB_OUT	Out	None	Y	N	Y	N	Y	N	Y
Te Miko Reef	TMR	In	LC	Y	Y	Y	Y	Y	Y	Y
	TMR_B	In	LC & SD	Y	Y	Y	Y	Y	Y	Y
	TMR_OUT	Out	None	Y	N	Y	N	Y	N	Y

At each site two 25 m transects were laid out at right angles to the anchor line, creating a single 50 m transect. On each transect, a random point quadrat (RPQ) (after Foster et al. 2007) was used at eight points, 5 m apart, to record the biogenic substrate type (rhodoliths, algae, microalgal mat and sponge/tunicate or bare substrate) under each of 10 knots. The point quadrat was a 1 m long bar with a 120 cm long string attached like a loose string on a bow. Five knots on the string were used as points and the organisms or substrate found on each side of the bar were sampled giving 10 points per quadrat (as detailed in Foster 1975).

At sites within the rhodolith beds (i.e., KWB, TMR, TMR_B) all live rhodoliths and associated biodiversity from within a 25 by 25 cm quadrat were collected into plastic bags at each of the eight random points on the transect. These quadrat samples were tipped out into large plastic containers and then thoroughly sorted. All associated epibiota were removed and preserved appropriately for each taxon group for later identification to species level where possible. The rhodoliths were identified to

species, assessed for vitality (proportion of dead and live rhodoliths), and measured to obtain size frequency at each site. Rhodoliths were collected from both locations and transported to Wellington for experimental work on vulnerability to stressors (Objective 2).

Infauna were assessed using a cylindrical 10 cm diameter by 10 cm depth core to remove substrate under each quadrat. A sample of sediment was kept from each core for subsequent grain size analysis (i.e., one sediment sample per quadrat). Core samples were sieved through a 5 mm mesh. All macrofauna (approximately 5 mm and above) were sorted to phylum and preserved appropriately for later identification to species level where possible.

For analysis of cryptofauna, five to ten of the largest available rhodoliths were collected, placed separately into individual bags, and preserved for subsequent analysis. If rhodoliths displayed more than one morphology or if more than one species was present in the transect, five of each morphology/growth form/species were collected. Individual rhodoliths (six *Lithothamnion* from each of TMR and TMR_B and six *Sporolithon* from each of KWB and TMR_B) were weighed using the buoyant weight technique (Potin et al. 1990), and measured (x, y, z axes) before being preserved in 10% v/v formalin in seawater.

Along the full length of each transect, divers counted surface macrofauna in a 1 m wide swath. In addition, opportunistic collections of macroalgae were made by phycologically trained divers over an area of 500 m^2 in both beds, and in adjacent areas outside of the beds. Data from these dives were analysed and interpreted as quantitative observations as they involved the same diver, covering the same area, for dives of equal duration. Macroalgae were either fresh-pressed onto herbarium paper or preserved in 4–5% v/v formalin in seawater for subsequent detailed morphological investigations. Some specimens were sub-sampled for molecular analysis with small pieces of tissue removed and dried in silica gel.

Fish collections were made using targeted rotenone sampling to qualitatively sample small fish closely associated with the three-dimensional structure provided by the rhodoliths. Fish were collected by laying four small-scale rotenone traps per bed. A plastic box 30 cm by 30 cm by 15 cm was laid over the substrate and anchored with weights. Rotenone solution was prepared by mixing 500 g of rotenone powder with approximately 15 ml of concentrated detergent and 1 L of seawater, resulting in a thick slurry. The Rotenone slurry was introduced into the enclosed space which served as a trap for fish. After 30 minutes all the visible fish were collected from within the enclosed space by inserting a flat metal sheet under the trap and the surface rhodoliths, and collecting the sample in a plastic bag for subsequent sorting through supernatant and rhodolith components for fish. Fish were also collected opportunistically, for example, fish attached to macroalgae or within the crevices of the rhodoliths.

2.1.2.2 Processing of samples

a. Rhodolith species composition and attributes

Rhodoliths collected in the quadrats were characterised in the following ways:

i. Number. Rhodoliths were counted after sorting by species and size frequency (grouped into 1 cm size classes using the longest axis of each rhodolith, i.e., 1 - 2 cm, 2 - 3 cm, 3 - 4 cm, etc), and by whether they were dead or alive (based on the presence of pigmentation). Individuals under 1 cm were discarded and not counted.

ii. Size and shape. Rhodoliths were measured in three dimensions and their maximum projection sphericity was calculated after methods of Sneed & Folk (1958). Sphericity is an expression of how closely a shape resembles a sphere, and it can be determined by examining the relation between the long (L), intermediate (I), and short (S) axes of the particle, the maximum projection sphericity, Ψ, being given by the expression $\Psi = \sqrt[3]{(S^2/LI)}$. For a perfect sphere, $\Psi = 1$. Values less than one relate to increasingly less spherical shapes.

For each quadrat sample, at least 20 rhodoliths were measured, with rhodoliths from each size class found within the quadrat, where possible a minimum of 5 rhodoliths per size class. For each of the rhodoliths measured, the number of branch tips within a 1 cm by 1 cm square was measured in five

positions (after Steller et al. 2003). The volume and surface area of rhodoliths were calculated by assuming the rhodoliths were spherical and using the average of the x, y, z measurements to obtain an estimated diameter. The volume and surface area were then calculated ($V= 4/3\pi r^3$, $A = 4 \pi r^2$). Relative volume of interstitial (external) water contained in monospecific rhodolith beds was estimated by covering 1000 cm^3 of both species of rhodoliths with seawater, then by decanting off and measuring the interstitial water volume. These values were used when considering cryptofaunal diversity and abundance.

iii. Growth in the field. Rhodoliths of both species were collected in February and transported in seawater back to our field laboratory. Intact, whole individual rhodoliths were weighed, cleaned, labelled (by tying one end of an approximately 30 cm long length of monofilament fishing line to each rhodolith, and the other end of the monofilament to a length of numbered plastic surveyors' tape as well as a numbered plastic cow tag). The rhodoliths were then placed in an aerated 0.025% (w/v) alizarin red seawater solution overnight. Each stained rhodolith was weighed (buoyant weight method), its diameter (longest axis) measured, then secured via its leader line to a horizontal main string line. Between 35 and 45 rhodoliths were attached to a single line.

The stained rhodoliths arrayed on the lines, maintained in cool damp conditions, were then returned to the field and deployed at both locations, with rhodoliths returned to the site from which they had been collected (Figure 3). Each horizontal line was attached to stainless steel stakes secured in the substrate so that the rhodoliths were suspended immediately above the rhodolith bed. Four horizontal lines were deployed at each location. Each line had attached to one of its end stakes a HOBO light and temperature logger. In September these lines and loggers were retrieved from the field sites, the individual rhodoliths cleaned, weighed and measured, and then dried individually for further study.

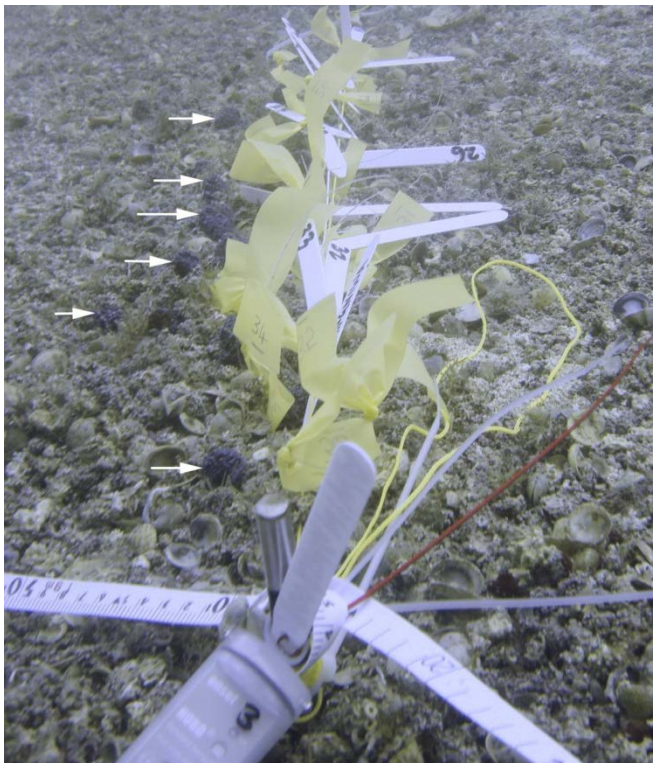

Figure 3: Example of a main growth line pinned to the seafloor between February and September 2010. The alizarin stained rhodoliths (white arrows) are darker than the surrounding rhodoliths, and each rhodolioth is individually tagged. A HOBO logger is visible in the foreground.

b. Identification of associated diversity

i. Invertebrates. Invertebrates collected opportunistically or in cores, transects and algal searches, were identified by taxon specialists to species level where possible. Voucher material was deposited in NIWA's Invertebrate Collection (NIC) to enable future taxonomic research. In the laboratory the rhodoliths preserved for cryptofaunal analysis were rinsed of formalin and transferred to 80% ethanol. Subsequently, branch tips were counted, then each rhodolith was inspected under a dissecting

microscope and any surface biota were removed. The rhodoliths were then broken up using pliers and forceps to allow the removal of any internal biota. All biota were sorted by phylun and counted.

ii. Macroalgae. Where possible, macroalgae were identified to species level. Some samples that were preserved in the field were examined microscopically with the preparation of permanent slides to examine specific diagnostic anatomical and morphological features. Vouchers of macroalgal collections were retained and representative herbarium specimens were lodged in the Herbarium of the Museum of New Zealand Te Papa Tongarewa.

Molecular sequencing was carried out on a small subset of the collections to confirm identifications. In particular, sequencing was used for species where current taxonomic knowledge is limited, where only sterile material was available, for novel material, and to add to our existing phylogenetic database and extend our documentation of biodiversity. Target taxa that were sequenced included non-geniculate coralline algae, crustose red and brown algae, species of *Dictyota* and bladed red algae ('*Tsengia*', *Grateloupia*, '*Halymenia'*). DNA was extracted from four coralline algal specimens using a Qiagen Blood and Tissue DNA Extraction Kit with a modified protocol as described in Broom et al. (2008). DNA was extracted from the remaining silica-gel dried target specimens using the CTAB protocol of Zuccarello & Lokhorst (2005). Extracts were diluted 1:100 with 0.1 by TE buffer, and 3 µl was used in subsequence PCR amplifications. The *psb*A gene was amplified from specimens using standard primers (Yoon et al. 2002). The plastid-encoded large subunit of the ribulose bisphosphate carboxylase/oxygenase gene (*rbc*L) was amplified using primer combinations appropriate to the taxa. Amplified products were checked for correct length, purity and yield on 1% agarose gels stained with ethidium bromide. PCR products were cleaned using ExoSAP-IT (USB, Cleveland, Ohio) or with Exonuclease I/ Alkaline phosphatase digest, and sequenced using standard methods. Sequences were compared to sequences held in GenBank using NCBI BLAST (http://blast.ncbi.nlm.nih.gov/) and to sequences of New Zealand specimens obtained previously using phylogenetic analysis. All sequences obtained, as well as related sequences selected from GenBank, were compiled in Se-Al version 2a11 (Rambaut 1996).

iii. Fish. Specimens were fixed in 10% v/v formalin, preserved in 70% v/v ethanol in glass vials, identified and deposited in the National Fish Collection at the Museum of New Zealand Te Papa Tongarewa.

2.1.2.3 Data analyses

Biodiversity was assessed using PRIMER (Clarke & Gorley 2006; Clarke & Warwick 2001; Anderson et al. 2008). For the infaunal and epifaunal samples, the number of taxa, number of individuals, Pielou's evenness, Shannon-Weiner diversity and Simpson's Index (Table 2) were calculated for individual replicates, and then averaged to give site estimates and compared using PERMANOVA (Table 2). The average Bray-Curtis similarity was calculated to give a measure of within-site variability and compared using PERMDISP (Table 2). For the macroalgal presence/absence data, number of taxa and Bray-Curtis similarities were assessed. Nonmetric multi-dimensional scaling ordinations (MDS) of square root transformed data were used to calculate Bray-Curtis similarities and assess similarities in community composition. Other data (e.g., biogenic substrates, swath counts) were analysed using ANOVA or General Linear Models as appropriate, followed by Bonferroni pairwise comparisons if overall differences were detected.

a. Diversity and community comparisons

Purely qualitative and non-repeated samples were removed (i.e., the data from the rhodolith field growth lines, anchor, dredge, hand-collected and opportunistic sampling) and then analyses of between site temporal data were carried out for:

a. Infauna - Core data (Five sites: KWB, TMR, TMR_B, KWB_OUT, TMR_OUT),
b. Epifauna - Transect data (Three sites: KWB, TMR, TMR_B),
c. Macrofauna – Diver swath counts (Five sites: KWB, TMR, TMR_B, KWB_OUT, TMR_OUT)
d. Macroalgae - Algal searches (Five sites: KWB, TMR, TMR_B, KWB_OUT, TMR_OUT).

Table 2: PRIMER Indices and tests used in analyses of biodiversity data.

Univariate:	
Pielou's Evenness	A measure of diversity which quantifies how equal the community is numerically. It is constrained between 0 and 1. A high evenness value indicates a more equal distribution of individuals between taxa.
Within-site Bray-Curtis similarity	The similarity of an invertebrate community within a site.
Simpsons	Accounts for the richness and the proportion of each taxon from a biodiversity sample within a site. It is the probability that two randomly selected individuals in a site belong to different taxa.
Multivariate:	
PERMANOVA	tests the simultaneous response of one or more variables to one or more factors in an ANOVA design on resemblance measures, using permutation methods.
PERMDISP	tests for the homogeneity of multivariate dispersions, based on any resemblance measure, i.e., Bray-Curtis similarity (Anderson et al. 2008).

b. Rarity

Using a reduced dataset (excluding collections made opportunistically from dredges, anchor recovery and rhodolith field growth lines recovery) the number of taxa rare in frequency (S_{RF}) was also investigated using the following index:

$S_{RF} = \Sigma Sj$, where Sj occurs only at 1 site.

Rarity was calculated at several levels for all taxa, and subdivided between algae, fish and invertebrates:

- Overall rarity – taxa that only occurred once across all seasons, sites, methods
- Seasonal rarity – taxa that only occurred once within a season, regardless of site and method
- Site rarity – taxa that only occurred once at each site (not directly comparable between inside and outside beds)
- Method rarity – taxa that only occurred once in cores and algal searches (directly comparable between inside and outside beds).

2.2 Objective 2

2.2.1 Techniques for measurement of rhodolith growth and condition

Rhodolith growth and health were evaluated periodically and in most cases non-destructively over the course of the experiments, using a combination of approaches (Steller et al. 2007b).

a. Buoyant weight

Water displacement was used to assess weight (buoyant weight, mg) of the rhodoliths as a proxy for growth due to the irregularity of rhodolith shape/form following the method of Potin et al. (1990).

b. Alizarin red

Staining with alizarin red (refer 2.1.2.2.a.iii for method) and then examination of growth has been documented for both non-geniculate and geniculate coralline algae (e.g., Andrake & Johansen 1980; Blake & Maggs 2003). Rhodoliths retrieved from the field growth lines (six rhodoliths of each of *Lithothamnion crispatum* and *Sporolithon durum*) and rhodoliths grown in culture for an equivalent time period under the control conditions of the light experiment (refer 2.2.3.1) (eight samples of each rhodolith species) were examined for growth. From each rhodolith five branch tips were selected, mounted in dental wax, the tips cut longitudinally with a razor blade. To distinguish between natural and stained bands, the natural pigment was removed by leaving tips in tap water in daylight for 12 h, which did not affect the Alizarin stain (following the methods of Blake & Maggs 2003).The amount of growth of the tip subsequent to staining was measured from digital images captured using a Pixelink PL-A686C camera and software, connected to a Leica MZ12 stereomicroscope.

c. PAM fluorometry

The health or condition of rhodoliths was assessed using PAM fluorometry. This technique is used widely in studies of photosynthetic rates in marine algae (Wilson et al. 2004). Its advantages are that it is non-invasive and non-destructive. However, it provides only relative photosynthetic rates.

2.2.2 Experimental conditions

The two species of rhodoliths (*Sporolithon durum* and *Lithothamnion crispatum*) collected in February and September 2010 from the field locations in the Bay of Islands were transported to NIWA's Mahanga Bay Aquaculture Facility in Wellington. In addition rhodoliths from a bed in the low intertidal zone at Army Bay, Whangaparaoa Peninsula (36° 36' S 174° 48' E), and from a subtidal bed in Catherine Cove, D'Urville Island (40° 51' S 173° 53' E) were collected and transported live to Wellington. The rhodoliths were maintained in a seawater holding system to acclimate them to local conditions prior to commencement of the experimental programme.

Seawater was supplied to the holding system and the experimental culture chambers from the facility's filtered (nominal 5 µm) continuous flow seawater system. For the period of all experiments conducted at Mahanga Bay salinity values were around 34.1 ‰ (similar to those recorded at the Bay of Islands at the collection sites at around 35 ‰ - Chiswell et al. 2010). In preparation for all experiments rhodoliths were acclimated at low light (less than 5 µmol quanta m^{-2} s^{-1}) for at least one week. In addition, all rhodoliths were brushed and thoroughly hand-cleaned of epiphytes before being stained with alizarin red for 24 hours (following Steller et al. 2007b), and weighed for subsequent analyses.

Long-term (approximately 10 month) experiments were set up to examine the effects of differing regimes of light, burial and fragmentation on the growth and physiology of *Lithothamnion crispatum* and *Sporolithon durum*. In addition a shorter comparative experiment was carried out to assess the impacts of temperature and ocean acidification on growth of the two species of rhodolith.

For the light, fragmentation and burial experiments a modified polypropylene hydroponic tank (dimensions 104 cm (w) by 350 cm (l) by 20 cm (d), or volume 711 L) was used. Replicate containers consisted of 2 L plastic milk bottles with top halves removed (dimensions: 10 cm (w) by 10 cm (l) by 15 cm (d), or 1500 cm^3, 1.5 L). The system was semi-recirculating and pumped by a 20 000 L/hour Hailea pump (model H20000, China). Flow rates were set at approximately 1–2 L/minute. A constant flow of seawater was supplied via a 40 mm manifold with a series of smaller (20 mm) manifolds supplying water to the individual tubs via small 3 mm Whisper™ irrigation tubes directed onto the bottom of the chambers.

The whole experimental culture system was set up inside a commercial shade-house (70% neutral density plastic) but was also covered with an additional green shade cloth to reduce natural light to around 6.7% of natural irradiance and encapsulate the range of irradiance levels recorded with HOBO loggers (Section 2.1.1.2) on the seafloor at the Bay of Islands field sites between February and September 2010.

2.2.3 Experiments

2.2.3.1 Light

The growth of the two Bay of Islands species of rhodoliths under different irradiance levels was assessed over 10 months. Experimental irradiances were set up to reflect levels measured *in situ* at the two study sites, and were achieved by covering the whole tank with a single layer of green shade cloth and adding neutral density screen to individual treatment levels as follows: (1) control of no neutral density screen giving 6.7% of natural irradiance, (2) one layer of neutral density screen giving 4.2 % of natural irradiance, (3) two layers of neutral density screen giving 2.7 % of natural irradiance, (4) three layers of neutral density screen giving 1.5 % irradiance and (5) black polythene cover resulting in 0.7% of natural irradiance. These treatments are referred to as control, single, double, triple and black.

Two additional smaller scale experiments were set up using *Sporolithon durum* rhodoliths from Whangaparoa and D'Urville Island. In each case there were only enough rhodoliths available to run two treatments, control and single shading.

For each species and treatment level, there were three replicates tubs/units containing three weighed, cleaned and stained rhodoliths, i.e., a total of 45 rhodoliths of each species. Each tub was placed inside a black plastic planter bag to limit incident light. Light and temperature were measured over each period of the experiment by placing an Onset HOBO Pendant® temperature/light data logger (UA-002-64, 64K) in randomly selected representative chambers from each of the 5 treatment levels. As described for field measurements all light level values derived from the HOBO loggers (in lux) were converted to give approximate estimates of PAR (Herrera et al. 2009).

2.2.3.2 Disturbance

a. Burial

Rhodoliths of both species were buried using two different types of sediment: (1) farm sediment - collected from underneath a fish cage (approximately 10 m depth) at Mahanga Bay, and (2) sediment - collected from an area adjacent to the fish cage at the same depth. Controls treatments were tubs with no sediment added, whereas burial treatments were tubs with 100 mL of either farm sediment or sediment added to completely or almost completely bury the rhodoliths.

The burial experiment was set up as a removal experiment. Each treatment was replicated three times for each species, and then five times for each assessment period giving a total of 45 replicates for each species. Rhodoliths were removed and assessed at 7, 14, 28, 55 and 84 days after establishing the experiment. At each assessment the rhodoliths were cleaned, weighed and assessed with PAM.

b. Fragmentation

The impact of fragmentation on rhodolith growth and health was assessed by breaking cleaned and stained rhodoliths in half before weighing them using the buoyant weight method. A corresponding number were left intact to serve as controls. For each treatment (broken or control) three rhodoliths were allocated to each of three replicate tubs giving nine rhodoliths of each species per treatment.

2.2.3.3 Ocean acidification and temperature

The response of rhodoliths to increased ocean acidification was examined for two pH levels and three temperatures in an orthogonal design as outlined below (Table 3). Values were chosen to represent current physical conditions (pH 8.05), and possible future conditions with ocean acidification using values predicted to occur in 100 years (pH 7.65) (Orr et al. 2005). Water temperatures were chosen to give a gradient (in 5° C increments) that encapsulated current winter temperature (15° C) through to future predictions of summer temperatures for northern New Zealand (25° C).

Figure 4: Experimental array of tubs containing rhodoliths and HOBO loggers in the shade house at the Mahanga Bay facility.

Table 3: Temperature and pH values tested in ocean acidification experiment.

		Temperature (° C)		
pH	8.05 (current)	15	20	25
	7.65 (100 years)	15	20	25

The experiment was conducted in a purpose built insulated cold room using an experimental CO_2 diffusion system developed at NIWA's Mahanga Bay Aquaculture Facility and was run for 36 days over October/November 2011. The system has previously been described by Cummings et al. (2011) but was modified by the addition of extra 70 L header tanks in which to manipulate both pH and/or temperature. Filtered seawater (1 micron) was supplied to the header tanks via agricultural Apex® Space Saver ballcock valves to control the level in each header tank. Temperature was controlled in each of six header tanks by Omega CN740 controllers controlling 2000 W submersible heater elements to warm water from a controlled 15° C stock seawater supply to either 20° C or 25° C. Similarly Omega PHCN-37 pH controllers with WTW Multi 340i with sensorex glass probes were used to control pH in the header tanks by dosing CO_2 to diffusion coils made of 10 meters of 4 mm ID silicon tubing immersed in seawater in the header tanks. Dosing of CO_2 to the silicon diffusion coils was switched by two way pinch-valves supplied by a Bio-Chem® 075P3MP12 valve. From each pH/temperature system automatic measurements of pH (six per day) were made spectrophotometrically using an automated system as described by Cummings et al. (2011) (see also McGraw et al. 2010 for further details) with temperature and pH values recorded in a dedicated LabView® system.

Water from each header tank was recirculated by pumping (with a small 1000 L. hr^{-1} Haliah pump) through a 20 mm seawater supply ring-manifold running around the perimeter of the room and then back to its respective header tank. This was done to minimise temperature gradients in the supply manifold and also to ensure good mixing of seawater temperature and pH characteristics within each treatment system. Water from the supply ring-manifolds was then gravity-fed through black 4 mm ID tubing to individual replicate culture chambers constructed from 450 mL PET jars (with their rims removed) at a rate of 140 $ml.min^{-1}$ (Figure 4). Pipette tips (200 µL) functioned as flow regulating

nozzles to fix pre-calibrated flow rates given a constant head of seawater supplied from the header tanks.

There were three replicates established for each of the experimental treatments. Hand-cleaned, weighed and alizarin stained rhodoliths were added to replicate tubs; one *S. durum* rhodolith and two *L. crispatum* rhodoliths were added to each container (to give approximately equal biomass for each species) giving a total of nine rhodoliths for each treatment in the experiment. The ocean acidification and temperature experiments ran for 36 days over October/November 2011. Rhodoliths were maintained for the duration of the experiments under dimmable fluorescent lights with a 12:12 h light/dark cycle adjusted to give an average daily photon flux density of 25 µmol m^{-2} s^{-1} [photosynthetically active radiation] using Phillips New Generation TLD 36 W / 86 500° Kelvin colour temperature fluorescent tubes. Light levels were chosen to reflect maximum (summer) light levels measured at the field sites. Rhodoliths were assessed as described above at approximate weekly intervals. On each occasion individual rhodoliths were hand-cleaned and weighed and on some occasions individuals were also assessed with PAM.

2.2.4 Other observations

a. PAM - a test of the method

During experiments at Mahanga Bay a bloom of diatoms entered the experimental system, covering the rhodoliths in a fine filamentous film. We conducted an experiment to investigate various treatments for controlling/eradicating the diatoms and the impact on PAM readings. All rhodoliths were brushed under running seawater to remove the film of diatoms and other detrital material. Rhodoliths were then placed in one of 12 treatments (Table 4), with three rhodoliths per treatment. Weights of all rhodoliths used in the experiment were taken initially by measuring buoyant weight before treatment. The oven drying treatment was only conducted at the initial set-up, and not repeated at each measurement date as the rhodoliths were bleached white and initial PAM fluorometry yields were zero indicating that the rhodoliths were dead.

Table 4: Experimental treatments to investigate the efficacy of treatments for removing or reducing diatom growth on rhodoliths.

Treatment #	Percentage bleach	Time
1	1	30 seconds
2	1	2 minutes
3	1	10 minutes
4	2	30 seconds
5	2	2 minutes
6	2	10 minutes
7	5	30 seconds
8	5	2 minutes
9	5	10 minutes
10	0	brushed only
11	0, freshwater immersion	60 seconds
12	0, dried at 60° C initially	overnight

Using PAM fluorometry, yield measurements were taken in steady state illumination after the rhodoliths had been treated. Measurements were made every two weeks to minimise handling and time out of the experimental system, then a final PAM fluorometry reading was made after fifteen weeks. Four measurements were made from each rhodolith, with the rhodolith turned 45 degrees between measurements. For each treatment a total of twelve measurements were made and then these were pooled to give an average yield per treatment. The yield parameter reflects the efficiency of the overall photochemical energy conversion within the photosynthetic process.

b. Species-specific responses to growth in culture - apparent antifouling effect

After rhodoliths were transported to Mahanga Bay from the field and placed in tanks prior to the experimental work it was observed that there was an apparent difference between the two species in the degree of fouling by epiphytes (predominantly diatoms). This occurred despite the fact that both

species were in the same common tank and had a shared seawater supply. The *Lithothamnion* rhodoliths and also the plastic plumbing adjacent to them were less fouled than the *Sporolithon* rhodoliths and their adjacent plastic plumbing (Figure 5).

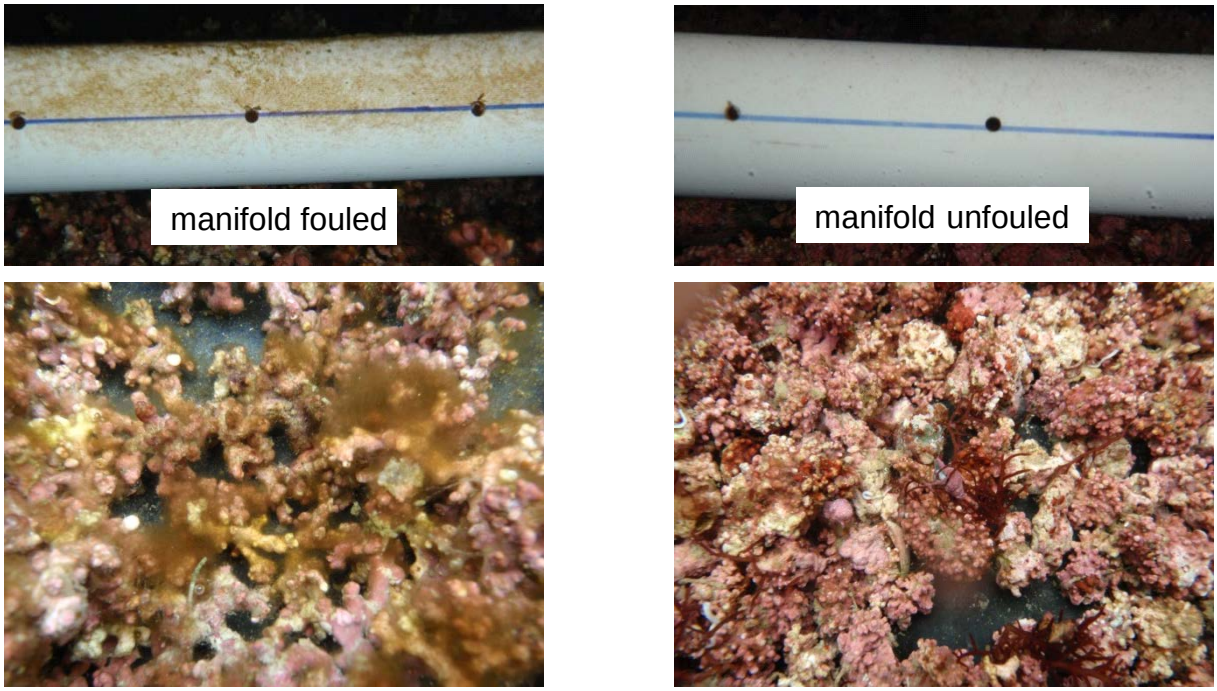

Figure 5: Observed differential antifouling effect of two species of rhodoliths – *Sporolithon durum* (left), *Lithothamnion crispatum* (right).

We set up a simple experiment to more formally test these observations by exposing clean Perspex fouling plates to different amounts of rhodolith biomass. Small tanks were set up in a random array with either *Sporolithon* or *Lithothamnion* rhodoliths, and with the control tanks containing no rhodoliths. We randomly assigned either 50 g or 100 g of either species to separate culture chambers and the control treatments contained no rhodoliths. Into each chamber we placed two Perspex fouling plates that were cut to fit into the cuvette holder of a CECIL 1010 single beam spectrophotometer. There were four separate replicates for each treatment and the control. After two weeks we determined the degree of transmittance through the Perspex fouling plates as a relative proxy for the degree of fouling.

2.2.5 Associated species

Data from the field collections in February and September 2010 were reviewed to identify whether there were any rhodolith-associated species (e.g., ephemeral, seasonal red algae; filamentous green algae) that would be able to be experimentally manipulated to determine their sensitivity to disturbance.

Although we were able to identify some species that appeared to belong to these categories (e.g., ephemeral – *Dudresnaya*; seasonal – *Asparagopsis armata*, *Delisea compressa*, *Laingia* sp.; filamentous green algae – *Cladophora* spp., *Derbesia novae-zelandiae*) the difficulty of obtaining material for culture and maintaining species for experimental work was beyond the scope of the time and resources available. The recirculating seawater system at Mahanga Bay also would not have been suitable for this work, as the potential for cross contamination with Wellington species was high, and the water temperatures available for experiments were not suitable for northern species.

