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The Trophic Ecology of *Psammechinus miliaris* in Scottish Sea Lochs

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A thesis submitted to the Open University in fulfilment of the requirements of the Degree of Doctor of Philosophy

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Abstract

Understanding the trophic relationships between organisms is crucial to understanding ecosystem functioning and as such regular echinoids have been termed keystone through the action of their grazing. Much research has focused on this group's action as herbivores, but as a group omnivory is common. The aim of this study was to investigate the trophic ecology of the locally super abundant regular echinoid species *Psammechinus miliaris* within Scottish sea lochs. To do this the study used manipulative field experiments combined with biochemical analysis of trophic proxies. The manipulative field experiments involved either the hand removal or the caging of *P. miliaris* to determine the impact the sea urchin grazing has on benthic community structure. These studies revealed that grazing of *P. miliaris* can have a major influence on the biomass and structure of the benthic invertebrate communities. The biochemical analysis of trophic proxies was used to quantify the spatial and temporal variations in the trophic interactions of *P. miliaris.* These studies focused on the urchin gonad and compared differences in the gonadal somatic indices (reflecting nutritional and reproductive state) with the fatty acid biochemistry and stable isotope ratios of Carbon and Nitrogen. These studies revealed high levels of spatial and temporal heterogeneity in the trophic interactions of *P. miliaris* and suggested that the populations exhibited significant levels of omnivory. The combination of these studies showed that *P. miliaris* plays an important role in structuring benthic invertebrate communities and in the flow of energy through ecosystems and through this, ecosystem functioning.

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As no man is an island entire of itself, no thesis is created in a vacuum. Even behind this driest of tomes due for a dusty shelf is a rich tapestry of human stories and relationships, without which there would be no book. So here I say a few thank yous to all those that made it possible.

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"Two drifters off to see the world….

..my huckleberry friend, moon river and me"

"I wondered why it is that we're all such bloody fools. Why don't people instead of the idiocies they do spend their time on, just walk round and look at stuff? That pool for instance – all the stuff that's in it. The mystery of their lives, down there underwater. You could spend a lifetime watching them, ten lifetimes, and you still wouldn't have got to the end of that one pool. And all the while the sort of feeling of wonder, the peculiar flame inside you. It is the only thing worth having, and we don't want it."

George Orwell 1939

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Chapter 1

INTRODUCTION

"Feeding is such a universal and common place business that we are inclined to forget its importance. The primary driving force of all animals is the necessity of finding the right kind of food and enough of it. Food is the burning question in animal society, and the whole structure and activities of the community are dependant upon questions of food supply."

Charles Elton 1927 Animal Ecology

This description and its placing of trophic ecology at the centre of ecological theory has been affirmed by a number of subsequent defining works (Linderman 1942, Fretwell 1987, Hessen 1992, Menge 2000). The description highlights the dual facets of the subject; the influence of trophic interactions on community structure through predation and trophic cascades (Connell 1961, Paine 1980), and the mapping of energy flow through ecosystems principally using food webs as a descriptive and quantitative tool (Paine 1966, Polis 1991). There is, however, an increased emphasis on the integration of these two subjects, and an understanding of the role of individual organisms and their behaviour in shaping the functioning of ecosystems (Moe et al. 2005, Ptacnik et al. 2005). Many studies on the trophic ecology of sea urchins have concentrated on their grazing activities, and the way in which this grazing acts to structure benthic communities.

THE ROLE OF URCHINS IN REGULATING INVERTEBRATE COMMUNITY STRUCTURE

Echinoid grazing has long been understood to play a crucial role in the structuring of many intertidal and subtidal habitats (see (Lawrence 1975, Elner & Vadas 1990) for reviews), and echinoids have been described as keystone herbivores in some environments (Lessios et al. 2001). Through their action as herbivores they have the ability to profoundly alter the algae composition of their habitats leading in many cases to 'urchin barrens'. These phenomena

are described as the removal of erect macro-algae from rocky reefs to leave only bare rock and encrusting algae (Lawerence 1975).

Table 1-1 Examples of documented occurrence of urchin barrens from around the world

Location	Habitat	Species	Reference
Northern Norway	Subtidal	*Strongylocentrotus droebachiensis*	(Hagen 1983)
Southern California, USA	Subtidal	*Strongylocentrotus franciscanus*	(Tegner & Dayton 1991)
Norway	Subtidal	*Strongylocentrotus droebachiensis* and *Echinus esculentus*	(Sivertsen 1997)
Chilean coast	Intertidal and subtidal	*Strongylocentrotus droebachiensis*	(Vasquez & Buschmann 1997)
Japan Sea coast of southwestern Hokkaido	Subtidal	*Strongylocentrotus nudus*	(Mine et al. 2001)
Southern New Zealand	Subtidal	*Evechinus chloroticus*	(Villouta et al. 2001)
Mediterranean Sea	Subtidal reefs	*Paracentrotus lividus* and *Arbacia lixula*	(Bulleri et al. 2002)
Northern Gulf of St. Lawrence, Canada	Rocky subtidal zone	*Strongylocentrotus droebachiensis*	(Gagnon et al. 2003)

The phenomena of barrens have been recorded from a wide range of habitats and sea urchin species (Table 1-1). In addition to their role as herbivores, a number of sea urchin species are known to be omnivorous (Himmelman & Steele 1971, Lawerence 1975, Briscoe & Sebens 1988). It has been shown that within the marine environment, predation can control the community structure of sessile epibenthic species. Such control by predation can be in the form of reducing the density and/or limiting the range of distribution of prey species as a result of direct consumption (Connel 1961). Predation also acts to alter community composition by causing changes in successional processes. This usually involves the removal of the competitively dominant species by the predators resulting in a subsequent increase in species diversity (Castilla & Duran 1985). However the interaction between predators and the benthic community may be complicated and involve a series of trophic cascades (Sala et al. 1998).

The impact of sea urchins on benthic epifaunal populations through trophic interactions has mainly been studied by observing changes in community composition following a change in sea urchin density. Such changes in density are normally a result of an uncontrolled variation in the natural population; for example through disease, fishing or as a result of experimental manipulation. The nature and direction of this variation in the community, following changes in the urchin population seems to be highly variable, and dependent on a number of factors. These factors have been shown to include: the species of the urchin; their interaction with other urchin species (Bulleri et al. 1999); variability in the recruitment of epibenthic invertebrates (Prince 1995); the 'natural' urchin density prior to the experiment (Karlson 1978); the urchin density with respect to the original density (Andrew & Underwood 1993); and the duration of the study (Bulleri et al. 1999). Given the variability in the nature of this response it is difficult to discern a pattern of interaction between echinoid grazing and epibenthic invertebrate communities. However, a review of the literature has indicated that it is possible to split the interactions into two categories, direct and cascade effects.

THE DIRECT EFFECTS OF URCHIN GRAZING ON SPECIES DIVERSITY

Paine (1971) postulated that the removal of the dominant grazer would be associated with a reduction in species richness. However the impacts of urchin removal in some cases have not resulted in reduced richness. Significant changes in invertebrate composition followed the removal of *Centrostephanus coronatus* from subtidal rock reefs south of Los Angeles, California (Vance 1979). After 27 months ungrazed areas supported more sponges, hydroids, sea anemones, erect bryozoans and tunicates. Vance (1979) concluded that urchin grazing reduces diversity at a small scale but may act to increase diversity on larger scales. Descriptive studies have also documented the influence of urchins following natural variation in populations. Intensive grazing by large aggregations of *Strongylocentrotu*s on the New England coast caused a major shift in the dominance of epifauna and denuded the

substrate of nearly all encrusting invertebrates (Witman 1985). The author states that urchins grazing at high densities can cause significant reductions in the species richness and diversity of all functional groups of benthic invertebrates, except mussel bed infauna.

As well as altering species diversity, urchin grazing has been shown to significantly decrease the biomass of prey species. Cages were set up to exclude *Strongylocentrotus droebachiensis* from parts of a rocky subtidal wall in northern Norway (Sanders & Gulliksen 1980). After 18 months in the caged exclusion areas there was conspicuous settlement of the barnacle *Semibalanus balanoides*. Barnacles settling in non-caged areas were quickly removed by urchin grazing. During the experiment one of the cages was damaged in a storm leading to five urchins entering the experimental area. These urchins rapidly removed the established barnacle population. The impact of the urchin *Arbacia pundulta* on invertebrate populations was similarly studied. Subtidal rail bridge pilings in North Carolina were either denuded of urchins, artificially stocked to increase the density or left as a control (Karlson 1978). It was found that eight species of sessile invertebrates were significantly affected by these treatments. Three species of bryozoan (two arborescent and one mat-forming) showed reduced levels of abundance in the presence of elevated urchin densities. In treatments with increased urchin density the hydroid *Obelia*, the sponge *Haliclona* and the crested oyster (*Ostrea equestris*) all showed increased abundance. In addition to the effects on specific species, *A. pundulata* also had a major effect on total epifaunal cover and subsequently the availability of space on the substrate. Removal sites had the greatest epifaunal cover and those sites with urchin populations elevated above the norm had the lowest amount of free space. Ayling (1981) concluded that urchin grazing could clear ascidians and sponges from some substrates following experimental exclusion of *Evechinus chloroticus* from the subtidal of a site in New Zealand.

Not all studies have shown that urchin grazing has a significant impact on the epibenthic fauna. The removal of two species of urchins (*Paracentrotus lividus* and *Arbacia lixula*) from shallow subtidal reefs of the Mediterranean (Benedetti-Cecchi et al. 1998) resulted in no significant changes in the abundance of barnacles or bryozoans over a period of 18 months. A similar result was obtained following the removal of *Echinometra mathaei* from intertidal rock platforms in Western Australia (Prince 1995). There was limited recruitment of invertebrates to the cleared areas and there was no evidence to suggest that macro invertebrates were prevented from occurring because of high densities of urchins.

INDIRECT OR CASCADING TROPHIC INTERACTIONS

In conjunction with direct grazing on invertebrates, sea urchin grazing can influence community composition by altering successional changes and by creating free space for settlement. This has been reported for limpet recruitment following a reduction in urchin density. Fletcher (1987) found that after the removal of *Centrostephanus rodgersii*, the extent of the limpet recruitment was dependent on the species and the depth of the treatment. Following an initially high recruitment in the absence of urchins, populations of all limpet species declined. It is possible that this was due to loss of habitat following over-growth by macro-algae.

Increased recruitment and reduced adult numbers have been associated with a reduction in the density of the urchin *Diadema antillarum* (Sammarco 1980, 1982). Experimental removal of *D. antillarum* led to greater coral settlement, followed by a reduction in coral cover due to algal overgrowth. In addition to this several species dependent effects were illustrated. The coral *Agraricia* was competitively less successful as the density of *D. antillarum* increased, and *Porites* (another coral genus) mortality was positively correlated to urchin density. There was also an observed decline of coral diversity in response to reduced echinoid density, although a greater number of coral genera recruited in the absence of urchins. Following

the recovery of the diademid numbers in the Caribbean there has been an associated reduction in algal cover and an increase in coral recruitment (Edmunds & Carpenter 2001).

SEA URCHINS AS HABITAT ENGINEERS

In conjunction with the direct and indirect effects of grazing, urchins have been shown to influence sessile invertebrates by acting as habitat architects or by providing shelter themselves. Sammarco (1974) found that high densities of *Diadema antillarum* could alter the microstructure of dead coral substrate, and linked urchin density with the species diversity of sessile invertebrates. Sea urchins have also been shown to have the ability to structure habitats at larger scale. It was estimated (Mokady et al. 1996) that bioerosion by the urchins *Diadema setosum* and *Echinometra mathaei* accounted for 13-22% of the reef slope calcification in the Gulf of Eilat, Red Sea. The spine canopy of some species of urchins can provide shelter for other invertebrates. The sea urchin *Parechinus angulosus*, for example, has been shown to have a positive impact on the survivorship of abalone *Haliotis midae* (Day & Branch 2000). The mechanism behind this association may include the protective shelter from predators, the reduction in sediment build up due to the locomotion of urchins, and also the provision of additional nutrition from drift weed trapped by the urchin (Day & Branch 2002). A similar association has also been noted in the urchin *Anthocidaris crasspina* and the black Japanese abalone (Kojima 1981).

SEA URCHINS AND ECOSYSTEMS

The second facet of sea urchin trophic ecology, the placement of trophic interactions into a larger context of energy flow through an ecosystem, has received less attention. Sea urchins have been described as links in the transfer of both autochtonous and allochtonous energy through ecosystems. Their role in the transfer of autochtonous energy, as elucidated by food webs studies, has been shown for a range of polar through to tropical environments (Fredriksen 2003, Corbisier et al. 2004, Glynn 2004). In conjunction with this, sea urchins

can play an important role in integrating both naturally and anthropogenically derived allochtonous material into food webs. Drift algae can provide up to 68% of the energy for the intertidal sea urchin *Tetrapygus niger* on the central Chilean coast (Rodriguez 2003). Deep sea urchins, *Echinus affinis*, have been found to incorporate sewerage sludge from offshore dumping into their diets (Rieley et al. 1997). In addition to their role in energy transfer through direct trophic interactions (feeding and being fed upon) sea urchins can mediate energy flow through nearshore benthic ecosystems through their production of faecal pellets, providing a potential food source for suspension feeders and detrivores (Mamelona & Pelletier 2005).

PSAMMECHINUS MILIARIS

*Psammechinus miliari*s is a regular urchin reaching a maximum diameter of 50 mm, and with a distribution from Iceland to Morocco and the Azores, although it is absent from the Mediterranean. It ranges from the intertidal to a depth of approximately 100m (Hayward & Ryland 1995). In Scottish sea lochs, the sea urchin *Psammechinus miliaris* is frequently found at high densities, 200 – 300 sea urchin m^{-2} are not uncommon (Kelly & Cook 2001). This species is an opportunistic omnivore and will exploit a wide range of foodstuffs of animal origin including hydroids, worms, other echinoderms, crustaceans, molluscs and sponges (for a review see (Lawerence 1975)). Hancock (1957) reported that *P. miliaris* fed on cockles, barnacles and ascidians and also grazed boring sponges and worms from oyster shells. *Psammechinus miliaris* has also been found to remove biofoulers from a range of artificial substrata (Kelly et al. 1998, Ross et al. 2004) The nature of their diet influences their growth. *Psammechinus miliaris* fed omnivorous or carnivorous diets have higher levels of growth, both somatic and gonadal than those fed purely algal diets (Cook et al. 1998). Kelp, on its own, seems to be of limited nutritional value to *P. milaris* (Otero-Villanueva et al. 2004). Given the high abundances of *P. miliaris* in Scottish sea lochs, their omnivorous

feeding, and the nutritional advantage conferred by this omnivorous feeding, the current study was designed to investigate the trophic ecology of *P. miliaris* within Scottish sea lochs

Two principal approaches were taken during this study to investigate the trophic ecology of *P. miliaris*. Firstly through manipulative field experiments, the impact of grazing on invertebrate community structure was investigated. Secondly the biochemical composition of the sea urchins was examined using fatty acid and stable isotope ratio analysis. This can potentially enable use of certain biochemical trophic proxies to further elucidate the diet of the urchins and in doing so better understand the role that sea urchins play in the flow of energy through benthic ecosystems.

STUDY SITE

Three locations were chosen on the south side of Loch Creran, on the west coast of Scotland. Loch Creran is a silled sea loch, 12.8 km long with four basins which reach a maximum depth of 49m. It has been characterised as a well mixed system with a flushing time of three days (Black et al. 2000). The shores are mainly fucoid dominated rocky areas and more sheltered mixed stones and sediment flats (Connor 1990). The three sites were chosen to be qualitatively similar, had dense populations of the sea urchin *Psammechinus miliaris* (Kelly pers comm.) and to be less than 5km apart. The study sites were Rubha Garbh (RG), Sea Life (SL), and South Shian (SS) (Figure 1-1). These sites consisted of shoreline with a range of boulders, cobbles and stones on a mixed substrate of sand and mud. All three sites had similar topography in terms of boulder size and shore slope; however one site (RG) was possibly subject to greater freshwater input than the others in the form of run off from adjacent fields.

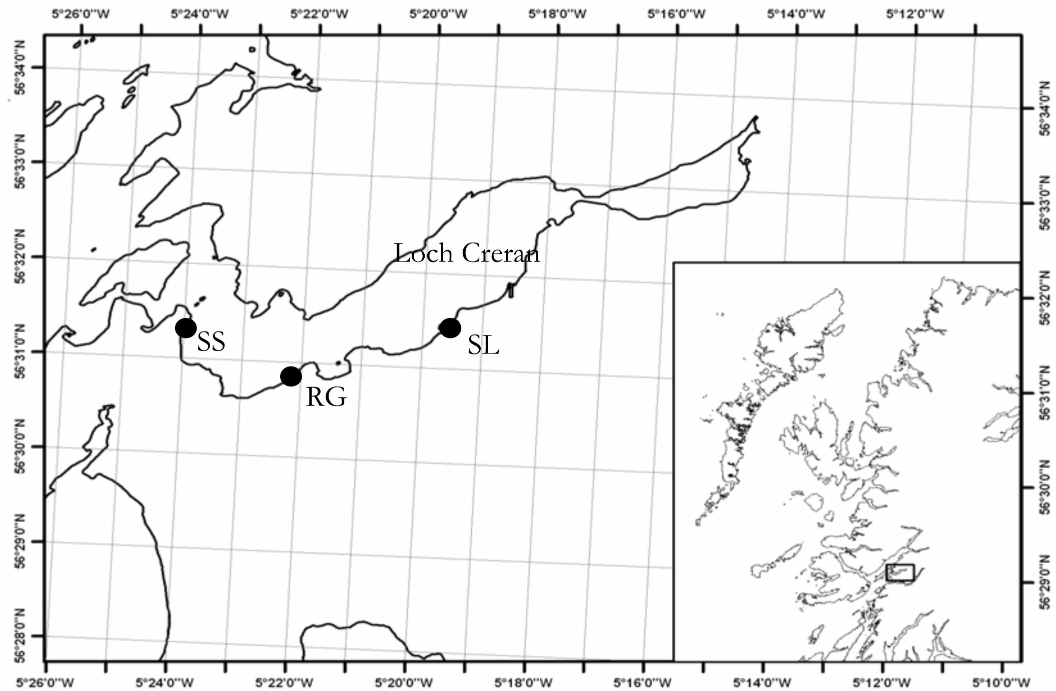


Figure 1-1 The location of Loch Creran on the west coast of Scotland with the three experimental sites used within this study marked. SS=South Shian, RG=Rubha Garbh, SL=Sea Life

The algal canopy on these sites was predominately a mixture of *Ascophyllum nodosum, Fucus serratus* and *Fucus vesiculosus*. The under-storey consisted of encrusting red algae (*Lithophyllum* sp.) and ephemeral green algae such as *Enteromorpha* sp. The sessile epifauna was dominated by serpulid and spirorbid (polychaete) worms. The most common elements of the mobile epifauna were *P. miliaris*, the common starfish *Asterias rubens*, the common whelk *Buccinum undatum,* and smaller grazing gastropods such as top shells (*Gibbula* sp) and periwinkles *(Littorina* sp.*)*.

Chapter 2

GONAD FATTY ACIDS AND TROPHIC INTERACTIONS OF *PSAMMECHINUS MILIARIS*

INTRODUCTION

Understanding the trophic relationships that exist between species and within species, and the way that these interactions vary at temporal and spatial scales has been a principal component of marine ecological research throughout its development as a science (Elton 1927, Linderman 1942, Fretwell 1987, Menge 2000). In order to gain a better understanding of these interactions, numerous techniques have been used to explore and quantify them. These techniques range from passive observation such as direct study of feeding behaviour (Zamon 2001) or examination of faecal pellets (Bonesi et al. 2004), through to invasive methods such as stomach content analysis (de la Moriniere et al. 2003) and biochemical sampling methods such as stable isotope analysis (Kharlamenko et al. 2001), carotenoid pigment analysis (Hudson et al. 2003) and fatty acid analysis (Pond et al. 1997).

Fatty acid analysis relies on the premise that when dietary lipids are ingested they are broken down into their constituent fatty acids. These fatty acids are at least partly conservative and can be reincorporated into the ingester's tissue (Iverson et al. 2004). These fatty acids can then be recovered from the tissue by extracting the lipid in the laboratory, and transestifing it into its component fatty acids. These fatty acids are then separated using gas chromatography and identified by comparing retention times with that of known standards. The suite of fatty acids produced for each sample is termed the fatty acid signature. Certain components of the fatty acid signature or ratios of these components can act as dietary indicators.

Fatty acid analysis has been used to study trophic interactions in a wide range of habitats including terrestrial (McWilliams et al. 2004), freshwater and marine (Go et al. 2002). One of the principal areas of study has been investigation of trophic ecology in species where direct observations have not been possible such as deep-sea seastars (Howell et al. 2003). Using fatty acid analysis it was possible to separate nine species of deep-sea seastars into three trophic groups based on their fatty acid signatures: suspension feeders had high levels of biomarkers characteristic of photosynthetic algae, the mud ingesters' fatty acid signature was characterised by those associated with heterotrophic bacteria, and the predators/scavengers occcupied a space between the other two. Variation in the fatty acid signature can be used to differentiate trophic niches of co-occurring species. A study into species of Cichlids from the great lakes of Africa (Kuusipalo & Kakela 2000) showed that species originally thought to show a high degree of plasticity in their feeding actually had stable and defined niches based on their fatty acid signatures. Marine mammals exemplify another group of organisms where direct observations of feeding can be difficult and fatty acid analysis has offered valuable insights into their trophic interactions (Iverson et al. 2004). Fatty acid analysis can also be used to determine the relative importance of different food sources, such as terrestrial, marine or bacterial to sessile filter feeding invertebrates. A study into the fatty acid signature of the mud clam *Geloina coaxans* from the mangrove forests of Japan (Bachok et al. 2003) revealed the importance of mangrove detritus and bacteria as a major food source.

As well as being used to establish broad scale patterns in trophic interactions, fatty acids can also reveal patterns of variation at a range of spatial and temporal scales. For example, it was shown that Antarctic krill (*Euphausia superba*) vary on a regional scale, with three regionally distinct groups (Cripps et al. 1999), and the authors concluded that regional variation in diet could have a larger effect on the fatty acid signature than sex or maturity stage. Fatty acid signatures from blubber samples of Harbour Seals (*Phoca vitulina richardsi*)

from Alaska were found to vary at both regional scales (100's km) and between individual haulouts within 9 to 15 km (Iverson et al. 1997). Iverson et al's study found that this spatial variation was also mirrored in a number of prey species, with the authors suggesting differences in seal fatty acid signatures were a result, not only of localized feeding patterns, but also of the composition of their prey. Differences in fatty acid signatures have also been linked to patterns of productivity in the squid *Moroteuthis ingens* (Phillips et al. 2003). This study showed that there were significant differences between sampling sites despite their relative proximity, and the authors concluded that these differences were a result of differences in the productivity of the water masses.

Few studies have examined the differences in the fatty acid signature of a species occurring in different habitats. An example of one such study where the effects of spatial and inter habitat variation were studied involved the limpet *Patella depressa* from the coast of Portugal (Morais et al. 2003). The authors concluded that limpets from an exposed site had different fatty acid signatures to those from a sheltered site during the summer, but no difference was detectable during the winter. This difference was attributed to wave and wind exposure causing a different algae community to develop. Another study reported that mussels collected from the intertidal exhibited significant differences in a number of fatty acids when compared to those from the subtidal (Freites et al. 2002).

In conjunction with spatial variation it has been shown that fatty acid signatures of a species or an individual vary over a range of temporal scales. Inter annual variation in the fatty acid signature was reported for one scottish colony of Grey Seal (*Halichoerus grypus*) but not from the other study population (Walton & Pomeroy 2003). Much more common is the reporting of intra annual variation, in particular when it is linked to the reproductive cycle. This link between fatty acid signature of the gonad, and the reproductive cycle has been demonstrated for the bivalves *Pecten maximus* (Pazos et al. 1997), *Ruditapes decussates*

(Ojea et al. 2004), and C*rassostrea gigas* (Soudant et al. 1999), the cephalopods *Octopus vulgaris* and *O. defilippi* (Rosa et al. 2004a), *Eledone cirrhosa* and *E. moschata* (Rosa et al. 2004b), and the crustaceans *Aristeus antennatus* (Rosa & Nunes 2003) and *Nephrops norvegicus* (Rosa & Nunes 2002). These studies have shown that there are major changes in the fatty acid signatures in the gonads during sexual maturation, and they highlight the importance of two specific fatty acids, 20.5(n-3), and 22.6(n-3), in this maturation cycle.

Studies of echinoid gonadal somatic indices (GSI), the index of gonad mass relative to whole organism size, have revealed considerable variability at both spatial and temporal scales. Echinoid gonads have a multifunctional role and are known to reflect both nutritive and reproductive status (Russell 1998). Kelly (2000) found the GSI was higher in intertidal compared to subtidal populations of *Psammechinus miliaris* in Scottish sea lochs. It was proposed that this difference was a result of better food quality and/or quantity found in the intertidal, and more specifically the greater abundance of invertebrate prey in the intertidal. It has been shown that *P. miliaris* individuals fed on salmon feed or mussel flesh (Otero-Villanueva et al. 2004) or grazing on the encrusting biota of scallop lines have significantly higher GSI's than those fed on algae (Cook et al. 2000).

This study addresses two main hypotheses. Firstly, for the study populations the GSI of *P. miliaris* found in the intertidal are higher than those in the subtidal and these differences are a result of depth and are not confounded by simple spatial variation. Secondly, we hypothesise that intertidal and subtidal populations can be differentiated in terms of their fatty acid signatures, reflecting a difference in diets between the two habitats. The study also explores for the first time the changes to the fatty acid signature of this echinoid's gonad over the spawning period.

METHODS

Study site

The study locations (separated by km's), Rubha Garbh (RG) and South Shian (SS) have been previously described in the Introduction.

Field protocol

Five *P. miliaris* individuals of approximately equal test diameter were collected from each of two replicate sites, each of two depths (intertidal and subtidal), and each of two locations (Figure 2-1). Those from the intertidal zone were collected from an area just above Extreme Low Water Springs (ELWS) which is exposed for approximately 2.5 hours for three consecutive days on spring tides. Those from the subtidal zone were collected using snorkel from permanently submerged areas at a depth of two to three meters. The specimens from each replicate were collected from within a one metre radius.