3. RESULTS

3.1 Objective 1

3.1.1 Characterisation of beds: qualitative and quantitative site characteristics

3.1.1.1 Mapping

The data on the percentage cover of rhodoliths were grouped into the following categories - 10–45, 50–75, 80–100 % - and then these were mapped to get an impression of the distribution of the rhodoliths at the sites (Figures 6 and 7). At Kahuwhera Bay poor visibility and the sediment which covered the seafloor, rhodoliths and associated species meant that interpreting the video taken at this site was particularly difficult. Ground truthing via dredge sampling enabled confirmation of the presence of rhodoliths. In contrast, high water clarity and absence of sedimentation at Te Miko reef enabled confident determination of the percentage cover of rhodoliths.

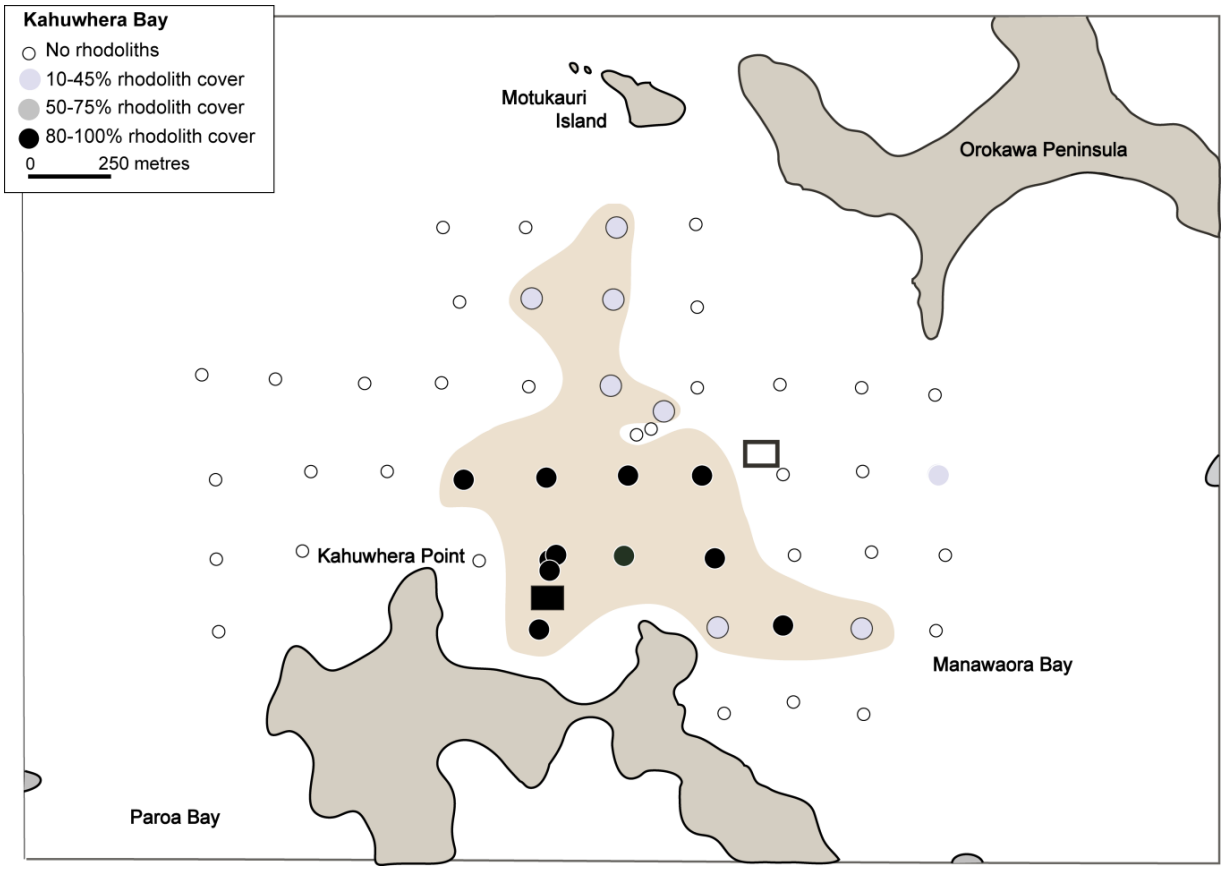

Figure 6: Map of Kahuwhera Bay showing the position of survey points (circles). Shaded area = approximate area of rhodolith bed. Black box = location of KWB, Open box = location of KWB_OUT.

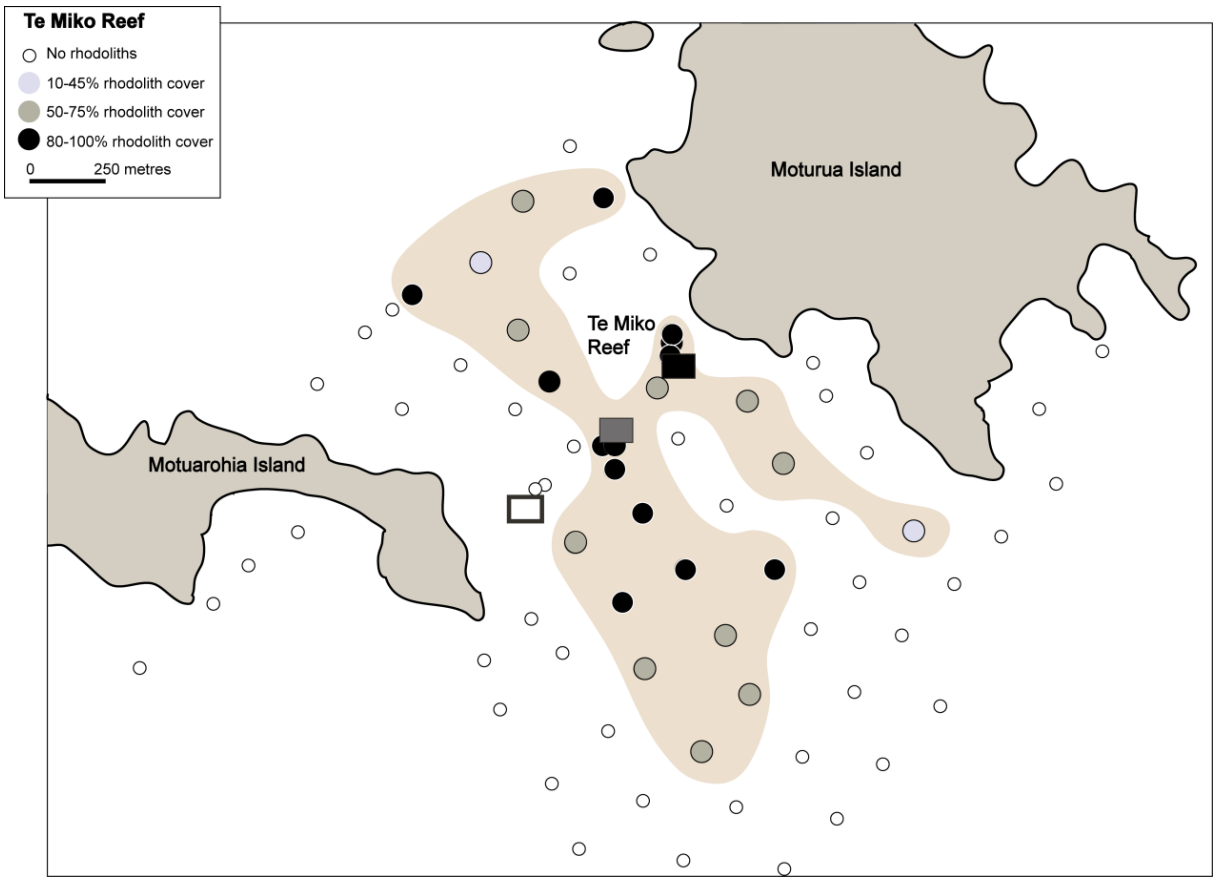

Figure 7: Map of Te Miko Reef showing the position of survey points (circles). Shaded area = approximate area of rhodolith bed. Black box = location of TMR, Shaded box = Location of TMR_B, Open box = location of TMR_OUT.

3.1.1.2 Physical characteristics of the locations

a. Sediment characteristics

At both the "inside" (Figure 8) and "outside" sites at Kahuwhera Bay there were fine sediments suspended in the water column resulting in turbid conditions. The rhodoliths and associated biota at KWB were covered in a layer of fine sediments. Live rhodoliths were in a more or less single layer overlaying grey to blackened rhodoliths in a darkly coloured rhodolith/sediment sublayer extending to a depth of at least 10 cm, presumably anoxic. Viable rhodoliths at Te Miko Reef (TMR and TMR_B – Figure 9) were clearly visible sitting on top of the substrate in a more or less single layer over rhodolith- and shell-derived gravel in contrast to the partial burial of rhodoliths at Kahuwhera Bay.

The sediments at both KWB sites contained a much greater fraction of mud than any of the sites at Te Miko Reef (Figure 10). At KWB there were equal proportions of gravel and mud with a lesser proportion of sand, whereas at KWB_OUT more sand was present and some scattered horse mussels were observed. The three Te Miko Reef rhodolith bed sites were dominated by sand and gravel with a very small percentage of mud (Figure 10). TMR_OUT was distinguished from other sites by the higher proportion of sand present. At both locations there was significantly more sand present outside than inside the beds, and also more gravel present inside than outside (Figure 10).

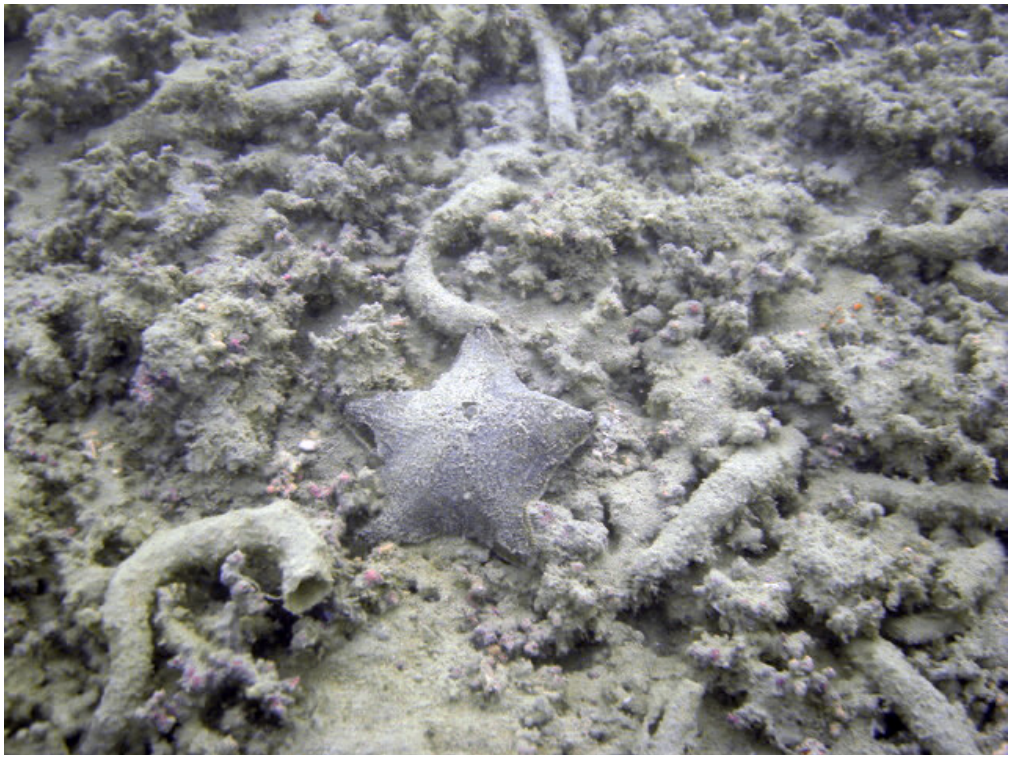

Figure 8: Kahuwhera Bay rhodolith bed showing the sediment cover and the associated macrofauna.

Figure 9: Te Miko Reef rhodolith bed showing the associated epiflora and shell fragments.

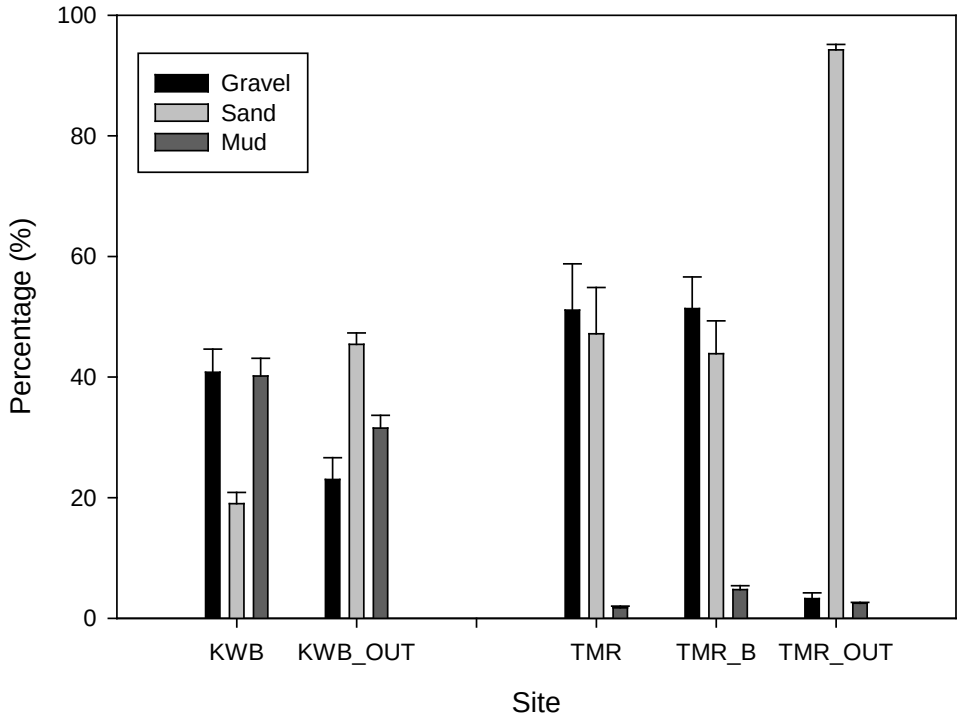

Figure 10: Sediment characteristics at the five Bay of Islands sites (KWB, KWB_OUT, TMR, TMR_B, TMR_OUT).

b. Water motion – current/tidal flow

The two rhodolith beds differed in current direction and strength (Figures 11–14). At Kahuwhera Bay there was no clear tidal signal whereas at Te Miko Reef there was tidally driven water movement. The current speeds recorded at Te Miko Reef were generally higher than those recorded at Kahuwhera Bay, particularly at peak flows (Figures 11, 12). There was no clear direction of water movement at Kahuwhera Bay (Figure 13) whereas Te Miko Reef water movement was along the northwest/southeast axis with a more dominant northwest tidal flow (Figure 14).

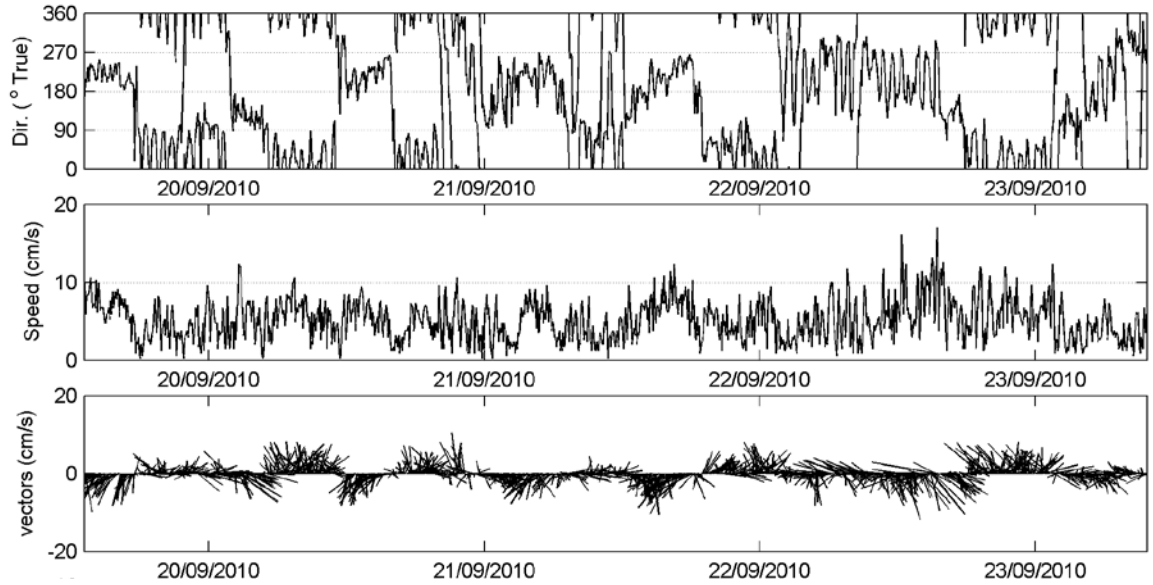

Figure 11: Kahuwhera Bay: current direction, speed, vectors measured by high resolution current meter deployed for 5 days to record current speed and direction.

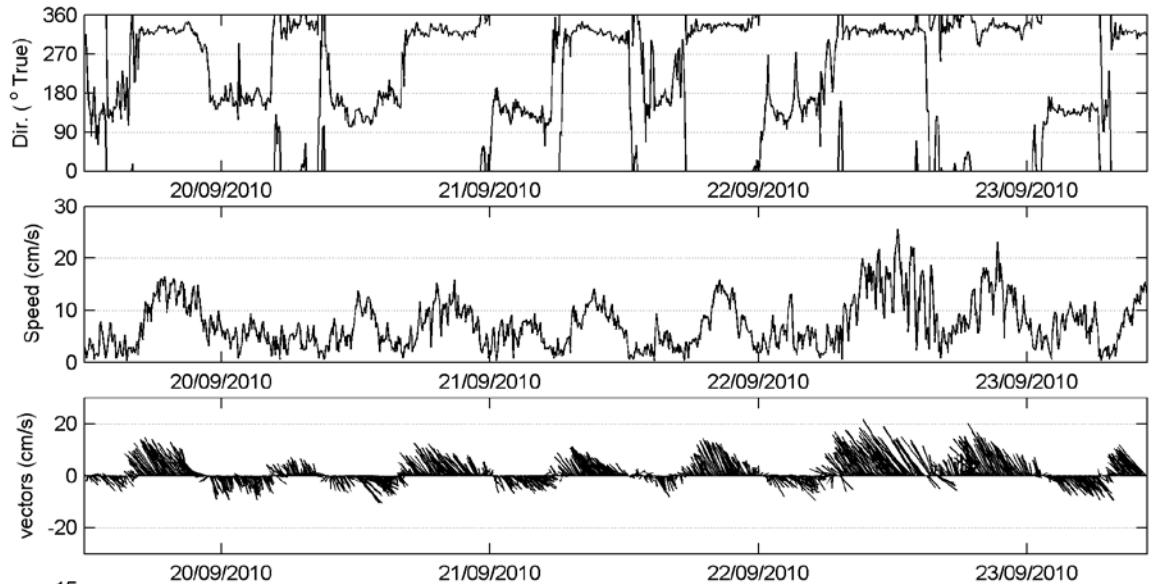

Figure 12: Te Miko Reef: current direction, speed, vectors measured by high resolution current meter deployed for 5 days to record current speed and direction.

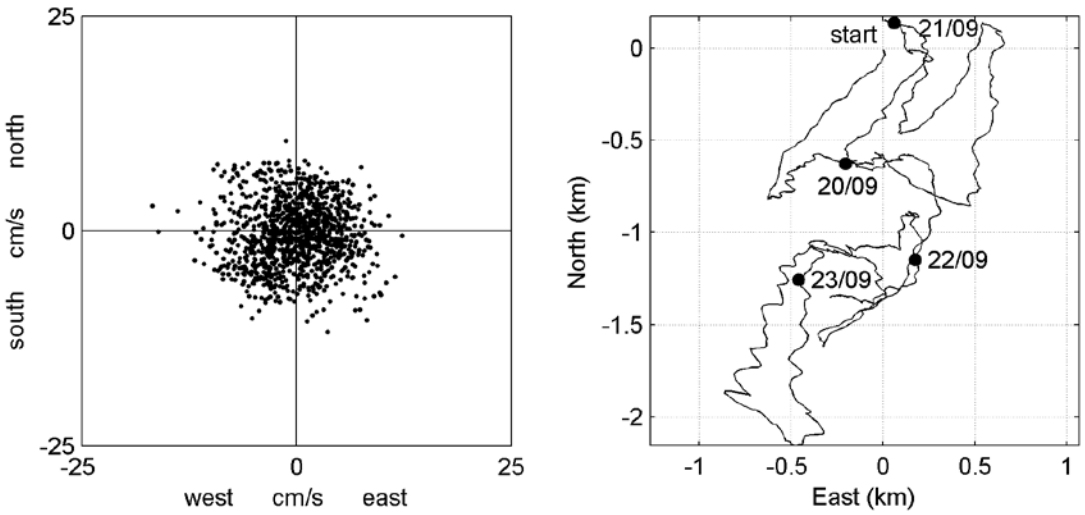

Figure 13: Kahuwhera Bay: current scatter (left) and progressive current vector (right).

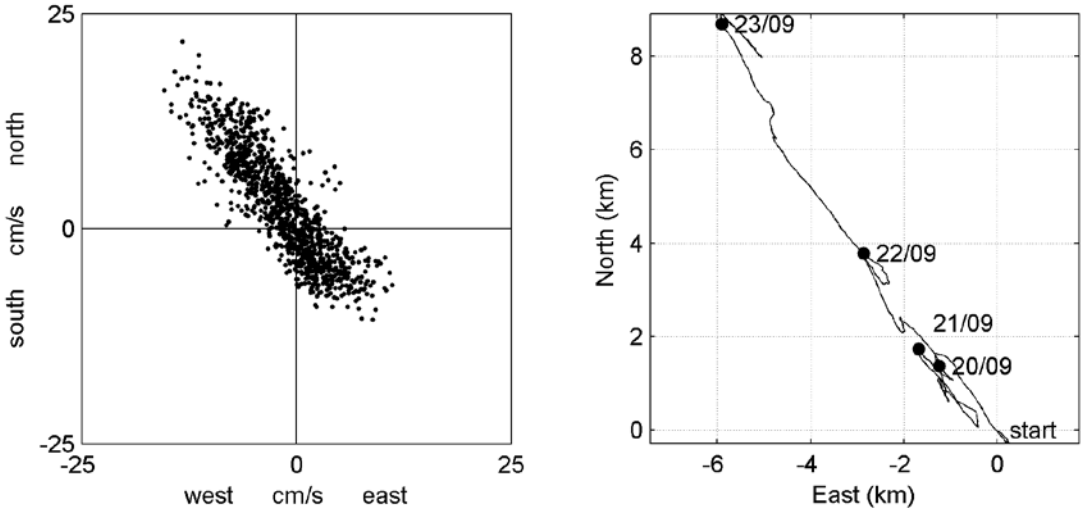

Figure 14: Te Miko Reef: current scatter (left) and progressive current vector (right).

c. Temperature and light

Maximum temperatures at the locations were recorded in February (approximately 21° C for both locations) and the winter minimum period was between late July and September (approximately 15° C for both locations) (Figure 15). The maximum and minimum light periods followed the same seasonal pattern. However, maximum light values at KWB were approximately half that of TMR (11 versus 22 μmol.m $^{-2}$.s^{-1}).

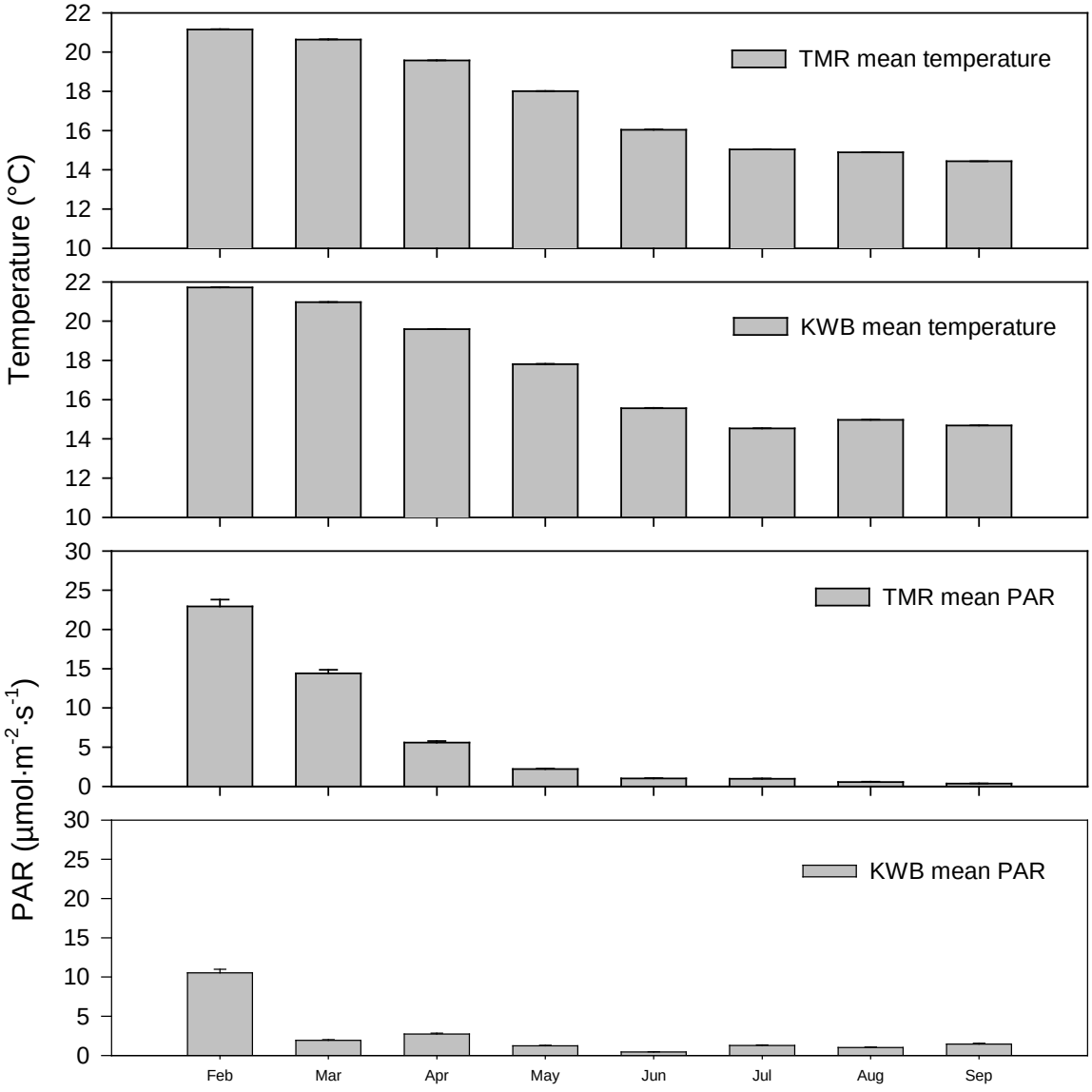

Figure 15: Monthly mean temperature and approximate PAR values recorded by HOBO dataloggers at Te Miko Reef (TMR) and Kahuwhera Bay (KWB).

3.1.2 Characterisation of biodiversity of the rhodolith beds

3.1.2.1 Biological/biogenic substrate

The percentage cover of rhodoliths occurring inside and outside the rhodolith beds did not vary seasonally (Table 5). Pairwise comparisons between all five sites were significant (p = 0.000) for all comparisons except KWB_OUT versus TMR_OUT and TMR versus TMR_B. Cover of macroalgae varied significantly by site and season with greater cover recorded at TMR_B than the other four sites.

Cover of microalgal mats varied significantly between site and season as did the amount of bare substrate present. Sponge/tunicate cover did not vary significantly (Table 5).

Table 5: Results of site and temporal analyses (General Linear Model) of the percentage cover of major biogenic substrate types recorded by random point quadrat inside and outside rhodolith beds at Kahuwhera Bay (KWB and KWB_OUT) and Te Miko Reef (TMR, TMR_B and TMR_OUT).

Variable	Source	SS	df	MS	*F*	*p*-value
Rhodoliths	Site	79 749.6	4	19 937.4	97.36	0.000
	Season	655.9	1	655.9	3.20	0.078
	Error	15 152.9	74	204.8		
Macroalgae	Site	6 352.2	4	1 588.0	13.14	0.000
	Season	1 324.2	1	1 324.2	10.96	0.001
	Error	8 940.8	74	120.8		
Microalgal mat	Site	906.5	4	226.6	5.02	0.001
	Season	420.1	1	420.1	9.30	0.003
	Error	3 342.1	74	45.2		
Sponge/tunicate	Site	6.9	4	1.7	0.74	0.567
	Season	0.01	1	0.01	0.004	0.947
	Error	171.2	74	2.3		
Bare substrate	Site	102 559.7	4	25 639.9	103.24	0.000
	Season	1 730.9	1	1 730.9	6.97	0.010
	Error	18 377.4	74	248.343		

3.1.2.2 Rhodolith characteristics - species composition and attributes.

a. Numbers and size

Sporolithon rhodoliths reached maximum sizes of 6–7 cm and were significantly larger than the largest *Lithothamnion* rhodoliths which attained 3–4 cm. The *Sporolithon* rhodoliths reached larger maximum sizes at Kahuwhera Bay than at Te Miko Reef B site. A higher proportion of *Sporolithon* than *Lithothamnion* rhodoliths were recorded as being dead at all sites (except Te Miko Reef). The density of rhodoliths at Kahuwhera Bay was higher than at the other two sites (Figure 16).

b. Rhodolith sphericity

Maximum projection sphericity varied between 0.81 and 0.92 in *Lithothamnion crispatum* (n = 83) and 0.75 and 0.83 in *Sporolithon durum* (n = 136) and these differences were not significant (Figure 17a). Branch counts varied between 11 and 27 in *Lithothamnion crispatum* (mean of 5 counts on each of 83 rhodoliths) and between 2 and 10 in *Sporolithon durum* (mean of 5 counts on each of 136 rhodoliths) and these differences were significant between species (Figure 17b). The surface area and volume of of the rhodoliths increased with size but did not differ significantly between species (Figure 17 c and d).

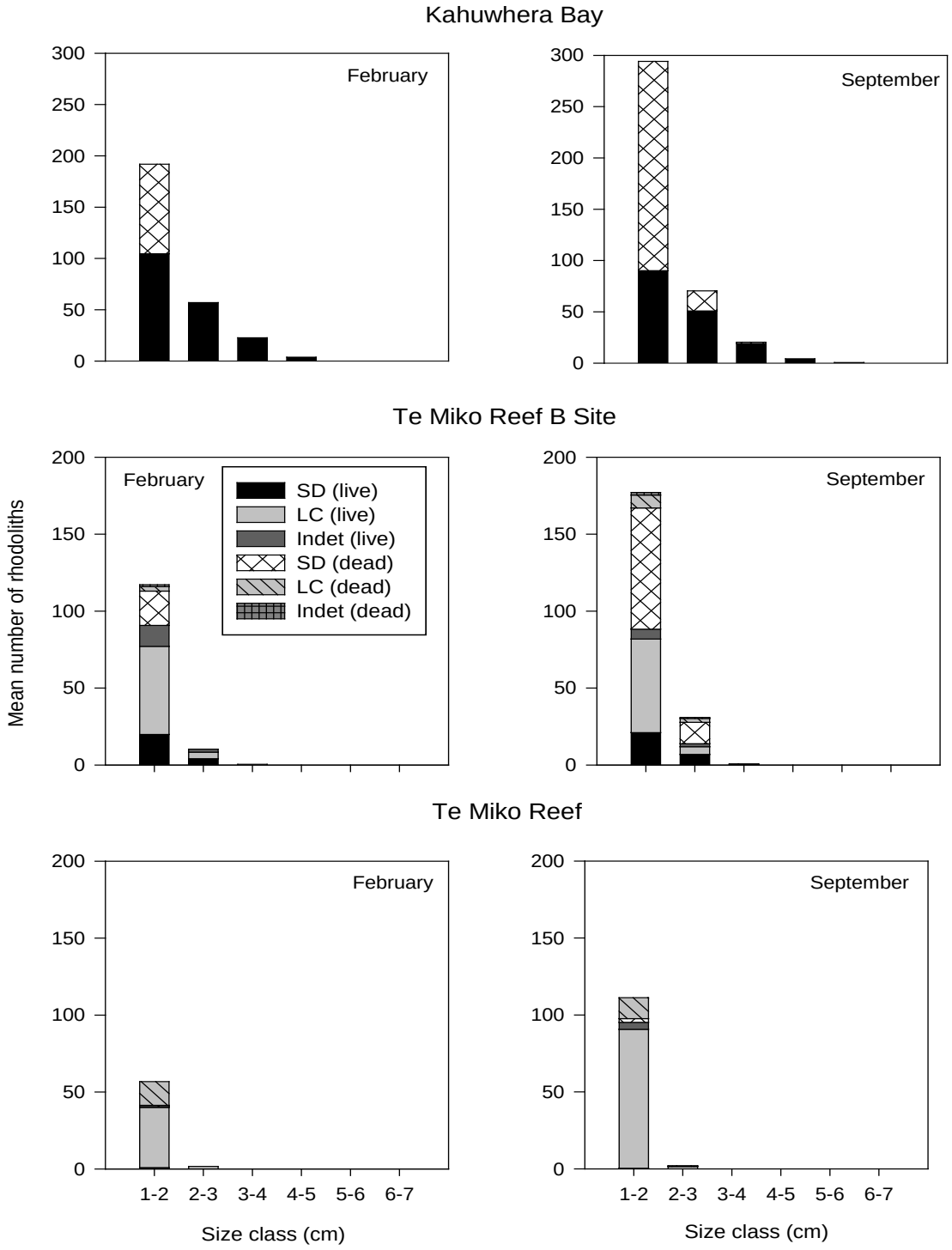

Figure 16: Number, state and size of two species of rhodoliths collected in quadrats at three study sites. SD = *Sporolithon durum*, LC = *Lithothamnion crispatum*. Indet = specimens unable to be identified when collected in the field.

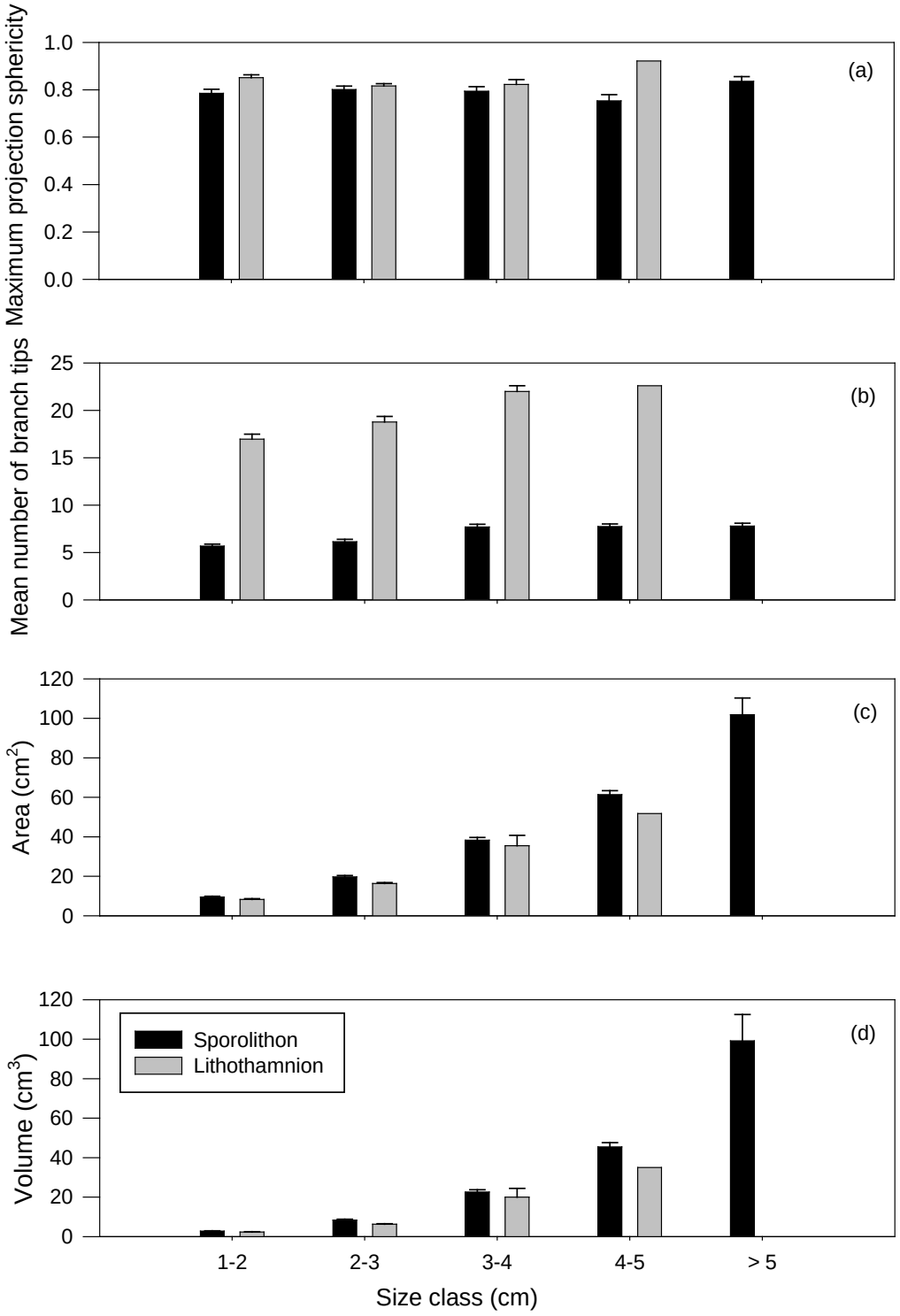

Figure 17: Characterisation of *Sporolithon durum* and *Lithothamnion crispatum* rhodoliths by size class: (a) maximum projection sphericity, (b) mean number of branch tips/cm^2, (c) surface area, (d) volume.

The relative volume of interstitial space was estimated as 77.0% (2.7 SD) and 62.7% (2.5 SD) for *Sporolithon durum* and *Lithothamnion* species, respectively.

3.1.2.3 Rhodolith growth in field

The majority of the rhodoliths placed on growth lines in the field in February were successfully retrieved in September with four lines found at TMR and three at KWB. When the rhodoliths were examined it was found that some had become significantly encrusted with epiphytic algae and invertebrates. Some of the rhodoliths appeared to have been eroded, possibly either having been abraded by moving against sediment or rhodoliths under the line, or damage resulting from abrasion by other neighbouring rhodoliths. It was apparent that in some cases there had been loss of branches

as negative growth (of the longest axes) was observed. The erosion and fragmentation appeared to have affected the *Lithothamnion* rhodoliths more than *Sporolithon*.

For measurement of growth of *Sporolithon* from Kahuwhera Bay we used 33 of the 38 rhodoliths retrieved, and from Te Miko Reef 27 of the 73 *Lithothamnion* rhodoliths retrieved were used (i.e., two thirds of TMR samples had negative growth). There was great variation in the amount of growth that occurred for specimens of both species. Because of the evidence of negative growth, high variability and poor correlation between x and y (Figure 18, R^2 = 0.0744, Figure 19, R^2 = 0.1055), it was concluded that this method to determine rhodolith growth rates in situ was unsuccessful, and growth was mostly assessed by buoyant weight (displacement method).

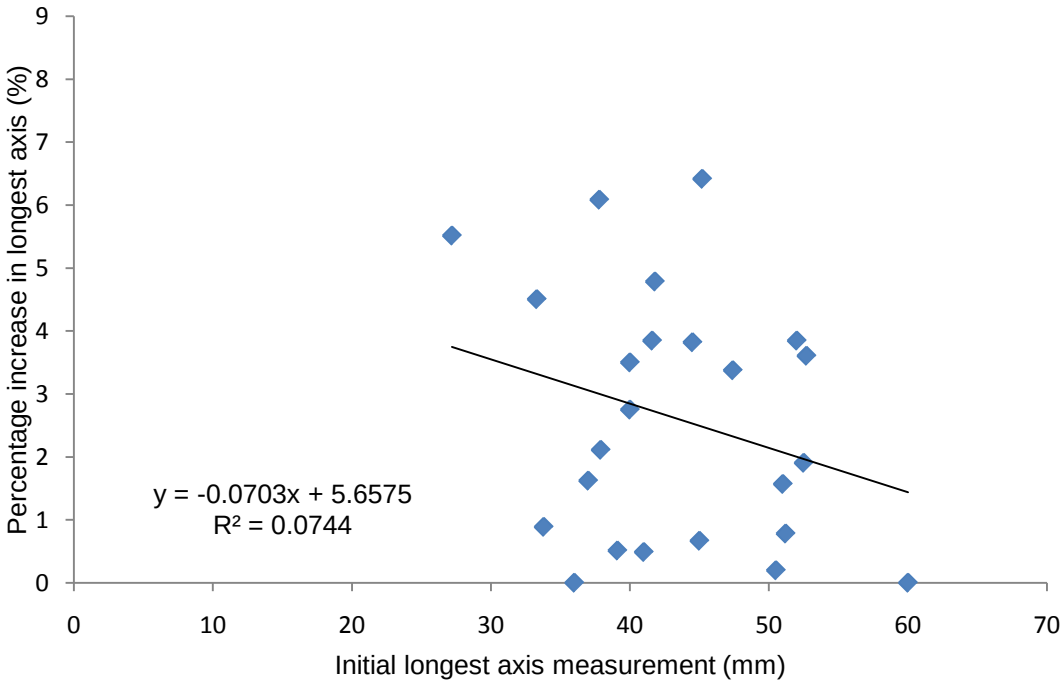

Figure 18: Kahuwhera Bay, *Sporolithon durum*: increase in largest axis measure (%) plotted against starting measure (mm), February to September 2010 (negative growth specimens removed).

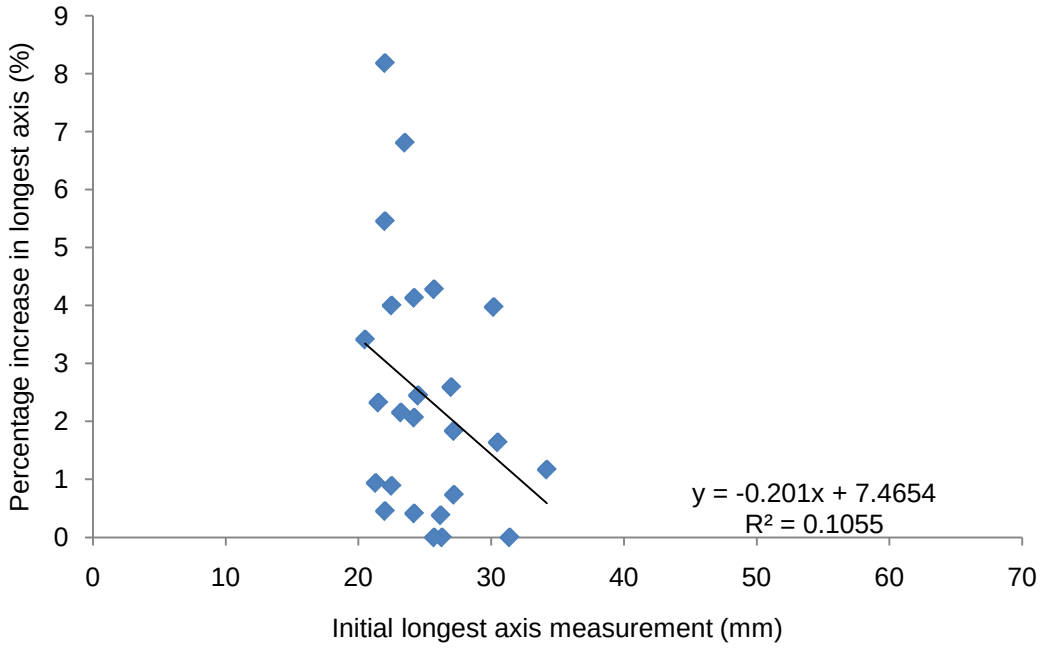

Figure 19: Te Miko Reef, *Lithothamnion crispatum*: increase in largest axis measure (%) plotted against starting measure (mm), February to September 2010 (negative growth specimens removed).

3.1.3 Associated biodiversity

3.1.3.1 Summary of Biodiversity Sampling:

a. Richness

The macroalgae, invertebrates and fish collected in this study are listed in Appendix 2, 3 and 4, respectively. The numbers of taxa collected by organism group for locations, sites, seasons and collection method are summarised in Tables 6, 7 and 8.

Table 6: Number of taxa recorded by site and organism grouping (invertebrates, algae (+seagrass), fish).

	Kahuwhera Bay			Te Miko Reef			
	KWB	KWB_OUT	Total	TMR_B	TMR	TMR-OUT	Total
Invertebrates	125	66	154	120	112	36	187
Algae (+ seagrass)	32	22	44	48	53	42 (+1)	88
Fish	2	1	2	3	2	1	5
Total	159	89	200	171	167	80	280

Table 7: Number of taxa recorded by organism group, month of collection and collection method.

	Total taxa	Month	
		February	September
Invertebrates	268	184	181
Algae (+ seagrass)	102 (+1)	75	67 (+1)
Fish	6	5	2
Total	377	264	251

Table 8. Number of taxa recorded by collection method.

All Taxa by Method	Total
Algal search	214
Transects	206
Cores	105
Opportunistic	96

b. Rarity

Of the 371 taxa considered in this dataset, 147 (40 %) were only collected on a single occasion (39 algae, 4 fish and 104 invertebrate taxa) (Figure 20). In February a slightly higher proportion of taxa were collected once (47%) than in September (41%). Figure 21 presents rarity data by field location, comparing numbers of taxa found once or more than once at each of the sites, and by organism group.

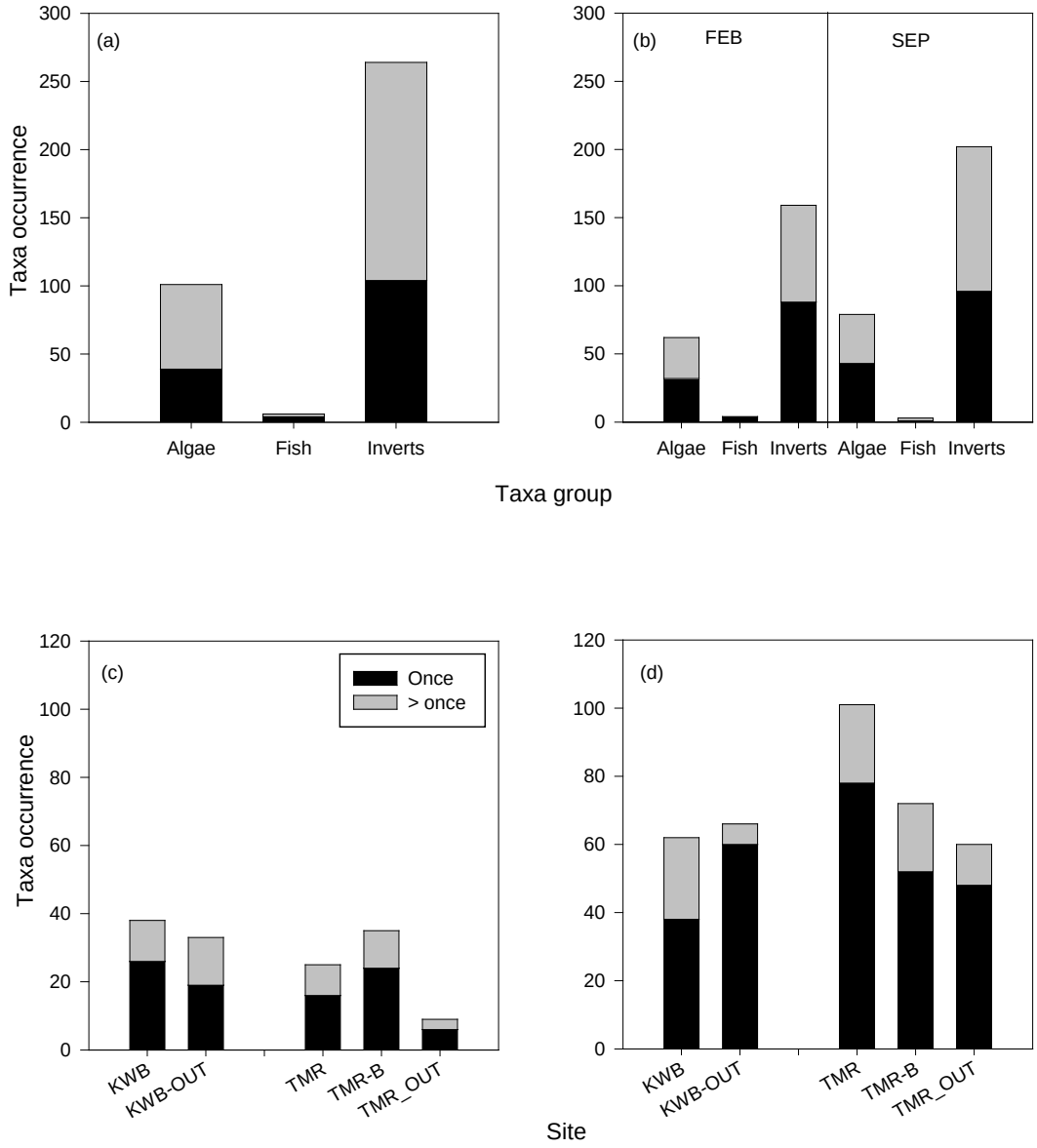

Figure 20: Rarity: (a) overall rarity, (b) rarity by season, (c) rarity by method – infauna (cores), (d) rarity by method – epiflora (algal searches).

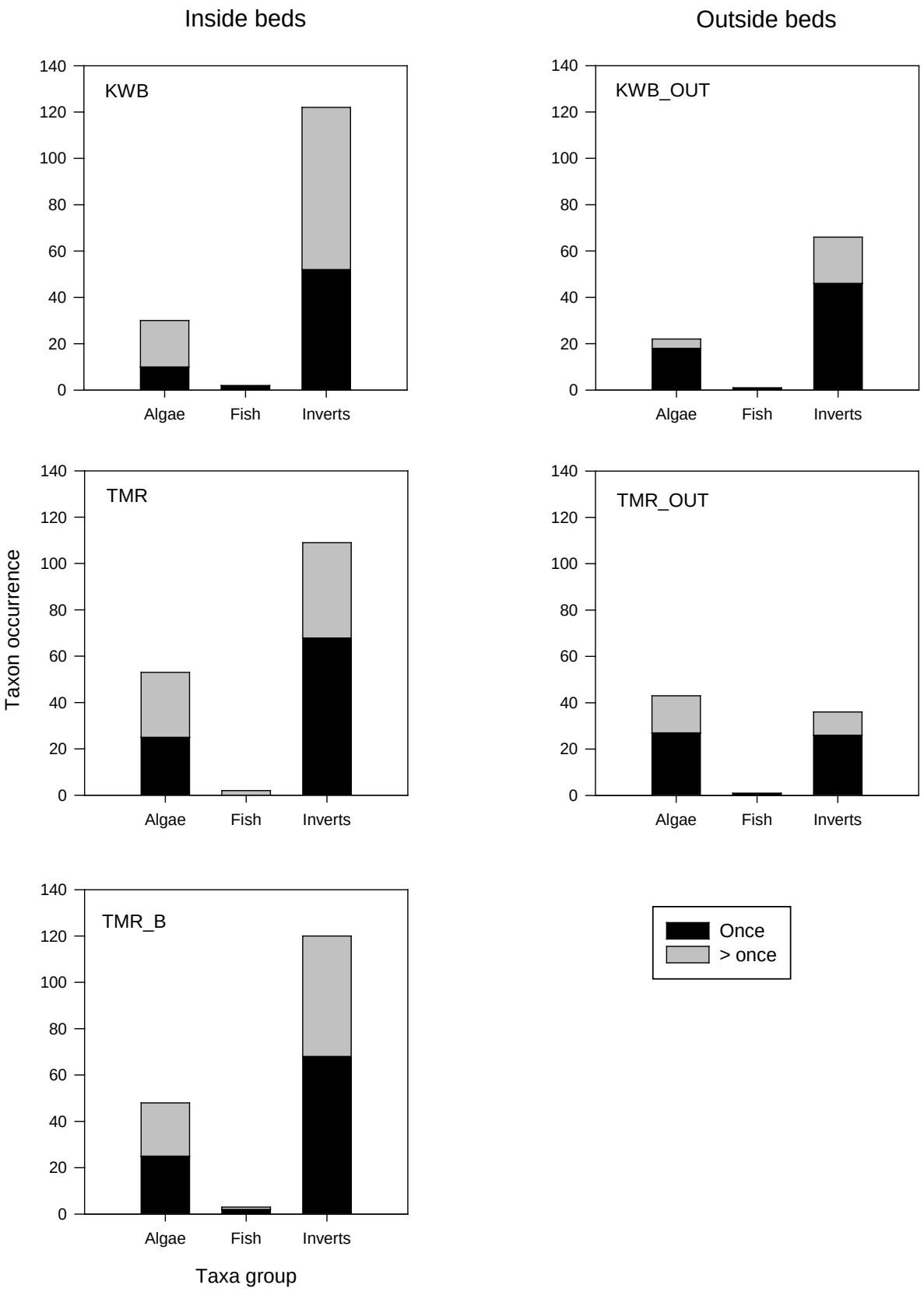

Figure 21: Number of taxa found once or more than once by site and organism group.

3.1.3.2 Invertebrates

A total of 1 088 invertebrate lots (2 093 individuals) were collected and 82% of lots and 87% of individuals were identified to species level (Table 9). Taxa found as cryptofauna on or inside rhodoliths were identified to phylum.

Table 9: Summary of the invertebrate samples collected, by lots and specimen numbers, and the level of identification achieved.

Identification level (%)	Lots	Specimens
Species	82.54	87.43
Genus	7.44	5.16
Family	3.22	2.01
Sub-order	0.74	0.53
Order	2.57	1.62
Class	1.10	0.57
Phylum	0.83	0.62
Unidentifiable	1.56	2.05

a. Infauna

Univariate analyses

The average number of infaunal taxa collected in cores ranged between 1 and 6, and PERMANOVA analyses indicated that they did not vary significantly between collection dates (February and September) but did between sampling sites ($p = 0.007$) (Table 10). Pairwise comparisons showed that KWB, KWB_OUT and TMR_B had significantly more taxa than TMR_OUT, and KWB_OUT also had more than TMR (Figure 22a, Appendix 5). The total number of individuals per core ranged between 1 and 41, and means did not vary significantly by time or site (Figure 22b, Table 10).

Evenness within sites was generally high (0.8 to 1.0) (Figure 22c) with the exception of TMR_OUT in September (Evenness = 0.5). Differences between sites were significant as were temporal differences (Table 10). There was also a significant interaction between site and time indicating that the effect of site on community evenness differed between seasons (Table 10).Within-site Bray-Curtis community similarities were generally low (below 55%) (Figure 22d) except for TMR_OUT in September (72%). The PRIMER PERMDISP test gave significant differences between sites ($p = 0.001$) and pairwise comparisons showed that TMR_OUT differed significantly from the other four sites (Appendix 5). The similarity of cores at KWB was also significantly different than those from KWB_OUT and TMR_B (Appendix 5). Both measures of diversity (Shannon-Weiner and Simpsons) varied significantly between sites (Table 10), and in both cases multiple comparison tests attributed this significance to the data from TMR_OUT in September (Figure 22e, f, Appendix 5).

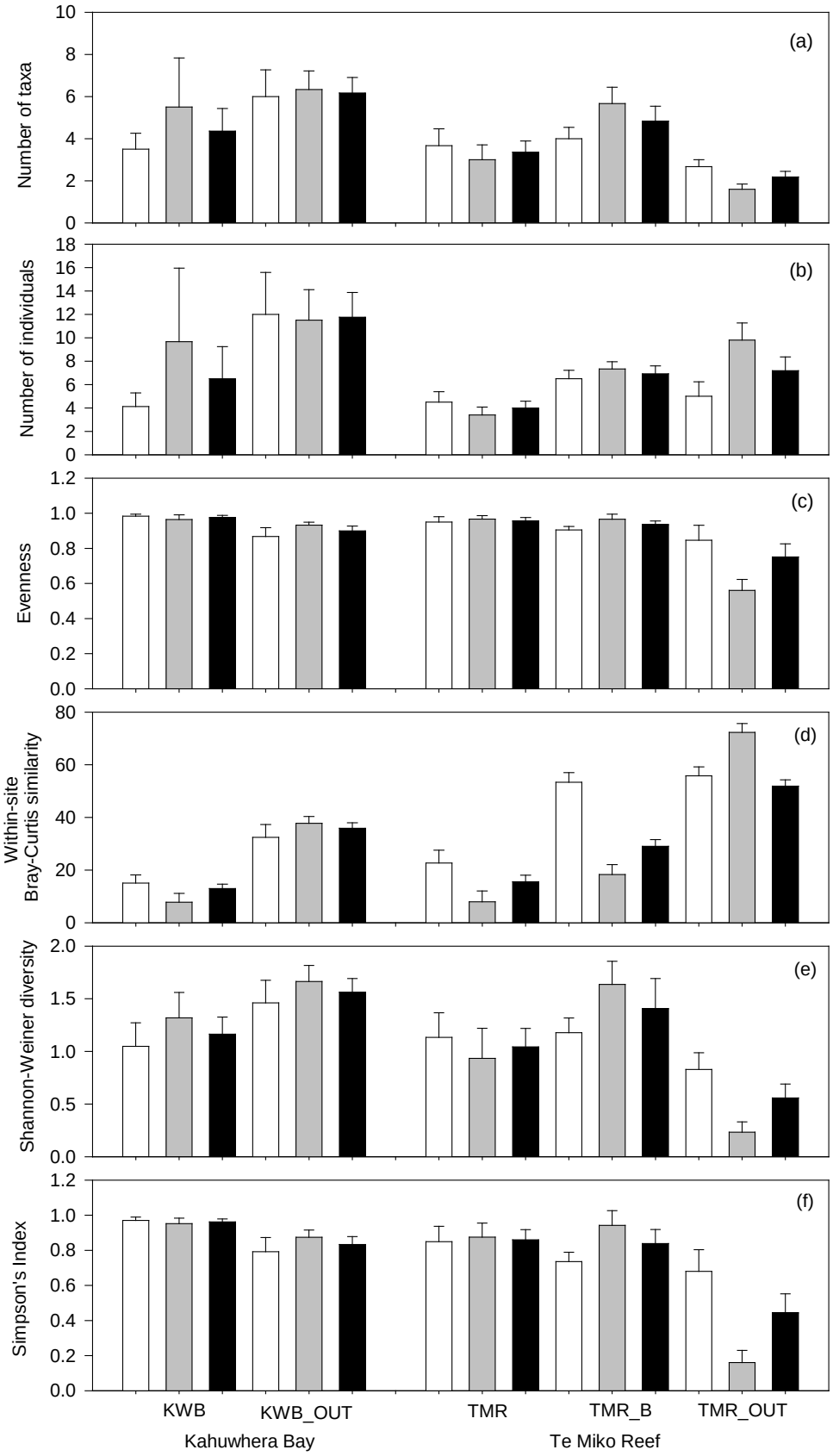

Figure 22: Infauna (a) Mean number of taxa, (b) mean number of individuals, (c) Pielou's evenness, (d) Bray-Curtis similarities, (e) Shannon-Weiner diversity, and (f) Simpson's diversity, per core at each site.

Table 10: Infauna: PERMANOVA analyses of taxa, individuals, evenness and diversity (Shannon-Weiner, Simpson) present in cores at the five sites.

Number of taxa

Source	df	SS	MS	Pseudo-F	P(perm)	Unique perms
Si	4	107.06	26.766	4.0551	0.007	999
Se	1	3.0371	3.0371	0.46012	0.513	998
SixSe	4	22.255	5.5638	0.84291	0.561	998
Res	50	330.03	6.6007			
Total	59	460.73				

Number of individuals

Source	df	SS	MS	Pseudo-F	P(perm)	Unique perms
Si	4	363.15	90.787	2.2317	0.069	998
Se	1	54.195	54.195	1.3322	0.287	998
SixSe	4	113.42	28.356	0.69703	0.633	999
Res	50	2034	40.681			
Total	59	2576.6				

Evenness

Source	df	SS	MS	Pseudo-F	P(perm)	Unique perms
Si	4	1.1462	0.28656	17.735	0.001	998
Se	1	0.0865	0.0865	5.3576	0.032	994
SixSe	4	0.66511	0.16628	10.291	0.001	999
Res	50	0.80787	0.0161			
Total	59	2.5608				

Shannon-Weiner

Source	df	SS	MS	Pseudo-F	P(perm)	Unique perms
Si	4	7.1727	1.7932	7.0219	0.001	999
Se	1	0.011	0.011	0.043	0.846	998
SixSe	4	2.0454	0.51136	2.0024	0.11	998
Res	50	12.769	0.25537			
Total	59	21.663				

Simpson

Source	df	SS	MS	Pseudo-F	P(perm)	Unique perms
Si	4	2.0293	0.50733	17.991	0.001	998
Se	1	0.0241	0.0241	0.85801	0.358	996
SixSe	4	0.87831	0.21958	7.7866	0.001	998
Res	50	1.41	0.0281			
Total	59	4.1676				

Multivariate analyses.

Ordination (MDS) analysis comparing the community composition of core samples from each site (Figure 23) showed reasonable separation between communtities from the two locations and between sites within locations; however there was more overlap between the Te Miko Reef sites and the cores from TMR_OUT are most similar to each other.