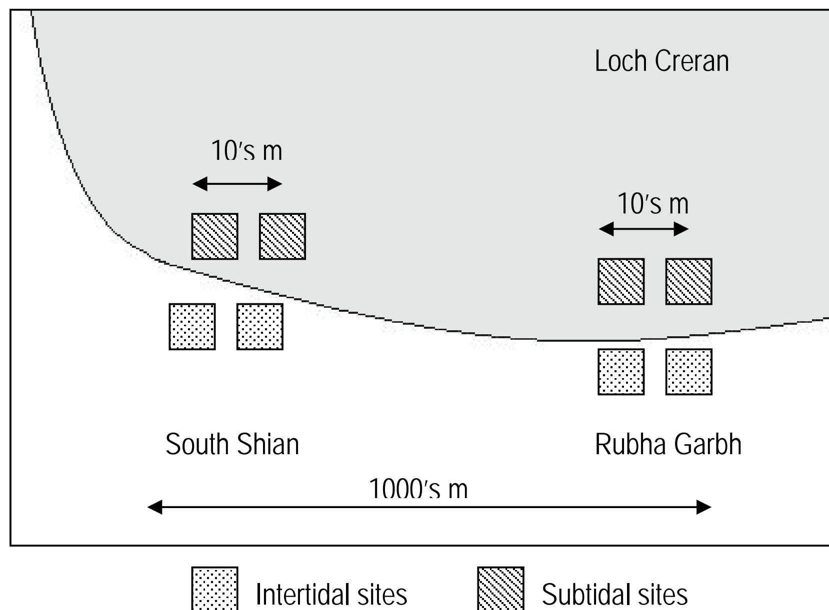


Figure 2-1 Diagrammatic representation of the experimental layout used in this study showing the approximate distance between different sampling units.

The replicate sites at each location were approximately 10m apart. The *P. miliaris* individuals were transferred to the laboratory and maintained in running sea water and without food until analysis (within 48 hrs). This was repeated each month, from July to September 2004.

Laboratory Analysis

Test diameter and wet mass for each individual *P. miliaris* was determined prior to the removal of the gonad. Test diameter was measured three times using adapted Vernier calipers, and the mean of the three measurements was used. The excised gonad was weighed, and a small sample was taken and used for microscopic determination of sex and maturity stage. The maturity stage was determined using a wet squash and scored on a six point scale (stage I recovery, stage II early growth, stage, III premature, stage IV mature, stage V partially spawned and stage VI spent (Kelly 2001)). The GSI's were calculated for each animal using the formula:

$$\mathbf{GSI=(wet\ mass\ gonad \div wet\ mass\ of\ whole\ test).100}$$

After dissection of the gonad, it was placed in chloroform:methanol (vol.2:1), and stored under nitrogen until ready for lipid extraction (>1 hour).

Lipid extraction and fatty acid analysis

The gonad was homogenized in chloroform:methanol (2:1 $10cm^{-3}$), and stored under nitrogen overnight at 4˚C. Prior to storage the fatty acid standard 23.0 (400µml, $0.5mg\ g^{-1}$) was added to each sample as an internal standard. The lipid was then extracted and transesterified to fatty acid methyl esters (FAMEs). The FAMEs were purified using thin layer chromatography and then stored in hexane under nitrogen at -16ºC for no longer than a week prior to gas chromatography.

The purified FAMEs were separated by a Perkin Elmer 8320 gas chromatograph equipped with split injector (100:1), flame ionization detector (FID) and a Zebron ZB-WAX fused silica capillary column (30m x 0.25mm i.d., 0.25µm film thickness). Helium was the carrier gas and the oven temperature was programmed to increase from 160ºC to 240ºC at $4ºC.min^{-1}$, then held for 10min. In order to store and integrate the chromatograms, the

detector output was coupled to a data system (Varian Star ™). The FAMEs were identified by comparison of their retention times with those of authentic standards with the exception of 16.0 dimethylacetal and 20.2 non-methylene interrupted dienes. These were identified using retention times from a previous study (Cook et al. 2000) All samples were run on the same column and under the same conditions. Individual fatty acids were identified and the relative content of each one was determined using peak areas and expressed as the percentage by weight of the total fatty acids characterized. This was then converted to relative mass per gram of wet weight of gonad tissue (mg g^{-1}), using the response of internal standard.

Statistical Analysis

Prior to investigation of GSI variability, the relationships between urchin test diameter and whole wet-mass, gonad wet-mass and GSI were examined using Pearson's product-moment correlation. Prior to all parametric univariate analysis, data normality was tested using the Kolmogorov-Smirnov test; any data found to be significantly different were log transformed to meet assumptions of normality. Data were also checked for homogeneity of variance using Cochrans test. Pearson product moment correlation values (rho statistic) and associated probabilities demonstrated a significant relationship between *P. miliaris* test diameter and a) whole wet mass (rho=0.834, p<0.001) and b) gonad wet (rho=0.416, p<0.001) mass as expected (Figs. 2-2a, 2-2b respectively). There was no significant correlation between test diameter and GSI (rho=0.132, p=0.15) thus establishing GSI as a size-independent metric (Figure 2-2c).

Differences in GSI were tested using a four factor ANOVA with month (M-3 levels, random and orthogonal), location (L-2 levels, random and orthogonal), depth (D-2 levels and fixed) and sites nested within location (S-2 levels). This produced the following ANOVA model:

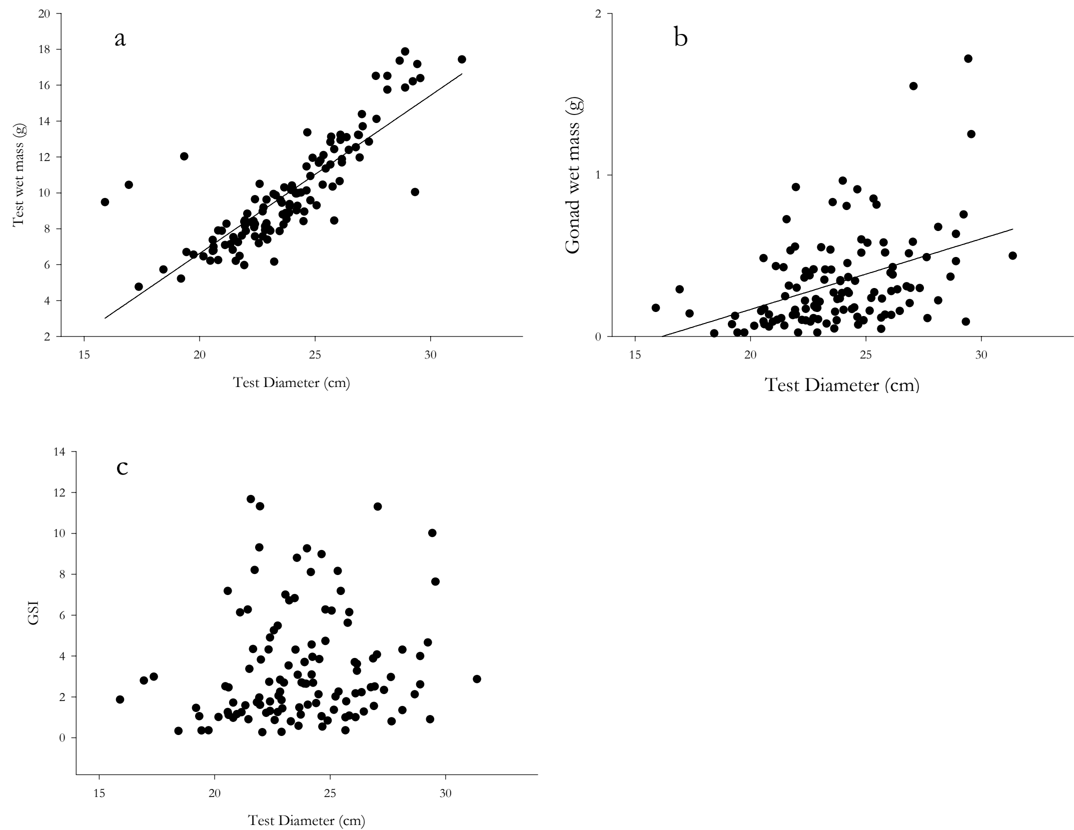
$$X_{ijklm}=\mu+M_i+L_j+D_k+S(L)_{l(j)}+ML_{ij}+MD_{ik}+MS(L)_{il(j)}+LD_{jk}+DS(L)_{kl(j)}+MLD_{ijk}+MLS(L)_{ijl(j)}+E_{m(ijkl)}$$

As this model does not allow for a direct comparison of depth, differences between the GSI at each depth were tested using three separate ANOVA's, one for each month:

$$X_{ijkl}=\mu+ L_i+D_k+S(L)_{k(i)}+LD_{ij}+DS(L)_{jk(i)}+E_{l(ijk)}$$

Differences between the proportion of each sex and maturity stage occurring at different locations, depths and sexes was tested using chi squared test.

Figure 2-2 Correlation plots between test diameter and a) test wet mass, b) gonad wet mass, c) G.S.I.%



All multivariate analysis was carried out using the PRIMER v6 (Plymouth Routines In Multivariate Ecological Research). The data were left untransformed (Howell et al. 2003) and converted into similarity matrices using Euclidean distances as the metric. A single outlier from the third month which had very low quantities of all fatty acids was removed

from the analysis. If the procedure required a balanced data set a dummy variable was created as the average of the other four fatty acid signatures from that replicate.

To visualise data similarity patterns non-metric multidimensional scaling (nMDS) was used. Permutation based ANalysis Of SIMilarity (ANOSIM) routines were used as the hypothesis testing framework. Differences in the fatty acid signatures with depth, sex, and maturity stage were explored using the SIMilarity PERcentages routine (SIMPER), both one way and crossed. To investigate the relationship between the fatty acid signatures of each month, a second stage MDS plot was used (Brown et al. 2002). This calculates the Spearman correlation coefficient between different similarity matrices, and uses this to generate another similarity matrix that reflects the differences between the different fatty acid signatures for each month, depth and location.

By matching the fatty acid signatures to the environmental variables for each sample it is possible to find which of the environmental variables are correlated (Spearman rank) with the fatty acid signatures (BIO-ENV). Five environmental variables were used: depth, site, month, sex, and maturity stage.

RESULTS

The gonadal somatic indices were calculated for each location, depth and month (Figure 2-3). There is a clear downward trend, for all sites and locations, with the largest magnitude of decline seen at the intertidal SS site which was also had the highest initial value, from an average GSI of 8.1% in July to 2.0% by September. The averaged reduction between July and September averaged over location was 71% for both intertidal and subtidal.

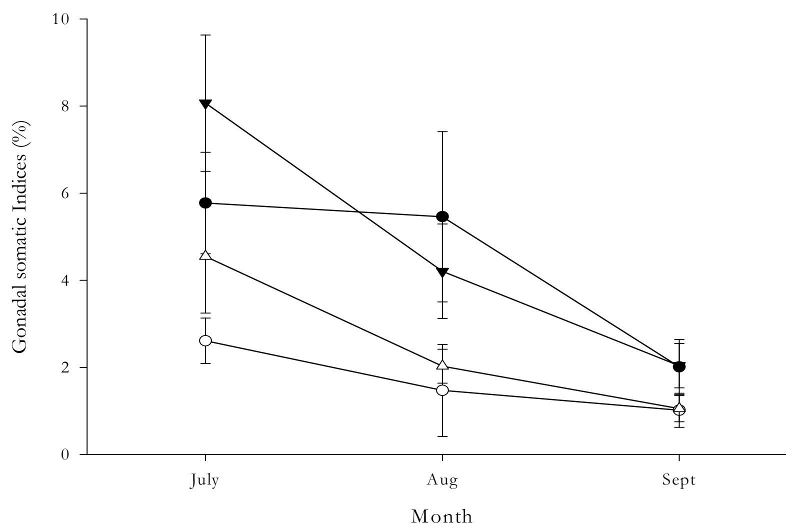


Figure 2-3 The mean GSI for each location and depth from July to September. Error bars show 95% confidence intervals. ● RG Intertidal, ○ RG Subtidal, ▼ SS Intertidal, ∇ SS Subtidal

The difference in GSI at each depth was tested separately for each month, prior to analysis the data were square root transformed to meet assumptions of heterogeneity of variance. Post hoc pooling of the Location x Depth term was performed on all three months to increase the power of the test. All data met the assumption that the F-ratio of the pooled term was sufficiently large (>0.25) to reduce the probability of type II errors (Underwood 1997). There were significant differences in the GSI between the intertidal and subtidal for all months (Table 2-1)

Table 2-1 Analysis of variance of the GSI for each month.

		July			August			September		
Source	df	MS	F	P	MS	F	P	MS	F	P
Location	1	1.74	18.0	0.051	0.00	0.01	0.947	0.00	0.02	0.90
Depth	1	4.61	133.1	0.001	5.53	12.7	0.037	0.95	166	0.001
Site(location)	2	0.10	0.64	0.535	0.054	0.32	0.726	0.15	3.17	0.056
[1]Loc. x Depth	1	0.00			0.486			0.00		
[1]Depth x Site(loc.)	2	0.05			0.411			0.01		
Residual	32	0.15			0.169			0.05		
Total	39									
Pooled	3	0.03	0.22	0.88	0.436	2.57	0.07	0.01	0.11	0.95

[1] pooled terms

There were no significant differences in the distribution of sex and maturity stage between the depths (Table 2-2), but there was however significant variation in the distribution of sex between the months and the maturity stage. In August there were more male samples than female and in September more females than male. The distribution of maturity stage showed a clear bias between sexes, with females having a higher proportion of stages three, five and six. The maturity stage for both sexes by month showed a clear progressive increase over the months (Figure 2-4).

Table 2-2 The chi square analysis of distribution of sex, and maturity stage between depth location and month

Month	Female	Male					
1	20	20					
2	16	24					
3	27	13					
	χ^2=6.316 p=0.04		Depth		Month		
Stage			Intertidal	Subtidal	1	2	3
3	18	1	9	10	15	4	0
4	12	26	23	15	25	13	0
5	19	18	18	19	0	14	23
6	14	12	10	16	0	9	17
	χ^2=23.67 p<0.01		χ^2=3.174 p=0.37		χ^2=112.7 p<0.01		
Depth							
Intertidal	34	26					
Subtidal	29	31					
	χ^2=0.836 p=0.36						

In July the majority of urchins were at maturity stage 4 as opposed to 3 for females. This trend for males to be developmentally ahead continued for the remainder of the study, with a modal stage of 5 for males, and 4 for female during August, and six and five respectively during September. There was a significant difference between the GSI for each maturity stage (df=3,116 f=22.81, $p<0.001$). Pair wise comparison showed that stages 3 and 4 were similar and significantly different from stages 5 and 6. The highest mean GSI was recorded for stage 4 with mean of 5.95% and the lowest was stage 6 with a mean GSI of 2.15% (Figure 2-5).

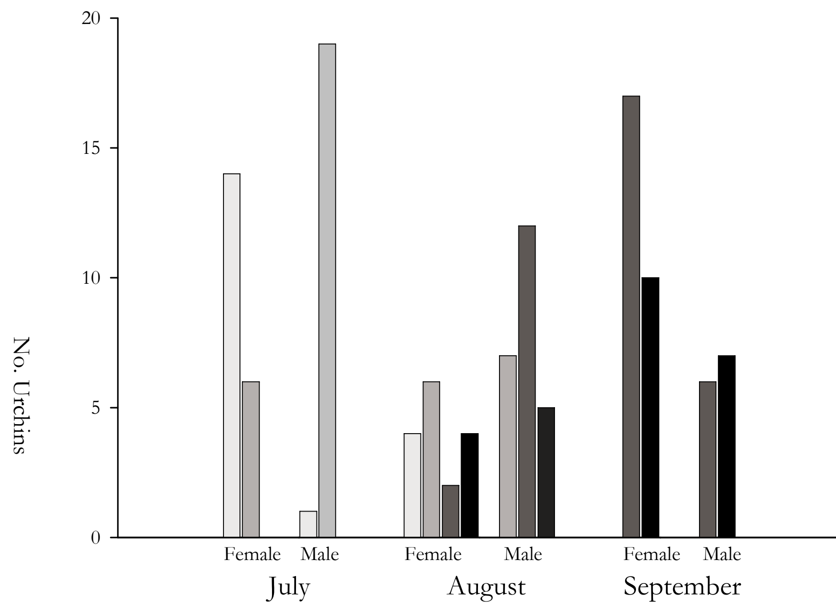


Figure 2-4 The number of urchins recorded at each maturity stage and sex for each month.
■ Stage 3, ■ Stage 4, ■ Stage 5, ■ Stage 6

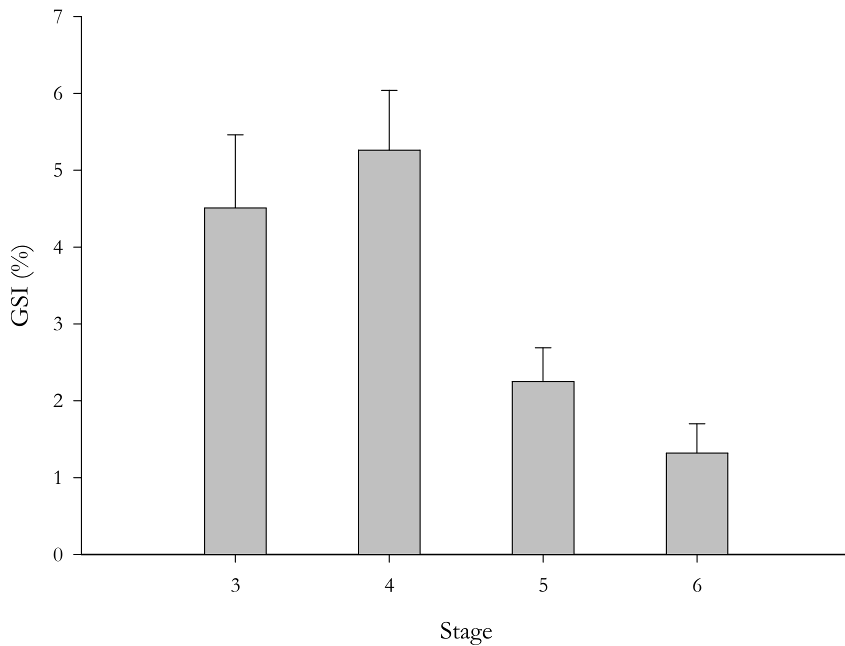


Figure 2-5 The mean GSI for each maturity stage (error bars=95% confidence interval)

Fatty Acid Analysis

The predominant fatty acid for all months and depths (Table 2-3) was 20.5(n-3) being highest in the subtidal in July (17.88%), followed by 20.4(n-6) and 16.0. Polyunsaturated

Table 2-3 The relative abundances (%) of the identified fatty acids from the sea urchin gonads, separated by month and depth. 1 dimethylacetal 2 non-methylene interrupted dienes

	July				August				September			
	Intertidal		Subtidal		Intertidal		Subtidal		Intertidal		Subtidal	
Fatty acid	Mean	95ci	Mean	95ci	Mean	95%CI	Mean	95%CI	mean	95ci	mean	95ci
14.0	3.32	0.55	3.60	0.57	2.10	0.41	3.04	0.46	3.02	0.28	3.52	0.83
a15.0	0.13	0.03	0.08	0.03	0.11	0.05	0.04	0.04	0.13	0.05	0.10	0.04
15.0	0.64	0.07	0.58	0.09	0.56	0.11	0.54	0.13	0.91	0.10	0.66	0.13
16.0	10.21	0.26	10.92	0.37	8.77	0.98	9.81	1.25	10.53	0.51	10.54	1.53
16.1(n-7)	2.37	0.52	2.81	0.74	1.19	0.29	1.64	0.45	1.73	0.25	2.17	0.45
16.2	0.58	0.16	0.32	0.14	0.49	0.17	0.11	0.09	0.82	0.62	0.19	0.09
17.0	0.37	0.08	0.27	0.23	0.51	0.10	0.16	0.07	0.47	0.26	0.15	0.06
16.0 DMA[1]	3.36	0.31	4.39	0.48	4.04	0.53	3.98	1.07	4.14	0.64	4.13	0.90
16.4	0.19	0.08	0.19	0.10	0.12	0.05	0.04	0.04	0.10	0.04	0.04	0.03
18.0	3.49	0.52	3.76	0.53	4.51	0.62	4.49	0.48	3.43	0.35	2.86	0.44
18.1(n-9)	1.50	0.20	1.57	0.33	0.91	0.14	1.95	0.36	3.41	1.01	5.29	2.26
18.1(n-7)	2.76	0.20	2.59	0.20	2.53	0.16	3.09	1.68	2.55	0.21	2.57	0.66
18.2(n-6)	1.19	0.15	1.39	0.17	0.82	0.13	1.23	0.19	1.61	0.26	1.75	0.66
18.2(n-3)	0.08	0.05	0.10	0.11	0.07	0.04	0.01	0.01	0.41	0.11	0.30	0.11
18.3(n-6)	0.52	0.06	0.42	0.13	0.60	0.09	0.39	0.12	0.56	0.07	0.63	0.30
18.3(n-3)	1.52	0.27	1.44	0.32	1.07	0.33	0.89	0.21	1.96	0.38	0.86	0.33
18.4(n-3)	2.92	0.87	2.81	0.87	1.23	0.49	1.40	0.47	2.57	0.56	1.65	0.58
20.0	0.49	0.04	0.55	0.09	0.51	0.04	0.60	0.16	0.45	0.11	0.56	0.17
20.2 nMID[2]	6.06	0.47	6.52	0.84	6.84	0.44	8.55	0.62	6.32	0.76	7.53	1.25
20.1(n-9)	1.89	0.16	2.60	0.66	1.53	0.13	2.68	0.43	1.91	0.24	2.19	0.41
20.1(n-7)	1.18	0.08	0.87	0.15	1.30	0.10	0.85	0.13	1.12	0.18	0.96	0.18
20.2(n-6)	2.42	0.24	2.71	0.30	2.67	0.33	2.56	0.27	2.43	0.21	1.65	0.24
21.0	0.25	0.03	0.16	0.07	0.21	0.06	0.14	0.06	0.27	0.07	0.20	0.08
20.4(n-6)	11.32	0.89	13.35	1.62	11.61	0.89	16.57	1.16	11.60	1.25	12.64	1.92
20.3(n-3)	1.99	0.20	1.78	0.19	2.04	0.37	1.33	0.15	2.01	0.49	0.82	0.20
20.4(n-3)	0.71	0.14	0.49	0.16	0.56	0.14	0.34	0.10	0.71	0.19	0.40	0.16
20.5(n-3)	15.70	1.18	17.88	1.16	16.57	1.25	16.16	1.18	13.26	0.94	12.28	1.95
22.1(n-11)	0.15	0.04	0.07	0.04	0.17	0.05	0.15	0.08	0.27	0.11	0.22	0.08
22.1(n-9)	3.68	0.30	3.80	0.25	3.07	0.38	4.03	0.41	3.25	0.24	3.66	0.65
22.2(n-6)	0.09	0.02	0.12	0.12	0.14	0.03	0.01	0.02	0.51	0.32	0.46	0.36
22.5(n-3)	1.27	0.22	0.85	0.18	1.61	0.21	0.70	0.22	1.11	0.18	0.50	0.17
24.1	0.00	0.00	0.00	0.00	0.02	0.02	0.01	0.01	0.01	0.01	0.07	0.12
22.6(n-3)	4.84	1.44	2.15	0.33	6.55	1.30	2.53	0.52	4.10	1.15	1.94	0.53
Σ MUFA	13.52	0.98	14.31	1.14	16.97	0.55	13.76	2.40	14.25	1.26	17.05	1.97
Σ PUFA	44.58	1.87	45.49	1.97	45.20	2.45	41.77	4.59	42.86	1.54	35.88	3.23
Σ SFA	18.18	0.43	19.85	0.74	17.17	1.02	17.87	2.29	19.07	0.66	18.48	1.85
Total FAMEs (mg/g)	16.59	3.48	15.17	3.46	8.37	2.05	7.73	2.88	7.48	1.47	8.90	1.89

fatty acids (PUFA, n-3, n-6) formed the largest class ranging between 35.88 to 45.49%. The saturated fatty acids (SFA, 14.0, 15.0, 16.0, 17.0, 18.0, 20.0, 21.0) was the second largest class ranging between 17.07 to 19.85%. Monounsaturated fatty acids (MUFA, n-9, n-7) were the smallest class. The total FAMEs varied little between habitats but decreased between July and August, and remained stationary after that.

Prior to further analysis a series of pair wise comparisons (ANOSIM) were performed on the fatty acid signatures from each replicate. These showed that there was no significant difference between the replicates for any location, depth or month (Table 2-4).

Table 2-4 Pairwise multivariate (ANOSIM) test for the difference between replicates at each location, depth, and months

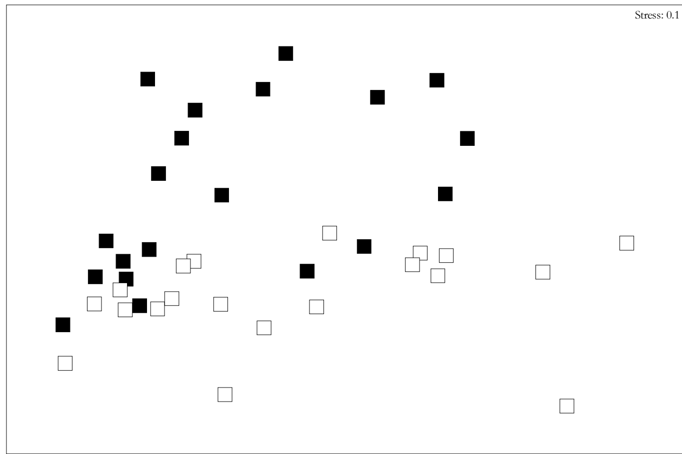
Month	Replicates	r value	p
July	South Shian Intertidal	-0.024	0.43
July	South Shian Subtidal	-0.112	0.83
July	Rubha Garbh Intertidal	-0.104	0.83
July	Rubha Garbh Subtidal	0.048	0.26
August	South Shian Intertidal	0.076	0.25
August	South Shian Subtidal	-0.112	0.88
August	Rubha Garbh Intertidal	-0.068	0.55
August	Rubha Garbh Subtidal	-0.024	0.50
September	South Shian Intertidal	0.064	0.25
September	South Shian Subtidal	0.069	0.27
September	Rubha Garbh Intertidal	0.056	0.24
September	Rubha Garbh Subtidal	0.032	0.39

The nMDS plots for each month (Figure 2-6) show that there is a clear separation between the fatty acid signatures of the intertidal and subtidal populations for all the months. This separation is supported by the crossed analysis of similarity results (Table 2-5). There were significant differences between the depths over all months, when averaged over sites. The difference between sites when averaged over depth shows a significant difference for July, but not for August or September. Although the separation between depths is stable

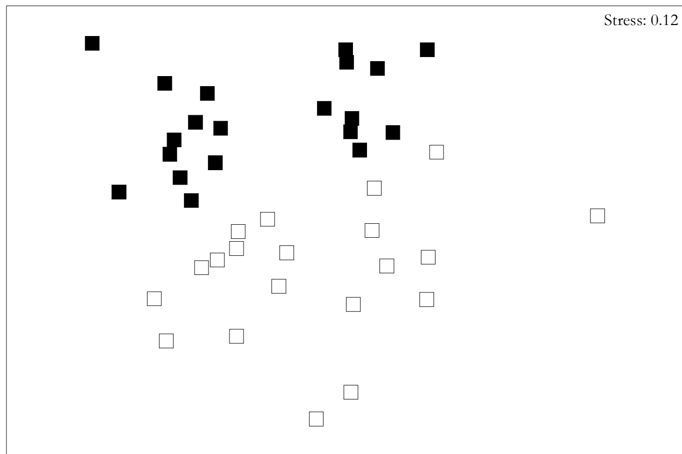
between the months, the fatty acids that are responsible for these differences vary between the months as shown by the SIMPER analysis (Table 2-6). For all months 20.4(n-6) is the predominant fatty acid in driving the differences between the two depths, and for all months the levels were higher in the intertidal than the subtidal. The percentage contribution of this fatty acid did change over the months being highest in August with 25.31% and lowest in September with 15.39%. The fatty acid 20.5(n-3) is the second most important fatty acid for July and September, and the third for August. There is however a difference between the months in the level of this fatty acid between the depths. For July the percentage contribution to the fatty acid signature is higher in the subtidal than in the intertidal (17.9% and 15.7% respectively). In August and September the percentage values are higher in the intertidal. For all months the fatty acid 22.6(n-3) is higher in the intertidal than the subtidal, and for all months occurs in the top four rank by percentage contribution. For the month of September 18.1(n-9) contributes 11.68% and ranks third; it does not however rank amongst the 90% cumulative contribution in the other two months.