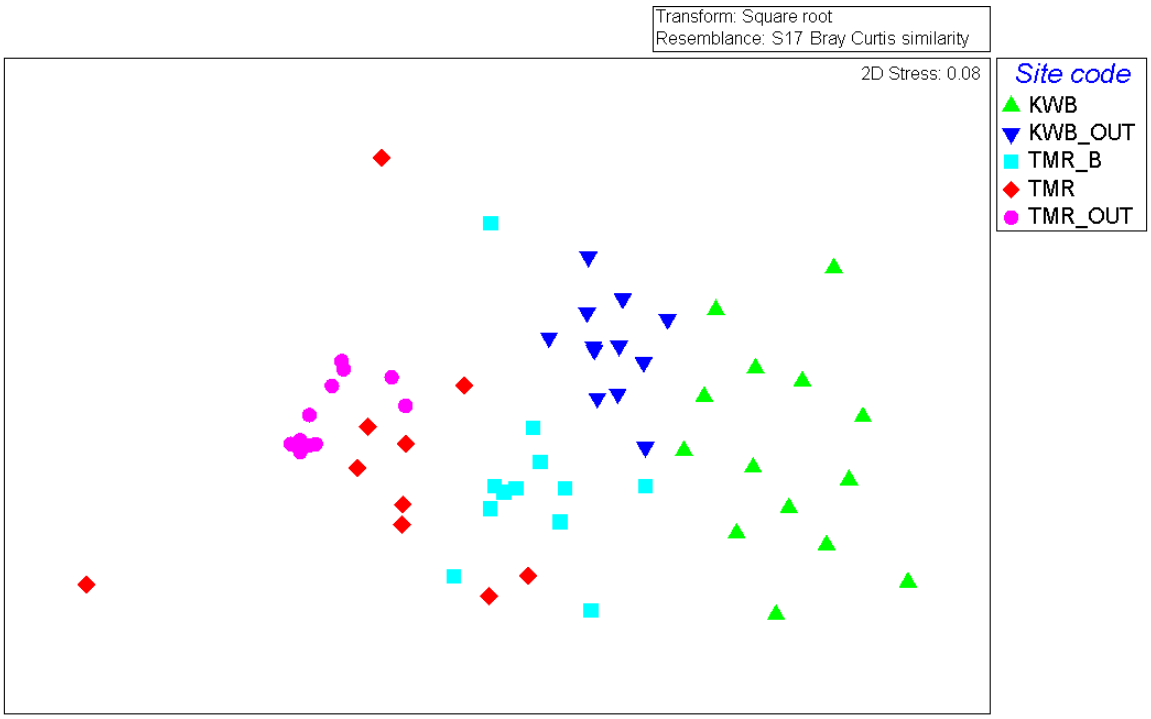

Figure 23: Non-metric multidimensional scaling based on infaunal taxa in cores collected in February and September inside and outside rhodolith beds at Kahuwhera Bay (KWB and KWB_OUT) and Te Miko Reef (TMR, TMR_B and TMR_OUT). Points closest together represent assemblages that are most similar. One outlier from TMR was removed.

Table 11: Infauna: PERMANOVA analyses of infaunal composition in cores from the five sites at Kahuwhera Bay and Te Miko Reef.

Source	df	SS	MS	Pseudo-F	P(perm)	Unique perms
Si	4	99 464	24 866	9.5594	0.001	998
Se	1	4 888.6	4 888.6	1.8793	0.005	999
SixSe	4	17 141	4 285.2	1.6474	0.001	997
Res	49	1.27×10^{-5}	2 601.2			
Total	58	2.497×10^{-5}				

Significant differences existed in the species composition found in cores from the five sites (Table 11). There was a significant difference in the species composition found in cores between sites inside and outside the rhodolith bed at Te Miko Reef, and also significant temporal differences were observed. There was also a significant interaction between site and time of sampling indicating that the effect of site varied with time of sampling. Pairwise comparisons (Appendix 5) indicated that all five sites were significantly different from each other (all p = 0.001). Assessment of the significant interaction between site and sampling date found that the interactive effect is attributed to temporal variation at TMR_B and TMR_OUT.

b. Epifauna – transects

Univariate analyses

Averages of between 6 and 20 taxa and 8 and 36 individuals were recorded from quadrats at each site. PERMANOVA indicated significant differences in number of taxa between sites and collection dates

(Table 12) and pairwise comparisons showed TMR and TMR_B both having significantly fewer taxa

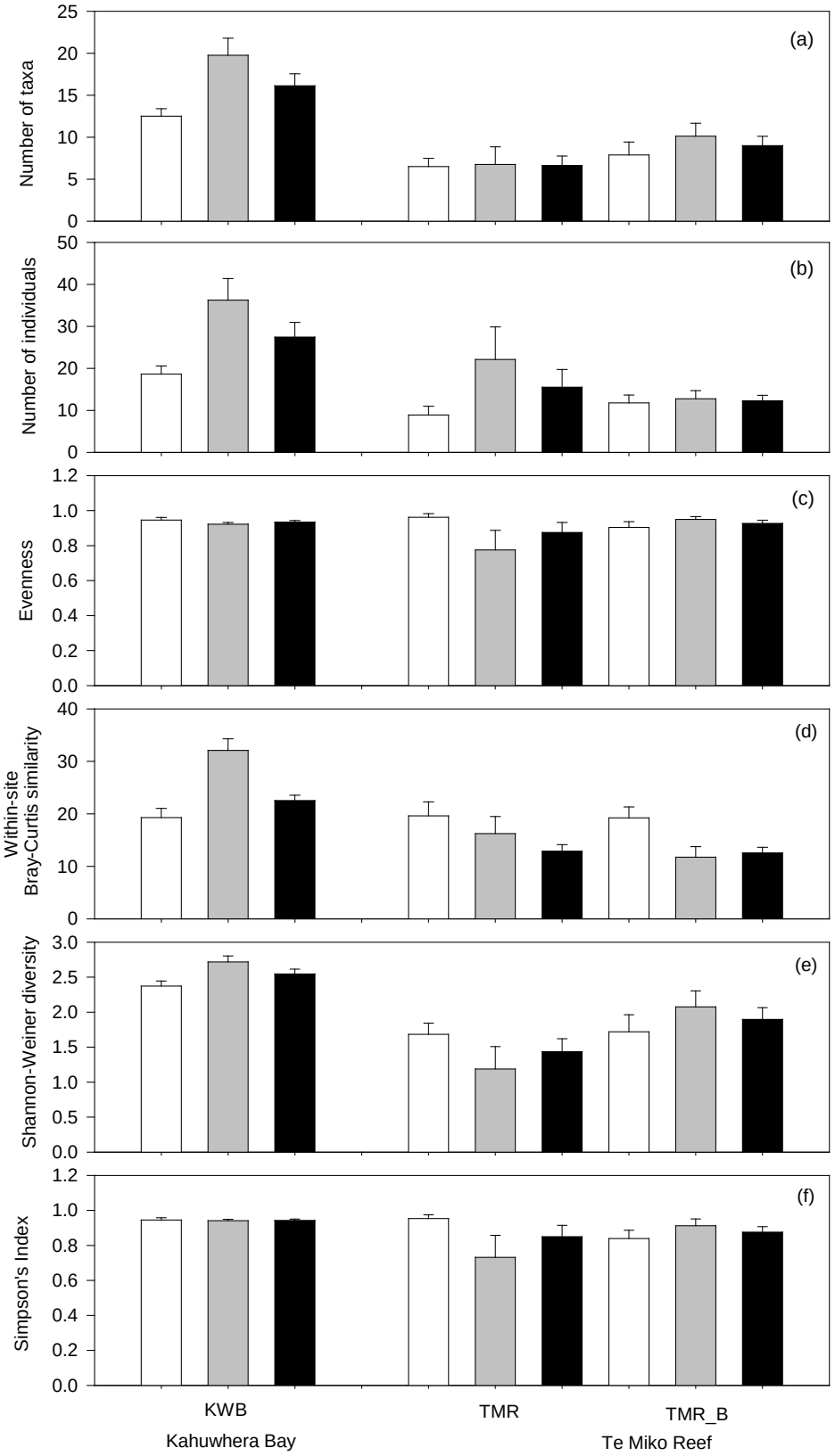

Figure 24: Epifauna (a) Mean number of taxa, (b) mean number of individuals, (c) Pielou's evenness, (d) within site Bray-Curtis similarities, (e) Shannon-Weiner diversity, and (f) Simpson's diversity of invertebrates per quadrat at each site.

than KWB (Figure 24a, Appendix 5). The same pattern occurred in number of individuals (Table 12, Appendix 5). Evenness of epifaunal communities in rhodolith beds was high (greater than 0.9) except at TMR in September (0.77). Although site or temporal differences were not significant, there was a

significant interaction between the two factors (Table 12). Within-site Bray Curtis similarities were very low (less than 20% with the exception of KWB in September at 32%) and significantly different (PERMDISP, p = 0.012, Appendix 5) between sites but seasonal differences were not significant (PERMDISP, p = 0.463) (Figure 24d).

The Shannon-Weiner measure of diversity varied significantly between sites (Figure 24e, Table 12), but not between seasons, and multiple comparison tests attributed this significance to KWB being more diverse than either TMR or TMR_B (p = 0.001 for both) (Appendix 5). In contrast the Simpson diversity index did not vary significantly between sites or seasons (Figure 24f) but there was a significant interaction between site and season with significant differences between TMR_B and the other two sites in February but not in September (Table 12, Appendix 5).

Table 12: Epifauna: PERMANOVA analyses of taxa, individuals, evenness and diversity (Shannon-Weiner, Simpson) in quadrats collected at the three sites within rhodolith beds (KWB, TMR and TMR_B).

Number of taxa

Source	df	SS	MS	Pseudo-F	P(perm)	perms
Si	2	782.17	391.08	19.398	0.001	999
Se	1	126.75	126.75	6.287	0.012	991
SixSe	2	104	52	2.5793	0.076	999
Res	42	846.75	20.161			
Total	47	1859.7				

Number of individuals

Source	df	SS	MS	Pseudo-F	P(perm)	perms
Si	2	2046.5	1023.3	7.5556	0.002	999
Se	1	1354.7	1354.7	10.003	0.003	995
SixSe	2	594.13	297.06	2.1935	0.127	998
Res	42	5688.1	135.43			
Total	47	9683.5				

Evenness

Source	df	SS	MS	Pseudo-F	P(perm)	perms
Si	2	0.0391	0.0195	1.2444	0.321	998
Se	1	0.0353	0.0353	2.2458	0.16	997
SixSe	2	0.1091	0.054	3.4701	0.025	998
Res	41	0.6445	0.0157			
Total	46	0.81699				

Shannon-Weiner

Source	df	SS	MS	Pseudo-F	P(perm)	perms
Si	2	9.9486	4.9743	14.796	0.001	998
Se	1	0.0570	0.0570	0.16969	0.656	995
SixSe	2	1.908	0.95402	2.8377	0.076	998
Res	42	14.12	0.33619			

Simpson

Source	df	SS	MS	Pseudo-F	P(perm)	perms
Si	2	0.0815	0.0407	1.8312	0.155	999
Se	1	0.0298	0.0298	1.3417	0.259	998
SixSe	2	0.17982	0.0899	4.0377	0.018	998
Res	41	0.91298	0.0222			
Total	46	1.1905				

Multivariate analyses

Ordination (MDS) analysis comparing the communities found within rhodolith beds at each site (Figure 25) showed distinct groupings for site (ANOSIM Global R = 0.608, p = 0.001), and samples from KWB appear more closely grouped (more similar) than those from the two sites at Te Miko Reef.

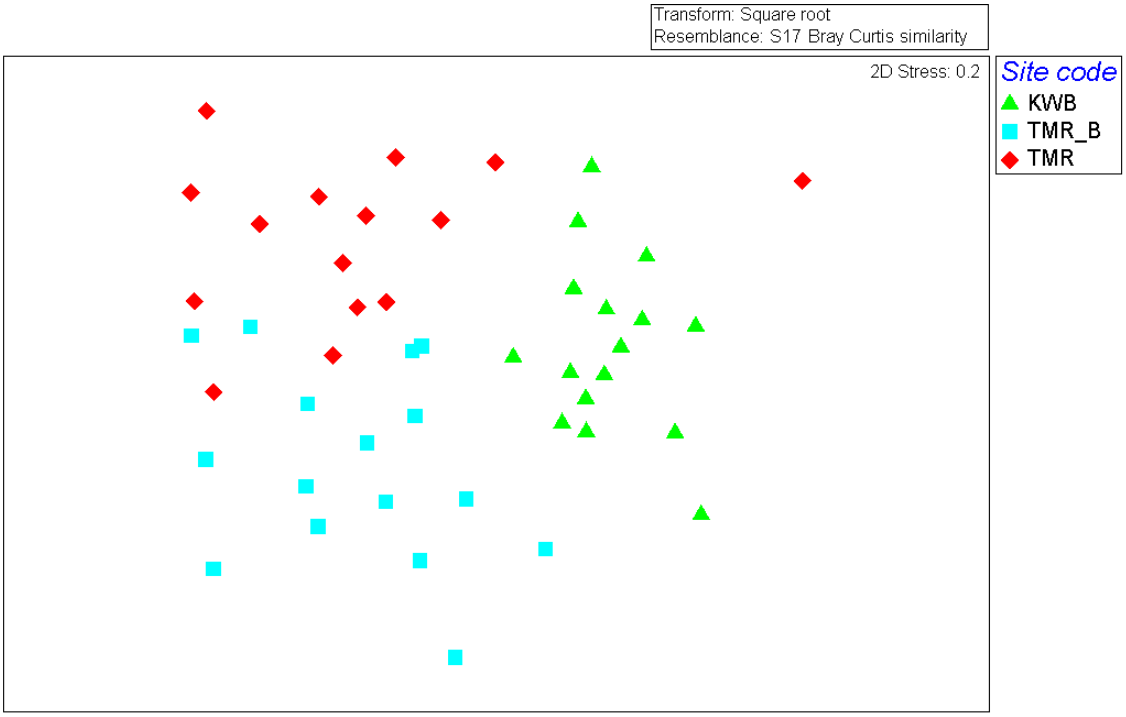

Figure 25: Non-metric multidimensional scaling (MDS) based on epifaunal taxa collected in transects in February and September inside rhodolith beds at Te Miko Reef (TMR and TMR_B) and Kahuwhera Bay (KWB). Points closest together represent assemblages that are most similar. One outlier from TMR was removed.

Table 13: Transects: PERMANOVA analyses of epifaunal composition in quadrats from the five sites at Kahuwhera Bay and Te Miko Reef.

Source	df	SS	MS	Pseudo-F	P(perm)	Unique perms
Site	2	36395	18198	5.4828	0.001	996
Season	1	8769.8	8769.8	2.6423	0.001	998
SixSe	2	14311	7155.5	2.1559	0.001	998
Res	42	1.394×10^{-5}	3319			
Total	47	1.988×10^{-5}				

PERMANOVA analyses found significant differences in the epifauna community composition between sites within the rhodolith beds at Te Miko Reef and Kahuwhera Bay, and there was also significant temporal variation in the epifauna present (Table 13). Pairwise comparisons showed that all the epifauna at all three sites (TMR, TMR_B and KWB) were significantly different from each other at both sample dates (Appendix 5), however the significant interaction term between site and time (Table 13) indicates that the effects of time differ between sampling dates.

c. Surface macrofauna

The number of large invertebrates observed during the swath count transects varied widely.

Pairwise comparisons between sites and seasons showed that there were significantly more molluscs inside the rhodolith beds at Te Miko Reef (TMR and TMR_B) than at the other sites and that numbers were significantly higher in February than September ($p = 0.019$). The rhodolith bed at Kahuwhera Bay (KWB) contained significantly more polychaetes than two of the three sites at Te Miko Reef (TMR and TMR_OUT), particularly in September. There were also more polychaetes at the KWB_OUT site than any of the three Te Miko Reef sites. One of the polychaetes found at both KWB and TMR was *Chaetopterus chaetopterus*-A, a species believed to be introduced and which forms binding mats (Figure 26). Significant differences were found between sites in molluscs, polychaetes, flatworms, echinoderms and sponge abundance (Figure 27, Table 14). There was also significant temporal variation in mean abundance of molluscs, polychaetes, and flatworms.

Flatworms were only sighted in February, outside the rhodolith bed at Kahuwhera Bay and inside one of the rhodolith beds at Te Miko Reef (TMR). There were significantly more echinoderms inside the rhodolith bed at Kahuwhera Bay (KWB) than any of the other four sites in either season ($p = 0.000$ for all four comparisons). The number of sponges counted did not vary significantly between seasons, but there were significant differences between sites, with more sponges found inside the rhodolith bed at Kahuwhera Bay than at any of the three sites within the Te Miko Reef location.

Figure 26: Introduced polychaete *Chaetopterus* collected from Kahuwhera Bay, showing how it binds rhodoliths.

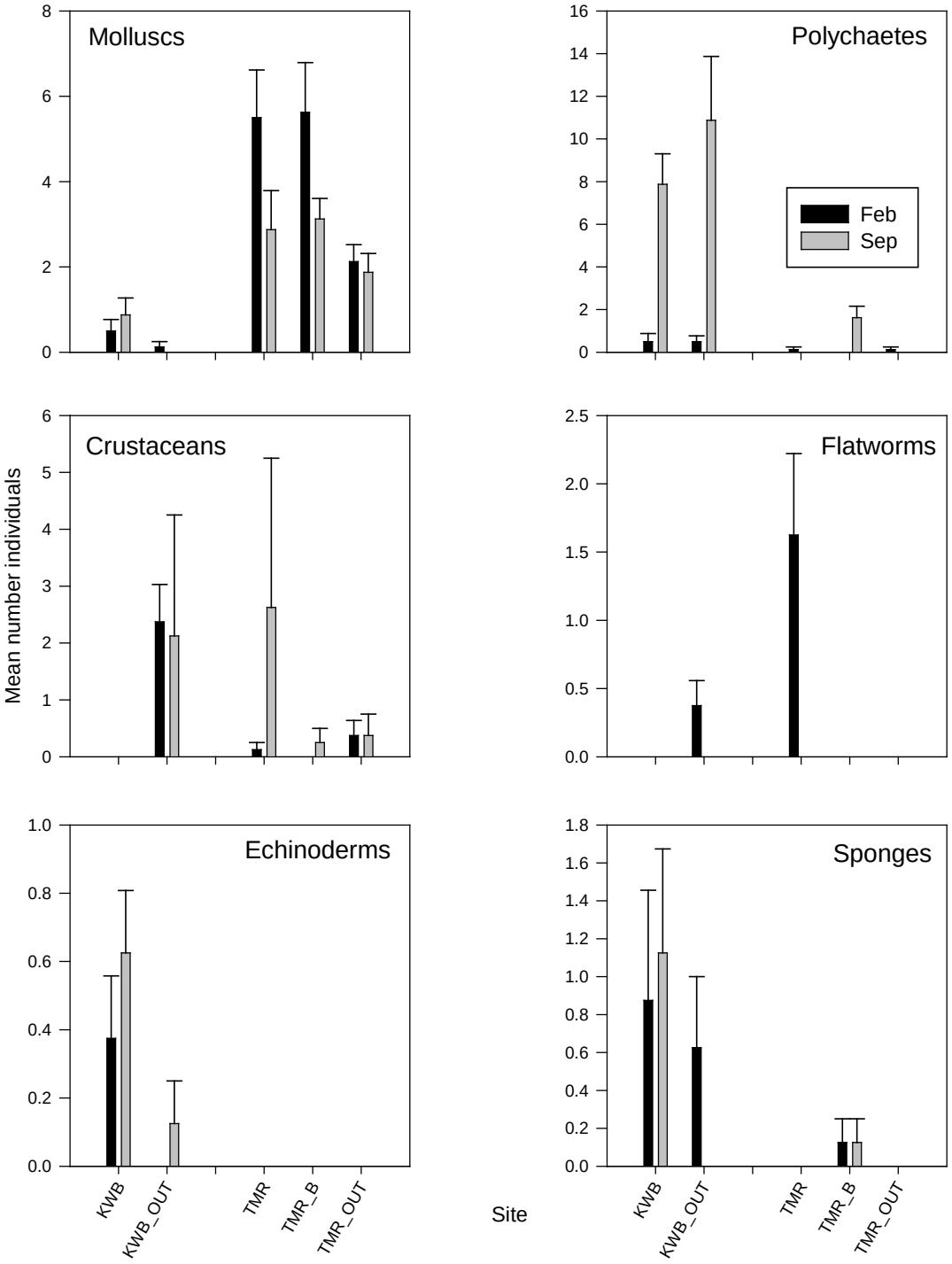

Figure 27: Mean number of surface macrofauna by major taxonomic group and sites collected in February and September.

Table 14: Results of site and season analyses (General Linear Model) for the average number of surface macrofauna observed during diver swath counts at Kahuwhera Bay (KWB and KWB_OUT) and Te Miko Reef (TMR, TMR_B and TMR_OUT).

Variable	Source	SS	df	MS	*F*	*p*-value
Molluscs	Site	248.95	4	62.23	16.96	0.000
	Season	21.01	1	21.01	5.73	0.019
	Error	271.55	74	3.67		
Polychaetes	Site	434.70	4	108.67	7.93	0.000
	Season	292.61	1	292.61	21.36	0.000
	Error	1013.57	74	13.70		
Crustaceans	Site	59.30	4	14.82	2.39	0.059
	Season	5.00	1	5.00	0.81	0.372
	Error	459.25	74	6.21		
Flatworms	Site	7.92	4	1.98	4.94	0.001
	Season	3.20	1	3.20	7.98	0.006
	Error	29.67	74	0.40		
Echinoderms	Site	3.05	4	0.76	11.69	0.000
	Season	0.11	1	0.11	1.72	0.193
	Error	4.82	74	0.06		
Sponges	Site	11.20	4	2.80	4.40	0.003
	Season	0.11	1	0.11	0.18	0.675
	Error	47.07	74	0.64		

3.1.3.3 Cryptofauna

The rhodoliths analysed for cryptofauna were a subset of the rhodoliths collected. Twelve rhodoliths of each of *Lithothamnion crispatum* (six from each of TMR and TMR_B) and *Sporolithon durum* (six from each of KWB and TMR_B) were measured, weighed and branch tips counted (following methods outlined earlier).

The total mean number of invertebrates per rhodolith was greatest in *Sporolithon durum* collected from Kahuwhera Bay (74 individuals per rhodolith), followed by *Lithothamnion crispatum* from Te Miko Reef B site (49), *S. durum* from the Te Miko Reef B site (44) and *L. crispatum* from Te Miko Reef (34 individuals per rhodolith). The phyla comprising the cryptofauna were dominated by polychaete worms and Ciliophora, accounting for between 68% and 91% of taxa found (Figure 28).

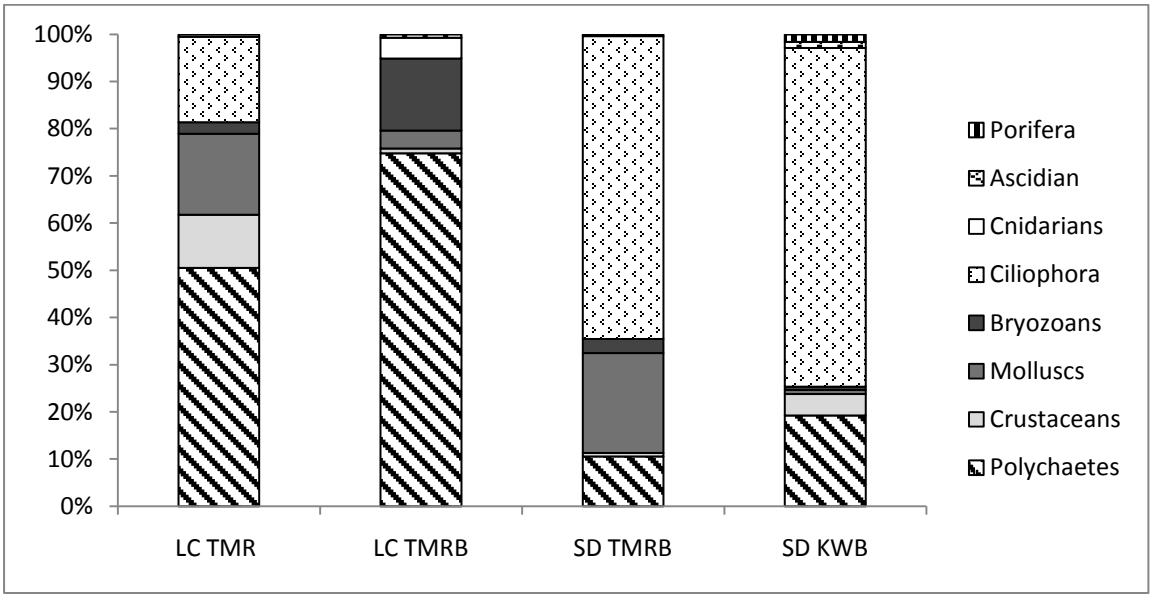

Figure 28: Average percentage of phyla found as cryptofauna on or within rhodoliths collected from the three sites inside rhodolith beds. (LC = *Lithothamnion crispatum*, SD = *Sporolithon durum*).

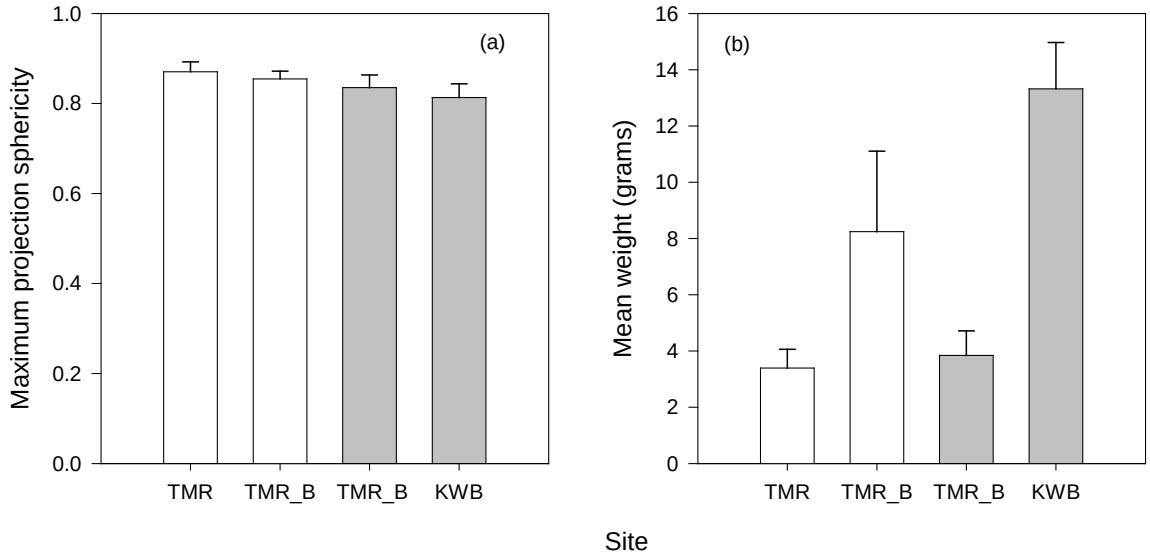

Figure 29: (a) Maximum projection sphericity of rhodoliths sampled for cryptofauna, (b) Buoyant weights of rhodoliths sampled for cryptofauna. (white bars = *Lithothamnion crispatum*, grey bars = *Sporolithon durum*).

The shape and size of the two rhodolith species studied are clearly different, and the size of rhodoliths of each species varied by site (Figure 29b). Although the species are quite similar in terms of their maximum projection sphericity (Figure 29a), the density of branching between species is significantly different (Figure 17b). The relative volume of interstitial space, estimated as 77.0% for *Sporolithon* and 62.7% for *Lithothamnion*, will have a bearing on the available surfaces for encrusting cryptofauna. However, no strong relationships were found between the surface area of the rhodoliths and the mean number of cryptofauna recorded (Figure 30). Cryptofauna also live within the rhodoliths, boring holes within the coralline matrix.

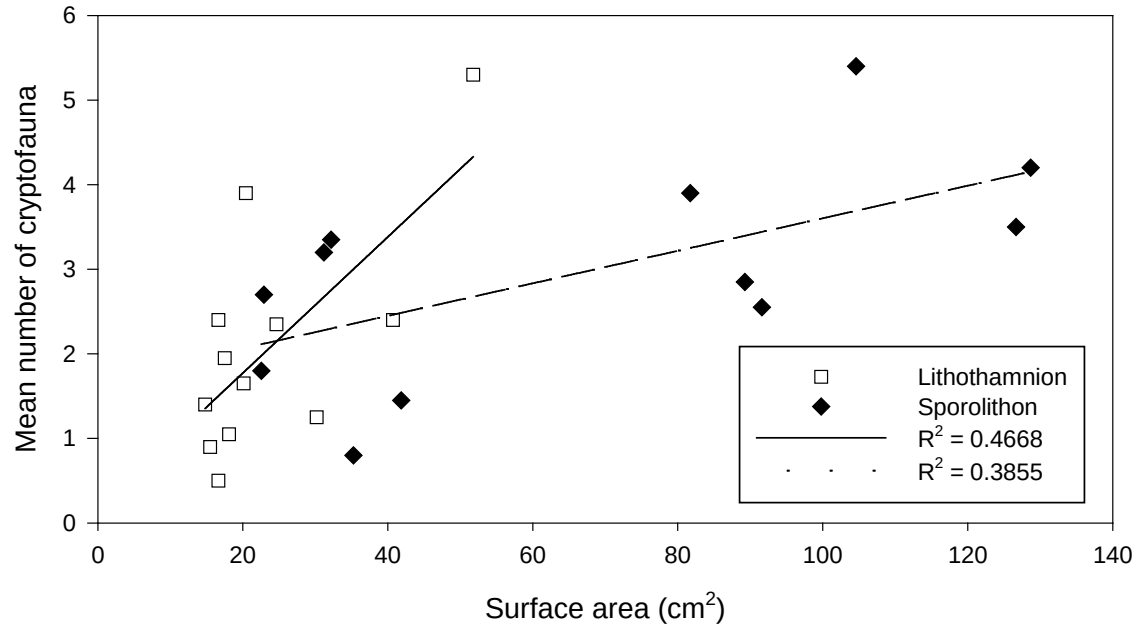

Figure 30: Mean number of cryptofauna recorded from *Lithothamnion crispatum* and *Sporolithon durum* rhodoliths in relation to estimated surface area.

3.1.3.4 Epiflora – Macroalgae

Three hundred and ninety two samples of macroalgae were collected. From these collections 102 distinct taxa were identified, with 63 identified to species level, 37 to genus (some of these provisional) and a further two provisionally to order - 12 green algae, 24 brown algae and 66 red algae (Appendix 2). Based on data from our other research programmes, we are aware of new genera that will have to be described for New Zealand species, but at present there are insufficient data for formal descriptions (e.g., species recorded here as '*Halymenia*' sp., '*Tsengia*' sp.).

Within the green algae, *Caulerpa flexilis* was a particularly conspicuous part of the flora recorded from all the Te Miko Reef sites and also in the Kahuwhera Bay rhodolith bed. The stoloniferous growth of this species means that it is well anchored in coarse sediment habitats, and forms erect stands that are c. 15 - 25 cm high (Figure 31). In the rhodolith beds this species may provide additional habitat for bottom-dwelling fauna.

Figure 31: Stolons of *Caulerpa flexilis* attached to *Lithothamnion* rhodoliths, collected at TMR.

This research revealed the presence of multiple species of the brown algal genus *Dictyota*. Four different sequences were obtained from five specimens. Three of these (listed here as *Dictota* sp. 1–3) have not previously been obtained from other New Zealand isolates, while the fourth species is conspecific with samples of a species of *Dictyota* (here referred to provisionally as *D. papenfussii*), previously collected from a range of sites, including the Cavalli islands, Stephenson Island and the Okahu Channel. In addition crustose Dictyotales were recorded in the field. The molecular sequencing results also revealed that amongst the crustose brown algae there were two species of *Cutleria*, present as the heteromorphic "*Aglaozonia*" crustose phase. One of these can be confirmed to be *Cutleria multifida*, a new record for the Bay of Islands, but the other species remains unidentified and is different from all sequence data in GenBank. Within the Scytosiphonaceae two genera were recorded, *Colpomenia* (with three species confirmed to be present at the field sites), and *Hydroclathrus clathratus* (considered to be native to the Kermadec Islands but introduced to northern New Zealand). Both *Ecklonia radiata* and *Sargassum sinclairii* were common and were collected from five and four of the sites respectively.