Table 2-5 The multivariate difference between the depths, averaged over the sites, and the sites averaged over the depth, for each month. The differences were generated using a crossed ANOSIM based on the untransformed data using Euclidean distance as the metric

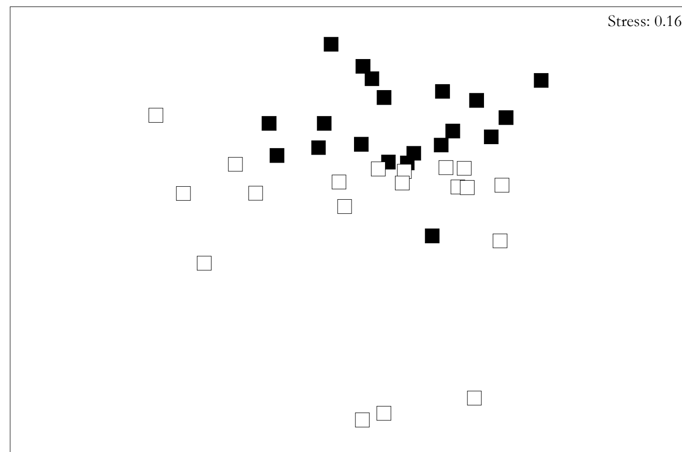
Month	Between depth averaged over site	Between site averaged over depth
1	$r=0.235$ $p<0.001$	$r=0.235$ $p=0.003$
2	$r=0.479$ $p<0.001$	$r=0.086$ $p=0.060$
3	$r=0.193$ $p=0.001$	$r=0.064$ $p=0.095$



JULY



AUGUST



SEPTEMBER

Figure 2-6 nMDS plots for each month showing the difference between the fatty acid signatures of urchins at different depths, based on untransformed data, using euclidean distance as the metric. ■ Intertidal □ Subtidal

Table 2-6 SIMPER analysis of the fatty acids contributing to the difference between the fatty acid signatures of intertidal and subtidal

	Intertidal		Subtidal			
July						
Variable	Mean	95% CI	Mean	95% CI	Contrib%	Cum.%
20.4(n-6)	11.3	091	13.3	1.62	22.27	22.27
20.5(n-3)	15.7	1.21	17.9	1.16	19.64	41.91
22.6(n-3)	4.84	1.48	2.15	0.33	19.61	61.52
18.4(n-3)	2.92	0.89	2.81	0.87	8.02	69.54
20.2 nMID	6.06	0.49	6.52	0.84	5.11	74.64
16.1(n-7)	2.37	0.53	2.81	0.74	4.54	79.18
14.0	3.32	0.56	3.6	0.57	3.38	82.56
18.0	3.49	0.53	3.76	0.53	2.99	85.55
20.1(n-9)	1.89	0.16	2.6	0.66	2.94	88.49
16.0 DMA	3.36	0.32	4.39	0.48	2.87	91.36
August						
20.4(n-6)	11.6	0.90	16.6	1.16	25.31	25.31
22.6(n-3)	6.55	1.30	2.53	0.52	18.51	43.81
20.5(n-3)	16.6	1.25	16.2	1.12	10.60	54.41
18.1(n-7)	2.53	0.16	3.09	1.68	10.35	64.76
16.0	8.77	0.98	9.81	1.25	9.68	74.44
16.0 DMA	4.04	0.53	3.98	1.07	5.08	79.52
20.2 nMID	6.84	0.4	8.55	0.62	4.15	83.67
18.0	4.51	0.62	4.49	0.48	2.19	85.86
14.0	2.1	0.41	3.04	0.46	1.99	87.85
22.1(n-9)	3.07	0.04	4.03	0.41	1.78	89.63
18.4(n-3)	1.23	0.49	1.4	0.47	1.66	91.29
September						
20.4(n-6)	11.6	1.25	11.9	1.37	15.39	15.39
20.5(n-3)	13.3	0.94	11.4	1.05	11.90	27.28
18.1(n-9)	3.41	1.00	4.3	1.21	11.68	38.96
22.6(n-3)	4.1	1.15	2.05	0.52	11.12	50.08
20.2 nMID	6.32	0.76	7.93	1.03	9.62	59.69
16.0	10.5	0.51	9.99	1.16	7.21	66.90
16.0 DMA	4.14	0.64	4.34	0.83	4.91	71.81
18.4(n-3)	2.57	0.56	1.74	0.58	3.52	75.33
14.0	3.02	0.28	3.7	0.78	3.44	78.77
20.3(n-3)	2.01	0.49	0.861	0.19	2.46	81.23
18.2(n-6)	1.61	0.26	1.84	0.67	2.31	83.54
18.3(n-3)	1.96	0.38	0.908	0.34	2.19	85.73
16.2	0.822	0.62	0.201	0.09	2.16	87.89
18.1(n-7)	2.55	0.21	2.71	0.63	1.98	89.87
22.1(n-9)	3.25	0.24	3.85	0.56	1.97	91.84

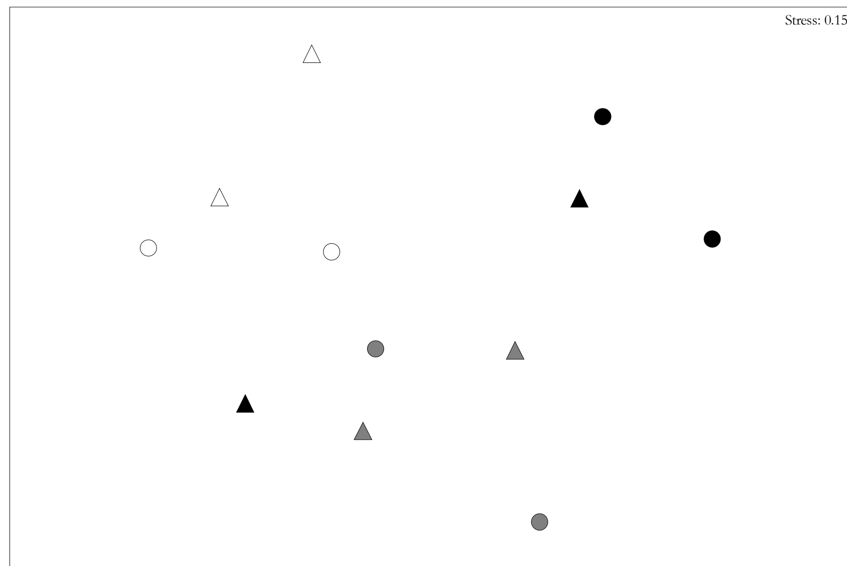


Figure 2-7 Second stage MDS plot showing the multivariate differences in the fatty acid signatures , between depth, and months using spear rank correlation to generate the similarity matrices. △Intertidal ○Subtidal □Month1 ■Month2 ■Month 3

The relationship between the fatty acid signatures of each month is represented in the second stage MDS plot (Figure 2-7). There is segregation in the signature by month, with this pattern being confirmed by the ANOSIM results (Table 2-7). Post hoc pair wise testing show that there is a difference between all pairs of months.

Table 2-7 One way ANOSIM with post hoc pair wise tests for differences between fatty acid signature for each month

Month	r	p
All months	0.172	P<0.001
1&2	0.111	P<0.001
1&3	0.184	P<0.001
2&3	0.228	P<0.001

Table 2-8 One way ANOSIM of differences between the sexes for each maturity stage based on the fatty acid signatures.

Stage	r	p
3	0.168	p=0.233
4	0.398	P<0.001
5	0.167	p=0.0900
6	0.055	p=0.220

In order to examine the role that the recorded change in maturity stage had on the fatty acid signature, a crossed ANOSIM was used to test for significance differences in the fatty acid signature of each sex at each maturity stage averaged over the months (Table 2-8). The test confirmed there was a significant difference between the months when averaged across the sex and maturity stage ($r=0.157$, $p=0.014$), and a significant difference between sex/maturity stage when averaged across months ($r=0.269$, $p<0.001$). Post hoc pair wise testing was used to examine the relation between the sexes at each maturity stage, when averaged over the months. Only at maturity stage four was there a significant difference in the fatty acid signatures between the sexes. A two-way SIMPER was used to see which specific fatty acids were contributing to this difference (Table 2-9). It was shown that 20.5(n-3), 20.4(n-6), and 22.6(n-3) were all found in higher proportions in the males, and contributed a total of 59% of the difference between them.

Table 2-9 Two way SIMPER analysis of the fatty acids contributing to the difference between the fatty acid signatures of the sexes at maturity stage four when averaged over the months

	Mean			
Variable	Female	Male	Contrib%	Cum.%
20.5(n-3)	14.8	18.6	22.65	22.65
20.4(n-6)	10.3	14.3	22.24	44.89
22.6(n-3)	3.66	4.94	14.21	59.10
18.4(n-3)	3.48	1.13	8.19	67.30
16.1(n-7)	2.98	1.41	5.14	72.44
18.0	2.84	4.91	4.71	77.15
14.0	3.91	2.24	4.14	81.29
16.0 DMA	3.65	4.07	3.58	84.87
20.2 nMID	6.4	6.86	2.97	87.83
20.1(n-9)	1.54	2.49	2.35	90.19

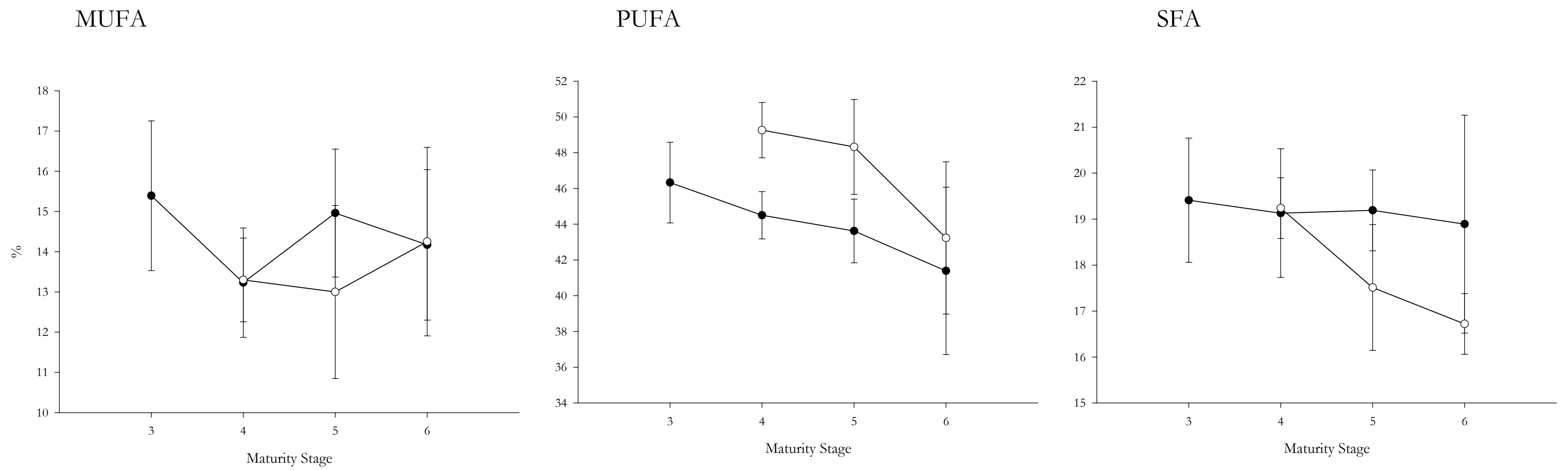
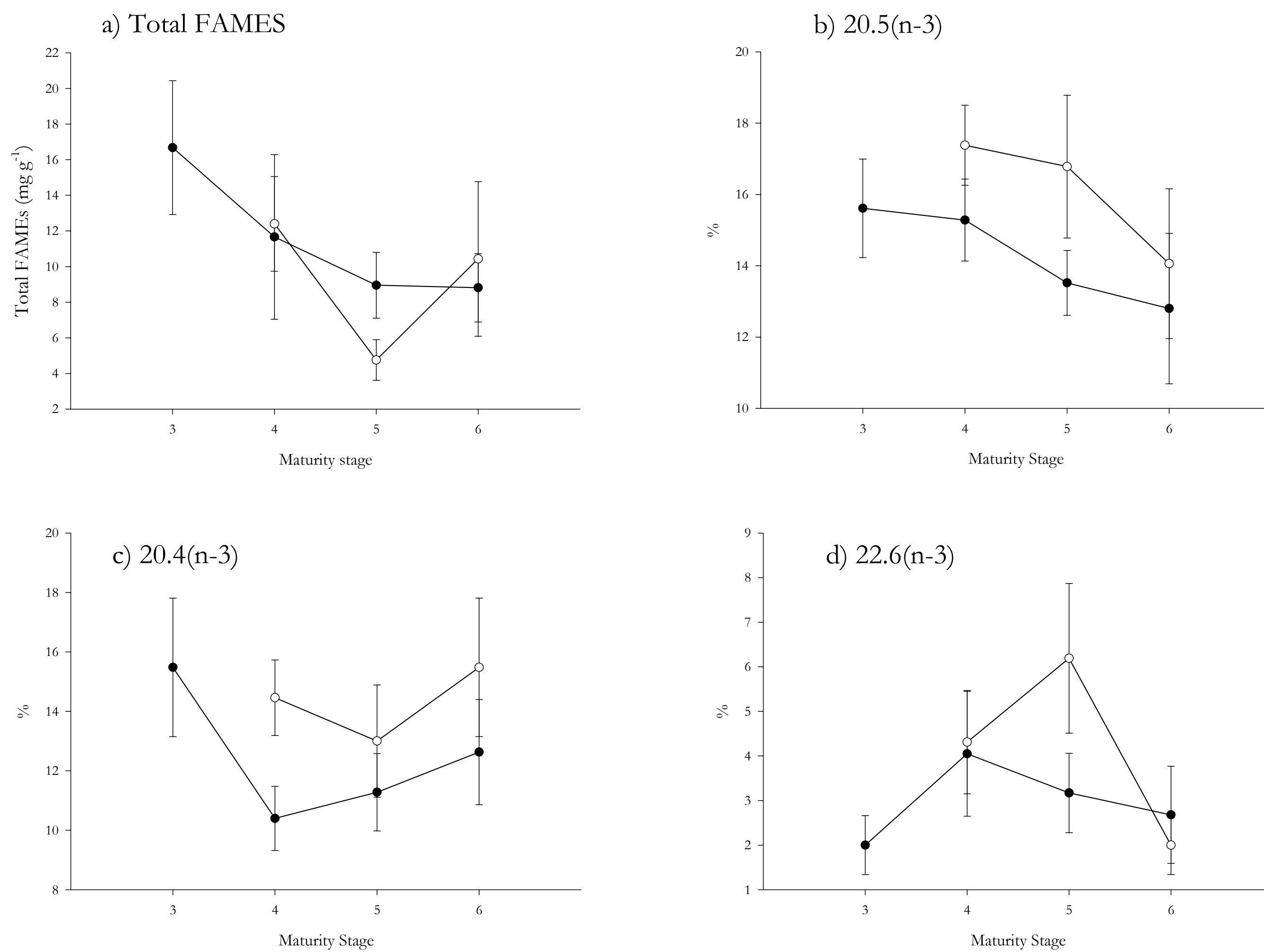


Figure 2-8 The variation in classes of fatty acid between sexes at each different maturity stage.

●Female ○Male

(MUFA Mono-unsaturated fatty acids, PUFAs Polyunsaturated fatty acids, SFA Saturated fatty acids)

Figure 2-9 The mean percentage (error bars =95% confidence intervals) of the a) Total FAMEs (Fatty Acid Methyl Esters) and the fatty acids b) 20.5(n-3), c) 20.4(n-6), d) 22.6(n-3) ●Female ○Male



The different fatty acid classes showed considerable variation in their mean levels for each maturity stage and sex (Figure 2-8). The total of the PUFA shows a clear reduction over the maturity stages for both sexes, but the SFA showed only a reduction for the males. There are only show three data points for the male as there was only one stage three male. Both sexes show a reduction in the levels of 20.5(n-3) with increasing maturity stage (Figure 2-9). The female urchins show a rapid reduction in the fatty acid between stage four and five, while males showed a dramatic reduction between stage five and six. For 20.4(n-6) there is a dramatic reduction between stage three and four and then a subsequent increase, the males showed a reduction between stage four and five followed by an increase at stage six. The inverse pattern is seen for 22.6(n-3), with an increase for females between stage three and four followed by a reduction between stage four and six. For the males there was an increase from stage four to five, and a reduction from stage five to six.

Of the five environmental variables designated for each urchin it was the variables depth and sex which provided the highest correlation (rho=0.305) with the fatty acid signatures. A permutation based hypothesis test showed that this correlation was significant (p=0.01%).

DISCUSSION

Echinoid gonadal indices

As the gonad is the primary nutritive storage organ, GSI's are used as the standard indicators of individual and population nutritive and reproductive status. In the current study, clear and significant variability of GSI with depth is demonstrated in the highly abundant species, *P. miliaris*, at the study sites on the west coast of Scotland. Individuals occurring in the intertidal zone had a significantly higher GSI than those just a few meters deeper in the subtidal zone. Study locations were chosen such that the spatial distance between replicates was equivalent to that between depths. Thus the differences between the GSI at the two depths were not attributable to patchiness at the scale of 10's of meters. So the first hypothesis that variation in the GSI is a result of depth and is independent of spatial variation can be accepted. This difference between the nutritional state of urchins from different habitats has been recorded for a number of different species. *Anthocidaris crassispina* from *Sargassum* beds had a higher GSI when compared to those from areas dominated by coralline algae (Yatsuya & Nakahara 2004), and *Centrostephanus rodgersii* from barren areas had a lower GSI compared to those from algal dominated areas (Byrne et al. 1998). Both studies found that gonad development was synchronous between the habitats, and this was also the case in the current study. The intertidal has been described as an environmental gradient of increasing harshness from low to high tide level (Underwood & Chapman 2000). *Psammechinus miliaris* in the intertidal zone are exposed for 1-2 hours every tidal cycle during spring tides for periods of up to 3-5 days. During these periods of exposure individuals were observed to be inactive and showed no evidence of feeding, although the period of inactivity was sometimes greater than the periods of exposure. Even when immersed diurnally the intertidal individuals rarely occurred away from their cryptic refuges (around the bases of boulders). Birds are known to be predators of intertidal sea urchins (Wootton 1995, Pacheco & Castilla 2000, Hori & Noda 2001), though

the importance of this has been debated (Barnes & Crook 2001). In addition the high incidence of UV radiation in the intertidal zone has been found to promote cryptic behaviour (Verling et al. 2002). The combination of these factors could well lead intertidal urchins to seek refuges even when immersed in shallow water. Given those animals in the intertidal have less time to forage, and are subject to higher levels of environmental stress, why should individuals in the intertidal zone have higher GSI values than those in the subtidal?

As well as a clear difference in the GSI between the two depths there is a progressive reduction in the GSI over the three month course of the study at both depths. This is coupled with a progressive increase in maturity stage of the urchins from the study population. A similar pattern was recorded for this species by Kelly (Kelly 2000), who noted a dramatic reduction in GSI during the month of August, and all of the females had reached maturity stage five. Although GSI has been questioned as a reliable predictor of maturity stage (Lozano et al. 1995), the study population showed a significant difference between pre spawned (stage 3&4), and post spawned (5&6).

Fatty Acid Analysis

The fatty acid composition of *P. miliaris* from this study was similar to that found in other studies of echinoids. Using all soft body parts of the urchin *Paracentrotus lividus* Serrazanetti et al.(1995) found the dominant fatty acids to be 14.0, 16.0 and 20.5(n-3). Also using all the soft tissue the fatty acids of *Tripneustes gratilla* (Floreto et al. 1996), found that PUFA was also the dominant class, and that 16.0, 20.4(n-6), 20.5(n-3) were the primary fatty acids. The testis of *Echinus esculentus* collected from southern England again showed a similar pattern but showed much higher levels of 22.6(n-3) than those of *P. miliaris* in our study (Allen 1968). A previous study of the fatty composition of *P. miliaris* from the west coast of Scotland (Cook et al. 2000) showed very similar patterns to those observed in this study.

There was however a difference in the total PUFA, with wild populations having values lower than reported in the current study.

Spatial Variation

The difference between the fatty acid signature of intertidal and subtidal urchins was constant over the course of the experiment, although there was a degree in variation in the fatty acids that drove this difference over time. The fatty acid 20.4(n-6) which contributed more to the difference between intertidal and subtidal for all months and was consistently higher in the subtidal has been associated with brown algae (Khotimchenko et al. 2002) and specifically with *Laminaria* sp (Cook et al. 2000). The fatty acid 20.5(n-3), which has been associated with brown algae through predominant for all months, changed from being higher in the subtidal in July to being higher in the Intertidal by September. The increase in this fatty acid in the intertidal could be a result of an increase in the availability of drift kelp to the intertidal as a result of early autumnal storms. The elevated levels of 18.4(n-3) in the intertidal urchins indicate consumption of ephemeral green algae (Mai et al. 1996). The presence of higher quantities of 22.6(n-3) in the intertidal is indicative of a diet that is higher in animal derived material (Kharlamenko et al. 1995). These results would indicate that the intertidal urchins were eating a diet richer in ephemeral green algae and filter feeding invertebrates while those in the subtidal had a diet richer in brown algae namely *Laminaria* spp.

The spatial heterogeneity in the fatty acid signatures shows variation over the course of the study. For all months there was no variation at the scale of 10s m, but for July there is a significant difference at the scale of 1000s m. However for August and September there is no significant difference at this scale. It is therefore important when looking at spatial variation to examine it over a range of temporal scales before drawing conclusions as to its importance. In this case the spatial heterogeneity observed in July could have been a result

of community differences in the biota of the sites, that became less pronounced through time, as in July the primary differences between the sites are a result of fatty acids associated with filter feeding invertebrate which were higher at South Shian, and ephemeral algae which was higher in Rubha Garbh. The site at Rubha Garbh has been noted for high levels of ephemeral algae, due to run off from a nearby field (Kelly pers. Comm.).

Temporal variation

It is important to understand that all temporal variation observed in the fatty acid signature of wild population of urchins is confounded by the dual role of the urchin gonad. Because the organ is both the reproductive and nutritive store (Russell 1998), any observed differences in the fatty acid signature over time may be a result of both changes in diet and changes in the reproductive maturity of the animal. The current study showed clear differences between the months in the fatty acid signature and this was consistent over depth, and location. An unknown proportion of the difference can be attributed to differences in diet, as when averaged over the maturity stage there are still significant differences between the months.

Sex based differences in the fatty acid signature of the gonad have been reported for a number of marine invertebrates. For this study there was only a significant difference at maturity stage 4 just prior to spawning. As there was only a single male stage 3, it makes it difficult to draw any conclusions for this maturity stage. That significant difference exist between the sexes prior to, but not post spawning is suggests that the difference in the fatty acid signature between male and female gonads at stage four is the result of the presence of high levels of gametes, and that these gametes have different fatty acid composition. For the difference reported between the sexes there is a degree of concordance with reported differences in other marine animals. The female gonads of molluscs (the limpet *Patella depressa* (Morais et al. 2003), the Scallop *Argopecten purpuratus* (Caers et al. 1999)) and a fish

(the smelt *Plecoglossus altivelis* (Jeong et al. 2002)) had higher proportions of 14:0, 16:1(n-7) and 18:1(n-9) and the male gonads had higher proportions of 20:4 (n-6) and 22:6(n-3). Such inter sex differences were also observed in the gonads of *P. miliaris*. However the male limpet had higher levels of 18:3(n-3) and 18:4(n-3), and the opposite was found in *P. miliaris* gonads. This difference between the sexes is an important consideration when using the fatty acid signatures to study the trophic ecology of natural populations that may, unlike *P. miliaris*, exhibit sex bias in spatial distribution (Guettaf et al. 2000).

There were distinct changes in the fatty acid composition of gonads with increased maturity stage. PUFAs have been associated with reproductive capacity in shrimps (Rosa & Nunes 2003) and specifically with structural elements in the phospholipid membranes that have been shown to be prominent components in spermatozoa of marine invertebrates (Morais et al. 2003), and were found in higher levels in the gonad of male limpets compared to females. This was supported in the current study with males having higher levels of PUFA and showed a dramatic reduction with maturity stage 4 through 6. It could be speculated that this was as a result of reduction of male gametes in the gonad during spawning In contrast MUFAs have been linked to energy reserves necessary for vitellogenesis, and significantly higher concentrations have been reported for female gonads (Brazao et al. 2003). The pattern for *P. miliaris* in this study does not show a clear pattern, with males and females showing similar levels, and no noticeable reduction with maturity stage. It is possible that the multifunctional role of the gonad may lead to a more complicated interruption of the variation in the fatty acid signature of the gonad. The fatty acids 20.5(n-3) and 22.6(n-3) have been linked to energy storage in teleost eggs (Fraser et al. 1988), and as an energy source during embryogenesis (Xu et al. 1994). It has also been hypothesised that 22.6(n-3) plays an important role in the membrane of eggs (Pazos et al. 1997). There was a rapid reduction in these fatty acids from stage 4 to 5 in the female

gonads of *P. miliaris*. It could be speculated that this reduction is as a result of release of oocytes rich in 20.5(n-3) and 22.6(n-3).

Further studies are required to differentiate the role of diet and sexual maturation in influencing the biochemical composition of the gonad. Ojea (2004) concluded that changes in the fatty acid composition of the clam *Ruditapes deccusatus* appeared to be more closely related to diet than to endogenous factors. The current study showed that the environmental variables of depth and sex were the best correlated with the fatty acid signature. This suggests that the duality of function of the gonad is reflected in its fatty acid signature, with habitat related diet differences combined with sex differences giving the best correlation with the fatty acid signature.

NOTE

Aspects of this chapter have subsequently been published:

Hughes A.D., Kelly M.S., Barnes D.K.A., Catarino, A.I and K. D. Black. (In Press). The Dual Functions of Sea Urchin Gonads are reflected in the temporal variation of their Biochemistry. Marine Biology

Hughes A.D., Catarino, A.I., Kelly M.S., Barnes D.K.A., and K. D. Black. (2005). Gonad Fatty Acids and Trophic Interactions of the echinoid *Psammechinus miliaris*. Marine Ecology Progress Series 305 p101-111

Chapter 3

RAPID CHANGE IN SEA URCHIN GONAD BIOCHEMISTRY FOLLOWING A CHANGE IN DIET

INTRODUCTION

In order to fully understand trophic interactions of a species and the role that a species plays in regulating community structure, it is important to resolve the spatial and temporal scales at which these interactions occur. Temporal heterogeneity in trophic interactions can have important consequences for food-web structure; temporally explicit food-webs are better predictors of predator prey ratios and levels of omnivory than those without a defined temporal component (TavaresCromar & Williams 1996). Such heterogeneity in temporal elements can also lead to changes in the primary carbon source on which the food-web is based. For example, in a tropical aquatic food-web study there was a seasonal shift in the primary carbon source from algae to prokaryotes and fungi (Winemiller 1990). Ontogenetic shifts in diet can similarly lead to dramatic shifts in trophic position (Deudero et al. 2004) and as such can mediate predator-prey interactions (Olson et al. 1995).

Biochemical proxies for trophic interactions have been an important tool for ecologists (Fry & Sherr 1984, Kleppel & Pieper 1984, Pond et al. 1997). As the techniques have developed there has been an increasing appreciation that the relationships between these biochemical proxies and the trophic interactions, for which they are estimates, are not simple (Post 2002, Dalsgaard et al. 2003). Many factors other than trophic interactions can cause significant variation in the biochemical proxies. Matthews & Mazumder (2005) recently showed that the lipid content of zooplankton (a trophic level independent change) could cause more variation in δ13C than trophic changes. Variation between metabolic pathways and rates have also been shown to result in significant differences in the values of trophic proxies between tissues from the same organism (Vanderklift & Ponsard 2003).

In light of this variability, it is desirable when using trophic biomarkers, to explore these sources of variation in the specific context of the investigation. In chapter 2 fatty acid analysis was used as a proxy for trophic interactions of *Psammechinus miliaris*. The current study was explicitly designed to look at temporal variation in the fatty acid signature at the scale of months. Temporal variations in fatty acid signature have been recorded within a number of species, most notably in changes linked to the annual reproductive cycle. In the bivalve *Ruditapes decussatus*, maximal content of the polyunsaturated fatty acids corresponded with oocyte maturation (Ojea et al. 2004). Temporal variations in the fatty acids signatures of predators have also been linked to variation in the type and composition of available prey (Phillips et al. 2003).

In order to validate conclusions drawn from our study (chapter 2) it is essential to determine if fatty acid analysis is capable of detecting trophic related changes in gonad biochemistry at the scale of months. The objective of this study was to define the possible temporal resolution when using fatty acid signatures of sea urchin gonads to investigate trophic interaction.

METHODS

Experimental set up

Fifty sea urchins were collected from a single intertidal location (SS) and within 10m of each other, on the shores of Loch Creran on the west coast of Scotland. The urchins were selected to be of similar test diameter (25 mm Ø). They were returned to the laboratory and held in aquaria with running sea water and without food for 17 days to standardize their nutritional status. Individuals were then randomly split into two groups of 25. The *P. miliaris* were distributed amongst 10 experimental aquaria, five urchins in each and five aquaria for each of the treatments.

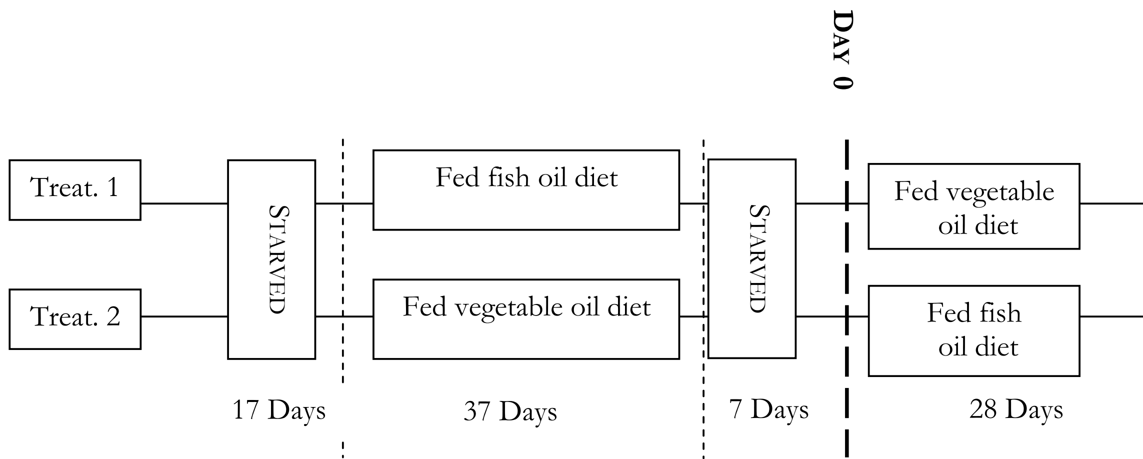


Figure 3-1 Schematic representation of the experimental procedure for each treatment.

The experiment consisted of two treatments: each group was fed a diet either rich in fish oil (cod liver oil) or vegetable oil (olive oil). The urchin feed was made by dissolving 50 mg of gelatin into 250 ml of warm water (60°C), then adding 250 ml of either olive or cod liver oil. This was then blended to form a homogenate and poured into Petri dishes to set. This was then cut into 1cm^2 sections and fed to the urchins. They were maintained on their diet for 37 days after which they were starved for a week before changing diet (Figure 3-1). Those previously fed on fish oil were swapped to diet two, the vegetable oil diet (Treatment 1) and *vice se versa* (Treatment 2). The *P. miliaris* were starved for seven days prior to the change to ensure active feeding upon the new diet. The day that the diet was

exchanged was termed day zero. Five individuals from each group (one from each aquarium) were selected at random, without replacement, on days zero, four, seven, fourteen, and twenty eight. The individuals were held without food for between 48 and 72 hours, to ensure the gut was empty prior to removal of the gonad (reducing the risk of sample contamination from ingested food from the gut). The gonads of these individuals were then removed and used for fatty acid analysis; during this procedure great care was taken to make sure that there was no contamination between the gut and the gonad. Samples of each diet were also analysed.

Lipid extraction and fatty acid analysis

This procedure was carried out as described in chapter 2.

Statistical Analysis

Using the relative proportion of each fatty acid in the sample as a variable, multivariate analysis using the PRIMER v5 package was used to test for differences in the fatty acid signatures between the two treatments. ANalysis of SIMilarity (ANOSIM) was used as a hypothesis testing framework to test for differences between treatment 1 and 2 at days 0, 4, 7, 14, and 28.

A stepwise procedure (BV step) was used to reduce the number of variables (fatty acids) prior to ordination of the data in a principal component analysis. This step-wise algorithm aims to select a sub set of variables which match the multivariate pattern for a full set of variables. For this study, the stop value for the correlation was set at rho=0.95. Five variables were selected with a rho=0.953. These variables were then used for a PCA analysis of those data where the ANOSIM results indicated a significant difference between the treatments. One way ANOVA was used to test the difference between the 16.1(n-7)/16.0 ratio between each treatment.

RESULTS

The primary constituents of the fish oil diet (Table 3-1) were 18.1(n-9), (16.78%), and 16.0 (14.46%), but it was also rich in 16.1(n-7), 20.1(n-9), 20.5(n-3), 22.6(n-3). The vegetable oil diet was primarily (76.25%) made up of 18.1(n-7), but was also rich in 16.0 (10.75%). At day zero Treatment 1 had higher levels of 20.5(n-3) constituting 12.78%, followed by 20.4(n-6) comprising 11.46%. For Treatment 2 these two fatty acids also made up the largest percentage, although their ranking had been swapped with 20.4(n-6) being the primary fatty acid with 13.72% and 20.5(n-3) with 10.56%. By day 28 this pattern was maintained although the values had altered and 18.1(n-9) had become the primary fatty acid (16.42%) of Treatment 2. Over the course of the experiment (Figure 3-2) there was little change in the GSI of treatment 2 while there appears to been a reduction in GSI of treatment 1 although day zero values remain within the confidence interval of day 28.

Multivariate analysis of the differences (ANOSIM) between the fatty acid signatures of each treatment at each sampling date showed that there were significant differences between the treatments at days zero and four (Table 3-2). After seven and 14 days there was no significant difference but by day 28 there was again a significant difference at the 5% level. The R value, which is the test statistic for the ANOSIM test, can be used as a measure of multivariate dispersal, with higher values indicating a greater difference between the two treatments. There was an increase in R from day zero to four and then a rapid decrease until day 14 (Table 3-2) followed by an increase to a value of 0.320 by day 28. As well as the variability between treatments it is possible to look at the inter treatment variability by examining the relative dispersal (rd) value. Over the course of the experiment the inter-sample variation for each treatment followed a similar pattern, increasing from day zero to a peak at day seven and then decreasing to day 28. There was however a lag for Treatment 1 with little change between day zero and day four (Figure 3-3).

Table 3-1 The relative abundances (%) of the identified fatty acids from the sea urchin gonads, separated by day and treatment. 1 dimethylacetal 2 non-methylene interrupted dienes

	Diet				Day Zero				Day 4			
	Cod Liver		Olive		Treatment 1		Treatment 2		Treatment 1		Treatment 2	
	Aver	CI	Aver	CI	Aver	CI	Aver	CI	Aver	CI	Aver	CI
14.0	4.07	0.09	0.00	0.00	3.04	0.91	1.67	0.39	1.96	1.31	2.25	0.99
14.1	0.10	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
a15.0	0.00	0.00	0.00	0.00	0.02	0.04	0.00	0.00	0.06	0.08	0.02	0.04
15.0	0.40	0.00	0.00	0.00	0.75	0.18	0.63	0.26	0.51	0.17	0.67	0.21
16.0	14.5	3.14	108	0.10	7.84	1.63	7.39	0.40	8.08	1.74	8.23	0.63
16.1(n-7)	7.43	1.39	0.50	0.78	2.93	0.67	1.24	0.36	2.83	1.49	2.31	0.88
a17.0	0.15	0.10	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
16.2	0.70	0.20	0.00	0.00	0.34	0.28	0.07	0.09	0.33	0.29	0.12	0.24
17.0	0.25	0.10	0.00	0.00	0.16	0.09	0.20	0.18	0.16	0.09	0.04	0.08
17.1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
16.3	0.60	0.01	0.10	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
16.0 DMA[1]	0.00	0.00	0.00	0.00	4.93	1.74	5.46	0.78	3.57	1.48	3.16	2.71
16.4	0.20	0.20	0.00	0.00	0.00	0.00	0.00	0.00	0.13	0.17	0.08	0.16
18.0	2.71	0.00	1.00	0.98	3.46	0.60	3.90	0.80	4.49	1.70	4.00	0.97
18.1(n-9)	16.8	0.41	0.00	0.00	5.46	2.56	9.69	2.90	2.93	0.70	9.98	5.46
18.1(n-7)	4.12	0.00	76.3	1.86	2.60	0.51	1.78	0.17	2.78	0.27	2.27	0.32
18.2(n-6)	1.66	0.10	5.70	0.20	0.21	0.41	0.00	0.00	0.83	0.47	2.40	1.39
18.2(n-3)	0.20	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
18.3(n-6)	0.20	0.00	0.00	0.00	0.24	0.23	0.20	0.16	0.12	0.24	0.10	0.20
18.3(n-3)	1.05	0.10	0.05	0.10	0.90	1.00	0.58	0.44	0.88	0.70	0.69	0.81
18.4(n-3)	2.81	0.19	0.00	0.00	2.19	1.92	0.60	0.71	1.90	1.81	1.17	2.00
20.0	0.10	0.00	0.40	0.00	0.36	0.28	0.11	0.14	0.38	0.19	0.16	0.19
20.2 nMID[2]	0.00	0.00	0.00	0.00	7.08	1.96	7.88	1.56	7.38	1.17	7.37	1.28
20.1(n-9)	8.89	0.10	0.00	0.00	2.57	0.90	3.64	0.25	2.12	0.45	3.76	1.10
20.1(n-7)	0.30	0.00	0.00	0.00	0.62	0.15	0.43	0.23	1.16	0.18	0.98	1.20
20.2(n-6)	0.30	0.00	0.00	0.00	1.37	0.21	2.10	0.40	2.60	0.79	2.03	0.28
21.0	0.00	0.00	0.00	0.00	0.15	0.14	0.11	0.10	0.13	0.12	0.06	0.12
20.4(n-6)	0.40	0.00	0.00	0.00	11.5	1.95	13.7	1.38	13.2	2.00	12.1	1.61
20.3(n-3)	0.20	0.00	0.00	0.00	0.83	0.73	1.24	0.38	1.64	0.97	1.11	0.59
20.4(n-3)	0.70	0.00	0.00	0.00	0.64	0.73	0.23	0.12	0.48	0.28	0.26	0.36
20.5(n-3)	9.59	0.48	0.10	0.00	12.9	1.18	10.6	1.28	173	3.31	11.6	2.77
22.1(n-11)	6.23	0.20	0.00	0.00	0.89	0.54	0.11	0.10	0.12	0.13	0.64	0.56
22.1(n-9)	0.65	0.09	0.00	0.00	3.28	0.54	3.33	0.57	4.24	0.57	3.46	0.34
22.2(n-6)	0.00	0.00	0.00	0.00	0.14	0.27	0.00	0.00	0.42	0.56	1.48	1.17
22.5(n-3)	0.90	0.00	0.00	0.00	0.50	0.26	0.78	0.45	1.23	0.55	0.53	0.44
24.1	0.00	0.00	0.00	0.00	0.02	0.04	0.02	0.04	0.00	0.00	0.00	0.00
22.6(n-3)	11.3	0.19	0.00	0.00	3.18	1.30	2.03	1.09	3.48	1.48	2.04	1.28

	Day 7				Day 14				Day 28			
	Treatment 1		Treatment 2		Treatment 1		Treatment 2		Treatment 1		Treatment 2	
Acid	Aver	CI	Aver	CI	Aver	CI	Aver	CI	Aver	CI	Aver	CI
14.0	2.16	0.70	2.34	0.34	2.78	0.43	2.23	0.38	2.46	0.69	2.30	0.20
14.1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
a15.0	0.00	0.00	0.00	0.00	0.02	0.04	0.00	0.00	0.04	0.05	0.00	0.00
15.0	0.45	0.34	0.40	0.33	0.87	0.55	2.39	3.06	0.86	0.19	0.95	0.17
16.0	6.91	2.31	8.36	1.14	8.53	0.51	6.26	3.09	7.96	1.61	7.56	0.19
16.1(n-7)	1.98	0.83	2.62	0.71	2.10	0.62	1.44	0.81	1.75	0.62	2.57	0.40
a17.0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
16.2	0.00	0.00	0.08	0.16	0.09	0.18	0.07	0.13	0.11	0.13	0.07	0.13
17.0	0.08	0.10	0.04	0.08	0.04	0.09	0.03	0.06	0.09	0.11	0.04	0.09
17.1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
16.3	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
16.0DMA[1]	4.39	2.21	2.92	3.08	6.44	2.76	6.41	1.76	6.88	2.68	6.49	0.58
16.4	0.00	0.00	0.04	0.08	0.07	0.13	0.07	0.13	0.04	0.08	0.00	0.00
18.0	5.05	1.05	3.43	1.89	4.72	1.00	5.01	1.05	4.04	1.33	4.29	0.57
18.1(n-9)	9.79	6.78	18.5	9.19	6.75	5.15	6.13	5.41	5.74	2.90	16.4	6.49
18.1(n-7)	2.62	0.61	2.53	0.40	2.38	0.53	1.34	0.70	2.46	0.42	2.41	0.22
18.2(n-6)	0.95	0.65	2.90	0.62	0.53	0.47	3.12	2.60	0.89	0.56	2.38	0.79
18.2(n-3)	0.00	0.00	0.02	0.04	0.02	0.04	0.00	0.00	0.02	0.04	0.00	0.00
18.3(n-6)	0.10	0.13	0.10	0.20	0.11	0.22	0.03	0.06	0.20	0.18	0.07	0.13
18.3(n-3)	0.25	0.21	0.47	0.59	0.34	0.66	0.54	0.47	0.51	0.48	0.31	0.26
18.4(n-3)	0.51	0.66	1.08	1.40	0.92	1.80	0.87	0.88	1.07	1.15	0.64	0.56
20.0	0.17	0.20	0.06	0.12	0.09	0.18	0.17	0.21	0.17	0.20	0.04	0.09
20.2nMID[2]	8.62	1.99	8.29	0.95	11.2	2.94	9.49	2.31	9.75	1.97	8.33	0.22
20.1(n-9)	2.57	0.45	4.36	0.80	3.00	0.65	2.29	1.31	2.69	0.67	3.71	0.56
20.1(n-7)	0.46	0.38	1.21	1.95	0.25	0.48	0.48	0.39	0.89	0.61	0.39	0.50
20.2(n-6)	1.53	1.39	1.26	0.92	1.04	1.05	1.08	0.88	1.95	1.24	1.34	0.62
21.0	0.00	0.00	0.06	0.12	0.07	0.13	0.13	0.16	0.20	0.21	0.00	0.00
20.4(n-6)	11.8	1.02	8.91	4.79	14.9	2.92	16.2	3.44	12.6	2.23	11.9	1.12
20.3(n-3)	1.25	1.09	0.46	0.90	1.01	1.09	1.08	0.94	1.76	1.23	0.47	0.64
20.4(n-3)	0.18	0.16	0.19	0.37	0.25	0.48	0.23	0.31	0.42	0.29	0.09	0.17
20.5(n-3)	14.3	3.57	10.9	3.55	15.0	1.43	14.6	1.95	15.0	4.02	9.70	1.80
22.1(n-11)	0.25	0.39	0.45	0.74	0.22	0.33	0.21	0.20	3.81	0.91	2.88	0.72
22.1(n-9)	3.91	1.45	2.16	1.49	2.20	1.89	3.60	0.81	0.00	0.00	0.71	1.39
22.2(n-6)	0.59	1.10	2.60	3.20	0.41	0.66	1.28	2.20	0.09	0.08	0.16	0.31
22.5(n-3)	0.85	0.77	0.25	0.49	0.31	0.62	0.47	0.54	1.13	0.75	0.34	0.47
24.1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
22.6(n-3)	2.77	1.42	1.31	1.05	2.75	1.70	1.19	1.14	2.98	0.60	2.81	0.78

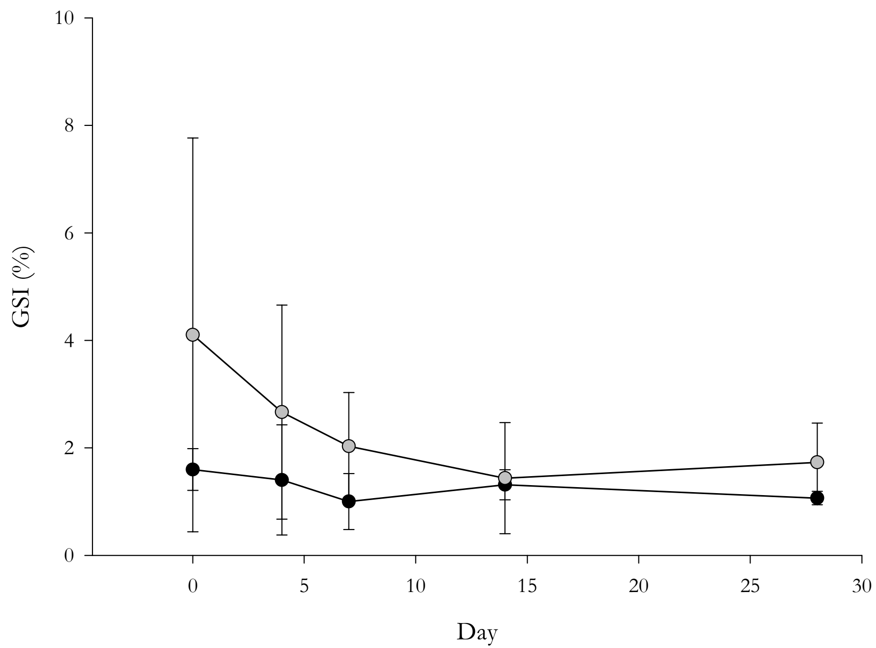


Figure 3-2 Variation in gonadal somatic indices after the swapping of diet. Error bars indicate 95%CI around the mean. ● Treatment 1● Treatment 2

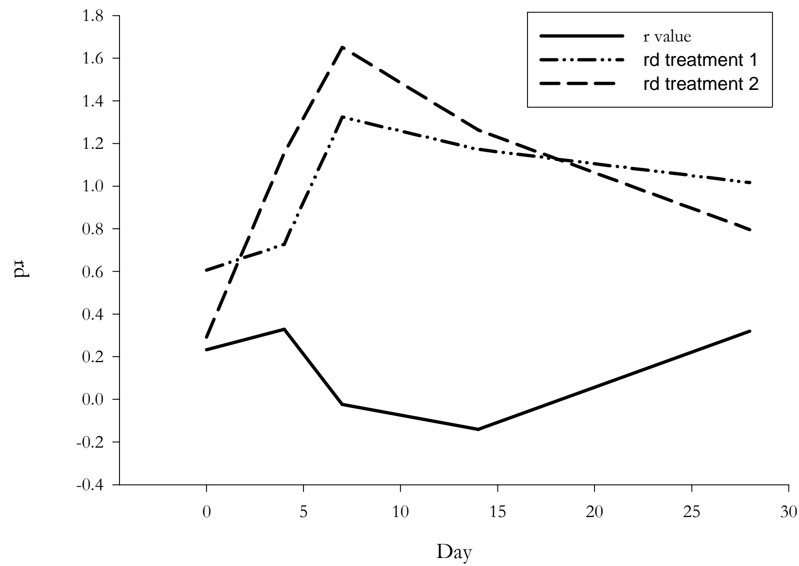


Figure 3-3 The multivariate dispersal between sample (r value) and within the samples (rd) over the course of the experiment

Covariance-based principle component analysis of days zero and 28 was carried out (other days were omitted for clarity). Prior to PCA the number of variables (Fatty Acids) were

reduced using a step-wise procedure to maximise the correlation between the new subset and existing 4 matrix. This resulted in the selection of five fatty acids (16.0, 18.1(n-9), 20.2NMID, 20.4(n-6), 20.5(n-3)) that gave a rho=0.953.

Day	0	4	7	14	28
R statistic	0.232	0.328	-0.024	-0.140	0.320
Probability (%)	4.0	4.8	45.2	88.9	4.8
rd treatment 1	0.606	0.727	1.323	1.172	1.016
rd treatment 2	0.291	1.154	1.651	1.263	0.796

Table 3-2 The multivariate difference between the treatments (R statistic), and their associated probabilities generated by the ANOSIM routine. The rd values are an indicator of intra sample variability.

The first two axes accounted for 88.3% of the variation (Table 3-3), with PC1 being primarily composed of 18.1(n-9) and 20.5(n-3). The PCA shows a complex pattern of interaction between the days and treatments. For Treatment 2 there was a clear shift along the primary axis between day zero and 28, denoting an increase in 18.1(n-9), and decrease in 20.5(n-3). The pattern for Treatment 1 was less clear, with a weak shift down PC (Figure 3-4).

The ratio of specific fatty acids shows a clearer pattern over the course of the experiment. The 16.1(n-7)/16.0 ratio of the diet A and B was 0.51 and 0.05 respectively (Figure 3-5) At day zero the ratio of these fatty acids in the gonads of individuals reflected these diet differences with Treatment 1 having a value of 0.38 and Treatment 2 of 0.17. By day 28 Treatment 1 had fallen to 0.21 and 2 had risen to 0.34. At day zero and day 28 there were significant differences in these ratios.