Amongst the red algae two species, *Corallina officinalis* and *Chondracanthus chapmanii*, were recorded at all five sites. *Chondracanthus* appeared to be stabilising the rhodolith beds in some places. This species has both creeping as well as upright growth, and branches from a single individual were found to be anchored to multiple rhodoliths (Figure 32). Another group of taxa were also common and present at four of the five sites: *Pterocladia lucida*, *Gigartina atropurpurea*, *Peyssonnelia* spp., *Aeodes nitidissima*, *Tsengia* sp., *Sarcodia montagneana*, non-geniculate coralline algae. The identification of some species of non-geniculate coralline algae was confirmed by sequence data but

other species were recorded as 'ngc' in the field. Three species of *Scinaia* were recorded, two present only at Te Miko Reef sites and a third species only at Kahuwhera Bay. The species C*ladhymenia oblongifolia* was found commonly at Te Miko Reef but was not found at Kahuwhera Bay.

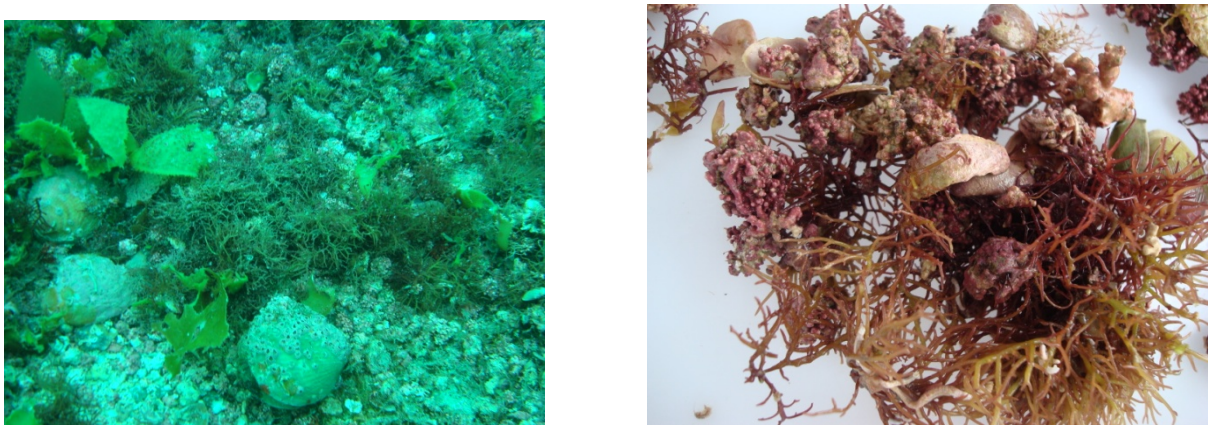

Figure 32: *Chondracanthus chapmanii* in the rhodolith bed at Te Miko Reef (left) and showing its attachment to individual rhodoliths (right).

The confirmation of the identification of *Dudresnaya capricornii* represents a new genus and family record for New Zealand. Specimens of *Dudresnaya* have previously been collected during field work in the north eastern North Island in 2006 and also during the Oceans 20/20 field programme in the Bay of Islands. The species recorded here as '*Halymenia*' sp. is distant from other genera in the order and it is highly probable that it represents an undescribed genus. There are two species of *Tsengia* recorded and one of these, listed here as '*Tsengia*' sp., in our opinion is a member of an undescribed genus. Two species of *Grateloupia* are recorded here, and these can be distinguished by sequence data but are not currently aligned with species described from the New Zealand region. The diversity of species within the Peyssonneliaceae in the sites studied was very high (increasing the taxa recognised in New Zealand), with at least five distinct species identifiable by sequence data, one of which we have identified as *P. boudouresquei* on the basis of sequence data.

The analysis of macroalgae inside and outside rhodolith beds is based on material collected in algal searches. These had equal collecting effort in terms of time, and had the same phycology expert as a collector for all sampling. For many algae it is not possible to count the number of individuals, therefore all analyses are on presence/absence data, and consequently the use of diversity indices that incorporate abundance was not possible.

Univariate

The number of taxa collected in the algal searches varied between 12 and 28 and varied significantly between sites but not seasons (Table 15).

Table 15: Epiflora: PERMANOVA analyses of number of macroalgal taxa from algal searches at Kahuwhera Bay and Te Miko Reef.

Source	df	SS	MS	Pseudo-F	P(perm)	Unique perms
Si	4	223.4	55.85	14.506	0.018	857
Se	1	25.6	25.6	6.6494	0.077	631
Res	4	15.4	3.85			
Total	9	264.4				

Multivariate

The ordination (MDS) analysis comparing the composition of the macroalgal communities found that there were distinct groupings within rhodolith beds at each site (Figure 33) and PERMANOVA analyses indicated that there was significant separation between sites (p = 0.003) and seasons (p = 0.047) (Table 16).

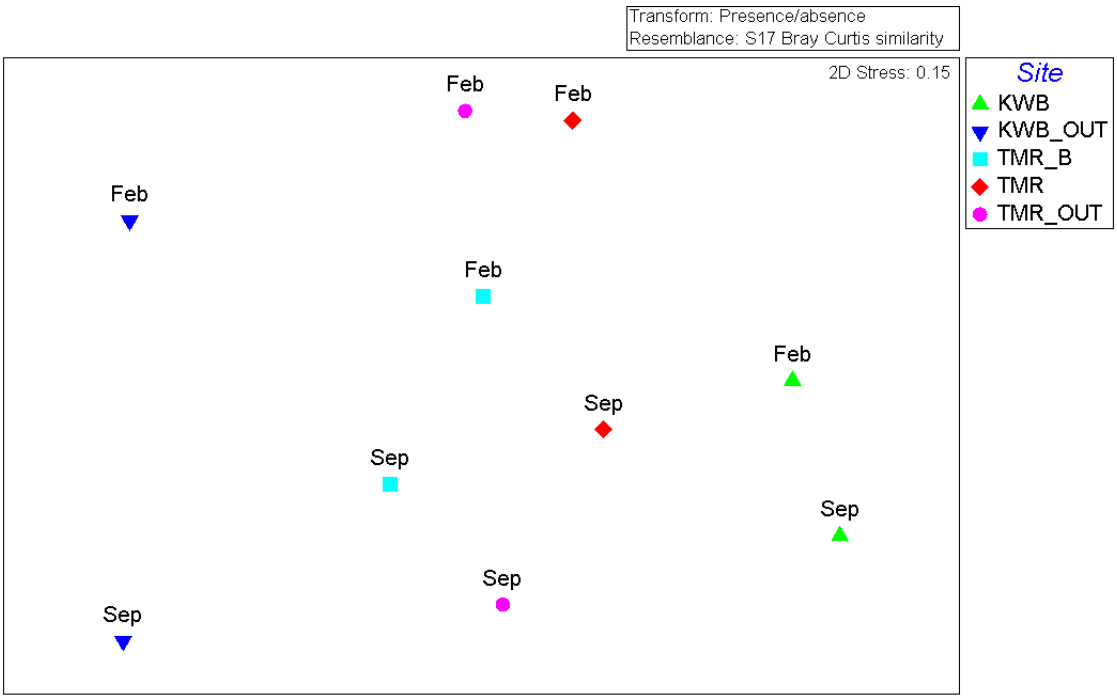

Figure 33: Comparison of macroalgal community composition sampled by algal searches at each site. Points closest together represent assemblages that are most similar.

Table 16: Algal searches: PERMANOVA analyses of algal community similarity between sites and collection dates.

Source	df	SS	MS	Pseudo-F	P(perm)	Unique perms
Si	4	13 164	3 291	2.2875	0.003	971
Se	1	4 011.2	4 011.2	2.788	0.047	929
Res	4	5 754.8	1 438.7			
Total	9	22 930				

3.1.3.5 Fish

Nineteen lots and twenty two individual fish were collected, and over 70% of these were clingfish in the genus *Trachelochismus* (Appendix 4). Fourteen fish were collected in February and eight in September. Of the twenty two individuals, nineteen were able to be identified to species, two to genus and one to family, and they were collected by the following methods: algal searches – 6 lots (8 individuals); core – 2 (2); Rotenone – 2 (3); Transects – 6 (6); on rhodoliths – 1 (1). Divers also recorded the presence of snapper (*Pagurus auratus*), gurnard (*Chelidonichthys kumu*), leatherjacket (*Parika scaber*) and stingrays in the vicinity of the rhodolith beds.

3.1.4 Overall community structure

Between rhodolith beds

Multivariate

Ordination (MDS) analysis comparing the overall invertebrate community structure collected by all methods in rhodolith beds at each site (KWB, TMR and TMR_B) showed some grouping by site and method, however there was also considerable overlap in community composition (Figure 34). Macroalgae communities sampled by all methods within rhodolith beds in February and September showed no clear pattern of method-specific or site-specific groupings (Figure 35).

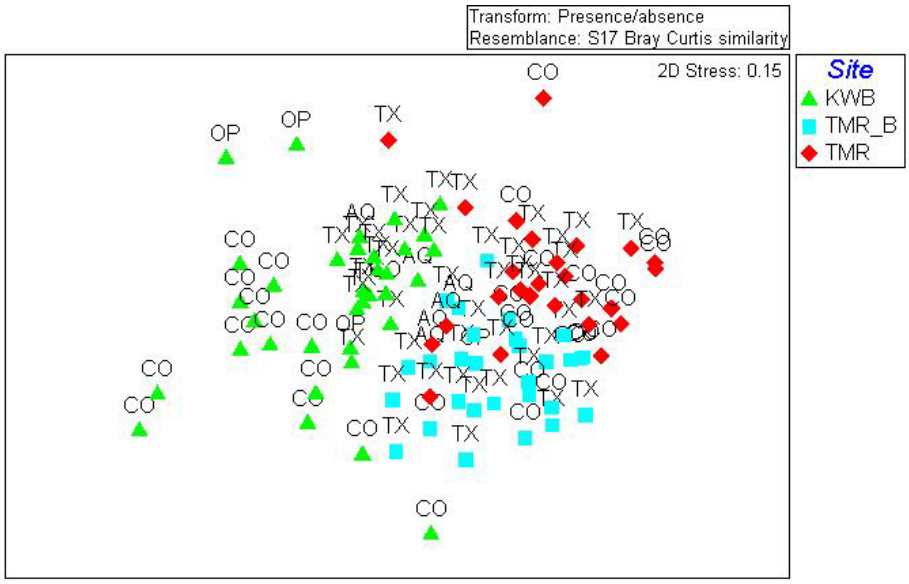

Figure 34: All invertebrates: Non-metric multidimensional scaling (MDS) based on invertebrate taxa collected by all methods in February and September inside rhodolith beds at Kahuwhera Bay (KWB) and Te Miko Reef (TMR, TMR_B). Points closest together represent assemblages that are most similar. Several outliers were removed to enable visual comparison.

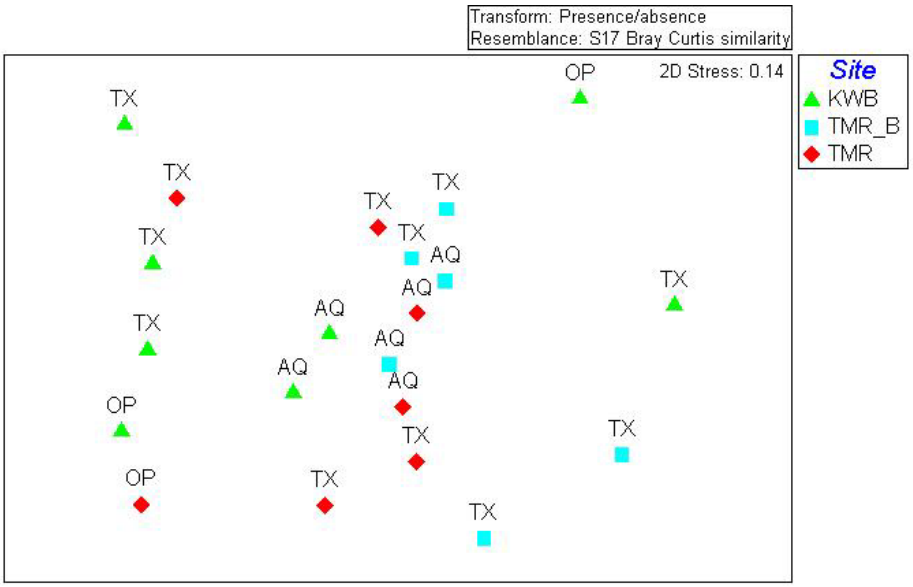

Figure 35: Macroalgae: Non-metric multidimensional scaling (MDS) based on macroalgal taxa collected by all methods in February and September inside rhodolith beds at Kahuwhera Bay (KWB) and Te Miko Reef (TMR, TMR_B). Points closest together represent assemblages that are most similar.

Outside rhodolith beds

Analysis of invertebrates collected by all methods outside rhodolith beds shows a clear difference for invertebrates collected at TMR_OUT in the taxa collected during the sampling of infauna and the taxa collected opportunistically or in algal searches, whereas the taxa collected from KWB are more closely related across collection methods (Figure 36). The infauna collected in the cores are more similar to each other than invertebrates collected by other methods.

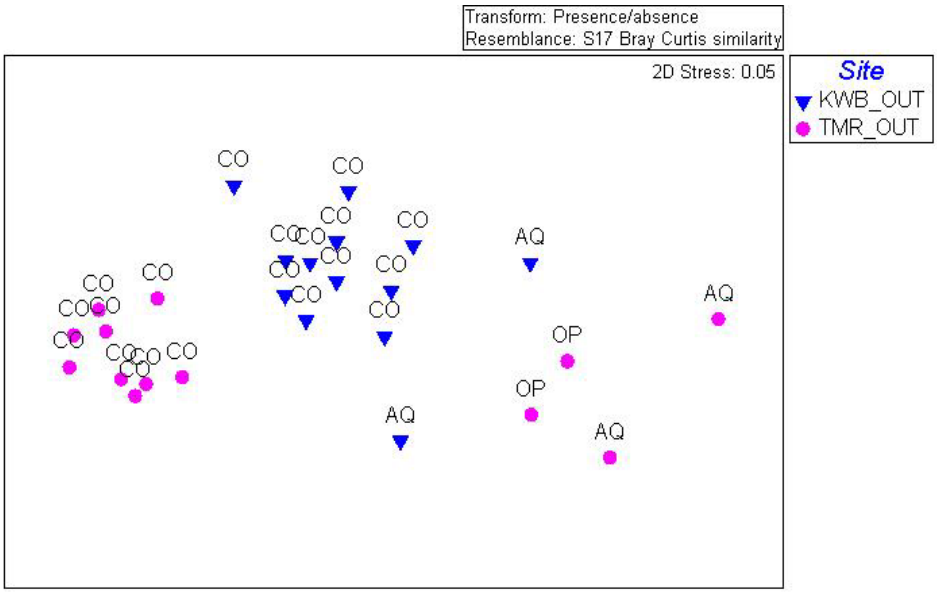

Figure 36: Non-metric multidimensional scaling (MDS) based on invertebrate taxa collected by all methods in February and September outside rhodolith beds at Kahuwhera Bay and Te Miko Reef. Points closest together represent assemblages that are most similar.

Macroalgae community structure was dissimilar between sites and methods. There were both method-specific and site-specific differences in the macroalgae sampled at these sites (Figure 37).

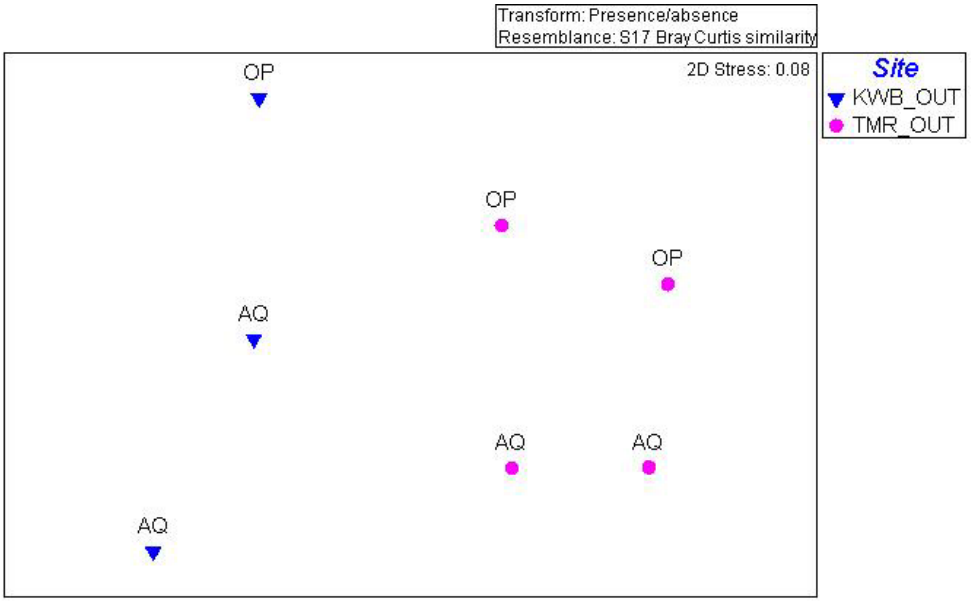

Figure 37: Non-metric multidimensional scaling (MDS) based on macroalgal taxa collected by all methods in February and September outside rhodolith beds at Kahuwhera Bay and Te Miko Reef. Points closest together represent assemblages that are most similar.

3.2 Objective 2

3.2.1 Techniques for measuring rhodolith growth/condition

a. PAM

Pulse amplitude modulation fluorometry (PAM) was used to assess the health or stress levels of all rhodoliths in the culture experiments. The results we obtained raised issues about the interpretation of the yield measurements (refer sections 3.2.2.1 (light) and 3.2.2.2 (disturbance) below).

b. Measurement of growth - Alizarin red and buoyant weight

Measurement of growth employing the alizarin staining method was not uniformly successful. Not all branches appeared to have taken up stain, a number of the branches on the rhodoliths showed signs of erosion or damage, and determining the main axis of growth was difficult. Measutrements of growth are presented in Table 17.

Table 17: Total growth (µm) measured on rhodoliths retrieved from field growth lines and grown in culture after alizarin red staining for seven months.

	Sporolithon field	*Sporolithon* culture control	*Lithothamnion* field	*Lithothamnion* culture control
Average	261	196	244	190
Maximum	450	371	483	226
Minimum	141	111	146	146
n	14	15	15	3

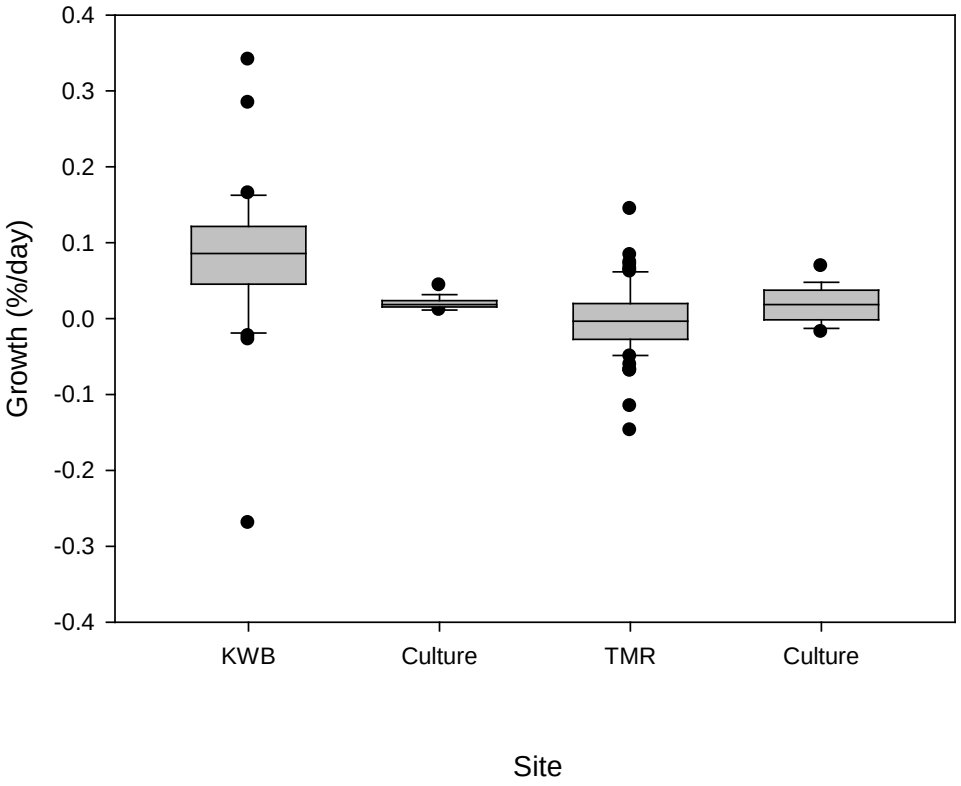

Figure 38: Comparison of growth of rhodoliths, measured by the buoyant weight method, recorded in the field (217 days) for *Sporolithon durum* at Kahuwhera Bay (KWB) (n = 38) and *Lithothamnion crispatum* at Te Miko Reef (TMR) (n = 73) with growth of the control rhodoliths held at the Mahanga Bay aquaculture facility in Wellington (228 days) (n = 18 for each species).

Growth of individual S*porolithon durum* rhodoliths tethered at Kahuwhera Bay ranged between -0.27 and 0.34 %/day (average 0.08 %/day) (Figure 38). In contrast, *S. durum* grown in culture in Wellington showed an average growth of 0.02 %/day, and ranged between 0.01 and 0.04 %/day. Tethered *Lithothamnion crispatum* rhodoliths grew between -0.15 and 0.14 %/day (average -0.001 %/day), whereas *L. crispatum* in culture averaged 0.02 %/day and ranged between -0.02 and 0.07 %/day (Figure 38).

3.2.2 Experiments

3.2.2.1 Light

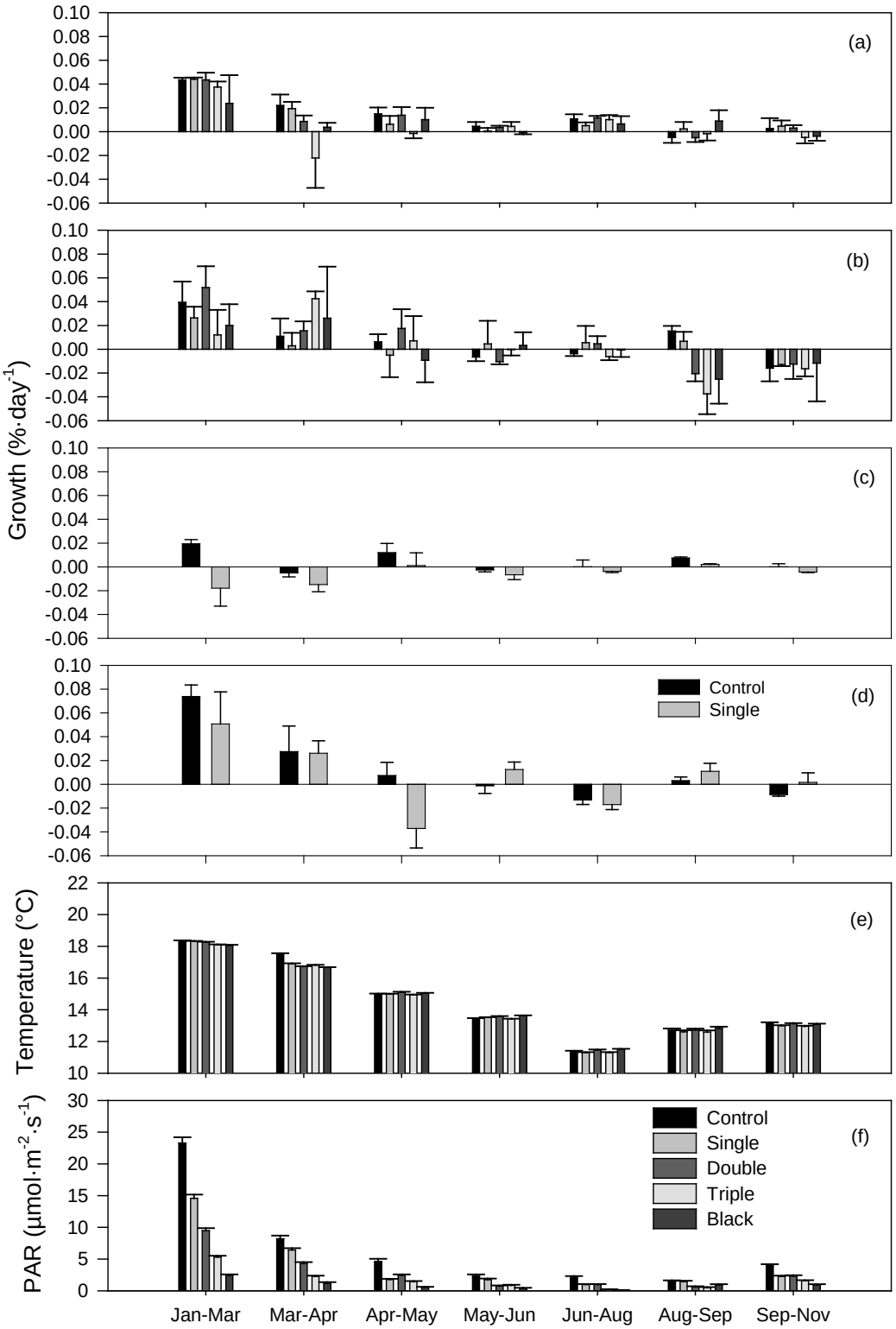

Figure 39: Light experiment: Growth of rhodoliths in culture over 10 months assessed by buoyant weight method. (a) *Sporolithon*, Bay of Islands, (b) *Lithothamnion*, Bay of Islands, (c) *Sporolithon*, Whangaparaoa, (d) *Sporolithon*, D'Urville Island, (e) temperature and (f) irradiance (as approximate PAR) recorded by HOBO loggers in shade-house tank in the culture system over the same period.

Rhodoliths from the Bay of Islands (*Sporolithon* and *Lithothamnion*), as well as *Sporolithon* from Whangaparoaoa and D'Urville Island, were grown in culture under five light levels providing a 6-fold

range of PAR (Figure 39f). Both *Sporolithon* and *Lithothamnion* from the Bay of Islands (Figures 39a and 39b respectively) showed no significant response to light levels but did show significant declines in growth over the course of the experiment. The cultures were being maintained under ambient temperatures at Mahanga Bay (Figure 39e) which declined over the seasonal course of the experiment. The *Sporolithon* rhodoliths from D'Urville Island (Figure 39d) followed a similar pattern to the Bay of Islands rhodoliths, whereas the rhodoliths from the intertidal bed at Whangaparaoa (Figure 39c) showed an initial reduction in growth associated with shading. All the rhodoliths had been acclimated to the conditions prior to placement in the experiment and this response differs from the other rhodoliths in this experiment.

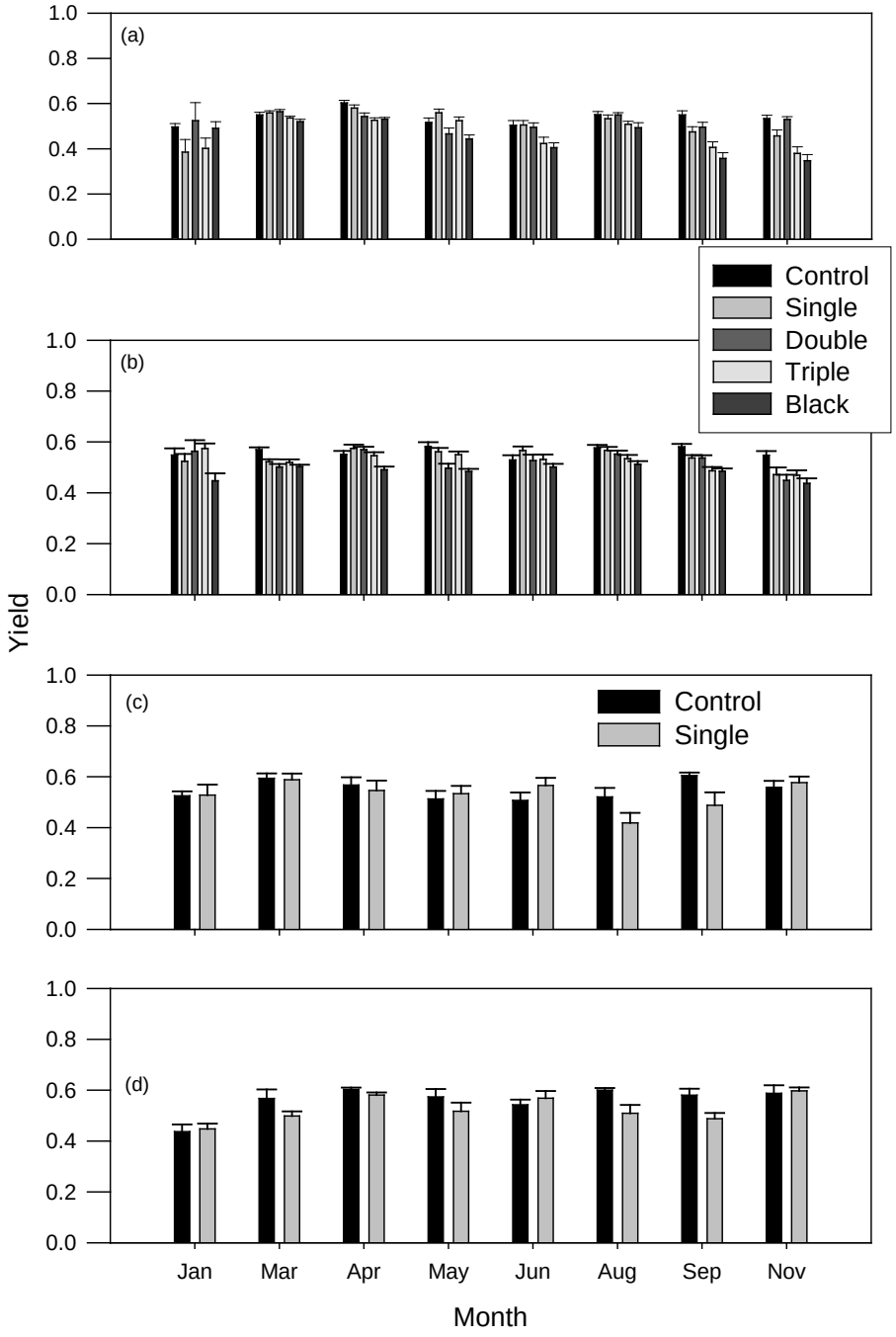

Figure 40: PAM yield of rhodoliths under varying light regimes (a) *Sporolithon*, Bay of Islands, (b) *Lithothamnion*, Bay of Islands, (c) *Sporolithon*, Whangaparaoa, (d) *Sporolithon*, D'Urville Island.

PAM yield measurements did not vary significantly between treatments or species (Figure 40).

3.2.2.2 Disturbance

a. Burial

Growth of both species varied considerably under all three treatments (Figure 41). While *Sporolithon* showed a general pattern of positive growth under the control treatment and negative growth under the two sediment treatments, the pattern for *Lithothamnion* (Figure 41b) was less clear with the control rhodoliths initially showing negative growth. However, by the third removal period (day 28) *Lithothamnion* was showing a similar pattern to *Sporolithon*.