Table 3-3 The eigenvalues and eigen vectors for the covariance based PCA, using the five fatty acids selected by the stepwise procedure BV step

PC	Eigenvalues	% Variation	Cum % Varaition
1	45.05	77.1	77.1
2	6.55	11.2	88.3
3	4.32	7.4	95.7
4	1.51	2.6	98.3
5	1.00	1.7	100.0

Variables	PC1	PC2	PC3	PC4	PC5
16.0	0.000	0.133	-0.308	0.502	-0.797
18.1(n-9)	0.917	-0.194	0.315	0.004	-0.151
20.2 NMID	-0.008	-0.661	-0.218	0.619	0.364
20.4(n-6)	-0.069	-0.633	-0.347	-0.595	-0.347
20.5(n-3)	-0.394	-0.328	0.799	0.103	-0.299

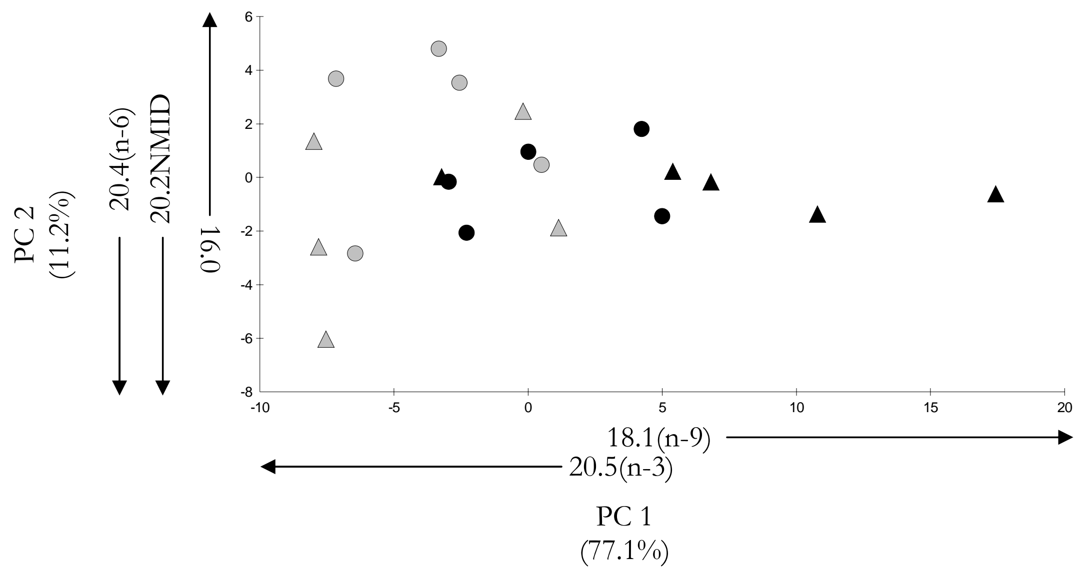


Figure 3-4 PCA of the fatty acid signature of each treatment at day zero and 28. ● Day zero ▲Day 28, ■Treatment 1 ■Treatment 2

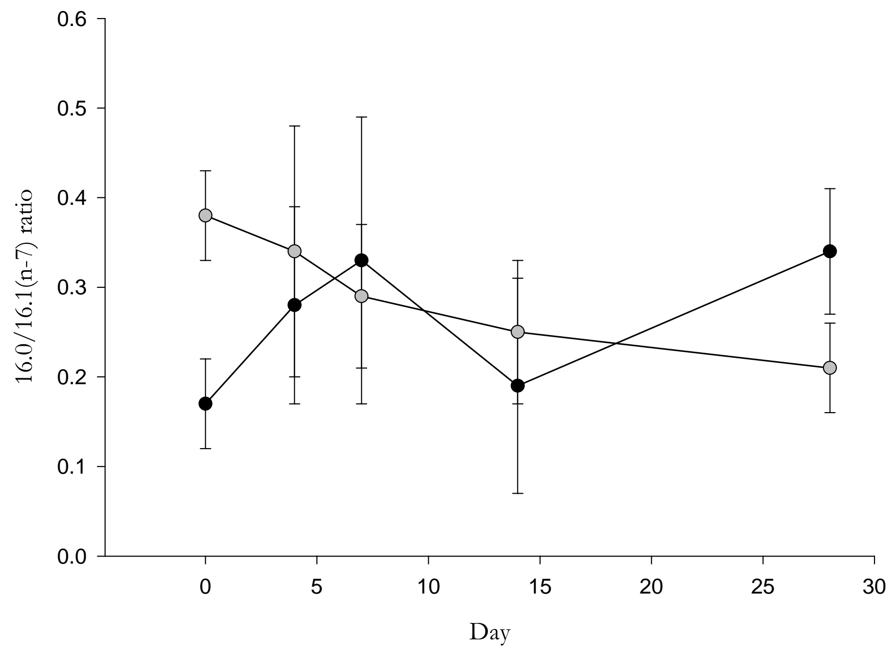


Figure 3-5 The ratio of fatty acids16.1(n-7)/16.0 found in the gonads of the two treatments . ● Treatment 1. ● Treatment 2

DISCUSSION

Echinoid gonads have the potential for rapid response to changes in diet. Russell (1998) showed that three weeks after diet manipulation there was a rapid and dramatic change in mass of the gonad of *Strongylocentrotus droebachiensis*. A similar rapid change was also recorded in the current study species, *P. miliaris*, with significant increases in the GSI of urchins fed salmon food compared to those fed an algal diet over a period of four weeks (Cook et al. 1998). The current study demonstrated that gonad of *P. miliaris* is capable of rapid shifts in fatty acid composition following a change in diet. Four days after the diet change there was a significant shift in the 16.1/16.0 ratio towards the ratio of the new diet. Rapid changes in the biochemistry of urchin gonads following a change in diet have also been found in other species. Six weeks after a change of diet, the gonads of *S. droebachiensis* exhibited a different colour to that of the control (Vadas et al. 2000). Furthermore, Liyana-Pathirana et al. (2002) showed that after nine weeks on an artificial diet there were significant differences in biochemical composition of the urchin gonad.

The current study found a significant difference in the multivariate fatty acid signature between the two treatments after 44 days on different diets. Although the fatty acid signatures prior to experimental treatment were not analysed, previous studies have shown no significant variation at the scale of 10's m (Hughes et al, 2005), and therefore it is unlikely that such a difference was present in this case. After 44 days on the first diet, the ratio of 16.1(n-7)/16.0 reflected that of the diet on which individuals in each treatment had been fed. Seven days after the diet was swapped there was no longer a significant difference in the fatty acid signature between the treatments, the multivariate inter-sample difference had increased, and the 16.1/16.0 ratio was no longer significantly different between the treatments. However after 28 days there was again a significant difference in the fatty acid signature between the two samples, the inter-sample variation had reduced and the 16.1(n-7)/16.0 ratio was again significantly different and reflected the new diet.

Fatty acid composition of diets can be reflected in the fatty acid composition of the gonads (Cook et al. 2000). This was supported in the current study: the ratio of 16.1(n-7)/16.0 at days zero and 28 reflected that of the diet the *P. miliaris* had been fed. However there was no evidence to suggest that the multivariate fatty acid signature of the study *P. miliaris* came to resemble those fed previously on the diet (Treatment 1 at day zero did not resemble Treatment 2 at day 28 and vice versa). The clearest direction of movement was shown in Treatment 2, the group of individuals swapped from a vegetable oil diet to the fish oil diet. The direction of the changes, as illustrated by the PCA, was along the primary axis that was characterised by increasing values of 18.1(n-9). This fatty acid was absent from the vegetable oil diet, but occurred in high concentrations in the fish oil diet. As such the more pronounced change in treatment 2 than in treatment 1 following the change in diet is likely to be a result of the accumulation of essential fatty acids from the fish oil diet in the gonad, whereas the vegetable oil diet was poor in such fatty acids and there was little assimilation of it.

It is likely that the diet containing fish oil would be of greater nutritional value, as it contained a broader range of essential fatty acids. Although both groups were fed *ad libitum*, how much of each diet was ingested was not measured. At day zero Treatment 1 individuals had the higher GSI, which decreased when they swapped onto the vegetable oil diet. This indicated that the vegetable oil diet was nutritionally poorer for the urchins. The greater shift in fatty acid signature for Treatment 2 could be a result of their greater ingestion, absorption or integration of the diet.

Two models have been put forward to describe the way in which the fatty acid profiles may change following a change in diet (Jobling 2003). The wash out model hypothesises that a change in fatty acids would be primarily as a result of metabolism and turn-over of fatty

acids, while the dilution model hypothesises that existing stores of fatty acid become diluted as the organism grows and deposits increasing amounts of the fatty acid from the new diet. In the current study, there was no growth of *P. miliaris* gonads during the course of the experiment, and in fact there was a reduction in the GSI. This provides evidence that when animals are nutrient limited and not increasing tissue mass, it is still possible for there to be a turnover in the composition of fatty acids of that tissue. The changes in the fatty acid profile during this experiment may thus be best described by the wash out model.

The aim of the current study was to investigate the temporal scales at which fatty acid signatures could be used to investigate changes in fatty acid signatures in wild populations. This aim was clearly met for the model species: the study has shown that *P. miliaris* is capable of rapid change in fatty acid composition. Measurable differences in the fatty acid signature were recorded after seven days, and within a month of a change of diet it was possible for significant differences to develop in the gonad of *P. miliaris*. Although the diets used for this experiment were artificial and rates of change in a wild population may be different, it has shown the possible temporal resolution when examining trophic interactions using fatty acid signature to be at the scale of weeks or months.

Chapter 4

OMNIVORY IN THE ECHINOID *PSAMMECHINUS MILIARIS* DETERMINED BY SCALE-DEPENDENT PROCESSES

INTRODUCTION

It has recently been argued that the ability of animals to feed from more than one trophic level should confer on that animal an advantage when living in a variable environment (Agrawal 2003). Conversely, many previous theoretical studies have predicted that omnivory should be rare (Hairston et al. 1960) *sensu* (Menge 2000). Over many decades a range of studies have demonstrated that omnivory is common across a wide range of terrestrial, aquatic and marine habitats (Linderman 1942). This ability to feed at multiple trophic levels has been used to define the concept of omnivory, and can have profound influences on the way that ecosystems function by changing benthic community composition (Dorn & Wojdak 2004), influencing prey diversity (Diehl 1992), and altering food web structure (Kling et al. 1992).

The use of the ratios of stable isotopes of carbon to elucidate trophic interactions showed that some groups of primary producers could be differentiated in terms of their $^{13}C/^{12}C$ ratio ($\delta^{13}C$) (Parker 1964) and that this difference was at least partially conservative between trophic levels. Salt marsh animals were found to reflect the carbon isotope value of their presumed diet (Smith & Epstein 1970, Haines 1976), with algivores having markedly different values to animals feeding on marine monocots. Although these values are conserved between trophic levels, there is a relatively small enrichment per trophic level. There seems to be, on average, an increase in 1‰ per trophic level, though it may be as high as 3‰ (Deniro & Epstein 1978). There has also been a reported sequential increase in the ratio of $^{15}N/^{14}N$ ($\delta\ ^{15}N$) between consumers and their prey. Estimates for the

magnitude of the ($^{15}N/^{14}N$) ratio increase range from 2.4 to 3.4‰, but it may be as low as 2.3‰ (McCutchan et al. 2003). The use of stable isotope analysis, and specifically $\delta^{15}N$, has led to the trophic position of organisms being considered as on a continuum rather than at discrete trophic levels (France 1997). The recognition of this continuum has come with the increasing identification of omnivory in marine food webs from a range of latitudes (Marguillier et al. 1997), and from a range of habitats (Hobson et al. 2002).

High levels of omnivory have been reported for regular echinoids (Echinodermata) as a group. In his extensive review of regular echinoid diet, Lawerence (1975) reported that of 201 species covered, 103 (51%) species included animal material in their diet (as well as algae). There is also extensive evidence that an omnivorous diet promotes higher levels of somatic and reproductive growth in sea urchins (Meidel & Scheibling 1999). It is crucial to understand the degree of omnivory within wild populations of echinoids given that: omnivory is relatively common in this taxon; that an omnivorous diet confers a substantial nutritional benefit to the individual; and that through their trophic interactions, some echinoids have been recognized as keystone species in some environments (Lessios et al. 2001). Using stable isotopes of nitrogen, this study aimed to determine the variation in trophic position for two contrasting, but linked, populations of a model species: the sea urchin *Psammechinus miliaris* (Hughes et al 2005). Secondly, the role of scale dependent processes in driving any spatial and temporal variation in trophic ecology is investigated.

METHODS

Study Site

Three sites within the lower basin of Loch Creran were chosen: Rubha Garbh (RG), Sea Life (SL), South Shian (SS). These sites have been previously described.

Experimental Design

The experimental protocol was designed for a multifactorial, mixed-model ANOVA. There were four sampling events nested into two seasons within a single year(2005) (Winter (January) and Summer (June)). Each sampling event consisted of collecting ten *P. miliaris* from each site. Of these, five *P. miliaris* were collected from the low intertidal zone, and five from the shallow subtidal. The subtidal *P. miliaris* were collected using snorkel from a depth of between two and three meters directly adjacent to the location of the intertidal collections. All *P. miliaris* were collected from a 10 m^2 area within each site, and the animals were selected to be similar in test diameter. On each occasion sampling was completed at all three sites in three consecutive days. The *P. miliaris* were returned to the laboratory, and kept in sea water aquaria without food for 24-36 hours. This reduced the probability of contamination during dissection by allowing time for passage of organic material through the digestive tract. Two control populations of *P. miliaris* were kept within the laboratory and fed either an exclusively vegetable diet of cleaned kelp, or an exclusively animal diet of mussel flesh. Both groups were fed *ad libitum*. The control *P. miliaris* were sampled in April between the two seasons. The test diameter was measured using callipers, the animal weighed, and the gonad excised. The gonad was then weighed. This allowed the calculation of the gonadal somatic indices (GSI) using the formula:

GSI= (wet mass of gonad/whole wet mass of urchin) x 100

The gonad was then homogenised in chloroform methanol in order to extract the lipid. This was done to normalize lipid content amongst samples, as lipid is known to be ^{13}C

depleted (Deniro & Epstein 1977). The samples were stored overnight, filtered on to glass-fibre filters, and then dried to a constant weight at 50ºC prior to further analysis.

Stable Isotope Analysis

Carbon and nitrogen isotope analyses were carried out for all samples by continuous flow isotope ratio mass spectrometry (CF-IRMS), using a Costech (model ECS 4010) elemental analyser (EA) interfaced with a ThermoFinnigan Delta Plus XP mass spectrometer. Approximately 0.7 mg of material was loaded into a 4 x 6 mm tin capsule and combusted in the EA at 1020°C for simultaneous determination of carbon and nitrogen isotope ratios. Three internal laboratory standards were analysed throughout the run to allow corrections to be made for potential linearity effects or instrument drift. All isotope abundances were expressed in δ notation as parts per thousand (‰) deviation from the international standards V-Pee dee belemnite (carbon) and AIR (nitrogen), according to the equation: $\delta X = [(R_{sample}/R_{standard})-1] \times 1000$, where X is ^{15}N or ^{13}C and R is the corresponding ratio $^{15}N/^{14}N$ or $^{13}C/^{12}C$. Measurement precisions of both $\delta^{15}N$ and $\delta^{13}C$ were estimated to be 0.3‰.

Statistical Analysis

The multifactorial mixed-model ANOVA used had four factors. Of these factors, Season (S) had two levels (Summer & Winter) and was fixed; Time (T) had two levels and was nested within Season; Site (Si) had three levels (RG, SL & SS) and was random and orthogonal; Depth (D) had two levels (Intertidal and Subtidal) and fixed and orthogonal; and there were 5 replicates. This resulted in the following model:

$$\boldsymbol{X_{ijklm}=\mu+S_i+ T(S)_{j(i)}+Si_k+D_l+SSi_{ij}+SiD_{kl}+SiT(S)_{kj(i)}+DT(S)_{lj(i)}+SiD_{kl}+SSiD_{ikl}+DSiT(S)_{lk\,j(i)}+E_{m(k\,j(i))}}$$

Prior to all analysis, homogeneity of variance was determined using Cochran's test; where significant variation existed the data were square-root transformed. Where the ANOVA indicated a significant difference existed between the factors, or a significant interaction existed, post-hoc Student-Newman-Keuls (SNK) tests were performed. In order to investigate the variation in $\delta^{15}N$, independent of seasonal changes in biochemistry, the data were standardized by subtracting the lowest value measured for a sampling event. This retained the inter site and depth variation but removed an element of temporal variation.

RESULTS

There was significant variation in the GSI over the course of the study (Figure 4-1). The

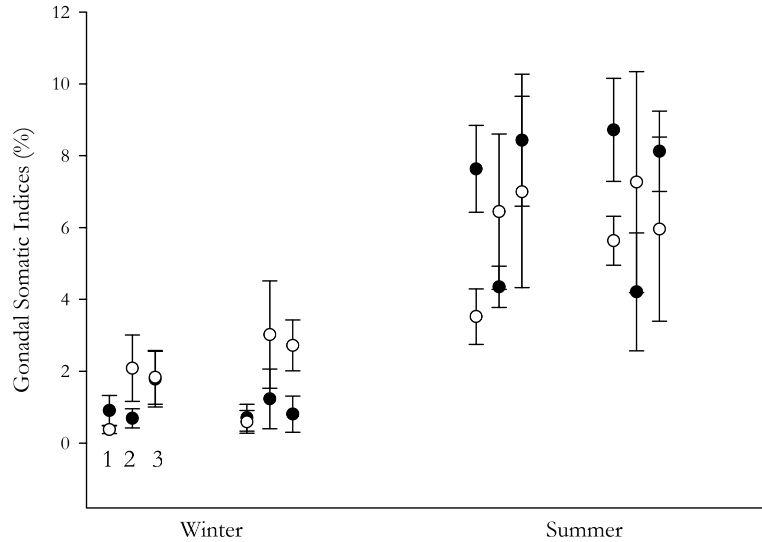


Figure 4-1 Variation in the GSI with season and depth (error bars =95% confidence intervals). The data are grouped by sampling event (2 per season). The symbols are ● (Intertidal) and ○ (Subtidal).

1=SS, 2=SL, 3=RG

Source	DF	SS	MS	F	P
Season	1	41.4246	41.4246	73.7	0.035 [1]
Time(Season)	2	0.1341	0.0671	0.41	0.686
Site	2	1.5298	0.7649	4.73	0.088
Depth	1	0.0079	0.0079	0.00	0.955 [1]
Season*Site	2	1.3135	0.6567	4.06	0.109
Season*Depth	1	1.2014	1.2014	3.04	0.201 [1]
Site*Time(Season)	4	0.6471	0.1618	1.33	0.265
Depth*Time(Season)	2	0.3012	0.1506	1.95	0.257
Site*Depth	2	3.7889	1.8944	24.49	0.006
Season*Site*Depth	2	0.6441	0.3221	4.16	0.105
Site*Depth*Time(Season)	4	0.3094	0.0774	0.63	0.639
Error	96	11.697	0.1218		
Total	119	62.9991			

Table 4-1 Analysis of variance for the GSI between Season, Sites, and Depth. 1 denotes not an exact F test

variation in GSI spanned two orders of magnitude: the lowest recorded GSI was 0.11% whilst the highest was 12.52%. There was a significant interaction between the factors depth and site (Table 4-1). Post hoc SNK testing revealed a significant depth-dependent difference between the sites (in the intertidal the GSI at site SL was lower than at the sites RG or SS, whereas in the subtidal the GSI at site RG was lower than those at SL or SS). There was a significant increase in the GSI between winter and summer and this was consistent between sites and depths.

The variation in $\delta^{13}C$ between depths differed between seasons (Figure 4-2), however the patterns were consistent between sampling events within each season. In winter there was

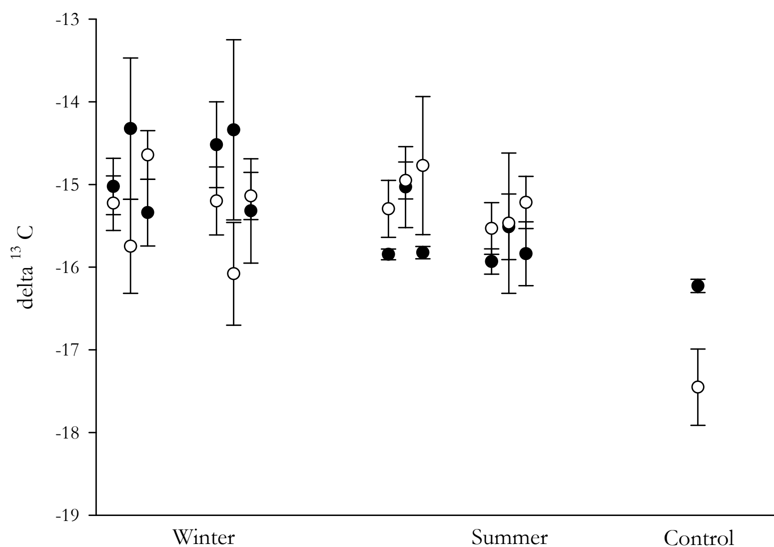


Figure 4-2 Variation in δ13C between season and depth (error bars =95% confidence intervals). The data is grouped by sampling event (2 per season). ● Intertidal ○ Subtidal. The control data represent experimental populations fed vegetable diets (●) or animal diet (○)

no consistent pattern between the sites. Two sites had a higher $\delta^{13}C$ in the intertidal in winter whereas in summer all subtidal sites had higher values than the intertidal. Urchins fed on a control diet of animal material had lower values of $\delta^{13}C$. Analysis of variance confirmed this complex pattern (Table 4-2). There was a significant interaction between

site, depth and season. Post hoc SNK showed that for two intertidal sites winter $\delta^{13}C$ were significantly higher when compared to the summer. Of the subtidal sites one was significantly higher and one was significantly lower. In the winter two sites (RG, SL) had significantly higher values in the intertidal compared to the subtidal, the other (SS) was significantly lower. For the summer the subtidal urchins at two sites had significantly higher $\delta^{13}C$ than those in the intertidal.

Table 4-2 Analysis of variance for the $\delta^{13}C$ between Season, Sites, and Depth, with post-hoc Student-Newman-Keuls test for differences in Depth and Season, = denotes no significant difference. 1 denotes not an exact F test

Source	DF	SS	MS	F	P	
Season	1	3.88	3.88	-	-	1
Time(Season)	2	1.35	0.68	2.53	0.195	
Site	2	0.40	0.20	0.74	0.533	
Depth	1	0.04	0.04	-	-	1
Season*Site	2	1.51	0.76	2.83	0.172	
Season*Depth	1	7.29	7.29	-	-	1
Site*Time(Season)	4	1.07	0.27	0.62	0.651	
Depth*Time(Season)	2	0.88	0.44	13.27	0.017	
Site*Depth	2	9.80	4.90	148.31	0.001	
Season*Site*Depth	2	1.96	0.98	29.66	0.004	
Site*Depth*Time(Season)	4	0.13	0.03	0.08	0.989	
Error	96	41.52	0.43			
Total	119	69.82				

Post-hoc SNK test

Site	Depth	Season		
RG	Inter	Winter	>	Summer
RG	Sub	Winter	=	Summer
SL	Inter	Winter	>	Summer
SL	Sub	Winter	<	Summer
SS	Inter	Winter	>	Summer
SS	Sub	Winter	=	Summer

Season	Site	Depth		
Winter	RG	Inter	<	Sub
Winter	SL	Inter	<	Sub
Winter	SS	Inter	>	Sub
Summer	RG	Inter	>	Sub
Summer	SL	Inter	=	Sub
Summer	SS	Inter	>	Sub

The relationship between GSI and $\delta^{13}C$ varied between depth and season (Figure 4-3). The only significant negative correlation was observed for the summer intertidal (spearman rank correlation df=28, r_s=-0572, p=0.001). Only the winter subtidal showed a weak and non significant positive correlation between the two variables.

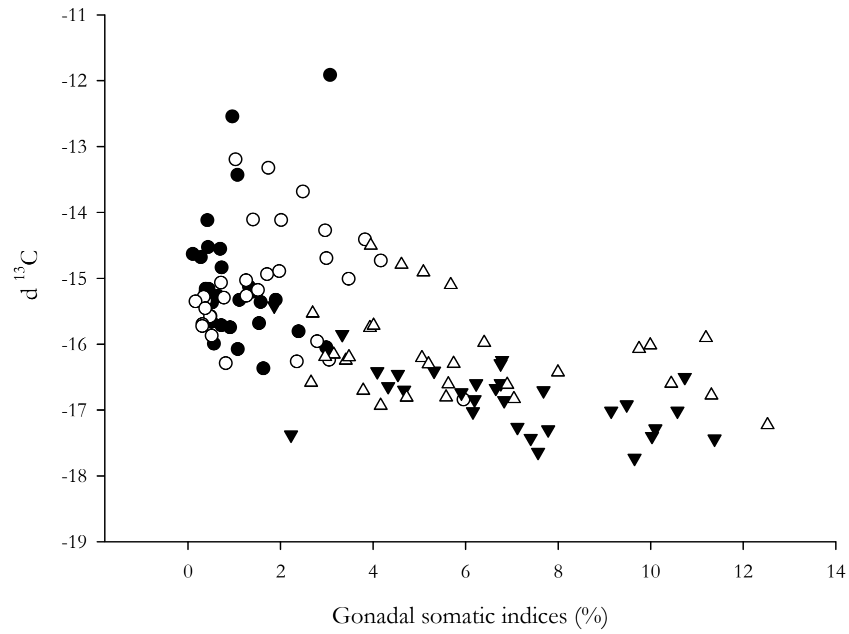


Figure 4-3 The relationship between GSI and δ13C, for each season and depth. The symbols are ● Winter Intertidal, ○ Winter Subtidal, ▼ Summer Intertidal, and ΔSummer Subtidal

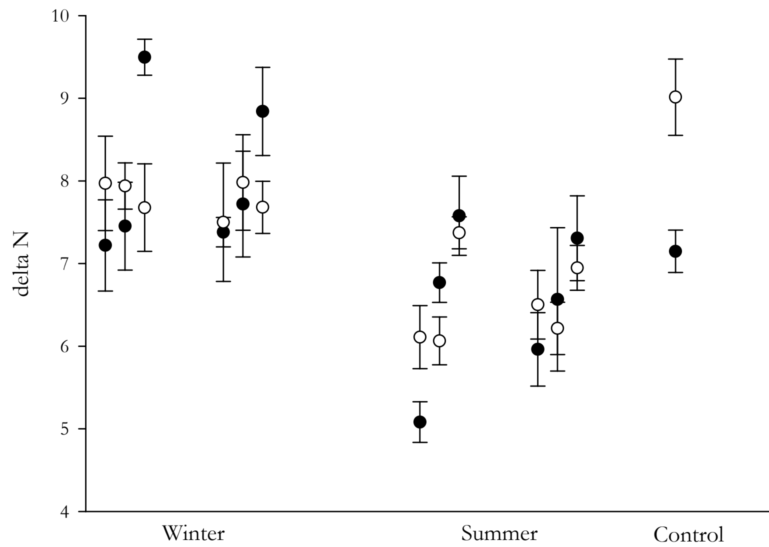


Figure 4-4 Variation in δ15N between season and depth (error bars =95% confidence intervals). The data is grouped by sampling event (2 per season). ● Intertidal ○ Subtidal. The control data represent experimental populations fed vegetable diets (●) or animal diet (○)

The analytical results of $\delta^{15}N$ determination of the wild *P. miliaris* population displayed a complex pattern of response (Figure 4-4). ANOVA revealed that there was a significant interaction between season, site and depth (Table 4-3). Post hoc SNK testing was again used to investigate interactions. For all sites and depths, except the subtidal zone of SS, the

Table 4-3 Analysis of variance for the δ15N between Season, Sites, and Depth, with post-hoc Student-Newman-Kuels test for differences in Depth and Season, = denotes no significant difference. 1 denotes not an exact F test, 2 denominator zero for F test

Source	DF	SS	MS	F	P	
Season	1	55.88	55.8804	[2]		
Time(Season)	2	0.29	0.1462	0.19	0.836	
Site	2	27.35	13.678	17.59	0.010	
Depth	1	0.41	0.417	0.08	0.808	[1]
Season*Site	2	1.15	0.5791	0.74	0.531	
Season*Depth	1	0.35	0.358	0.15	0.749	[1]
Site*Time(Season)	4	3.11	0.7777	2.22	0.072	
Depth*Time(Season)	2	0.04	0.0249	0.06	0.938	
Site*Depth	2	11.21	5.6054	14.55	0.015	
Season*Site*Depth	2	5.62	2.8119	7.30	0.046	
Site*Depth*Time(Season)	4	1.54	0.3853	1.10	0.360	
Error	96	33.58	0.3499			
Total	119	140.58				

Post-hoc SNK test

Site	Depth	Season			Season	Site	Depth		
RG	Inter	Winter	>	Summer	Winter	RG	Inter	=	Sub
RG	Sub	Winter	>	Summer	Winter	SL	Inter	=	Sub
SL	Inter	Winter	>	Summer	Winter	SS	Inter	<	Sub
SL	Sub	Winter	>	Summer	Summer	RG	Inter	<	Sub
SS	Inter	Winter	>	Summer	Summer	SL	Inter	=	Sub
SS	Sub	Winter	=	Summer	Summer	SS	Inter	=	Sub

winter values were significantly higher than the summer values. The difference between the depths varied between sites and seasons. In the winter the $\delta^{15}N$ values associated with the intertidal zone of the SS site were significantly higher than those in the subtidal, but at the other sites there were no significant differences. In the summer the $\delta^{15}N$ values associated with the subtidal at RG were significantly higher than those in the intertidal. For the other sites there was no difference between the depths. The largest magnitude of variation within a single site at a single depth and sampling event was 2.3‰. The control population, which were held in aquaria and fed either animal or plant diets, showed a significant difference (ANOVA df=1, f=38.24, p=0.003) in their $\delta^{15}N$ values. The *P. miliaris* fed on animal tissue

had a significantly higher $\delta^{15}N$ value than those fed on algae. The average difference between the two groups was 1.87‰.

The relationship between $\delta^{15}N$ and GSI of *P. miliaris* varied between seasons and, to a lesser extent, between depths (Figure 4-5). Only the summer subtidal showed a positive correlation between $\delta^{15}N$ and GSI (spearman rank order correlation df=28, r_s=0.477, p=0.08).

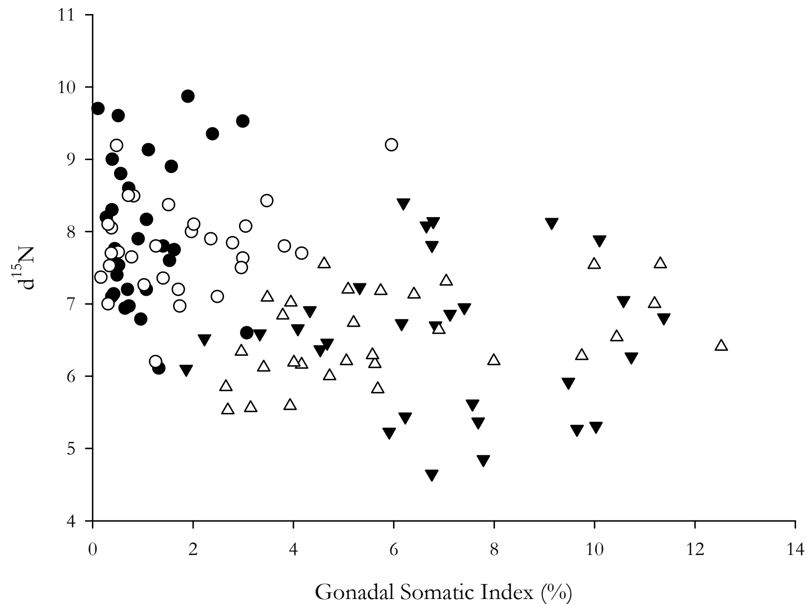


Figure 4-5 The relationship between GSI and δ15N, for each season and depth. The symbols are ● Winter Intertidal, ○ Winter Subtidal, ▼Summer Intertidal, and △ S Summer Subtidal

When the $\delta^{15}N$ values were corrected for seasonal difference they revealed significant variation between sites and depths and the differential response of these to season (Figure 4-6). This treatment retains the variation between site and depths, but removes the temporal variation. The maximum observed variation was 3.76‰. The standardization showed that the *P. miliaris* at the different sites responded differently with season. In winter *P. miliaris* from the intertidal zone of the SL site had distinctly higher values than those

in the subtidal. In the summer *P. miliaris* from the intertidal of the RG site were lower than those from the subtidal.

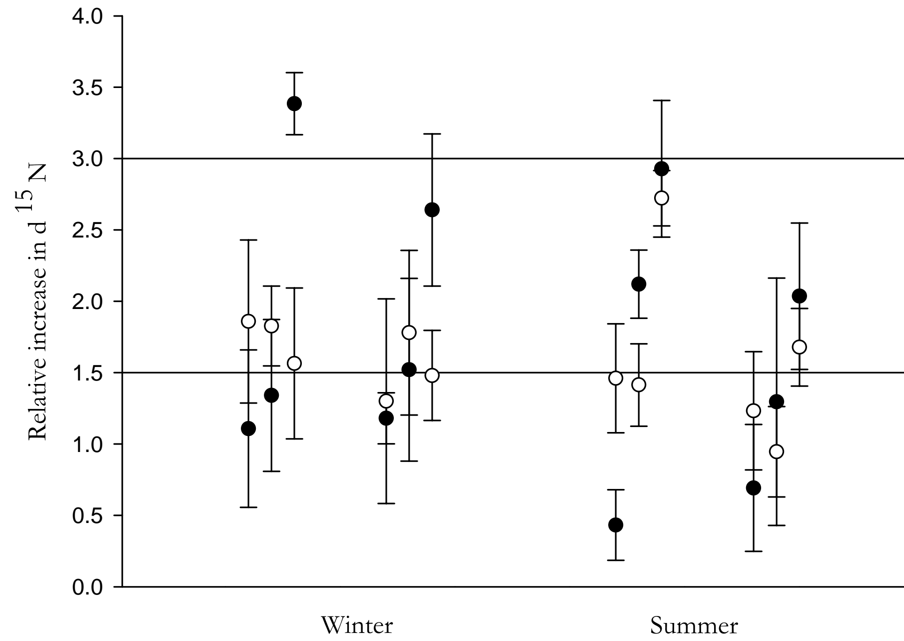


Figure 4-6 The relative increase in δ15N in *P. miliaris* with depth, when season effects are accounted for (error bars =95% confidence intervals). The data are grouped by sampling event (2 per season). The symbols are ● (Intertidal) and ○ (Subtidal). The lines represent approximate values for a half and a full shift in trophic level.

Discussion

Echinoids have long been thought of as pivotal animals to coastal communities through their feeding on algae. Recent studies have increasingly shown echinoids to graze animals as well, and the findings reported here reveal the taxon to be a good model for probing some complexities underlying ominvory. The current study showed a predictable pattern in the increase of GSI between the winter and the summer in the echinoid *P. miliaris*. GSI varies seasonally in many other species of echinoid (Konar 2001), and the pattern of increase observed at the study site (Loch Creran) follows that reported for *P. miliaris* elsewhere within the west coast of Scotland (Cook et al. 1998). The increase in GSI can signify two main processes which reflect the dual function of the gonad (Russell et al. 1998). Increase in GSI has been linked to both an increase in nutrient and gamete storage (Walker & Lesser 1998). As a result, any biochemical analysis which aims to investigate the trophic status of echinoids from investigation of gonads, is naturally confounded by this dual function (Hughes et al, in press), and as such needs careful interpretation.

There is a well established relationship of ^{12}C enrichment of the lipids faction relative to whole tissue or organism (Park & Epstein 1961, Smith & Epstein 1970). This relationship can be problematic if the tissue used has variable lipid content, and can lead to lipid-related variation masking variation derived from the ultimate source of the carbon or subsequent trophic enrichment (Gannes et al. 1997). Therefore when using tissue with a variable lipid content it has been recommended that the lipid be extracted prior to analysis (Jardine et al. 2003). However whether this is suitable for all studies has been questioned (Matthews & Mazumder 2005). In the current study in which lipids were extracted there is however evidence to suggest that the extraction has not been 100% efficient. The negative relationship between GSI and $\delta^{13}C$ during the summer provide evidence that there is sufficient lipid still within the sample to bias the results. It is, therefore, not possible to

determine how much of the difference observed is as a result of lipid and how much is as a result of different carbon sources. This problem draws into question the usefulness of using gonad tissue for $\delta^{13}C$ studies that aim to determine the sources of carbon within diet.

A marked decrease in the $\delta^{15}N$ between winter and summer for *P. miliaris* was also found here. As there is an incremental increase in $\delta^{15}N$ between predator and prey (Minagawa & Wada 1984), the observed change in $\delta^{15}N$ of *P. miliaris* could indicate a shift away from a (winter) carnivorous diet towards a more herbivorous one (in summer). However when these values were compared to the control population, the lowest values (which were observed in the winter) were comparable to those of the control population, that had only been fed on algae. It seems some of the wild population had a predominantly herbivorous diet. The lowest $\delta^{15}N$ values in the summer were, however, below that of the herbivorous control (but the control population were harvested for analysis in April). An explanation for such a finding could be that changes in gonad biochemistry (related to maturation of gametes) caused a differential isotopic accumulation between the seasons. Other taxa can have depleted $\delta^{15}N$. This is true in the scallop *Pecten maximus* gonad compared to its mantle and in summer compared to winter (Lorrain et al. 2002), in krill *Euphausia superba* oocytes (Schmidt et al. 2004), or in recently hatched decapod zoeae (up to 2.3‰ lower) compared to the parental tissue (Schwamborn et al. 2002). Echinoid gonads show a seasonal pattern in amino acid (Liyana-Pathirana et al. 2002) and protein content (Montero-Torreiro & Garcia-Martinez 2003). So a seasonal change in protein content, amino acid composition and the presence of gametes may account for the seasonal down shift in $\delta^{15}N$ values exhibited by the study population.

The $\delta^{15}N$ value can be used to determine the body condition as well as the trophic level of an animal. For example, animals that are starved exhibit an increase in $\delta^{15}N$ (Adams & Sterner 2000). This has been attributed to catabolism of body nitrogen leading to further

enrichment. Sea urchin populations can be nutrient limited (Himmelman 1986, Konar 2001), and it has been suggested that regular echinoids are well adapted to nutrient limitation (Andrew 1989). Evidence of starvation would be manifested as a negative relationship between $\delta^{15}N$ and GSI values. When data from *P. miliaris* from both seasons were pooled, such a relationship was found. However when the data were separated by season and depth, there was a weak positive relationship between GSI and $\delta^{15}N$ with the exception of the summer intertidal population, which showed no relationship. So it is unlikely that the higher values of the winter population can be explained as due to starvation, especially as the values correspond to the control populations that were fed *ad libitum*. Therefore, even though the GSI recorded in the winter were up to two orders of magnitude lower than those from the summer, no evidence of metabolic stress associated with starvation was found in the current study.

Scales of variability

Although the sexual maturation of organisms may strongly influence $\delta^{15}N$ values, the gonad development of *P. miliaris* can be synchronous between different populations at the scale of 10's kms (Kelly 2000) and between the intertidal and subtidal populations (Hughes et al, in press). As such it is possible to standardize the data such that levels of variation in an individual can be examined by comparing any elevation to the lowest recorded value for that sampling event. This will enable the relative level of omnivory to be gauged independently of changes in sexual maturation, but will consistently underestimate actual levels of omnivory.

The level of population omnivory, as indicated by relative increases in $\delta^{15}N$, varied between the individual, the sites, and the habitats, showing that both small and large scale processes can influence the trophic interactions of a species. Variability at the scale of the individual *P. miliaris* was high. The largest difference between *P. miliaris* at the same site, depth and

time was 2.3‰; close to values calculated for a complete trophic level. This inter-individual difference can reflect the individual variation in the foraging environment that a sea urchin is exposed to. An expected strategy to optimise foraging behaviour for an echinoid (MacArthur & Pianka 1966) would be to maximise its increase in trophic level. For sea urchins in general (Meidel & Scheibling 1999) and *P. miliaris* specifically (Cook et al. 1998), it has been demonstrated that a diet consisting of more animal material, leads to greater somatic and gonadal growth. The large variation (almost a complete trophic level) reported here in the levels of omnivory of individuals collected within 10 meters of each other indicates that individual *P. miliaris* experienced very different environments at the scale of meters. The role of habitat heterogeneity in mediating predator-prey interaction (Flecker & Allan 1984) and some evidence for the way they may vary over spatial scales have been documented (Forrester & Steele 2004). Small-scale habitat heterogeneity has been shown to mediate sea urchin grazing at scales of meters (Benedetti-Cecchi & Cinelli 1995). For *P. miliaris* this variation in levels of omnivory suggests that there is a significant interaction between habitat heterogeneity and their foraging behaviour.

Levels of omnivory within the population also exhibited large-scale temporal and spatial variation. Some sites had consistently higher relative $\delta^{15}N$ when compared to the other sites signifying that processes working at the scale of kilometres are driving these differences. Although the sites were chosen to be qualitatively similar, the differences in omnivory denote possible differences in parameters such as hydrography and productivity (Menge 2000) or the presence of distinct cryptofaunal assemblages (Barnes & Brockington 2003). At other sites there were strong seasonal effects indicating that temporal inter-seasonal variation was having a profound effect on the trophic ecology of *P. miliaris*.

Omnivory has a strong influence on both the population ecology of the omnivore and on the community structure in which it exists. This study has revealed a high degree of trophic

plasticity within a population of regular echinoids, a group for which omnivory is common. In order to fully understand the population and trophic ecology of this important group it is crucial to appreciate the factors that regulate the foraging behaviour of the individual, and the way these interact with scale dependent processes to drive regional and seasonal variation.

Chapter 5

ARE PASSIVE INTEGRATED TRANSPONDER TAGS A SUITABLE METHOD FOR PERMANENTLY IDENTIFYING INDIVIDUAL *PSAMMECHINUS MILIARIS*?

INTRODUCTION

The ability to record variation at the level of the individual is crucial to understanding many ecological processes such as trophic interactions, population dynamics, reproduction and recruitment, and behavioural ecology. As such sea urchin researchers have found it necessary to mark and identify individuals or groups of urchins in order to investigate a range of ecological questions such as growth (Ebert 1965), foraging behaviour (Dance 1987), movement (Tuya et al. 2004), migration (Agatsuma et al. 2000), and mortality (Russell 1987). There is however no accepted or universal method for tagging sea urchins and a brief history of tagging shows that a wide range of methods have been developed.

External marking

A wide range of techniques have been developed in order to tag urchins externally. One approach is to secure a band or tag around the circumference of the test. Moore (1935) commented that attempts to place elastic bands around the test of *Psammechinus miliaris* were unsuccessful as individuals easily removed their tags. The success rate of this method of tagging appears to be species dependent as 10% of *Paracentrotus lividus* retained elastic band tags around their tests 84 days later (Moore 1935).

Many researchers have tried drilling holes through echinoid tests in order to attach tags. In a study into the growth and age of *Hemicentrotus pulcherrimus,* Fuji (1963) drilled two ~1mm

holes into the interambulacral zones, threaded a piece of fishing line through the hole and attached a piece of coloured vinyl tube. He reported that from an earlier study this method of tagging caused reduced growth rates in tagged urchins relative to untagged individuals. A very similar technique was used by Ebert (1965) with *Strongylocentrotus purpuratus;* he observed that the test regeneration around the monofilament was rapid and the tag was secured within two weeks. Ebert did not, however, mention associated mortality, the level of tag retention or influences on growth relative to non-tagged individuals. Insertion of anchor or T tags into holes into the test of *Evechinus chloroticus* (Dix 1970) resulted in insertion holes failing to recalcify, although holes did become plugged with a tissue mass. These *E. chloroticus* showed tag loss, poor gonad development in tagged compared to non-tagged individuals. Nelson & Vance (1979) tried passing surgical stainless steel wire through the test, or inserting a barbed segment of stainless steel leader through the wall of the test, of *Centrostephanus coronatus*. The wire in both cases was coded with coloured beads. Duggan and Miller (2001) compared success rates associated with three external tags, all of which were attached through holes in the test. The first of these, Anchor tags, involved inserting a T bar into the hole and letting the hole recalcify around the tag. The high mortality associated with this method was linked to the failure of the hole to calcify in turn due to excess tag movement. The authors found that screwing steel bolts into the holes also led to high mortality, but in this case due to hole enlargement. The third and most successful method they tried utilised a nylon screw and resulted in no significantly greater mortality between the control and the tagged individuals. A novel technique, described by Tuya (2004), was to insert a small fishing hook into the periproctal membrane of *Diadema antillarum*. This hook was attached to a small numbered float with a length of fishing line, enabling individual identification for short periods.

Another common, but less intrusive, technique is to tag the spines. Carpenter (1984) placed numbered sections of surgical tubing around individual spines of the long spined

Diadema antillarum. As the podia are shorter than the spines of *D. antillarum* they cannot remove the tag. The authors found that if the urchin was followed and retagged when it looked likely that the urchin would drop the spine, it was possible to follow an individual urchin for periods of up to 12 months. Spine tags (sleeve insulations from electric wire) were also successfully used to track the diurnal movement of *P. lividus* (Crook et al. 2000).

Alternatively different coloured paints can be used to identify individuals from different groups. This involves the removal of the skin from the madreporites; drying this area and then painting with nail varnish and sealing with fast drying dental cement. The tags remained visible over the 12 month course of an experiment by Agatsuma (2000).

Chemical Tags

A large number of studies have used chemical markers or dyes to measure the growth of sea urchins (Gage 1992b, 1992a). These chemicals bind with calcium that is incorporated into the growing edges of ossicles and can later be detected in the laboratory under ultra-violet light. It is then possible the to measure the growth increment since the individual was tagged and so calculate growth rates over known periods. The chemicals are either injected into the peristomal membrane or the animal is immersed in a solution for a number of hours. Chemicals commonly used include tetracycline (Kalvass et al. 1998) and calcein (Lamare & Mladenov 2000). These techniques do not enable individual identification and require destructive sampling.

Internal tags

Duggan and Miller (2001) investigated the use of two internal tags for use with *Strongylocentrotus droebachiensis*. They either used two sizes of welding rod stamped with a unique number or a poultry tag that already had a unique number. These were either inserted through a cut in the peristomal membrane or through a hole drilled into the test.

They found that tags inserted through test holes had a higher proportion of retention than the tags inserted through the peristomal membrane so used the former for experiments. Following a four month growth trial they found that there was no significant difference between controlled and tagged individuals with respect to test diameter. In a field trial it was found that tagged animals were detectable at distances of 7-10 cm underwater using underwater metal detectors. Ultimately though tagged individuals had to be destructively sampled for the tag to be reclaimed and identifications made.

The problem of the need for destructive sampling associated with the internal tagging of sea urchins was addressed by Hagen (1996) when he used Passive Integrated Transponder (PIT) to tag *Strongylocentrotus droebachiensis*. The PIT tag is a microchip programmed with a unique identification number, attached to a copper wire antenna. This is normally encapsulated in a rounded glass cylinder. When the microchip is energised by a radio signal from the reader, it transmits its unique identification code. This signal is detected and decoded into a unique multi-digit identification number which is displayed by the reader. These tags have been used in a number of different aquatic animals: sea turtles (Parmenter 1993), crabs (Pengilly & Watson 1994), manatees (Wright et al. 1998), octopus (Anderson & Babcock 1999), penguins (Ballard et al. 2001), slipper lobsters (Bianchini et al. 2001), freshwater crayfish (Bubb et al. 2002), and teleost fish (Dare 2003). In contrast, a range of common Antarctic invertebrates have proved unsuitable for PIT tagging leading either to tag rejection or mortality. These include the nemertean *Parbolasia corrugatus*, the gastropod *Marseniopsis* sp., the holothurian *Cucumaria* sp., the sea star *Perknaster* sp. and the echinoid *Sterechinus neumayeri* (Brockington, Chapman & Peck unpublished data). Hagen (1996) injected 12x2mm tags into the peristomal membrane of *S. droebachiensis*. The only tag rejection observed was in an individual measuring 22 mm (test diameter), and no mortality of tagged urchins occurred in the first 76 days of the experiment. After day 76 no

significant difference in the mortality or growth was found between the tagged and the control group.

As the success of tagging methods has been shown to be highly species, and possibly region-specific the aim of the current study is to evaluate the use of PIT tags with the urchin *Psammechinus miliaris*, and to determine if the method is suitable for field studies to investigate short term patterns of activity in wild populations.

METHODS

The study consisted of three sequential experiments: the pilot study was an initial small scale trial of the method; the feasibility study was aimed to further refine the technique and the field trial aimed to examine the practicability of using the tags in the natural environment.

Tagging procedure

The tagging procedure was the same for all three studies. The tags were contained in sterile single-use 12 gauge canulae. Each individual sea urchin was removed from the aquarium, inverted and the tag was injected through the peristomal membrane. In small individuals the injection was made at an oblique angle to ensure the tag was fully inserted.

Pilot Study

A small scale pilot study was conducted to determine if internal PIT tagging of *P. miliaris* was viable. A number of tags and a reader were obtained on approval from the manufacturer (PetID). The pilot study was in some respects constrained by the number of tags obtained.

Sea urchins were collected from a single location within Loch Creran, returned to the laboratory, kept in running sea water aquaria for two weeks and fed a diet of *Laminaria saccharina ad libitum*. The animals were then divided into four treatments: Treatment 1 were tagged with 12mm tag (n=10); Treatment 2 were tagged with 8mm tag (n=5); Treatment 3 were injected through the peristomal membrane but no tag inserted (n=10); and Treatment 4 were a control with no tag (n=10). Post-treatment animals were held in separate aquaria with independent sea water supply and fed as before. Individuals were measured at monthly intervals for four months.

Feasibility Study

Fifty four individuals from four size classes ($s_1 \approx 17.8$mm, $s_2 \approx 20.3$mm, $s_3 \approx 23.9$mm, $s_4 \approx 26.8$mm) were collected from the South Shian site (SS). These urchins were collected from the intertidal zone and returned to a holding tank at the SAMS laboratory. The animals were held for two weeks to acclimatize in aquaria with running sea water and fed *Laminaria saccharina*. They were then transferred to the individual experimental aquaria. Of the 14 animals for each size class seven were designated at random as a control and seven were injected through the peristomal membrane and a PIT tag inserted. The tags used were AVID® 12mm tags, supplied in sterile single use disposable syringes. The 12mm tag was used as it was not possible to obtain a sufficient quantity of the 8 mm tags. The tags were scanned with an Avid® Power TracKer™II, prior to insertion to ensure that they were functioning correctly, and the animals were scanned after tagging. The animals were then kept in their individual aquaria and monitored over a period of four months. The individual aquaria were located in seven larger tanks using a randomised block design After four months, somatic growth was estimated as the growth increment as a percentage of the original size. Gonadal growth was calculated as the wet weight of the gonad divided by the wet weight of the test multiplied by 100. Tag loss and mortality was recorded throughout the experiment.

Field Trial

In the third trial 80 individuals were tagged with Avid 12mm PIT tags. These *P. miliaris* came from two sites at Loch Creran (Rubha Garbh (RG) and Sealife (SL): see Introduction for site descriptions and map), from the intertidal and subtidal zones at each site, and were chosen on the basis that they were the maximum size available (ranging between 19mm and 33mm). Individuals were collected and allowed to acclimatize as previously described.

After tagging individuals were returned to the aquaria where they were kept for three months, to allow any tagging related mortalities to occur before they were transferred to their original environments. Due to unexpectedly high mortality, it was not possible to return the urchins to the locations they had come from. Instead the surviving individuals were returned at random to the two sites and depths.

Individuals were returned to a single marked point at two sites, and at each site either in the intertidal or the sub tidal. Twenty-four hours later the *P. miliaris* were located by searching the substrate with the tag reader. The reader was housed in a flexible waterproof housing that allowed full access to its functions and displays. For the subtidal urchins the substrate was swept using snorkelling equipment. The searches started at the marked release point and radiated out from there. Due to difficulties caused by short range of the tag reader (approx 15 cm) and the rugosity of the habitat, the search process was slow and limited to a one meter radius. When the tagged urchins were recorded, their individual number and position was noted. The position was recorded as a polar coordinate from the point of release. This was done using a meter rule and an underwater compass.

RESULTS

Pilot Study

Over the four month course of the experiments the greatest mortality was observed for individuals implanted with the 12 mm tags (Figure 5-1). After the first month there was 30% mortality in this group. After the first month there was no further mortality recorded from any group.

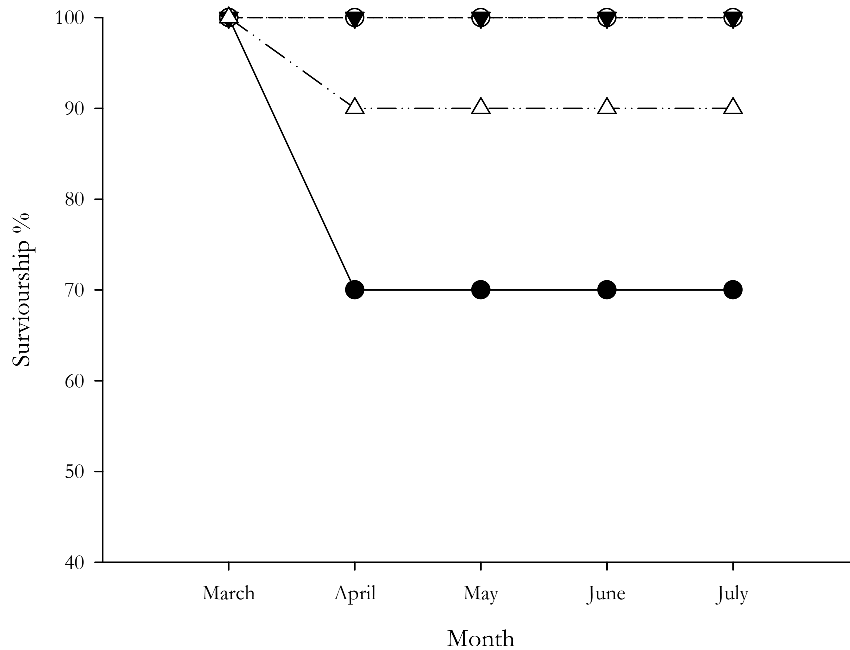


Figure 5-1 Relative surviourship for sea urchins in the pilot study.
12mm tags●, 8mm tags ○, procedural control▼, control Δ

There was little somatic growth during the course of the four months (Figure 5-2), and no difference between the growth rates was observed.