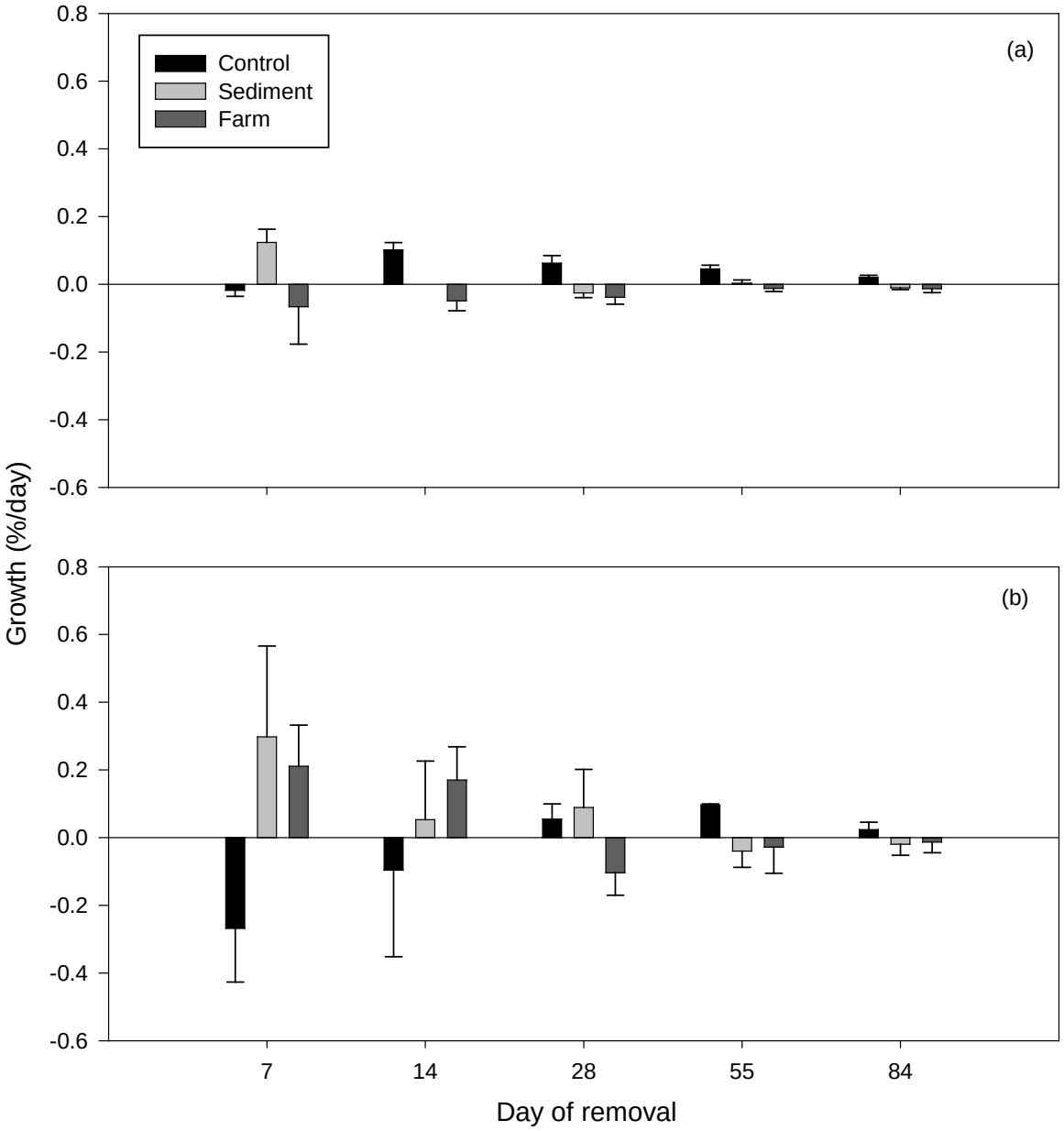

Figure 41: Response of (a) *Sporolithon durum* and (b) *Lithothamnion crispatum* to burial by sediment and marine farm sediment, measured by buoyant weight method at five intervals after establishment of the experiment.

b. Fragmentation

For both species, growth declined in both the fragmented and control treatments (Figure 42).

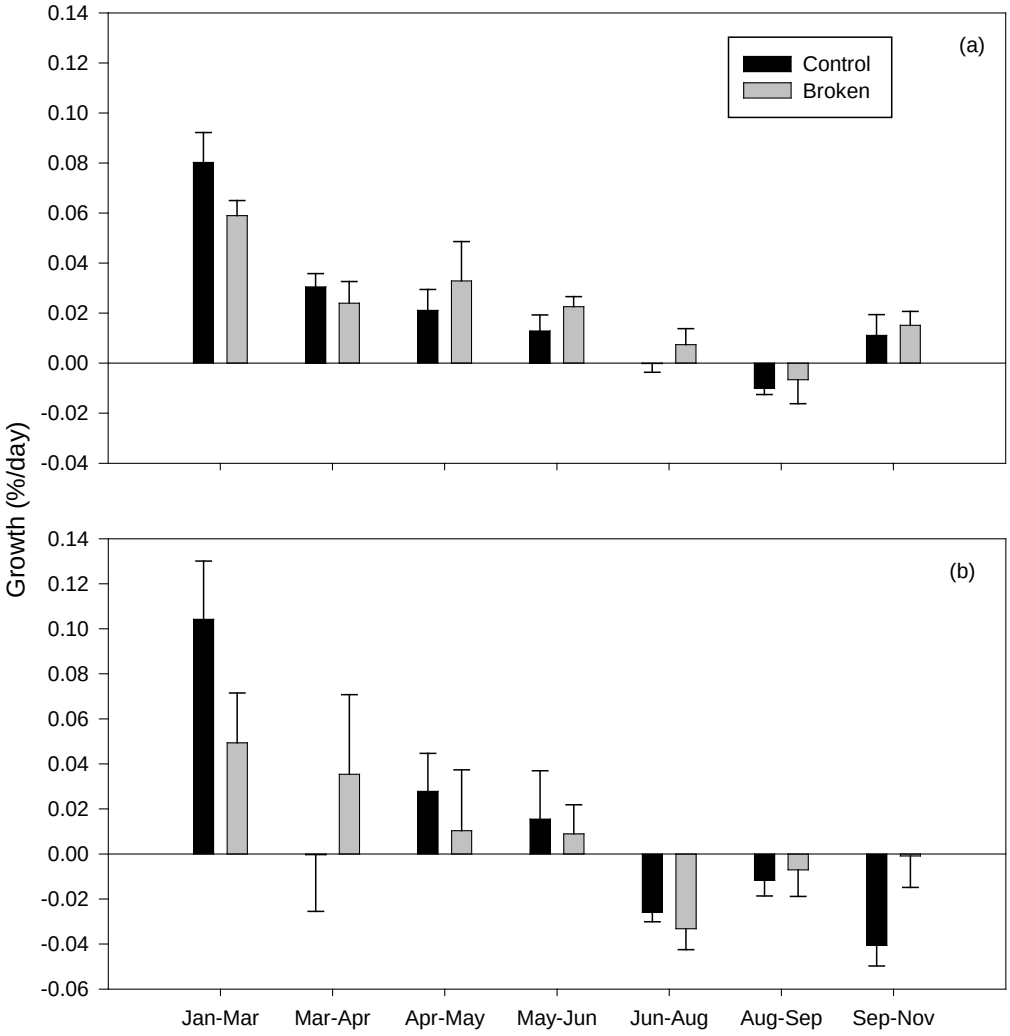

Figure 42: Response of (a) *Sporolithon durum* and (b) *Lithothamnion crispatum* to fragmentation, measured by buoyant weight method.

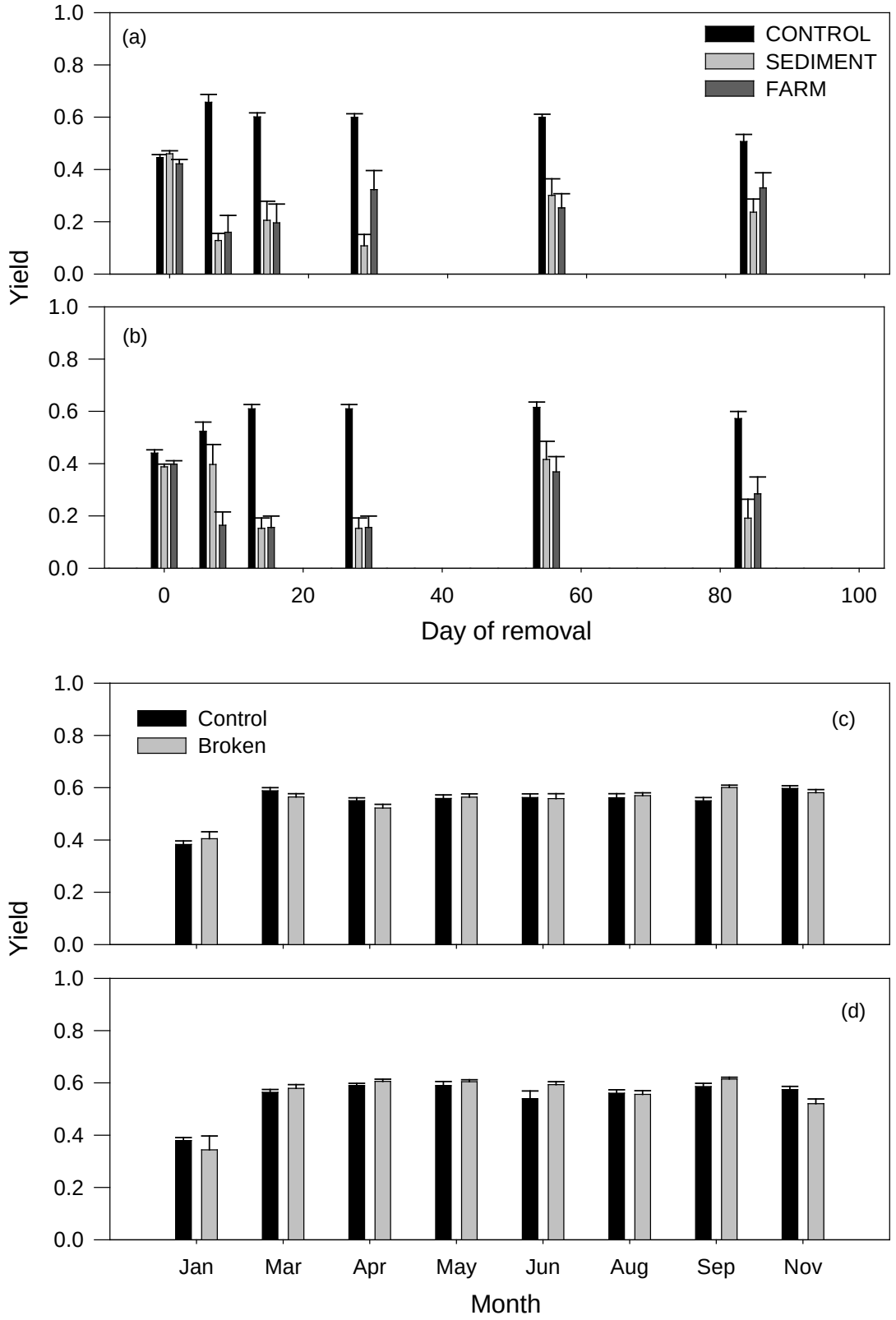

Figure 43: PAM: Response of rhodoliths to burial and to fragmentation assessed by PAM (a) burial *Sporolithon*, (b) burial *Lithothamnion*, (c) fragmentation *Sporolithon*, (d) fragmentation *Lithothamnion*.

PAM yield did not vary significantly between the treatments or species in the fragmentation experiment and there was no difference between the controls and the treated samples. However, the rhodoliths buried in sediment or fish farm sediment produced significantly lower yields than the unburied control treatments in both Spo*rolithon durum* and *Lithothamnion crispatum* (Figure 43).

3.2.2.3 Ocean Acidification

Figure 44 describes the conditions of the ocean acidification experiment.

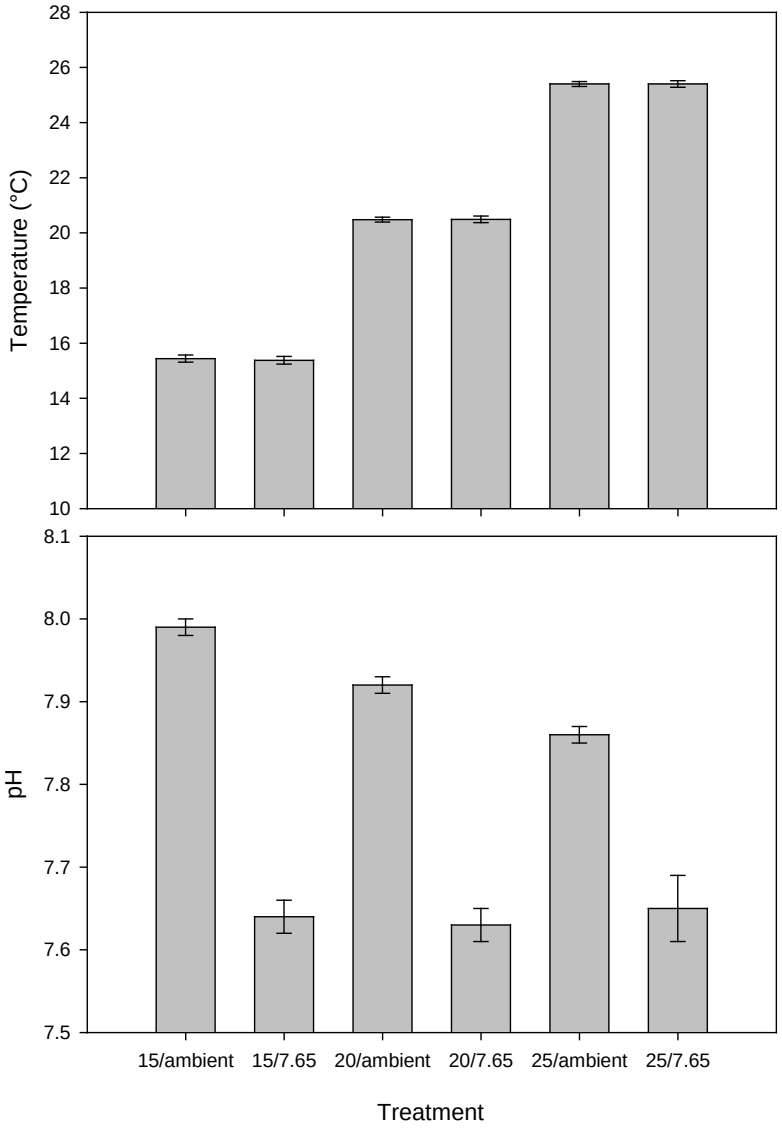

Figure 44: Mean temperature and pH measurements recorded for the duration of the experiment.

At ambient pH (8.05), increasing water temperature resulted in a decrease in growth of both species of rhodolith (Figure 45). However there was also an effect of water temperature on non-manipulated pH seawater which was presumably because of the effect of temperature on the solubility of CO_2 (Figure 44). Overall there was a significant effect of temperature on growth (p less than 0.001, Table 18) with the highest temperature (25° C) having the greatest effect. In addition, at the lowest water temperature tested (15° C) there were significant differences observed in rhodolith growth between pH levels (p less than 0.001, Table 18). As pH decreased growth of both rhodolith species also decreased with significant differences between the species at both pH levels (Figure 45). There was a significant difference between the effects of treatments on the two species ($p = 0.006$, Table 18) and further statistical analysis showed significant interaction between temperature and pH level ($p = 0.047$, Table 18) on growth. Overall the greatest effect on growth rate came with the combination of high temperature (25° C) and low pH (7.65) on *Lithothamnion crispatum* which showed negative growth.

Table 18: Ocean acidification:Three way ANOVA of the effect of pH, temperature on two species of rhodolith in culture.

Source of Variation	DF	SS	MS	F	P
Temperature	2	0.0737	0.0369	85.627	<0.001
pH	1	0.0271	0.0271	62.943	<0.001
Species	1	0.00382	0.00382	8.886	0.006
Temperature x pH	2	0.00300	0.00150	3.480	0.047
Temperature x Species	2	0.00257	0.00128	2.984	0.070
pH x Species	1	0.00139	0.00139	3.221	0.085
Temperature x pH x Species	2	0.00260	0.00130	3.021	0.068
Residual	24	0.0103	0.000430		
Total	35	0.125	0.00356		

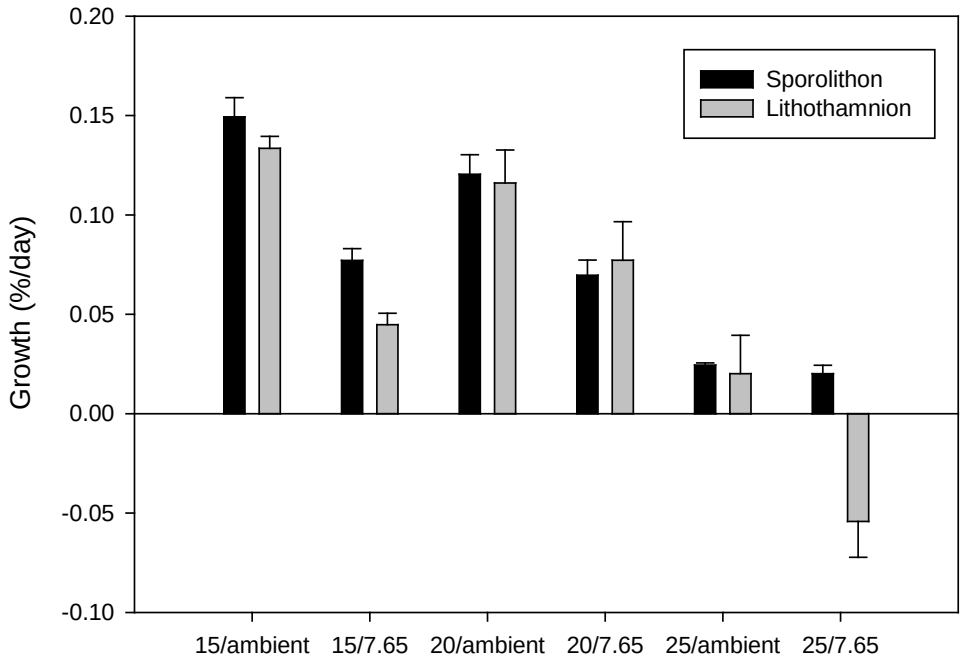

Figure 45: Growth of two species of rhodolith under varying conditions of pH and temperature.

3.2.2.4 Other observations

a. PAM – test of method

Yields of all treatments showed high variability over the experimental period (Figure 46) making assessment of the health of the rhodoliths difficult. Of concern was the fact that treated rhodoliths that had been brushed, dried at 60° C overnight and were bleached white, had PAM fluorometry yield measurements of close to zero at the initial measurement (Figure 46, 08-03-10), indicating they were dead, but the yield a month later (Figure 46, 03-04-10) was similar to results for all other treatments. A further three months later the PAM reading again was reduced to almost the initial measurement. We assume that this indicates that the PAM readings are not only measuring the state of the rhodolith but are also measuring epiflora.

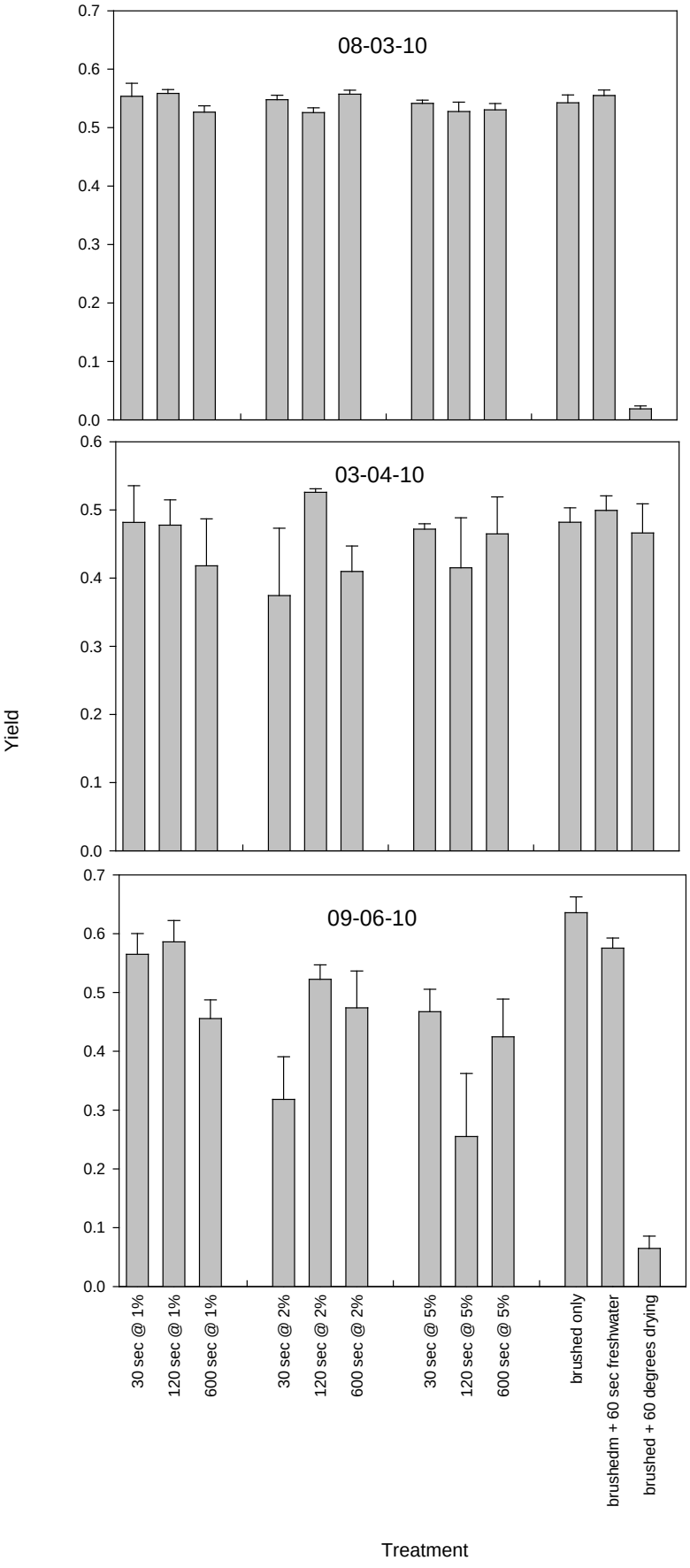

Figure 46: PAM fluorometry yield measurements for the 12 treatments over three measurement periods illustrating variability. Note, not all measurement periods shown.

b. Species specific responses to growth in culture – apparent antifouling effect

After two weeks of exposure of fouling plates to proximity to different amounts of rhodolith biomass there was a statistically significant difference (p equals 0.001) among the treatment groups. Perspex fouling plates showed less fouling (more light transmittance) when exposed to either 50 g or 100 g of *Lithothamnion* compared either the control, or with 50 g or 100 g of *Sporolithon* rhodoliths (Figure 47). This difference was also visually apparent. The difference in the level of fouling between the two species became less apparent with time, although no further measurements were made.

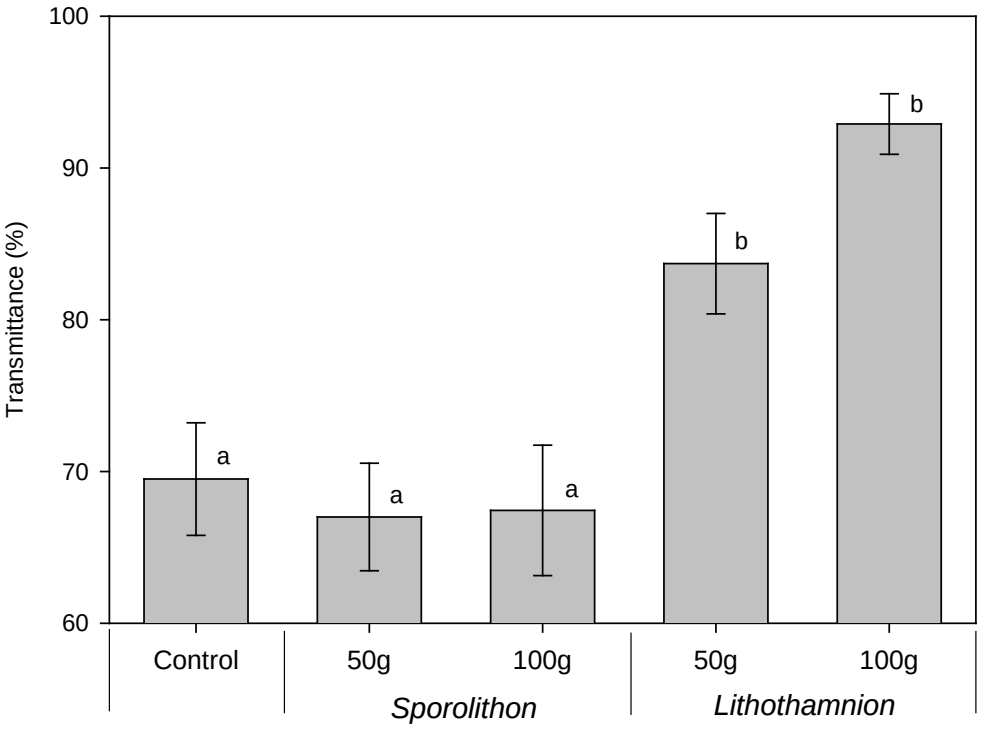

Figure 47: Measure of transmittance (using a spectrophotometer) through transparent perspex fouling plates as a relative measure of diatom fouling. Control = no rhodolith present; 50 g or 100 g *Sporolithon durum*; and 50 g or 100 g *Lithothamnion crispatum*. Bars labelled with the same lower case letter do not differ significantly (P less than 0.05) according to pairwise comparisons.

4. DISCUSSION

This is the first detailed field investigation of subtidal rhodoliths in New Zealand, characterising two species, *Lithothamnion crispatum* and *Sporolithon durum*, in terms of their size and shape, and examining the structure and physical characteristics of rhodolith beds at two locations and documenting their associated biodiversity. It is also the first study investigating the response of New Zealand species of rhodolith-forming non-geniculate corallines to environmental stressors when grown under culture conditions.

Recent research on the non-geniculate coralline algae of northern and central New Zealand has provided a good platform for this study (Harvey et al. 2005; Farr et al. 2009), enabling identification of the rhodolith-forming species in northern New Zealand. The selection of study sites for this current study was aided by field work carried out during 1) an earlier BRAG project on coralline algae of northern New Zealand (ZBD2004/07) when the locations of previously reported rhodolith beds were resampled, and 2) the Bay of Islands Oceans 20/20 Survey Programme in 2009/2010, when collections of *Sporolithon durum* and *Lithothamnion crispatum* (as *L. superpositum*) rhodoliths were

made from five sites within the Bay of Islands (and also from sites on the shelf outside the Bay of Islands).

The species composition of the two beds differed markedly, with respect to the rhodolith species present as well as the associated biota. Water motion and sediment characteristics of the beds were very distinct. The Te Miko Reef rhodolith bed has attributes typical of beds described elsewhere. It is in an area where there is consistent directional water movement, which apparently is not so strong as to damage the rhodoliths or dislodge and transport them to unfavourable habitats or out of the euphotic zone. The water was clear at Te Miko Reef and there was a conspicuous associated flora and macrofauna on the surface of the bed. The Kahuwhera Bay bed, however, is atypical of the majority of rhodolith habitats reported in the literature. It would be possible to overlook the rhodoliths present as the visibility was much lower than at Te Miko Reef and the seafloor was covered in a coating of fine sediments with a substantial proportion of the rhodoliths buried. The associated macrobiota was less conspicuous at Kahuwera Bay than at Te Miko Reef except for the presence of sponges. However the data presented here show that there was high diversity of both macroalgae and invertebrates at both rhodolith beds, but with different species composition.

Sediment is usually regarded as being deleterious for rhodolith growth and health although there are a few published reports of rhodoliths from areas of fine sediments in other parts of the world (e.g., Bosellini & Ginsberg 1971; Bosence 1983b; Perry 2005; Wilson et al. 2011). The key issue for rhodolith survival and health appears to be the amount of sediment transport and mobilisation that occurs and also the amount of water motion – that is, whether there is sufficient water motion to prevent burial and to enable adequate photosynthesis to proceed. Burial can result from sedimentation and also from disturbance resulting from water motion and storm events. Sediment mobilisation as a result of bioturbation has been suggested as playing a part in the turning of individual rhodoliths (Marrack 1999). We have diving observations at the Kahuwhera Bay site from September 2009 (Oceans 20/20 field work), and February and September 2010 (this study), and at these times the rhodoliths were covered by fine sediment. Swales et al. (2010) found that sedimentation rates in the Bay of Islands have increased markedly over the past century associated with land use changes and deforestation, and in particular, sedimentation in the Te Rawhiti Reach has increased over the past several decades. It is not clear if the bed at Kahuwhera Bay is threatened by this increasing sedimentation and the risk of burial or whether the conditions in the bed are well within the thresholds for survival of *Sporolithon* (and the associated biota). Further research on the functioning of the system and on productivity of the rhodoliths would be required to determine the health and state of the beds. It is interesting that the species present subtidally at Kahuwhera Bay, *Sporolithon durum*, which is able to withstand the sediment cover at that site, is also the species found at Te Miko Reef in the brightly lit subtidal bed, and has also been reported growing in the intertidal zone at Whangaparaoa Peninsula (Basso et al. 2009) where it is exposed to bright light conditions and with little sediment present.

The invertebrates collected in this study include a number of new records for this region as well as new collections of infrequently found species. Shallow water species of a number of invertebrate taxa are currently poorly represented in national invertebrate collections. This study has provided specimens from a rarely sampled habitat, such as the ophiuroid *Cryptopelta tarltoni*, previously thought to be uncommon or rare and known only from the type material. The sponges recorded here as *Axinella* n. sp. 9, *Haliclona* n. sp. 14, *Hamigera* n. sp. 2 are only known from the Kahuwhera Bay bed, and *Axinella* cf n. sp. 1 and *Clathria* (*Axosuberites*) n. sp. 1 are previously known only from Three Kings Islands and Northland, respectively. Three species of sponge were recorded in this study for the first time since they were described in 1924, and thus are considered to be rare species. These are *Hymerrhabdia* cf *oxeata* (Dendy, 1924), *Plocamione ornata* (Dendy, 1924), and *Rhaphidhistia mirabilis* (Dendy, 1924). The specimens were small, presumably recent settlements, indicating that the rhodolith beds may be important from a sponge settlement point of view (M. Kelly, NIWA – pers. comm.). The holothurian *Pseudocnus sentus* collected in this study is the first record of this species from the northern North Island, known previously only from southern New Zealand (N. Davey, NIWA – pers. comm.). In addition, specimens collected from Te Miko Reef contributed to a paper revising the amphipod genus *Mallacoota* in New Zealand (Kilgallen & Ahyong 2011). At least three undescribed species of polychaeta not seen elsewhere were amongst the collections made in the rhodolith beds as well as collections that will be valuable for resolving the taxonomy of several

known undescribed, or currently insufficiently investigated taxa yet to be formally reported for New Zealand (G. Read – pers comm).

Of the 2093 invertebrate specimens collected in this study 87.4 % were able to be identified to species level, but there remain taxa which were not able to be identified with certainty because of insufficient taxonomic knowledge of near-shore species or because of a lack of taxonomic experts for some groups (e.g., Isopoda). For example, the Ophiuroid family Amphiuridae needs revising (both morphologically and genetically) to sort out problems both at species and genus level. Species of *Amphiura* are highly morphologically variable and further research is required to confirm identifications (S. Mills, NIWA – pers. comm.).