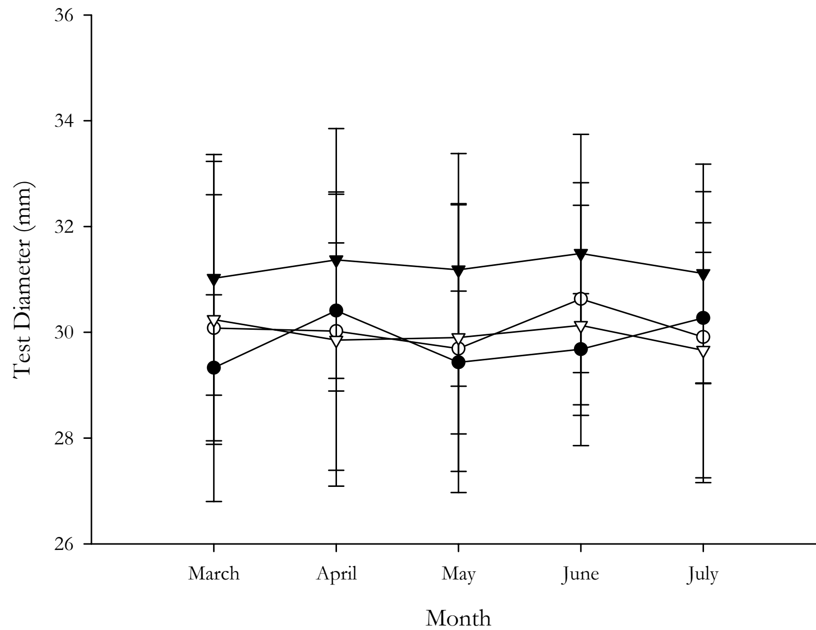


Figure 5-2 Test diameter of sea urchins averaged for each treatment. Error bars =95%CI 12mm tags●, 8mm tags ○, procedural control▼, control▽

Feasibility Study

The number of individuals that retained their tags was strongly related to their size. By the end of the experiment only a single case of mortality was recorded, from a Size 2 (~20.3mm Ø) tagged individual. Tag retention at four months (Figure 5-3) was zero for size 1 individuals (~17.8mm Ø), 14.3% for size 2, (~20.3 mmØ), 57% for size 3(~23.9mm Ø) and size 4 individuals (~26.8mm Ø).

In order to use a balanced ANOVA, a dummy variable was created for the single case of mortality. This showed that there was no significant difference in gonadal growth between treatments or size class. For the somatic growth there was no effect of tagging; however there was a significant difference in growth rates between the size classes (Table 5.1) Detectability of the tags was 100% in all size classes; there were no cases where the reader failed to detect tags that were later found on dissection.

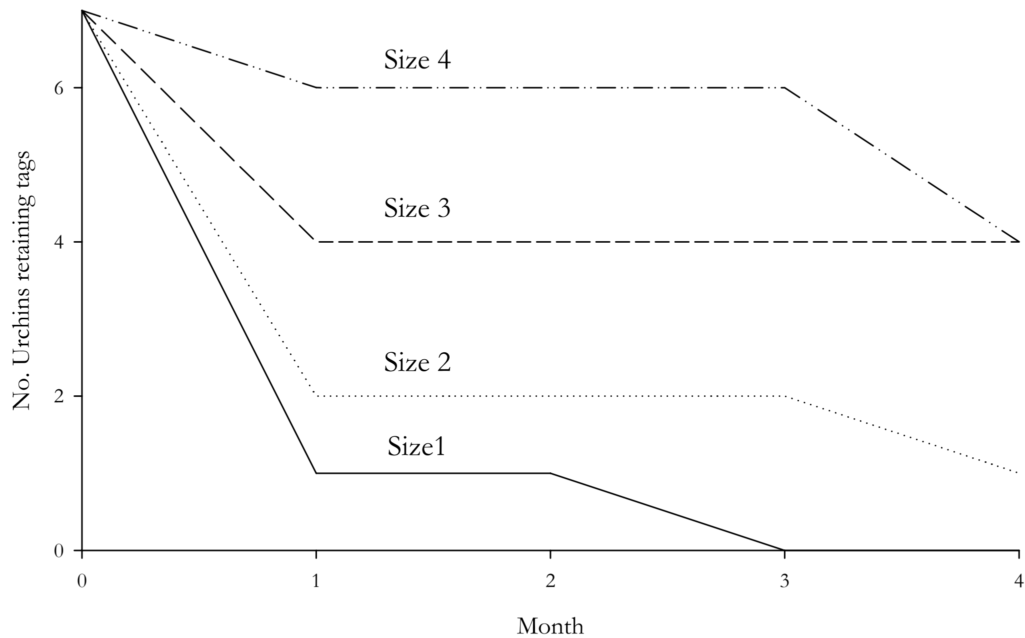


Figure 5-3 Tag retention for the different size classes of sea urchins

Table 5-1 Analysis of variance for GSI and percentage growth increment for tagged and non tagged urchins from four different size classes

		Gonadal Somatic Indices			
Source	SS	DF	MS	F	P
Size	38.08	3	12.69	1.55	0.21
Treatment	13.48	1	13.48	1.64	0.21
SizexTreatment	11.99	3	4.00	0.49	0.69
Residual	393.31	48	8.19		
Total	456.86	55			

		Percentage growth Increment			
Source	SS	DF	MS	F	P
Size	85.98	3	28.66	3.33	0.03
Treatment	22.00	1	22.00	2.55	0.12
SizexTreatment	28.52	3	9.51	1.10	0.34
Residual	413.53	48	8.62		
Total	550.03	55			

Field Trial

For this analysis no discrimination between mortality and tag rejection was made; both were grouped as failures. Of the 80 urchins tagged 32 were classed as failures. Logistic

regression was used to model the outcome of the tagging of urchins for the field trial. The model was used to describe the changes in the probability of a successful out come to the tagging over the size range of urchins used, and the location from which they came (Figure 5-4). Analysis of deviance showed that the model was a good fit to the data (deviance ratio=16.51, approx χ^2<0.01). The success of tagging operations varied strongly between the intertidal and the subtidal populations, for example an urchin of 24 mm had 25% chance of being successfully tagged if it came from the intertidal compared to 80% if it had originated in the subtidal.

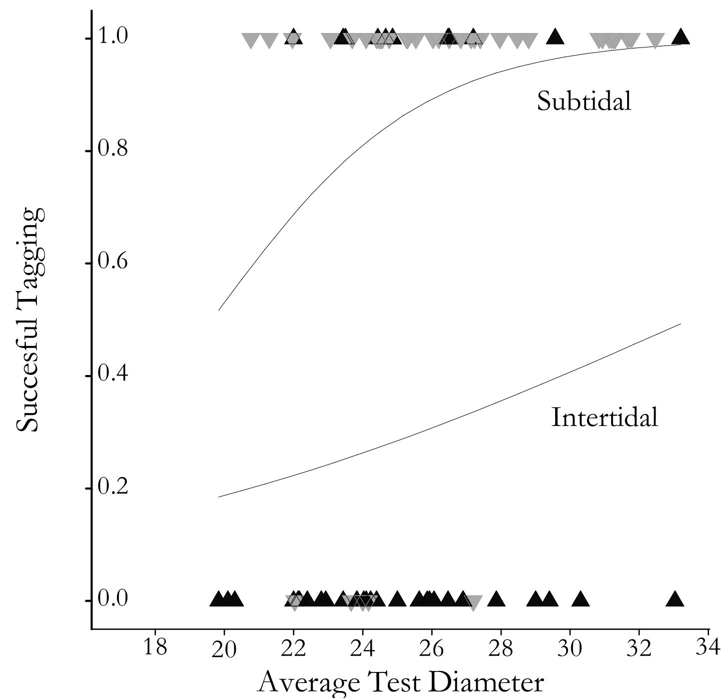


Figure 5-4 Logistic regression of successful tagging outcomes, showing the relationship between tagging successes and test diameter for the two populations.
▼Subtidal ▲ Intertidal

Relocation rates in the wild after 24 hours varied between the locations and depths. Of the 12 *P. miliaris* released at each site and depth, six were relocated at RG intertidal, seven at RG subtidal, six at SL intertidal and 11 at SL subtidal. There were also considerable differences in the distance traveled by individuals although there was no discernable difference between the sites and depths (Figure 5-5).

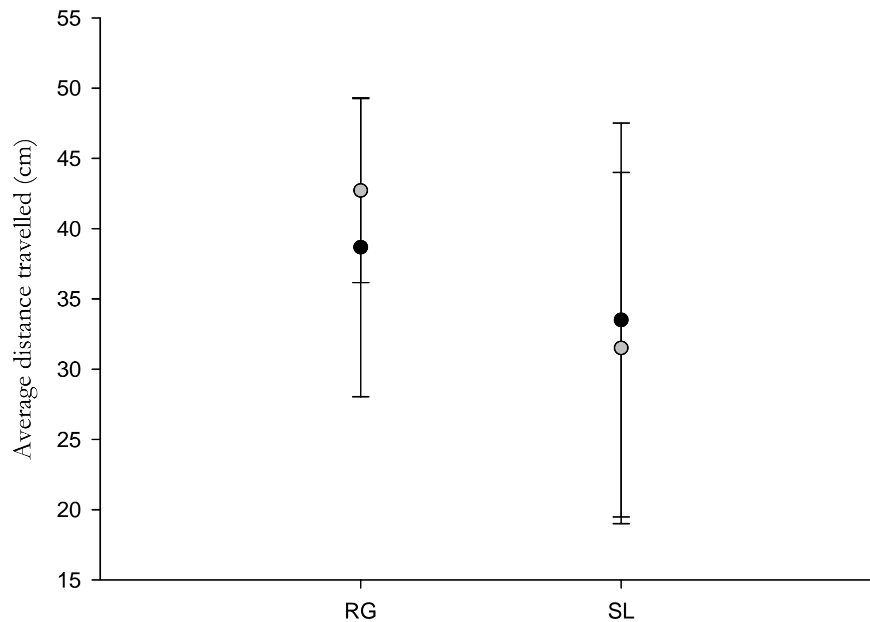


Figure 5-5 Average distance traveled by recaptured urchins. Error bars represent 95%CI
● Subtidal ● Intertidal

Polar plots were used to visualise the movements of individuals over the 24 hr study (Figure 5-6). These revealed motion patterns inconsistent with random movement, with possible bias in the direction of travel, although there was no consistent pattern between sites and depth. However due to the small sample size no statistical analysis was undertaken.

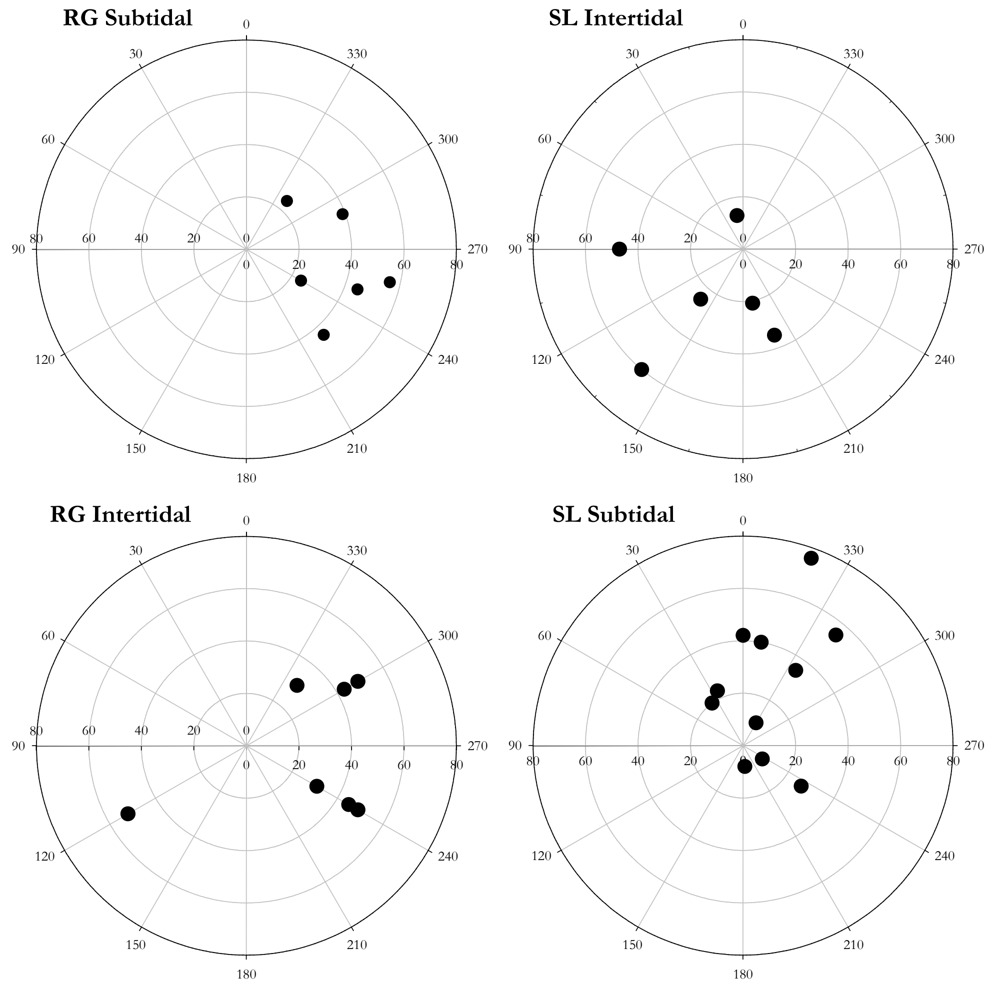


Figure 5-6 Polar diagrammes showing the distance and position of the recaptured urchins after 24 hours. 0° indicates an up shore movement.

DISCUSSION

Suitability of a tagging method depends on the parameters for which individual or group estimates are wanted. For example a tag which reduced somatic growth may be suitable if for the determination of hourly movement rates, but would be unsuitable for estimates of longevity. In general, desirable qualities in a tag include: ease of application, good tag retention by the animal, low tagging related mortality, normal levels of somatic and reproductive growth, no changes in behaviour, easy identification of tagged animals, and economically viable associated costs. Many of the methods in the past have failed to meet these criteria for reasons including: poor tag retention (Moore 1935), tagging related mortality (Duggan & Miller 2001), poor somatic (Fuji 1963) or gonadal (Dix 1970) growth or the requirement for destructive sampling to retrieve the tags (Gage 1992b).

The initial pilot study showed promising results with low mortality, 100% tag retention and identification, and no differences in somatic growth over the four month experiment. On the strength of these results a larger feasibility study was carried out. The study aimed to trial the tagging method amongst a larger group of animals, and over a range of sizes. This feasibility study showed that PIT tags meet many of the criteria required of a successful tagging methodology. The tagging caused no difference in somatic and gonadal growth, or mortality and the tag detection rate was 100%. There was however a large size dependent variation in tag retention. Hagen (1996) found that the only *Strongylocentrotus droebachiensis* individual to lose a tag was a small individual (22mm Ø). In the current study tag retention was higher than 50% for larger individuals with an average test diameter of 23.9mm or more. An important consideration if the tags are to be used for growth studies is that the minimum feasible size of tagging is not close to the maximum size of the animal. For *P. miliaris* this size dependent success would limit the use of these tags to older animals. However this experiment showed the maximum size obtained by *P. miliaris* is double that

of the minimum feasible tagging size. The exact mechanism behind the rejection of the tag is unknown, most of the tag loss occurred within the first month, and it seems probable that the tag was lost through the incision hole.

The field trial revealed an unexpected pattern of mortality. There was significantly higher mortality in individuals from the intertidal when compared to the subtidal zone. The pattern of size-related tagging mortality existed for both populations, but was much higher in the intertidal zone. Although the reason for this differential is unknown, one potential explanation is that intertidal individuals are more stressed than those in the subtidal and that tagging places sufficient additional stress on these to result in mortality. Another explanation is that it has been shown that urchins from the intertidal have higher GSI's than those from the subtidal. It is possible that the lack of space within the test related to the higher levels of GSI may have increased the failure rates. It has been shown that the two populations are different in terms of GSI, and in their gonad biochemistry (Hughes et al, in press). The difference in response to tagging related stress demonstrates a good example of the inherent problems of scaling up from pilot studies to larger experiments and the assumptions that can be made from a small trial.

Given the unexpected mortality it was not possible to continue the field trial as envisaged. A limited version was undertaken to provide proof of concept, and to test the appropriateness of the technologies. This showed that it was possible to successfully waterproof the reader and to use it underwater to a depth of approximately 1.5 m, whilst using a snorkel. The reader was able to detect urchins at a range of approximately 10-15 cms. This was sufficient for low rugosity areas, but in areas with a complex topography it was not possible to get the reader close enough to the urchin, such that the urchin had to be picked up and presented to the reader. This is obviously unsatisfactory if you wish to have zero disturbance to the urchin.

Previous trials have shown that *P. miliaris* is very difficult to permanently tag using external tags (Hughes unpub. data). In the light of this and studies that have questioned the accuracy of growth estimates obtained using chemical markers (Russell & Meredith 2000), as well as recent findings that some echinoid species are in fact much longer lived than previous estimates have suggested (Ebert & Southon 2003), there needs to be some new method that allows yearly growth measurements of urchins to be made so that growth parameters of wild populations can be estimated. The current study suggests that despite the relatively high tag loss, especially in smaller urchins, PIT tags remain a viable option for permanently marking *P. miliaris*. Given the important ecological role that *P. miliaris* plays in the ecology of sea lochs and the high degree of individual trophic plasticity exhibited there is a need for accurate information on movement, growth and population ecology. As such this method of tagging appears to be the most promising at the current time.

Chapter 6

HETEROGENEITY IN THE INFLUENCE OF SEA URCHIN GRAZING OF A SCOTTISH SEA LOCH

INTRODUCTION

The regulation of community structure in rocky intertidal habitats has been described by Menge (2000) as being 'dependent on the interdependence of small-scale, short-term ecological processes including top-down effects such as grazing and larger-scale processes that create variation in bottom-up effects'. Such a description highlights both the role of grazing and the way in which its effects can be mediated by factors acting at a range of spatial scales.

That grazing can have profound effects on intertidal and subtidal benthic communities has been well-established (Ayling 1978, Hawkins & Hartnoll 1983, Witman 1985, Fletcher 1987). With these studies came an understanding that these grazing related impacts can vary in both time and space. The effect of the removal of *Strongylocentrotus purpuratus* from rock pools, and its exclusion from subtidal rocks by cages on the Washington coast by Paine (1969) showed that subsequent changes to algal populations were related to tidal height. Successional changes occurred most rapidly in the subtidal or in the low intertidal. A similar urchin grazing impact relationship with depth was observed for the urchin *Evechinus chloroticus* from New Zealand (Villouta et al. 2001). The removal experiment, which ran for two years, resulted in conspicuous changes in the algal assemblages at all depths. The rate of colonisation of large brown algae (Phaeophyta) such as *Carpophyllum*, *Cystophora* and *Sargassum* was however, much slower at the deeper replicates. A study on the role of predation in controlling space utilisation in the rocky intertidal communities of New England showed it to be dependent on the degree of exposure of the shore (Menge 1976),

predation having the greatest role on sheltered shores. In conjunction with variation between different sites at spatial scales of kilometres and between tidal heights, several studies have reported a high degree of variation within sites and between replicates. Following the partial removal of the sea urchin *Centrostephanus rodgersii* from shallow subtidal reefs of New South Wales (Andrew 1993) there was considerable variation in the percentage cover of filamentous algae amongst replicate patches. Distances in the order of tens of meters separated these replicates and this variation was at times sufficient to swamp the treatment effects.

One source of spatial variation in the effects of sea urchin grazing has been attributed to habitat heterogeneity (Benedetti-Cecchi & Cinelli 1995). Experimental removal of *Paracentrotus lividus* and *Arbacia lixula* from littoral tide pools in the western Mediterranean showed that the grazing impact of these species was greater close to shelters used by the sea urchins. The study also found that there was a complex interaction between the brown algae and filamentous algae, and between the level of grazing and distance from the urchin refuges. It was suggested that the sea urchins' reliance on these shelters allowed for the coexistence of the algae and the urchins in close proximity. This study highlighted how spatial heterogeneity and habitat complexity may affect the outcome of interactions between grazers and algae.

Further work with these species in the same region identified another interaction that can result in increased spatial variation in impacts of urchin grazing. The study by Bulleri et al (2002) centred on shallow subtidal reefs where the two urchin species were the main herbivores. This habitat was characterised by a mosaic formed from patches of encrusting coralline algae and patches of erect turf forming algae. In order to separate the effects of urchin grazing and the presence of coralline algae on the recruitment of erect algae species, the effects of the removal of urchins and coralline algae were observed over a period of

eight months. The study found that the variability within and between patches was higher when encrusting corallines had been removed relative to where they were left untouched, irrespective of the presence of urchins. The authors argued this was evidence for the presence of coralline algae being the main source of variability.

Although sea urchin-algae interactions have been studied in a wide range of habitats (see (Lawerence 1975) for review), relatively little work has been conducted on intertidal populations. A number of studies have used littoral tide pools to study these interactions (Paine & Vadas 1969, Benedetti-Cecchi & Cinelli 1995); but the author is only aware of two other studies where intertidal urchin populations outside rock pools have been studied to determine the impact of their grazing on algal and invertebrate populations. One of the studies (Prince 1995) involved the removal of the intertidal populations of the urchin *Echinometra mathaei* from rock platforms at two sites on Rottnest Island, Western Australia. During this two year experiment there were distinct between-site differences. At one site the removal of urchins resulted in the predicted response of lush algal growth. At the other site the removal of urchins had little impact on the cover of non-encrusting algae. The author attributed this difference in response to a number of factors including differential food availability, wave exposure and platform topography at the sites. The other study examined successional changes caused by the caging of plots in the lower intertidal to prevent access by three species of birds that prey on the intertidal urchins (*Strongylocentrotus purpuratus*). The author (Wootton 1995) estimated that by reducing urchin grazing pressure these birds had a strong influence on algae abundance and diversity.

This current study is an attempt to look at the spatial variation in the impact that urchin grazing has on the encrusting communities of intertidal boulder fields that characterise the shores of Scottish sea lochs. Given its local abundance and dietary range the object of the

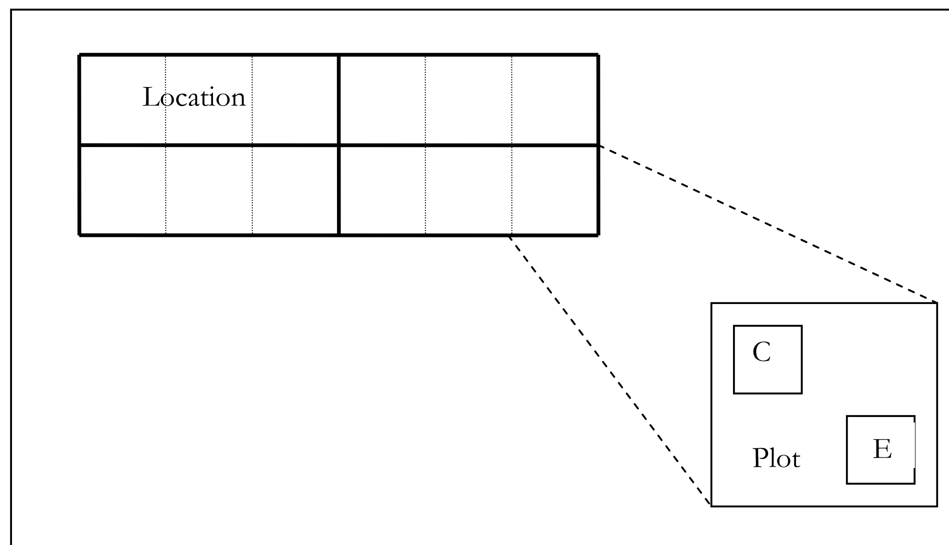
current study was to examine the influence of *P. miliaris* on the intertidal encrusting communities of Scottish sea lochs.

METHODOLOGY

Experimental Design

The experiment was sited within Loch Creran using three experimental sites; RG, SL, SS (see chapter 1) At each site, on the selected area of shore a 18x6m section was marked out using plastic pegs with the long axis parallel to the shore. This section was then divided into four sub sections (locations); each location was further divided into three 3x3m squares. One of these three squares was chosen at random to become the experimental plot. Within this area two 1.2x1.2 m quadrats were placed, one as a control and one as a treatment. Within this 1.2x1.2 m quadrat, an inner zone of 80x80 cm was used for all sampling. This was done to create a buffer zone in an attempt to reduce edge effects. The larger (1.2 m) quadrats were placed in an offset configuration to minimise the length of common border (Figure 6-1). The designation of control or treatment was made at random. This resulted in a total of four experimental and four control plots at each site

Figure 6-1 The experimental layout, showing the four locations within a site, and the position of the experimental (E) and control (C) plots within a location



Data Collection

- Removed Urchins

Following initial data collection (see section below), all the urchins were removed from the experimental plots. Two observers did this by systematically hand removing all visible urchins. Where possible, boulders and cobbles were carefully moved, to inspect the under surface, and replaced in their original position. This procedure was repeated on every spring tide, when the plots were uncovered. This meant the procedure was conducted every two weeks on average. The experiment was started in June 2003 and concluded in October 2003.

The urchins were removed from an area of 120x120 cm. These urchins were collected, counted and grouped into eight size categories. The grouping into size classes was done using a board made of marine ply into which eight holes of increasing size had been drilled. The diameter of each urchin test was estimated by eye, using the holes as a visual reference. This resulted in the urchins being split into the following eight size categories (Table 6-1).

Table 6-1 Size categories used to measure and group sea urchins from the three sites

Category	1	2	3	4	5	6	7	8
Size (mm)	>5	>10	>15	>20	>25	>30	>35	>40

This method was used instead of the traditional callipers to reduce handling time because of the large numbers of urchins involved.

Urchin Population Estimates

At the beginning of the experiment two 25x25 cm quadrats were used to make a population estimate of the urchins within the control and treatment areas. The quadrats were placed at random within an inner 80x80 cm area of the 1.2x1.2 m experimental plot and all the urchins found within were counted and measured, using the method outlined above. This was then repeated at two month intervals in August and October to test the difference between the populations found within the control and the treatment areas.

Following concerns over the degree of variance found, the number of quadrats used was doubled.

Data Analysis

To determine if at the start of the experiment prior to the first removal there was a significant difference in the number of urchins between the control and the experimental plots, the data were tested for homogeneity of variance (Levene's Test), and for normality (Kolmogorov-Smirnov Test); the data were square root transformed to meet assumptions of normality. Prior to further analysis the means were plotted to investigate possible interactions between the sites and treatments. As a result of obvious interactions between the factors, the sites were analysed separately using two way ANOVA. All univariate statistics were conducted using MiniTab 13. Subsequent data from following months were treated in a similar manner.

Photographic Quadrats

At the beginning of the experiment, and then at intervals of two months, three photographic quadrats were taken within the inner 80x80 cm area of the quadrat. The photographs were taken using an Olympus 5050 digital camera, in an Olympus T14 underwater housing. The images were taken when the experimental areas were underwater, to allow a better view of the under-story as the majority of the algae canopy was floating. It also increased the image quality by reducing glare and variability of light. The images were taken in JPEG format at a resolution of 2560x1920 pixels with a resultant image size of approximately 2-MB. The area of the photograph was kept constant using a scale bar that was attached to the underwater housing at a distance of approximately 15cm from the lens. This scale bar was placed against the substrate. As a result of the heterogeneity of the substrate, the angle of the photograph was at right angles to the plane of the largest surface area. This resulted in coverage of approximately 140cm^2.