In the study of soft sediment habitats during the Bay of Islands Oceans 20/20 Survey Programme, rhodolith beds were one of six habitat types distinguished (Hewitt et al. 2010). The rhodolith beds were found to harbour the highest number of infauna individuals and the highest number of taxa (using a finer meshed sieve than in our study). In this study we found significant differences between sites in the number of infauna taxa present but not in the number of individuals (which was highly variable), or in the seasonal presence of species. In this study the sediment characteristics at the two locations were distinct and may drive some of these differences. The site outside the rhodolith bed at Te Miko Reef had the lowest infaunal diversity and highest levels of within-site similarity perhaps because the sediments at this site were the most homogeneous, i.e., more than 90% sand.

Significant differences were found in the epifauna species composition between sites within the rhodolith beds at Te Miko Reef and Kahuwhera Bay, and there was also significant seasonal variation in the epifauna present. In the Kahuwhera Bay rhodolith bed there was a higher proportion of sponges present than at Te Miko Reef sites, as well as echinoderms and polychaetes. Heyward et al. (2010) described sponge gardens of Ningaloo Reef in Western Australia which consisted of sediments and rhodoliths with low densities of macroepibenthos but with locally dense and extensive filter feeding communities dominated by demosponges. Sponges have also been reported associated with rhodolith beds elsewhere, for example, in shallow rhodolith beds in Bahia Magdalena Mexico (Ávila & Riosmena-Rodriguez 2011), in Brazil (Leal et al. 2012) and as dominant taxa in the epibenthos of rhodolith beds in the Maltese Islands along with bryozoa (Sciberras et al. 2009).

The data obtained from the analyses of cryptofauna provide an initial insight into the composition of these communities. Cryptofauna not only use the interstitial spaces between the complex branching of the rhodoliths, but also some species are solely found boring in the coralline matrix of the rhodolith. There are insufficient data currently available from this research to analyse the cryptofauna into different functional or trophic groups or to analyse their use of the habitat space within or in close proximity to the rhodolith. This research has shown a difference in the total mean number of invertebrates per rhodolith, both by species and site. The numbers of cryptofauna individuals found per rhodolith in this study are very high when compared to studies elsewhere. This may be attributable to our recognition of Ciliophora, very small invertebrates which may have been overlooked elsewhere. However, international research on rhodolith cryptofauna communities has revealed considerable variation in terms of the taxa present and also the density of organisms. Steller et al. (2003) found that density of cryptofauna increased with both size and the branching density of the rhodolith. Harvey & Bird (2008) found that the cryptofauna inhabiting rhodoliths with very distinct fruticose and foliose morphologies, belonging to two different species in Western Port (Victoria, Australia) did not differ significantly. In the Gulf of California Hinojosa-Arango & Riosmena-Rodriguez (2004) reported that the cryptofauna of two distinct growth forms of a species of *Lithophyllum* did not differ significantly but in contrast, the cryptofauna of two different species within the same rhodolith bed were significantly different. Harvey & Bird (2008) observed that the number of individuals they found inhabiting the rhodoliths in the Western Port was lower than reported elsewhere in the literature, and that polychaete worms were the main taxon group present. Polychaetes also dominated in the cryptofauna analysed in Western Australia (56%) (Mathis et al. 2005) and also in cryptofauna found in a rhodolith bed in the Gulf of California (Steller et al. 2003). In other rhodolith habitats studied different taxa have been found to dominate (e.g., chitons, Konar et al. 2006). The cryptofauna in the rhodoliths from the Bay of Islands were dominated by polychaete worms and Ciliophora.

In the international literature, there are reports of distinct floras associated with rhodolith beds. In a series of papers on beds of Spain, Peña & Bárbara (e.g., 2008 a, b, 2010a, b) have reported on the diversity of species associated with rhodolith beds. They have observed strong seasonality in the species numbers and composition with more species present in spring/ summer than in autumn/winter, relating this to both photoperiod and temperature. This pattern was not seen in this study, where similar numbers of species (invertebrates and algae) were found in February and September, however, the composition of the algal communities differed significantly. In February a slightly higher proportion of taxa were collected once (47%) than in September (41%). Although our study had a temporal component, we were not able to fully explore seasonal responses within the beds.

The collections of macroalgae made during this study included both new records for New Zealand and Northland, but also new discoveries. A number of samples had crustose brown algae growing on the surface of the rhodoliths. Sequence data and sectioning revealed the presence of members of the Dictyotales which are currently undescribed, as well as the presence of the "*Aglaozonia*" sporophytic (crustose) phase of two species in the genus *Cutleria*, one of which is identical to sequence data for *C. multifida* in GenBank, and the other which matches no sequence data currently available in GenBank. Neither the sporophytic nor gametophytic phase of *C. multifida* has been found previously in the Bay of Islands. Whangarei Harbour has been the most northern location reported previously for this species. In 1980 a collection was made in the Leigh Marine Reserve of a species of *Cutleria* (gametophyte stage), provisionally identified as *C. mollis* Allender & Kraft, a species described from Lord Howe Island (Adams 1994). This species has not been collected subsequently in northern New Zealand. Further research is required to compare the *Aglaozonia* phase collected during this study with sequence data from *C. mollis*.

This study has revealed a number of new species of *Peyssonnelia* spp. *sensu lato*, increasing the known diversity of this order in New Zealand. However, further work is required to understand these species, their distribution and relationships to other species within the order/family. Our findings of members of the Peyssonneliales are consistent with discoveries in rhodolith beds in other parts of the world (e.g., Spain – Peña & Bárbara 2010a; Mediterranean – Ordines & Massuti 2009; Gulf of Mexico – S. Fredericq, University of Louisana, pers. comm.). Along with rhodolith beds, *Peyssonnelia* beds are recognised as also having high biomass and species richness where these have been studied in the western Mediterranean. Peña & Bárbara (2010a) list 23 different crustose seaweeds associated with subtidal rhodolith beds in northwest Spain, including five species of *Peyssonnelia* and also the *Aglaozonia* phase of *Cutleria multifida*. The European Atlantic and the Galician rhodolith beds are understood to be serving as refuges of crustose phases of heteromorphic species, the crustose phases providing "constant populations during unfavourable seasons" and enabling the later development of gametophytic stages (Birkett et al. 1998; Bárbara et al. 2004; Peña & Bárbara 2010a).

Two of the most common species found in the rhodolith beds in this study, the red alga *Chondracanthus chapmanii* and the green alga *Caulerpa flexilis*, appear to play a role in stabilising the rhodoliths or nearby sediments. *Chondracanthus chapmanii* grows amongst the rhodoliths, and attaches to them and to shell debris with rhizoidal pads, effectively consolidating clumps of rhodoliths. In the Bay of Islands Oceans 20/20 study it was observed that "The green alga *Caulerpa flexilis* was recorded by all five collection methods. In soft sediment habitats it stabilises the substrate as it has a stolon, or prostrate stem system, that anchors the alga to the substrate and produces upright branches which provide three dimensional structure for invertebrates and fishes. Several species were very common in assemblages found in soft sediment sites including *Cladhymenia oblongifolia, Gigartina atropurpurea*, and *Sarcodia montagneana*" (Nelson & D'Archino 2010). These latter species were also found within the rhodolith beds in this study. In some rhodolith areas in the northern North Island, *Caulerpa flexilis* has been observed to grow in rows on the seafloor, often along small ridges, with patches of rhodoliths growing in slight depressions between the clumps or lines of *Caulerpa*. Some of these ripple structures were seen by divers at Te Miko Reef. The sampling in this programme did not specifically examine the relationships between rhodoliths and other species that may play a role in structuring or stabilising the substrate.

The flora found in the rhodolith beds in this study includes the kelp *Ecklonia radiata*, typically found as a key species of rocky subtidal reefs in northern New Zealand. The *Ecklonia* plants require a

degree of stability to enable their development, indicating that the rhodoliths are not rolling continuously. The holdfast system of *Ecklonia* is hapteral and this is probably more suited to settlement on rhodolith-sized sediments than a discoidal holdfast by providing multiple points of contact with the substrate rather than a single discoidal pad.

Another group of species reported in association with rhodolith beds are gelatinous or fleshy species (Guimaraes & Amado-Filho 2008, Riul et al. 2009; Hernández-Kantún et al. 2010) for example belonging to the Acrosymphytaceae, Dumontiaceae or Halymeniaceae. Two large red blades belonging to the Halymeniales common in February on Te Miko reef require further attention based on the molecular data we have obtained. The only species of *Halymenia* previously reported from New Zealand has been *Halymenia latifolia* (type locality France). The '*Tsengia*' sp. from the Bay of Islands is not related to specimens of *Tsengia laingii* from the type locality in Brighton, and this newly found species probably represents a new genus. The recent discovery and recognition of *Dudresnaya* in northern New Zealand constitutes a new record for New Zealand both for the genus and the family (Dumontiaceae) (D'Archino & Sutherland submitted). Specimens were found in the rhodolith bed at Kahuwhera Bay in this study, as well as during the Oceans 20/20 field work in the Bay of Islands, and near the Cavalli Islands on cobble substrates.

The sampling protocols followed in this study followed recommended practices in international studies of rhodolith beds and enabled us to sample the biota present under, within and above the rhodolith beds. The collections in late summer and late winter/early spring have enabled us to obtain a snapshot of diversity within the beds. However, it is most unlikely that we have fully sampled the diversity in these beds (based on diversity indices). This study did not provide the scope for a closer examination of patchiness in terms of substrate, shelter /exposure to water motion within the rhodolith beds, and how such features may have an impact on the associated flora and fauna. Macroalgae collected during this study (102 taxa) greatly exceed the number collected during the Oceans 20/20 Survey in which only 40 macroalgal species were recorded across all the habitats defined as soft-sediment habitats, including several rhodolith beds (Nelson & D'Archino 2010). However, this may be a consequence of the fact that the sampling during that programme was focused primarily on invertebrates (Hewitt et al. 2010) while sampling in the current study included targeted macroalgal collection.

In many of the laboratory experiments in this study individual rhodoliths were measured and/or weighed. The experience gained from this study indicates that the variability inherent with the rhodoliths, and the small increments of growth within the experimental measurement intervals result in considerable noise in growth measurements. If further work is undertaken on these species we would recommend using larger quantities of experimental material. Although PAM fluorometry is recommended as a "non-invasive, rapid and repeatable measure of stress levels" in rhodoliths (Wilson et al. 2004) our tests on the impact of epiphytic films on PAM readings indicate that caution is required when interpreting results using this technique. The use of alizarin red staining in this study was relatively limited and we did not explore a range of concentrations or length of exposure to the dye, but rather followed published protocols. We found that the uptake of dye was inconsistent in the species we were studying. It is not clear to us whether this related to the species used in this study, or if the concentrations and length of exposure to the dye need to be modified for better or more consistent results. However, during this study experience of similar inconsistent results with alizarin red staining in *Sporolithon durum* were communicated to us (C. Payri, IRD Noumea – pers. comm.).

In some published studies rhodoliths have been very quickly damaged by burial, particularly when covered by anoxic sediments. Wilson et al. (2004) reported that burial in fine or anoxic sediments was lethal or caused significant stress, with rhodoliths dead within two weeks after exposure. In our study the impacts of burial were not as extreme as this, with burial resulting in a slowing of growth over time. The results of this part of the experimental programme are not surprising at least in the case of *Sporolithon durum*, a species clearly able to survive under the fine sediments at Kahuwhera Bay. It is interesting to note that in the burial experiment PAM readings clearly indicated stress in the sediment-treated rhodoliths, and it was also the experiment where diatom fouling was the least likely to be confounding the results. The results of this part of the study are not conclusive and further work is required to maintain the cultures under constant temperature conditions, and to test the interaction of variables, particularly in relation to photosynthetically available light, and available oxygen.

In the experiment in which rhodoliths were grown with a six-fold range in light intensity, there was no significant response to the light but there was a strong seasonal signal in the growth and condition of the rhodoliths, which we interpret as being a response to the lowered temperature. The light intensities chosen for this experiment were selected to reflect minimum and maximum light experienced by the rhodoliths in the field. Steller et al. (2007a) found in *Lithophyllum margaritae* that lab data "suggested that rhodolith growth is seasonally regulated by seawater temperature". They found buoyant weight was comparable at low temperatures but that at higher temperatures there was increased variability. The strong effects of temperature on photosynthetic, calcification and growth rates that they recorded led them to suggest that sea surface temperatures directly regulate bed production. We examined temperature in combination with the effects of lowered pH predicted to occur as a consequence of climate change and our experiments revealed the importance of temperature in the responses of both species of rhodolith.

Assessment of agents of change within the rhodolith beds

In the Ministry of Fisheries tender document, the incorporation of a qualitative assessment of agents of change within the specific rhodolith habitat and in the wider adjacent environment was requested. Internationally recognised drivers of change in biodiversity and ecosystems for coastal marine areas have been identified by the Millennium Ecosystem Assessment (http:// www.millenniumassessment.org/). In particular, habitat change and pollution (particularly in the form of excess phosphorus and nitrogen), followed by the impacts of invasive species and overexploitation have been identified, and climate change has been assessed as constituting a moderate but rapidly increasing driver of change.

Sediments. The sediment regime in the Bay of Islands was the focus of one of the elements of the Bay of Islands Oceans 20/20 Survey in 2009/2010. Sediment and modelling studies conducted as part of this programme demonstrated the potential significance of sedimentation as "a threat to water quality and benthic habitats in the Bay of Islands" (Morrison et al. 2010). The accumulation of fine-grained sediments in the shallow parts of the bay (less than 20 m depth) is occurring at "rates that are at the upper range of average sediment accumulation rates measured in North Island estuaries and coastal marine environments" (Swales et al. 2010) and the rates have increased in intensity over the past 100 years associated with land-use changes and deforestation. The majority of modern sediment is derived from the inflows to the bay of three main rivers, and then oceanographic processes (waves, tides and currents) re-suspend and disperse this material. The Te Rawhiti Inlet adjacent to the study sites is the largest sedimentary sink in the bay, and it appears that sediment accumulation rates in Te Rawhiti inlet have increased over the past several decades (Swales et al. 2010). As mentioned earlier in this report, the balance between sedimentation and water motion appears to be critical for rhodolith survival. If the sedimentation rate exceeds the re-mobilisation and movement of sediment, rhodoliths will be buried and unable to photosynthesise. Increasing sedimentation potentially poses a threat to rhodolith beds in the Te Rawhiti portion of the Bay of Islands. We do not have data on the fluxes or mobilisation of sediment at either site. Data on currents, tides and quantities of sediment influencing the rhodolith beds (including the variability in sediment depoits and dispersal over time) would enable a more informed evaluation of the potential risk of burial of rhodolith beds, of particular relevance at Kahuwhera Bay and potentially elsewhere in the Bay of Islands.

Nutrient enrichment. Both excess nutrients and anoxia have been identified as risk factors for rhodoliths. Hall-Spencer & Bamber (2007) investigated the effects of salmon farming on benthic diversity and found significant impacts of the farms on the rhodolith beds and associated benthos beneath the fish cages. Recent policies in the United Kingdom have encouraged the siting of sea cage fish farms away from sheltered areas and instead in areas with strong tidal flow in an attempt to reduce the impact of organic enrichment on the benthos beneath the farms. However this has resulted in farms being sited over rhodolith beds. Sanz-Lazaro et al. (2011) examined the impact of particulate wastes on rhodoliths near marine farms, and they recommended the need for "environmental protection agencies to define different aquaculture waste load thresholds for different benthic communities affected by finfish farming, according to their particular degree of sensitivity, in order to maintain natural ecosystem functions". Fish farming affects physico-chemical status of the sediment but also influences trophic functioning of the ecosystem. There is no evidence of eutrophication currently occurring within the Bay of Islands in the near shore region (Morrison et al. 2010) and in the

vicinity of the rhodolith beds there are no current plans for aquaculture developments. However nutrient enrichment impacts on the nearshore coastal region can also result from point sources such as effluent discharges, as well as more dispersed sources, for example resulting from changes in land use or catchment processes (such as development of intensive farming or horticulture resulting in enriched water draining into the coastal zone). The impact of land-derived enrichment can be hard to predict and will be strongly influenced by the receiving waters, their depth and the tidal and current regimes that they experience.

Ocean acidification. As calcified organisms, rhodoliths are known to be at risk from the consequences of rising atmospheric CO_2 and concomitant reductions in the pH of seawater. It is still not clear what the consequences of ocean acidification will be on calcified macroalgae but it is clear that these impacts will be complex and are likely to vary between species (Doney et al. 2009). There will almost certainly be flow on impacts on physiological and ecological fitness, affecting the efficiency of photosynthesis, growth rates, thallus rigidity and competitive ability (summarised in Nelson 2009).

The preliminary experiment we conducted clearly pointed to the potential effects of future ocean acidification and sea-surface warming on the survivability of both species. The lowest overall growth rates for both species were shown at 25° C, being approximately 2° C more than current summer time water temperatures experienced by the Bay of Islands populations. The effect of low pH at temperatures less than 25° C also resulted in lower growth rates (around 0.1 % day^{-1}). While the effect of either of these factors alone may not necessarily be lethal on the two rhodolith species we examined, the combination of low pH and high temperature had a clearly detrimental effect on *Lithophyllum crispatum*, with a negative growth presumably reflecting skeletal dissolution. These results are consistent with at least one other study on coralline algae in which it was concluded that the effect of pH alone may underestimate the long term effect on the survival of these algae (Diaz-Pulido et al. 2012).

Introduced/invasive species. The algae *Codium fragile* ssp. *fragile* and *Hydroclathrus clathratus* are considered to be non-indigenous species (Adams 1994). The genus *Hypnea* is almost certainly introduced in the New Zealand region with several species recorded from the northeastern North Island. The taxonomy of this genus is notoriously difficult and the New Zealand specimens have not been studied in detail. The specimens of *Grateloupia* collected in this study from northern New Zealand include material which differs in its sequence from samples previously collected. A detailed study of native *Grateloupia* species is needed before recognition of introduced species can be distinguished adequately. The genus *Grateloupia* includes a number of species from the north-western Pacific that have become invasive in other countries (e.g., *Grateloupia turuturu*, *G. asiatica* ,Verlaque et al. 2005; D'Archino et al. 2007). The Asian kelp *Undaria pinnatifida* has been introduced to north eastern New Zealand through the movement of infected marine farming equipment. The presence of *Ecklonia* in the rhodolith habitats indicates that *Undaria* would also be able to colonise these sites if introduced to the Bay of Islands. The reproductive output and the ability of *Undaria* to colonise a wide range of habitat types and at a wide range of depths suggests that rhodolith beds may be vulnerable to colonisation by *Undaria*: "Due to its ability to grow in a broad range of environments and to form dense monospecific stands, *U. pinnatifida* has the potential to strongly modify almost all rocky subtidal and intertidal communities in temperate locations" (Russell et al. 2008). *Undaria* would be able to colonise rhodoliths as its extensive and multiple hapteral holdfast system would be able to secure a strong foothold for thalli. It is highly probable that *Undaria* would have a major impact by shading the substrate and altering the light regime for the coralline algae and associated biota.

There were not many introduced or cryptogenic invertebrates found within the beds studied. The most conspicuous was *Chaetopterus chaetopterus* which was found at both KWB sites, and both TMR and TMR_B. This annelid worm is known to live in a variety of subtidal habitats and was first found in abundance in the Auckland region from 1997. Mats of tubes of this species can exclude the settlement of other species such as scallops.

Over exploitation/resource use. There is no history of use of rhodoliths in New Zealand as a source of lime for soils or for other industrial uses as found in both Europe and Brazil. The greatest threats to rhodolith beds in terms of resource use would be in dredging of the seafloor for increased marine

traffic or movement of vessels, or for fishing with gear that drags the bottom and disrupts the three dimensional structure of the beds. Because of the destructive impacts of various types of fishing gear on rhodolith beds the European Union has recently protected various rhodolith beds in the Mediterranean under fishing management regulations (Georgiadis et al. 2009). These authors point out that accurate maps of the location of rhodolith assemblages are essential for the application of the EU fishing regulations and the protection of these marine habitats. Similarly, in Panama acoustic mapping techniques have been used to characterise rhodolith beds, which are recognised there as important fish aggregation and nursery habitats and are being considered for protection under fisheries management plans (Harper et al. 2010). Acoustic mapping works well where the rhodolith cover can be differentiated from background substrates, but there can be difficulties with interpretation particularly distinguishing rhodoliths where they are small or present only in a thin layer, as well as where they are in shallow areas or narrow channels where survey vessels have difficulty operating (e.g., Hall-Spencer et al. 2008a).

The Bay of Islands is closed to commercial trawling and dredging providing some protection to the seafloor. However, recreational scallop dredging is permitted and there are large 'recreational take only' scallop beds in the coastal parts of the bay (Morrison et al. 2010). This type of dredging will be affecting the structure of the seafloor and potentially damaging rhodolith beds and associated biota. Recovery of rhodolith beds after disturbance will be very slow given the extremely slow growth rates recorded worldwide for coralline algae (Steller et al. 2009). In addition damage caused by anchoring of vessels can be significant. There is heavy boat traffic in the Bay of Islands and boat anchors can damage erect sedentary epifauna (e.g., sponges, mussels, tubeworms). The Department of Conservation has set up 'no anchor' areas to protect sensitive locations. Rhodolith beds would qualify for this classification.

5. MANAGEMENT IMPLICATIONS OF THIS STUDY

Identification, assessment and mapping of highly biodiverse marine habitats and ecosystems is a priority of the New Zealand Biodiversity Strategy. This study has documented high biodiversity in two subtidal rhodolith beds. This first study of subtidal rhodoliths in New Zealand targeted documentation and characterisation of biodiversity and was not able to also examine ecosystem functioning. The rhodolith beds studied were found to be more diverse than soft sediment areas outside the beds and it was also found that there are significant differences between beds sited in relatively close proximity (approximately 4 km apart). Given the substantial physical and biological differences between these beds, it is premature to generalise about rhodoliths in New Zealand and how rhodoliths may respond to stressors. The rhodolith beds that we have focused on are in the coastal zone. There are also rhodoliths found in the intertidal zone and also reported from greater depths offshore (e.g., from Oceans 20/20 Bay of Islands survey, Biogenic Reef sampling TAN1105, TAN1108). We do not know, for example, if beds in other parts of the country or in different habitat types are also characterised by unique species complexes and high diversity, and we do not know how differing beds and/or species will respond to the threats that are well-documented internationally but not tested in New Zealand. Information about the locations of rhodolith beds would provide valuable information for resource managers planning for multiple use of marine areas, for example, indicating sites where aquaculture developments or trawling activities would potentially be damaging to habitats harbouring high biodiversity.

Internationally rhodolith beds are generally regarded as fragile habitats that harbour high biodiversity. There is a strong consensus about the importance of protecting rhodolith beds, in particular from the impacts of sedimentation, eutrophication, physical disturbance and ocean acidification and these are all potential threats in the New Zealand context. There are various initiatives in different countries to protect these assemblages – the rhodolith-forming species and their associated biota. There is overwhelming evidence in the international literature that rhodoliths are sensitive to aquaculture impacts (involving eutrophication, sedimentation and physical disruption) with many studies emphasising the need for care with site selection for aquaculture facilities in the vicinity of rhodolith beds (e.g., Hall-Spencer et al. 2006; Peña & Bárbara 2009; Aguado-Giménez & Ruiz-Fernández 2012). The potential impacts of commercial developments such as mineral extraction, as well as fishing methods that affect the seafloor, need to be considered in the light of the contribution that

these beds may make to community functioning as well as the role the deeper beds may play as carbon sinks (Amado-Filho et al. 2012). Studies elsewhere are pointing to the probable impacts of ocean acidification on benthic community structure in shallow water carbonate ecosystems such as rhodolith beds (e.g., Kuffner et al. 2008). This study shows that there are species-specific responses in two New Zealand rhodoliths to changing pH and temperature conditions. There is a need to obtain more data about the vulnerability of New Zealand coralline algae to human-induced climate change.

One possible initiative would be the establishment of a reporting system to encourage the identification of locations where rhodoliths are sited. This would provide a valuable first step enabling potentially vulnerable sites to be mapped and documented in coastal planning documents, which would in turn assist with decisions on the appropriate use of specific coastal areas. This could potentially enable, for example, the establishment of no-anchor zones, as well as areas with a prohibition on activities that would disrupt these assemblages (whether recreational or commercial fishing, or other extractive activities, and whether at sites close to the coast or on the shelf). Incorporation of information about the location of rhodolith beds in planning documents would enable more informed decisions about uses of the marine environment. More information is needed about rhodolith beds in order to set standards for monitoring in order to measure natural variability as well as human-induced changes (including cumulative impacts of multiple uses). A greater understanding of the ecosystem functions played by these beds and their contributions to the health of nearshore systems would also provide a stronger basis for coastal management and conservation, and the protection of biodiversity.

Knowledge gaps

Distribution and mapping. Rhodoliths in New Zealand have been poorly documented to date and knowledge of the location of beds around the country remains very incomplete. Of the four rhodolith forming species reported from New Zealand, two species (*Lithothamnion proliferum* and *Lithophyllum* sp.) are known from single specimens, indicating that more targeted collections, documentation of the distribution of species and mapping of thodolith beds are required (Farr et al. 2009). The international literature strongly endorses the need for information on the distribution and attributes of the beds in order to manage both the specific habitats and species effectively There is guidance on methodologies for mapping and sampling beds (e.g., Steller et al. 2007b; Hall-Spencer et al. 2008a; Sciberras et al. 2009; Peña & Bárbara 2010b) with authors stressing the need for these to be done in a rigorous and comparable way.

Documentation and mapping rhodolith beds throughout the New Zealand would be an important step towards possible protection of the rhodolith species and their associated biodiverse assemblages. This would be consistent with international initiatives over the past decade as recognition of the importance of rhodolith beds has increased.

Diversity. This study has provided preliminary data on the diversity associated with two different rhodolith-forming non-geniculate corallines in the Bay of Islands. The list of associated species found during this study is impressive but is almost certainly considerably underestimating the range of associated species. Steller et al. (2007b) emphasise the need for critical monographic work to understand species present in rhodolith beds, and also the need to use a sampling method that partitions organisms by sub-habitats (i.e., cryptofauna, infauna, epifauna). It is clear from this study that the rhodolith beds support a diverse fauna and flora that is distinct from the surrounding 'bare' sediments; one of the arguments used to support their conservation as a unique habitat type. More intra-annual (seasonal) and inter-annual sampling is required to get a fuller picture and to address hypotheses about rarity and richness in these assemblages. Sciberras et al. (2009) state "for effective conservation management of rhodolith beds, in-depth studies on the distribution, biotic diversity and community structure of maërl beds are required" considering not only their spatial extent, and physical characteristics but also the taxonomic diversity within beds. Steller et al. (2007b) describe a basic methodology for surveying beds with one of the key steps to determine a sampling regime that "provides accurate taxonomic, distributional and diversity estimates". Hall-Spencer et al. (2008a) observed that rhodolith beds vary greatly in their composition and also in the range of threats they face. In order to monitor rhodolith beds and evaluate changes taking place within particular rhodolith beds they consider it is important to identify particular species known to be largely confined to rhodolith beds and which appear to be sensitive to disturbance. This requires a detailed understanding

of the particular beds and the associated biota. It is not known how the diversity and species composition of these beds in the Bay of Islands will compare with beds elsewhere in New Zealand (e.g., Kapiti Island, Marlborough Sounds, Fiordland, Foveaux Strait).

Functional and trophic relationships. Functional or trophic relationships within the assemblages were not investigated in this study. The role of associated biota in creating three dimensional habitat space needs to be examined in the light of research by Foster et al. (2007) on the relative contribution of rhodoliths to macroorganism diversity in a site also occupied by the large fucalean brown alga *Sargassum* (comparable to species that were recorded in our study). They argue for the need to expand frameworks for designing and planning for marine protected areas to include communities where there are multiple foundation species such as rhodolith beds which are also inhabited by large brown algae.

There has been no research done in New Zealand within these rhodolith beds on community structure in relation to food webs (see for comparison Grall et al. 2006) or on fluxes of carbon and nutrients (see for comparison Martin et al. 2007 a, b). Such research on the physiology of rhodolith-forming species and energy flows within the beds would provide valuable insights into the contribution these assemblages make to nearshore systems. Examination of calcification processes, the physiological responses of species to stress, for example in terms of internal pools of carbohydrates and pigments, would provide very useful data for understanding and interpreting the resilience/vulnerability of these species. We consider that we have obtained evidence of an anti-fouling response by *Lithothamnion crispatum* and that this warrants further attention particularly in terms of interactions within beds and assemblages and potential impacts on recruitment and settlement of associated biota within beds.

Mobility/stability/turnover. We have no data concerning the mobility of rhodoliths within beds over different time frames nor any information about the longevity of particular rhodolith beds, although in 2006 we sampled a rhodolith bed near Urupukapuka Island that had been identified by Hayward et al. (1981) and was still present 25 years later. A number of questions remain to be explored, for example, identifying the key drivers of stability/instability, the role of storms and the magnitude of disruption these cause, rates and impacts of sedimentation, whether bioturbation plays any role in New Zealand rhodolith beds. We have observed rhodoliths in ripple formations (which can be seen by aerial photography) where the rhodoliths are between ridges stabilised by *Caulerpa flexilis*, apparently providing shelter and longer term stability in areas of strong water motion. The role of such associated macroalgae, as well as various crustose algal species, and mat-forming invertebrates has not yet been explored.

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Appendices:

1. Field locations and sites in the Bay of Islands
2. Invertebrates collected from Kahuwhera Bay and Te Miko Reef
3. Macroalgae collected from Kahuwhera Bay and Te Miko Reef
4. Fish collected from Kahuwhera Bay and Te Miko Reef
5. Pairwise comparisons for PERMANOVA that showed significant differences between site and/or season

Appendix 1. Bay of Islands field locations and sites

Location	Site	Lat	Long	Depths sampled (m)	Observations
Te Miko Reef	TMR	35.22885 S	174.18349 E	7.7 – 11.5	Sandy, dead and live bivalves, flatworms, *Luidia*, Leatherjacket
	TMR_B	35.23237 S	174.18152 E	8 – 11.5	Sandy, *Dictyota*, live and dead bivalves, Octopus, Leatherjackets, *Halopteris*, *Zonaria*, *Caulerpa* and *Ecklonia*
	TMR_OUT	35.23280 S	174.17883 E	8 – 11.5	Broken shells, coarse sand, polychaetes, gurnard, stingray
Kahuwhera Bay	KWB	35.26265 S	174.18180 E	5.4 – 9.3	*Colpomenia* and *Chondracanthus*, wormtubes, muddy, abundant juvenile snapper, also spotties and triplefins around *Ecklonia*
	KWB_OUT	35.25924 S	174.18460 E	6.4 – 11.7	Horse mussels, dead gastropods and bivalves, seastars, hermit crabs, kina, filamentous algae

Appendix 2. Invertebrates collected from Kahuwhera Bay and Te Miko Reef (open circles = Feb, closed circles = September)

Taxa	KWB	KWB_ OUT	TMR	TMR_B	TMR_ OUT
Annelida					
Polychaeta			●	●	●
Capitellidae					
Notomastus sp.	●			●	
Notomastus unknown				○	
Maldanidae					
Macroclymenella stewartensis					○
Maldane theodori		○●			
Nicomache sp.	●				
Opheliidae					
Armandia maculata	○●			●	
Eunicida					
Dorvilleidae					
Dorvillea australiensis	○		●	○●	
Eunicidae					
Eunice ?australis		○			
Eunice australis	●		●		
Eunice cf. *vittata*	●	●	●	●	
Eunice sp.	○	○	○	○	
Marphysa unibranchiata				○	
Lumbrineridae			○●		
Onuphidae					●
Anchinothria unknown					○
Phyllodocida					
Glyceridae					
Glycera ovigera				●	
Glycera unknown				○	
Hemipodus simplex			●	●	
Hesionidae					
Ophiodromus angustifrons	○●	○●	○●	○●	
Nereididae					
Nereis falcaria	○●	●	●	●	
Nereis nereis-C			●		
Nereis sp.			●		
Nereis unknown			○	○	
Platynereis australis	●		○●	○	○
Phyllodocidae					
Eulalia Eulalia-NIWA-2			●		
Pterocirrus brevicornis	○		○		
Pisionidae					
Pisione oerstedii			○		

Polynoidae				●	
Harmothoe harmothoe-E	○				
Harmothoe macrolepidota	○				○
Harmothoe sp.			●		
Lepidonotus polychromus				●	
Lepidonotus sp.	●				
Syllidae			●		●
Eusyllis unknown			○		
Odontosyllis polycera	●				
Sabellida					
Oweniidae					
Owenia petersenae	○●	○●		○●	
Sabellidae					
Branchiomma curtum	○	○	○	○●	
Euchone pallida	○●	○●			
Pseudobranchiomma grandis	○				
Serpulidae			●	●	
Galeolaria hystrix			●		
Hydroides elegans			●		
Salmacina australis				○	
Serpula unknown					○
Spionida					
Chaetopteridae				●	
?*Spiochaetopterus* unknown		○			
Chaetopterus chaetopterus-A	○●	○	●	○	
Phyllochaetopterus sp.		●			
Spionidae					
Boccardia syrtis	●				
Polydora [indet]			●		
Polydora hoplura		●			
Prionospio multicristata	●				
Pseudopolydora paucibranchiata		○			
Terebellida					
Ampharetidae					
Amphicteis amphicteis-A		○●			
Cirratulidae					
Protocirrineris nuchalis	●				
Timarete anchylochaetus	●			○	
Flabelligeridae					
cf. *Brada* sp.	●				
Flabelligera affinis	○●		○		
Pherusa ?*parmata*	○				
Terebellidae			●	●	
?*Terebella* unknown		○		○	
Eupolymnia eupolymnia-A		●		●	

Lanice sp.			●		
Nicolea armilla	○		●	○	
Trichobranchidae					
Terebellides narribri	●			○	
Arthropoda					
Ostracoda				○	
Insecta					
Trichoptera					
Chathamiidae					
Philanisus plebeius					○
Malacostraca					
Amphipoda	○●	○	●		
?Ischyroceridae				●	
?Maeridae					
?? *Maera incerta*				○	
Amaryllididae					
Amaryllis c.f. *macrophthalma*		○	○		
Amaryllis sp. (nov?)	●		●		
Ampeliscidae					
Ampelisca chiltoni	○●	○●			
Ampithoidae					
Paragrubia sp.			○		○
Aoridae					
Aora c.f. *maculata*			○		
Aora sp. (aff. *maculata*)			●	●	
Caprellidae					
Caprella sp.			○		
Caprellina longicollis			●		
Ceinidae					
Ceina egregia			○		
Taihape karori			●		
Corophiidae					
Haplocheiridae lendenfeldi			○		
Dexaminidae					
Paradexamine houtete				○	○
Paradexamine pacifica			●	●	
Eusiridae					
? *Gondogeneia* sp. A					○
? *Gondogeneia* sp. B					○
Apherusa translucens				●	
Eusiroides monoculoides			○●		○
Eusirus sp.	●				
Gondogeneia sp. B					○
Oradarea novaezealandia			●	●	
Oradarea novaezealandia?		●	●		

Eusiridae ?					○
Isaeidae					
?*Gammaropsis* sp.	●			●	
Gammaropsis sp.				●	
Ischyroceridae	●				
Leucothoidae					
Leucothoe trailli		○			
Liljeborgidae					
Liljeborgia aequabilis	○	○			
Liljeborgia sp.				●	
Melitidae					
? *Melita* sp.	○				
Elasmopus wahine			●		
Maera mastersi	○●		●	○	
Mallacoota sp.			○	○	
Mallacoota petriei			●	●	
Melita awa	●				
Melita festiva	●			●	
Melita inaequistylis			○●	○	
Melphidippidae					
Hornellia sp.				●	
Podoceridae					
Podocerus c.f. karu			○		
Podocerus manawatu			●	●	
Podocerus sp.			○		
Decapoda					
Alpheidae					
Alpheus novaezealandiae	○●	●		●	
Alpheus socialis		○			
Diogenidae					
Paguristes setosus		○			
Dromiidae					
Metadromia wilsoni			○		
Hippolytidae					
Hippolyte bifidirostris				○	
Hymenosomatidae					
Elamena longirostris			○		○
Elamena producta			●	○	
Halicarcinus cookii	○●	○●	○●	○●	○
Halicarcinus sp.	○				
Majidae					
Leptomithrax longipes					○
Notomithrax minor	●	●		●	
Notomithrax peronii	○	○		○	○
Notomithrax sp.		●	○	●	

Paguridae			●	●	○
Diacanthurus spinulimanus					○
Lophopagurus (Australeremus) laurentae			○●	○	
Lophopagurus pumilus	○●			○	
Lophopagurus sp. 1				○	
Paguristes subpilosus				●	
Pagurixus hectori				●	
Pagurus ?*traversi*				○	
Pagurus traversi		●	○	○	○
Palaemonidae					
Periclimenes yaldwyni	●	●			
Porcellanidae					
Petrolisthes novaezelandiae	○●	○●	○	○	
Portunidae					
Liocarcinus corrugatus			●	○●	○
Isopoda	○		○	○	
Arcturidae					
Arcturidae sp. 1	○		●	○	○
Cirolanidae			●		
Gnathiidae				●	
Liljeborgidae	○				
Sphaeromatidae					
Cilicaea sp	○●	●		○	
Cymodoce sp.	●				
Stenetriidae					
Stenetrium fractum				○	
Stenetrium sp.	●		●	○	
Mysida		○			
Tanaidacea				●	
Maxillopoda					
Sessilia					
Archaeobalanidae					
Striatobalanus cf. *amaryilis*				○●	
Balanidae					
Balanidae	○		●		
Balanus trigonus	○●		○●		●
Brachiopoda					
Rhynchonellata					
Terebratulida					
Terebratellidae					
Calloria inconspicua				●	
Bryozoa					
Gymnolaemata					
Cheilostomata					
Bitectiporidae					

Schizosmittina cinctipora				●	
Celleporidae					
Celleporina hemiperistomata			○		
Celleporina sinuata			○●	●	
Lagenipora sp.				○	
Chaperiidae					
Chaperia n. sp.				●	
Chaperiopsis cervicornis				○●	
Cribrilinidae					
Figularia carinata	○●				
Lacernidae					
Rogicka biserialis	●				
Microporellidae					
Fenestrulina disjuncta		●		●	
Microporella speculum		●			
Phidoloporidae					
Rhynchozoon zealandicum			●	●	
Romancheinidae					
Escharoides angela			○●	○●	○●
Smittinidae					
Parasmittina delicatula	○●				
Steginoporellidae					
Steginoporella magnifica	○			○●	
Stenolaemata					
Cyclostomata					
Diaperoeciidae					
Diaperoecia purpurascens				●	
Diastoporidae					
Microeciella cf. *ridleyi*			○●		
Tubuliporidae					
Tubulipora sp.			○		
Cnidaria					
Anthozoa					
Actiniaria	●			○	○
Nyantheae	○●			●	
Hydrozoa					
Leptothecata					
Sertulariidae					
Amphisbetia minita			●		
Echinodermata					
Asteroidea					
Forcipulatida					
Asteriidae					
Astrostole scabra	○				
Coscinasterias muricata	○●	○			

Paxillosida					
Astropectinidae					
Astropecten polyacanthus					●
Valvatida					
Asterinidae					
Patiriella mortenseni	○●	○●		●	
Patiriella regularis	○				
Echinoidea					
Echinoida					
Echinometridae					
Evechinus chloroticus	○				
Temnopleuroida					
Temnopleuridae					
Amblypneustes elevatus	●		○●	●	
Holothuroidea	○●	○●	○		○
Apodida					
Chiridotidae					
Taeniogyrus dendyi	○●	●			
Taeniogyrus dunedinensis	●		●		
Dendrochirotida					
Cucumariidae					
Kolostoneura novaezealandiae	●	●			
Neocucumella bicolumnata			●	○●	
Plesiocolochirus ignava	○●	●	●	○●	
Pseudocnus sentus	○●				
Ophiuroidea					
Ophiurida					
Amphiuridae					
Amphipholis squamata	○●		○	○●	
Amphiura ?*alba*	○●				
Amphiura amokurae	○●				
Amphiura sp. cf. *alba/annulifera*	○				
Amphiura sp. cf. *alba/constricta*	○●	●			
Amphiura sp.cf. psilopora				●	
Amphiura spinipes	○●	○	○	○	
Ophiactidae					
Ophiactis resiliens	○		○●		
Ophiodermatidae					
Cryptopelta tarltoni	○●	○			
Ophiopeza cylindrica	○●				
Hemichordata					
Enteropneusta					
Enteropneusta					
Harrimaniidae					
Saccoglossus otagoensis	●		●	●	

Saccoglossus unknown	○		○		
Mollusca					
Bivalvia					
Arcoida					
Glycymerididae					
Glycymeris modesta			●		
Tucetona laticostata			○●	○●	
Myoida					
Corbulidae					
Corbula zelandica		○	○●	○●	
Hiatellidae					
Hiatella arctica			○		
Mytiloida					
Mytilidae					
Modiolus areolatus				○	
Nuculoida					
Nuculidae					
Linucula hartvigiana	○	○			
Pterioida					
Limidae					
Limaria orientalis	●	○●		○●	
Limatula maoria			○●	○●	
Pectinidae					
Talochlamys zelandiae	○●	●	○●	○●	
Tellinidae					
Macomona liliana		○			
Veneroida					
Carditidae					
Purpurocardia purpurata				○●	
Psammobiidae					
Gari stangeri		○		●	
Semelidae					
Leptomya retiara		○●			
Ungulinidae					
Felaniella zelandica		○●		●	
Veneridae					
Dosina crebra	○●				
Ruditapes largillierti	○●				
Tawera spissa			○●	●	○●
Gastropoda					
Nudibranchia	●	○		●	
Discodorididae					
Alloiodoris lanuginata	○				
Doridiidae					
Aphelodoris luctuosa	○				

Umbraculida					
Umbraculidae					
Umbraculum umbraculum			○	○●	
Trochidae					
Cantharidus cf *rufozona*			●		
Cantharidus purpureus			○●	○●	○
Coelotrochus tiaratus				○	
Turbinidae					
Cookia sulcata				○●	○
Modelia granosa	●		○●	○●	
Cephalaspidea					
Bullidae					
Bulla c.f. *quoyii*			○		
Bulla quoyii	○●		○	○●	
Littorinimorpha					
Calyptraeidae					
Maoricrypta costata			○●	○●	
Sigapatella novaezelandiae	○		○●		
Sigapatella spadicea			●		
Neogastropoda					
Buccinidae					
Cominella quoyana	○		○●	○●	
Penion sulcata	○				
Polyplacophora					
Acanthochitonina					
Acanthochitonidae					
Acanthochitona zelandica	○●		○●	○●	
Notoplax rubiginosa				●	
Pseudotonicia cuneata	●				
Ischnochitonina					
Chitonidae					
Onithochiton neglectus	○	●	●	○●	
Rhyssoplax aerea	○				
Rhyssoplax sp.			○		
Rhyssoplax stangeri	○	●	○●	○●	●
Ischnochitonidae					
Callochiton crocinus		○			
Ischnochiton maorianus	○●	●	○	○●	
Lepidopleurina					
Leptochitonidae					
Leptochiton inquinatus	○●	○●	●	●	○
Nemertea	○		○		
Phoronida					
Phoronis psammophila	●	○●			
Platyhelminthes		○	●		

Rhabditophora					
Polycladida	○		○		
Porifera	●	○			
Demospongiae					
Dendroceratida					
Dictyodendrillidae					
Dictyodendrilla dendyi	○				
Hadromerida					
Suberitidae					
Aaptos globosa	○				
Trachycladidae					
Rhaphidhistia mirabilis	●				
Halichondrida					
Axinellidae					
Axinella cf n. sp. 1	●				
Axinella n. sp. 9	●				
Bubaridae					
Hymerrhabdia cf *oxeata*	○●				
Halichondriidae					
Halichondria panicea		●			
Hymeniacidon hauraki	○●				
Haplosclerida					
Chalinidae					
Haliclona (Gellius) fragilis	●				
Haliclona n. sp. 14	○●				
Poecilosclerida					
Hymedesmiidae					
Hamigera n. sp. 2	○				
Microcionidae					
Antho (Acarnia) novaezelanica	○●				
Clathria (Axosuberites) n. sp. 1	○				
Plocamione ornata	○●				
Sipuncula			○	●	
Tunicata					
Ascidiacea					
Enterogona					
Ascidiidae					
Ascidiella aspersa		○			
Corellidae					
Corella n.sp.					○
Didemnidae					
Didemnum sp.	●				
Leptoclinides sp.				○	
Pleurogona					
Styelidae					

Asterocarpa coerulea	○				
Botrylloides leachii	○				
Cnemidocarpa bicornuta		○			

Appendix 3. Macroalgae collected from Kahuwhera Bay and Te Miko Reef

Taxa	KWB	KWB_OUT	TMR	TMR_B	TMR_OUT
Green algae (Chlorophyta)					
Bryopsidales					
Caulerpaceae					
Caulerpa flexilis	○		○●	○●	○●
Codiaceae					
Codium cranwelliae				○	
Codium fragile ssp. *fragile*	○	○			○
Codium gracile				○	
Codium sp.					○
Derbesiaceae					
Derbesia novae-zelandiae				○	
Cladophorales					
Cladophoraceae					
Chaetomorpha sp.			○●		
Cladophora feredayi		●			
Cladophora herpestica		○		○●	
Cladophora sericea	●				
Cladophora sp.			○	○	○
Ulvales					
Ulvaceae					
Ulva sp.		●	○		○●
Brown algae (Heterokontophyta)					
Dictyotales					
Dictyotaceae					
Dictyota 1	○				
Dictyota 2			●	○	●
Dictyota 3			●		
Dictyota crust			●	○●	○
Dictyota papenfussii				○●	○●
Dictyota sp.				●	
Distromium skottsbergii				○	
Zonaria turneriana			○●	○●	
Ectocarpales					
Scytosiphonaceae					
Colpomenia claytoniae			○		
Colpomenia ecuticulata	○●				
Colpomenia sinuosa	●	●			○
Colpomenia sp.	○●			○	
Hydroclathrus clathratus		○●	○●	●	
Fucales					
Sargassaceae					
Carpophyllum angustifolium				○	●
Carpophyllum flexuosum	○●				
Carpophyllum maschalocarpum	○●		○●	○	

Sargassum sinclairii		○	○●	○●	○
Xiphophoraceae					
Xiphophora chondrophylla			●		●
Laminariales					
Lessoniaceae					
Ecklonia radiata	○●	○	○●	○	○●
Sphacelariales					
Stypocaulaceae					
Halopteris paniculata				○●	○●
Halopteris sp.			○	●	○
Sporochnales					
Sporochnaceae					
Carpomitra costata				○	
Tilopteridales					
Cutleriaceae					
Cutleria multifida	○●		●	○●	
Cutleria sp. 2				●	
Red algae (Rhodophyta)					
Bonnemaisoniales					
Bonnemaisoniaceae					
Asparagopsis armata	○				○
Delisea compressa					○
Ceramiales					
Callithamniaceae					
Callithamnion colensoi		●		●	
Callithamnion sp.					●
Ceramiaceae					
Antithamnionella adnata					●
Ceramium sp.				○	
Pterothamnion lindaueri					○●
Delesseriaceae					
Hymenena sp.	●				
Hymenena variolosa				●	●
Laingia sp.					●
Schizoseris sp.		●		○	○
Rhodomelaceae					
Aphanocladia delicatula			○		
Chondria sp.			○		
Cladhymenia lyallii			●		
Cladhymenia oblongifolia			○	○	○
Laurencia distichophylla		○	○	○	
Laurencia thyrsifera	○●		○●		●
Pleurostichidium falkenbergii			●		
Vidalia colensoi					○●
Wrangeliaceae					
Anotrichium crinitum			●		

Griffithsia sp.					○
Corallinales					
Corallinaceae					
Corallina officinalis	○	○●	○●	○●	○●
Corallina sp.			●		
Jania sp.				●	
Jania verrucosa			○●		
Lithophyllum pustulatum			○		
Pneophyllum fragile	○		○		
Hapalidaceae					
Lithothamnion crispatum			○●	○●	
Mesophyllum sp.	●				
Sporolithales					
Sporolithaceae					
Sporolithon durum	○●			○●	
non-geniculate coralline	○●	○●	○●	●	
Erythropeltidiales					
Erythrotrichiaceae					
Erythrocladia sp.					○
Gelidiales					
Gelidiaceae					
Gelidium longipes		●			
Gelidium sp.		○			
Pterocladia lucida			○	○	○●
Gigartinales					
Dumontiaceae					
Dudresnaya capricornica	○				
Gigartinaceae					
Chondracanthus chapmanii	○●	●	○●	○●	○●
'Gigartina' atropurpurea	○●		○●	○●	○●
Gloiosiphonaceae					
Hypnea sp.			○		○
Kallymeniaceae					
Psaromenia berggrenii				○	
Phyllophoraceae					
Stenogramma interruptum			○		
Halymeniales					
Halymeniaceae					
Aeodes nitidissima	○		○	○	○
Grateloupia sp.	○●		●		
Grateloupia urvilleana			○		
'Halymenia' sp.	○		○	○	
Tsengiaceae					
Tsengia feredayae				○	
'*Tsengia*' sp.	●		○	○	○
Nemaliales					

Liagoraceae					
Liagora harveyana		○			
Scinaiaceae					
Scinaia australis			○	○	○
Scinaia berggrenii	●	○			
Scinaia firma				○	
Peyssonneliales					
Peyssonneliaceae					
Peyssonnelia boudouresquei	●	○		○	
Peyssonnelia sp.	●	●	●		●
Peyssonnelia sp. 1	○●		○●	○	
Peyssonnelia sp. 2			○		
Peyssonnelia sp. 2?	●				
Peyssonnelia sp. 3			○		
Peyssonnelia sp. 4			○●		
Peyssonnelia sp. 5	●				
undetermined red crust			●	●	
Plocamiales					
Plocamiaceae					
Plocamium angustum				●	○
Plocamium cirrhosum			●	●	●
Plocamium sp.			●		○
Sarcodiaceae					
Sarcodia montagneana	○●	●	●	○●	●
Rhodymeniales					
Champiaceae					
Champia laingii			●		○
Lomentariaceae					
Lomentaria sp.			●		
Lomentaria umbellata			●		
Rhodymeniaceae					
Rhodymenia sp.		●			
Plantae					
Alismatales					
Zosteraceae					
Zostera muelleri ssp. *capricorni*					●

Appendix 4. Fish collected from Kahuwhera Bay and Te Miko Reef

Taxa	KWB	KWB_ OUT	TMR	TMR_B	TMR_ OUT
Chordata					
Actinopterygii					
Gobiesociformes					
Gobiesocidae				○	
Trachelochismus melobesia	○	○	○●	○●	
Trachelochismus sp.	○				
Perciformes					
Clinidae					
Cristiceps aurantiacus					○
Plesiopidae					
Acanthoclinus sp.				○	
Leptocardii					
Amphioxiformes					
Epigonichthyidae					
Epigonichthys hectori			●		

Appendix 5. Pairwise comparisons for PERMANOVA that showed significant differences between site and/or season.

INVERTEBRATES:
INFAUNA:
Univariate
Cores – Number of taxa

Pairwise Groups	t	P(perm)	Unique perms
KWB, KWB_OUT	1.2107	0.248	999
KWB, TMR_B	0.26378	0.831	996
KWB, TMR	0.87889	0.428	996
KWB, TMR_OUT	1.8902	0.045	998
KWB_OUT, TMR_B	1.4557	0.158	880
KWB_OUT, TMR	2.9425	0.013	994
KWB_OUT, TMR_OUT	4.825	0.002	995
TMR_B, TMR	2.0406	0.052	994
TMR_B, TMR_OUT	4.8343	0.001	996
TMR, TMR_OUT	2.0432	0.06	996

Cores -Evenness
Term 'SixSe' for pairs of levels of factor 'Site'

Within level 'Feb' of factor 'Season'

Groups	t	P(perm)	Unique perms
KWB, KWB_OUT	2.6448	0.011	121
KWB, TMR_B	2.9707	0.013	121
KWB, TMR	1.2662	0.281	32
KWB, TMR_OUT	1.8906	0.016	90
KWB_OUT, TMR_B	0.64105	0.537	304
KWB_OUT, TMR	1.3895	0.179	116
KWB_OUT, TMR_OUT	0.21425	0.853	313
TMR_B, TMR	1.0654	0.374	47
TMR_B, TMR_OUT	0.65011	0.688	170
TMR, TMR_OUT	1.1399	0.304	65

Within level 'Sep' of factor 'Season'

Groups	t	P(perm)	Unique perms
KWB, KWB_OUT	1.0022	0.374	62
KWB, TMR_B	4.7898E-2	1	16
KWB, TMR	0.27079	0.923	12
KWB, TMR_OUT	4.7857	0.002	86
KWB_OUT, TMR_B	1.1499	0.282	112
KWB_OUT, TMR	1.6745	0.115	115
KWB_OUT, TMR_OUT	4.6124	0.004	304
TMR_B, TMR	0.24148	0.837	32
TMR_B, TMR_OUT	4.8262	0.002	148
TMR, TMR_OUT	4.4667	0.013	41

Cores - PERMDISP on within site Bray-Curtis similarities
DEVIATIONS FROM CENTROID
F: 10.637 df1: 4 df2: 54
P(perm): 0.001

PAIRWISE COMPARISONS

Groups	t	P(perm)

(KWB,KWB_OUT)	4.567	2E-3
(KWB,TMR_B)	2.7635	1.3E-2
(KWB,TMR)	1.3765	0.256
(KWB,TMR_OUT)	8.1938	1E-3
(KWB_OUT,TMR_B)	0.87746	0.464
(KWB_OUT,TMR)	2.2527	6.5E-2
(KWB_OUT,TMR_OUT)	2.3426	3.1E-2
(TMR_B,TMR)	1.1549	0.371
(TMR_B,TMR_OUT)	2.907	1.7E-2
(TMR,TMR_OUT)	4.5791	1E-3

Cores – Shannon-Weiner

PAIR-WISE TESTS

Term 'Si'

Groups	t	Unique P(perm)	perms
KWB, KWB_OUT	1.7494	0.092	998
KWB, TMR_B	1.04	0.284	997
KWB, TMR	0.60401	0.576	994
KWB, TMR_OUT	3.145	0.006	998
KWB_OUT, TMR_B	0.85102	0.386	998
KWB_OUT, TMR	2.3864	0.029	996
KWB_OUT, TMR_OUT	6.1904	0.001	995
TMR_B, TMR	1.702	0.101	997
TMR_B, TMR_OUT	5.3615	0.001	998
TMR, TMR_OUT	2.4317	0.035	995

Cores - Simpson

PAIR-WISE TESTS

Term 'SixSe' for pairs of levels of factor 'Site'

Within level 'Feb' of factor 'Season'

Groups	t	Unique P(perm)	perms
KWB, KWB_OUT	2.5371	0.015	105
KWB, TMR_B	3.2043	0.012	119
KWB, TMR	1.6114	0.156	32
KWB, TMR_OUT	2.7422	0.012	80
KWB_OUT, TMR_B	0.48531	0.617	180
KWB_OUT, TMR	0.47981	0.623	97
KWB_OUT, TMR_OUT	0.75992	0.473	231
TMR_B, TMR	0.93648	0.426	47
TMR_B, TMR_OUT	0.37192	0.727	114
TMR, TMR_OUT	1.1179	0.319	42

Within level 'Sep' of factor 'Season'

Groups	t	P(perm)	Unique perms
KWB, KWB_OUT	1.5123	0.161	63
KWB, TMR_B	0.22292	0.845	16
KWB, TMR	0.7566	0.563	10
KWB, TMR_OUT	11.059	0.003	88
KWB_OUT, TMR_B	1.2393	0.248	116
KWB_OUT, TMR	0.33689	0.778	118
KWB_OUT, TMR_OUT	9.1896	0.002	241
TMR_B, TMR	0.58307	0.63	31

TMR_B, TMR_OUT	10.537	0.002	148
TMR, TMR_OUT	7.6631	0.009	34

Multivariate

Cores – Bray-Curtis Similarities

PAIR-WISE TESTS

Term 'Si'

Groups	t	P(perm)	Unique perms
KWB, KWB_OUT	2.5918	0.001	998
KWB, TMR_B	2.6197	0.001	999
KWB, TMR	2.1626	0.001	998
KWB, TMR_OUT	3.4652	0.001	999
KWB_OUT, TMR_B	3.5461	0.001	998
KWB_OUT, TMR	3.05	0.001	998
KWB_OUT, TMR_OUT	4.9565	0.001	999
TMR_B, TMR	2.2684	0.001	999
TMR_B, TMR_OUT	4.7776	0.001	996
TMR, TMR_OUT	2.4566	0.001	999

PAIR-WISE TESTS

Term 'SixSe' for pairs of levels of factor 'Season'

Within level 'KWB' of factor 'Site'

Groups	t	P(perm)	Unique perms
February, September	0.87894	0.663	857

Within level 'KWB_OUT' of factor 'Site'

Groups	t	P(perm)	Unique perms
February, September	0.93624	0.552	415

Within level 'TMR' of factor 'Site'

Groups	t	P(perm)	Unique perms
February, September	1.0499	0.353	208

Within level 'TMR_B' of factor 'Site'

Groups	t	P(perm)	Unique perms
February, September	1.734	0.004	413

Within level 'TMR_OUT' of factor 'Site'

Groups	t	P(perm)	Unique perms
February, September	2.7953	0.007	417

EPIFAUNA:

Univariate

Quadrats – Number of taxa

PAIR-WISE TESTS

Term 'Si'

Groups	t	P(perm)	Unique perms
KWB, TMR_B	4.5668	0.001	987

KWB, TMR	5.9089	0.001	986
TMR_B, TMR	1.4898	0.161	980

Quadrats – Number of individuals
PAIR-WISE TESTS

Term 'Si'

Groups	t	P(perm)	Unique perms
KWB, TMR_B	4.9645	0.001	993
KWB, TMR	2.459	0.02	996
TMR_B, TMR	0.76902	0.457	993

Quadrats – Evenness
PAIR-WISE TESTS

Term 'SixSe' for pairs of levels of factor 'Site'

Within level 'Feb' of factor 'Season'

Groups	t	P(perm)	Unique perms
KWB, TMR_B	1.1645	0.286	791
KWB, TMR	0.64099	0.512	561
TMR_B, TMR	1.5017	0.16	572

Within level 'Sep' of factor 'Season'

Groups	t	P(perm)	Unique perms
KWB, TMR_B	1.4074	0.178	687
KWB, TMR	1.4046	0.258	852
TMR_B, TMR	1.6449	0.118	848

Quadrats - PERMDISP on within site Bray-Curtis similarities
DEVIATIONS FROM CENTROID
F: 5.3948 df1: 2 df2: 45
P(perm): 0.012

PAIRWISE COMPARISONS

Groups	t	P(perm)
(KWB,TMRB)	3.1575	1.3E-2
(KWB,TMR)	2.4823	3.7E-2
(TMRB,TMR)	0.14555	0.902

Quadrats – Shannon-Weiner
PAIR-WISE TESTS
Term 'Si'

Groups	t	P(perm)	Unique perms
KWB, TMR_B	3.6795	0.001	995
KWB, TMR	5.9189	0.001	998
TMR_B, TMR	1.8881	0.083	997

Quadrats – Simpson
PAIR-WISE TESTS
Term 'SixSe' for pairs of levels of factor 'Site'

Within level 'Feb' of factor 'Season'

Unique

Groups	t	P(perm)	perms
KWB, TMR_B	2.1439	0.042	796
KWB, TMR	0.34982	0.731	571
TMR_B, TMR	2.1918	0.039	589

Within level 'Sep' of factor 'Season'

Groups	t	P(perm)	Unique perms
KWB, TMR_B	0.7339	0.598	765
KWB, TMR	1.7861	0.081	886
TMR_B, TMR	1.4563	0.187	875

Multivariate

Quadrats – Bray-Curtis Similarities

PAIR-WISE TESTS

Term 'Si'

Groups	t	P(perm)	Unique perms
KWB, TMRB	2.5904	0.001	997
KWB, TMR	2.6468	0.001	998
TMRB, TMR	1.7368	0.001	998

Term 'SixSe' for pairs of levels of factor 'Site'

Within level 'Feb' of factor 'Season'

Groups	t	P(perm)	Unique perms
KWB, TMRB	1.9789	0.001	937
KWB, TMR	1.9738	0.002	916
TMRB, TMR	1.7587	0.001	917

Within level 'Sep' of factor 'Season'

Groups	t	P(perm)	Unique perms
KWB, TMRB	2.1879	0.002	933
KWB, TMR	2.2869	0.001	928
TMRB, TMR	1.5431	0.001	937

EPIFLORA:

Univariate

Algal searches – Number of taxa

Excluded terms

SitexSeason

PAIR-WISE TESTS

Term 'Si'

Groups	t	P(perm)	Unique perms
KWB, KWB_OUT	4.3333	0.2209	3
KWB, TMR_B	1	0.5388	2
KWB, TMR	4.3333	0.2248	3
KWB, TMR_OUT	3	0.2676	3
KWB_OUT, TMR_B	3	0.2137	3
KWB_OUT, TMR	4.3333	0.2358	3
KWB_OUT, TMR_OUT	3.5714	0.2367	3
TMR_B, TMR	11	0.2399	3
TMR_B, TMR_OUT	5	0.2625	3
TMR, TMR_OUT	1	0.494	2

Multivariate

Algal searches – Bray-Curtis Similarities

Excluded terms

SitexSeason

PAIR-WISE TESTS

Term 'Si'

Groups	t	P(perm)	Unique perms
KWB, KWB_OUT	1.5707	0.2462	3
KWB, TMR_B	1.981	0.2683	3
KWB, TMR	1.5426	0.2266	3
KWB, TMR_OUT	1.8477	0.2645	3
KWB_OUT, TMR_B	1.2803	0.2624	3
KWB_OUT, TMR	1.1251	0.2574	3
KWB_OUT, TMR_OUT	1.5604	0.2628	3
TMR_B, TMR	1.4806	0.2757	3
TMR_B, TMR_OUT	1.7086	0.2454	3
TMR, TMR_OUT	1.4362	0.2334	3

OVERALL COMMUNITY STRUCTURE:

Multivariate

Between rhodolith beds (presence/absence on combined invertebrates, algae and fish)

PAIR-WISE TESTS

Term 'Si'

Groups	t	P(perm)	Unique perms
KWB, TMR_B	2.068	0.001	999
KWB, TMR	1.9521	0.001	998
TMR_B, TMR	1.4182	0.025	999