The images were downloaded on to a PC then imported into the software package ArcView3.2 where a grid of ten by ten rectangles was overlaid. Each of the 100 areas were scored between zero and four according to the substrate type. The score was related to the percentage cover of that particular substrate type within the area such that 1=25%, 2=50%, 3=75%, 4=100%. The substrate was classified according to a modified Wentworth scale: however the largest size category used in this study was cobble as opposed to boulder on the original scale. This was done as the size of the photographed area did not allow resolution between the two categories. The area was then assessed for its biotic cover using the same percentage cover scoring system as used for the substrate type.

Table 6-2 Functional group used to describe the community assemblages for the photoquadrats

Algae	Invertebrates
Lithophyllum Sp	Serpulidae
Dead *Lithophyllum* Spp	*Pomatoceros* Sp
*Hildenbrandi*a Spp	*Serpula* Spp
Chlorophyceae	Spirorbidae
Erect Chlorophyceae	Balanomorpha
Encrusting fleshy green algae	Polyplacophora
Phaeophyceae	Patellacea
Erect Phaeophyceae	Trochidea
Encrusting fleshy brown algae	*Littorina Sp*
Rhodophyceae	Buccinidae
Erect Rhodophyceae	Mytilidae
Encrusting fleshy red algae	

Areas of the image that were out of focus, blurred or obscured were classified as obscured. Biotic cover was grouped into 20 functional groups. Use of these functional groups (Table

6-2) reduced the time spent analysing the images and often represented identification to the lowest taxonomic level possible from the images. For those organisms where specific counts could be made, such as spirorbid worms, the number per sub-area was counted and recorded. For encrusting algae or colonial fauna a percentage based system was used. The statistical techniques used are robust to the mixing of biomass and abundance data if appropriate consideration is given to the value of the two scales (Anderson & Underwood 1994).

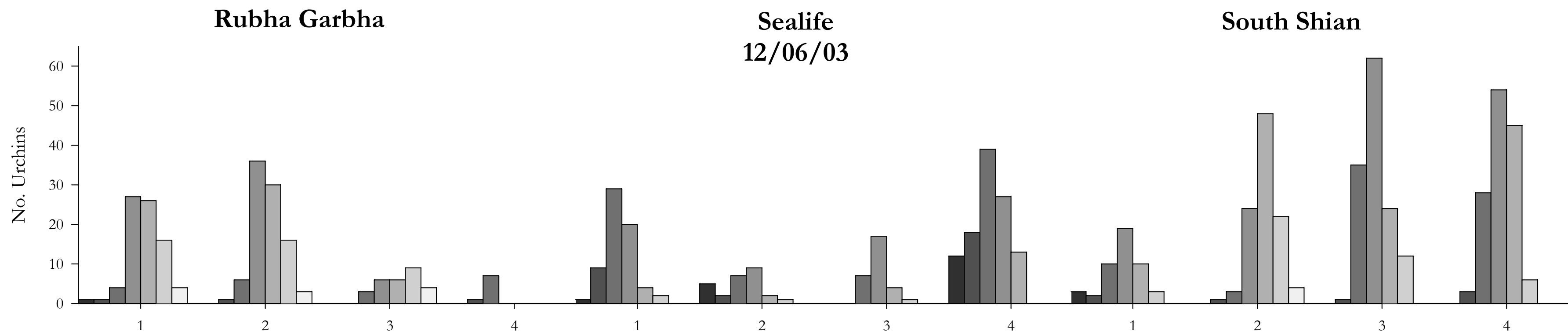
As the investigation focused on the effects of urchin grazing on encrusting communities, and also to reduce the variability caused by substrate heterogeneity, only those areas that were defined as cobbles were included in the analysis(photoquadrats that contained little or no cobble were removed from the analysis). As this resulted in unequal areas of substrate being compared between photoquadrats and sites, the data were converted into percentage cover and densities per unit area.

The species abundance data were used to generate species matrices for each photoquadrat. For groups where counts had been made this figure was converted into number per 140 cm^2. Where percent cover was used, this was multiplied by 100 to obtain an overall percentage. The species matrices generated were square root transformed to reduce the effect of rare species and then transformed into triangular similarity matrices based on the Bray-Curtis coefficient of similarity. These matrices were used to test for differences in community structure. Visual representation of the relationships between the samples was constructed using non-metric multidimensional scaling (nMDS) plots. To provide a hypothesis testing framework a permutation-based procedure, using the full dimensionality of the data, was used. This analysis of similarity (ANOSIM) is in many ways analogous to a univariate analysis of variance. All multivariate statistical analysis was carried out using PRIMER 5.3.

RESULTS

Urchin Removal

During the four months that the experiment was run a total of 5500 urchins were removed from the experimental plots. Figure 6-2 shows the size-frequency distributions of the urchins removed from each site and from each location within a site. It shows that there was considerable variation within sites as well as between sites and over time. On the first clearance a total of 855 urchins were removed from all three sites; this represented the highest number of removals from any one visit. The second highest total (725) came from the last removal. There were, however, some consistent trends throughout the period of the experiment. Each time fewer individuals were removed from the SL site compared to that of the other two. Location three at RG had very low numbers of urchins removed throughout the course of the experiment. There was a trend towards larger size classes over time, with the mode shifting from size class four on the first removal to size class six by the last.



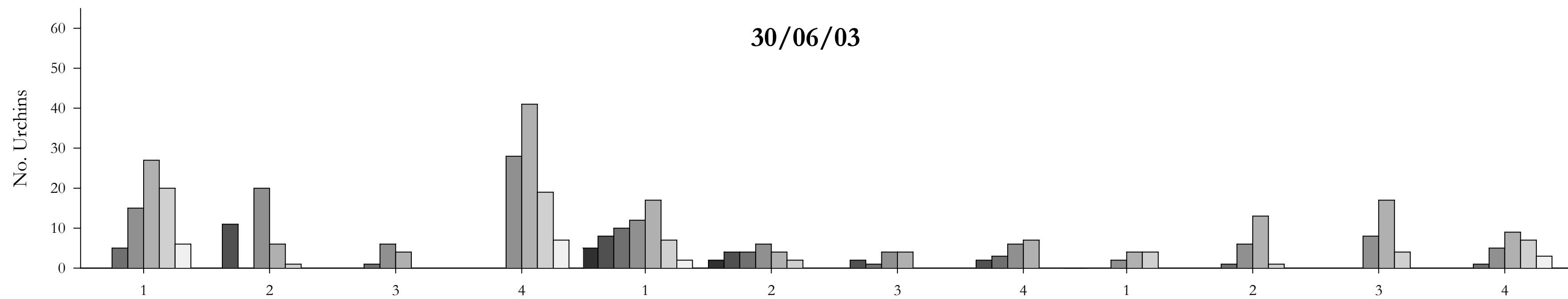


Figure 6-2 Size frequency distribution for urchins removed during the course of the experiment

Size 1 Size 2 Size 3 Size 4 Size 5 Size 6 Size 7 Size 8

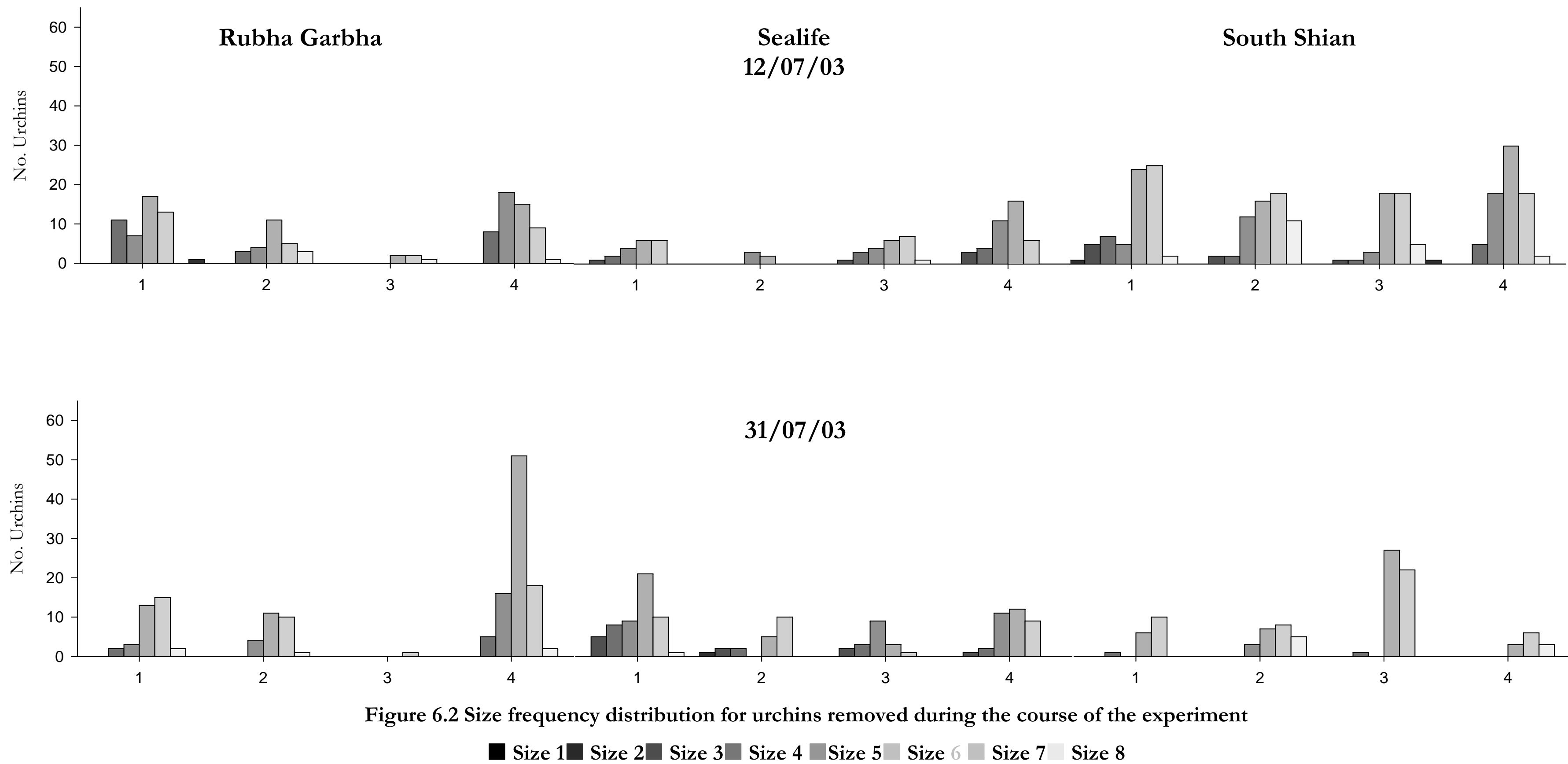
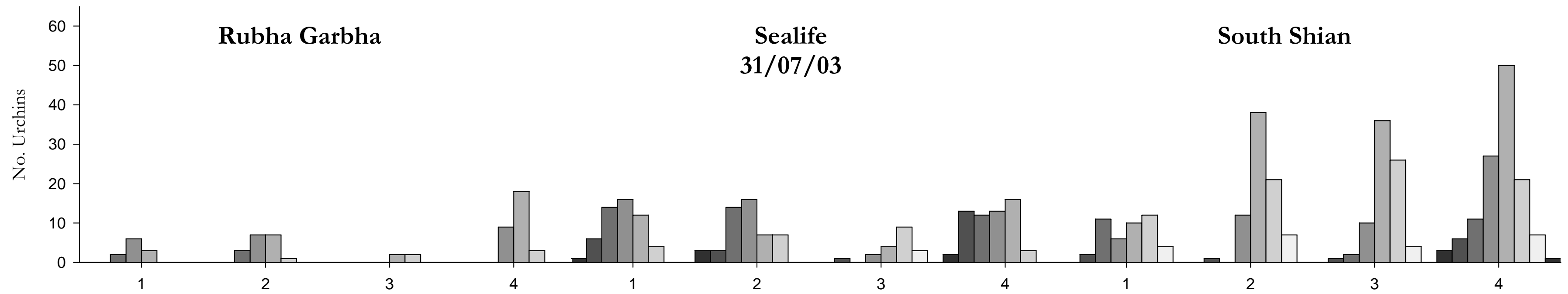


Figure 6.2 Size frequency distribution for urchins removed during the course of the experiment



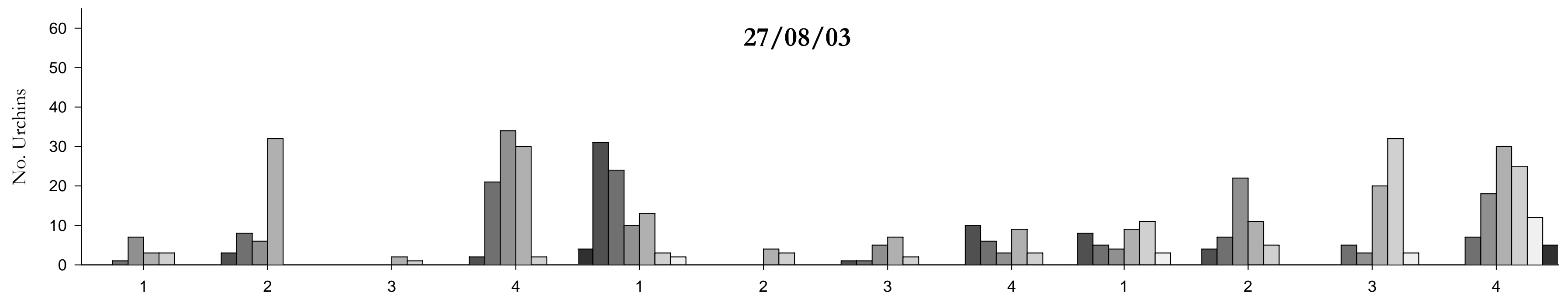


Figure 6.2 Size frequency distribution for urchins removed during the course of the experiment

Size 1 Size 2 Size 3 Size 4 Size 5 Size 6 Size 7 Size 8

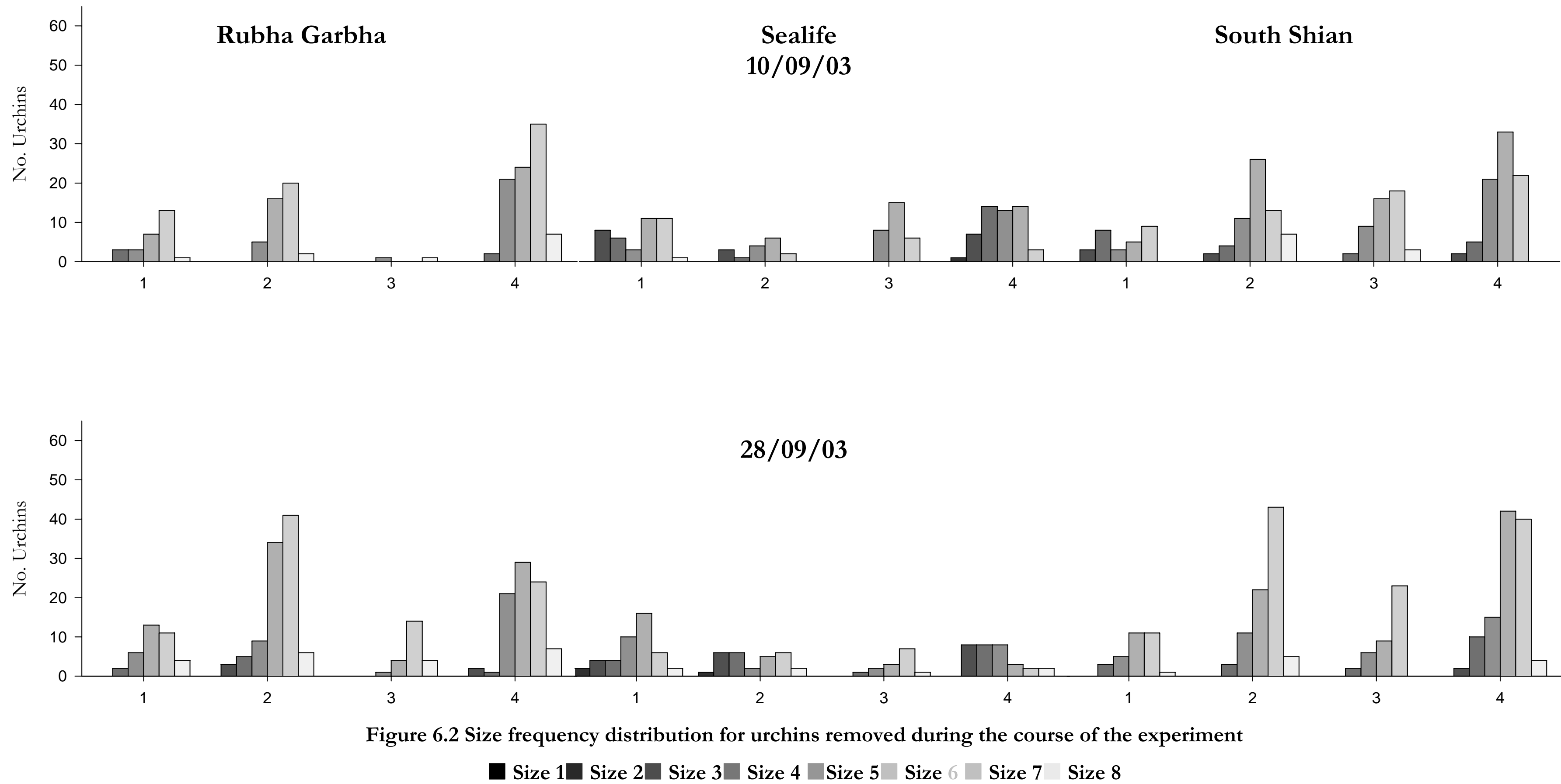
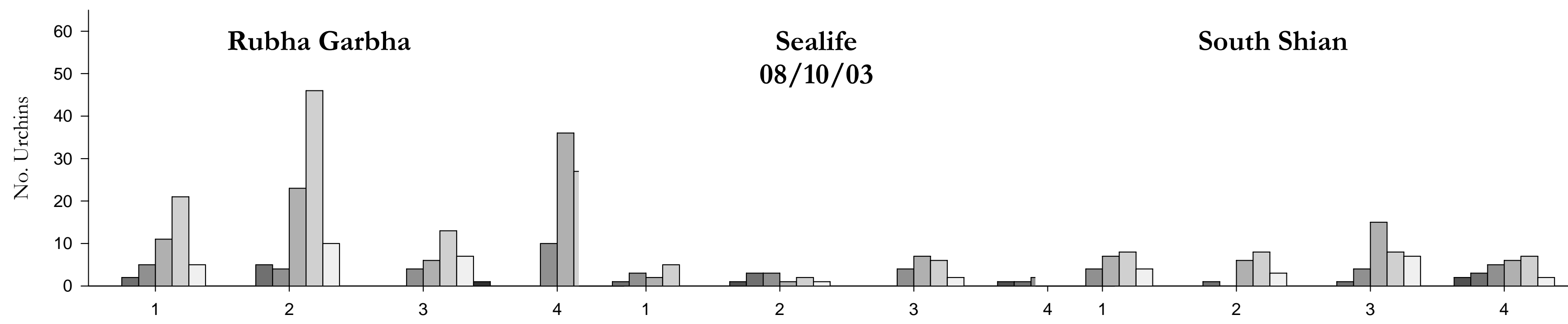


Figure 6.2 Size frequency distribution for urchins removed during the course of the experiment



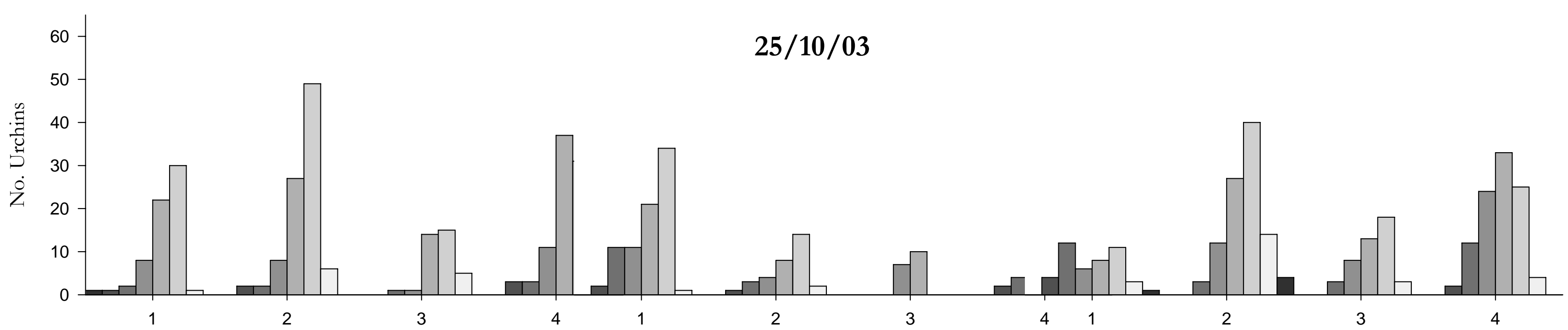


Figure 6.2 Size frequency distribution for urchins removed during the course of the experiment

Size 1 Size 2 Size 3 Size 4 Size 5 Size 6 Size 7 Size

Urchin Population Estimates

The number of urchins within each plot was compared for each treatment (control and experimental) to see how effective the removal of the urchins from the experimental plots had been (Figure 6-3). Using the Kolmorogov-Smirnov test the data were tested for their goodness of fit to a normal distribution and for homoscedasticity. Any data found to significantly differ from the normal were square root transformed and retested. All transformed data approximated to the normal distribution and had homogenous variance. At each site and date a two way ANOVA (Sokal & Rohlf 1995) was used to test for differences between locations and treatments (see Table 6-3).

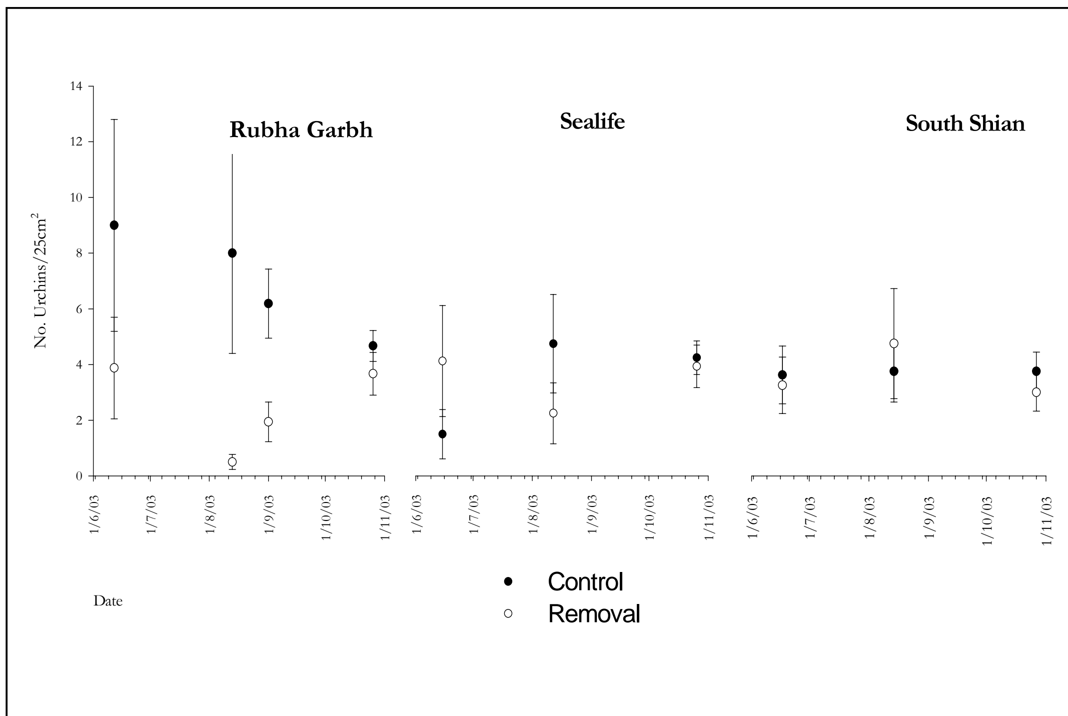


Figure 6-3 Population estimates of the number of urchins in the control and experimental plots at the three sites over the period of the experiment. Error bars represent the standard error of the mean.

Prior to any manipulation of the sites in June, there was a significant difference at the RG site between the number of urchins at each location and the treatments (Table 6-3). There was also a significant interaction between the two factors. At the other two sites there was no significant difference within the treatments, but there was a significant difference between locations at the SL site. After two months of regularly removing the urchins from

experimental plots, two of the sites (SL and SS) showed no significant difference between the number of urchins found in the control and experimental (urchin removal) plots, or between locations. At the RG site, there was a significant difference between the treatments, but not the locations.

After the August sampling the number of quadrats sampled was doubled to try and reduce the variability. An additional sample during September at the RG site showed that there were highly significant differences between urchin numbers at each location and within treatments.

By the end of the experiment (October) there was no significant difference in the estimated population between the control and the experimental plots at any site although the recorded number was lower in the experimental compared to the control at each site. There was, however, a significant difference between locations at two sites (RG and SL). After commencement of the experiment only once did the number of urchins in the experimental area exceed that of the control. This occurred in the August sample at the SS site.

Table 6-3 The difference between the population estimates of urchin density at control and experimental plots at the three sites. Tables show the result of a two way ANOVA , location Vs treatment. Significant differences at P<0.05 with *

RG	June					August					September					October				
Factor	DF	SS	MS	F	P	DF	SS	MS	F	P	DF	SS	MS	F	P	DF	SS	MS	F	P
Location	3	21.18	7.06	7.18	0.01*	3	6.59	2.20	1.71	0.241	3	17.76	5.92	11.68	0.00*	3	53.34	17.78	3.11	0.05 *
Treatment	1	5.21	5.1	5.29	0.05*	1	16.79	16.79	13.10	0.01*	1	15.13	15.13	29.84	0.00*	1	0.78	0.78	0.14	0.72
Interaction	3	12.32	4.11	4.17	0.05*	3	0.66	0.22	0.17	0.912	3	6.54	2.18	4.30	0.02*	3	37.34	12.45	2.18	0.12
Error	8	7.869	0.948			8	10.26	1.28			24	12.17	0.51			24	137.3	5.72		

SL	June					August					October				
Factor	DF	SS	MS	F	P	DF	SS	MS	F	P	DF	SS	MS	F	P
Location	3	14.825	4.942	7.51	0.010 **	3	81.5	27.2	1.80	0.226	3	53.34	17.78	3.11	0.045 *
Treatment	1	2.205	2.205	7.51	0.105	1	25.0	25.0	1.65	0.235	1	0.78	0.78	0.14	0.715
Interaction	3	3.977	1.326	2.01	0.190	3	40.5	13.5	0.89	0.486	3	37.34	12.45	2.18	0.117
Error	8	5.264	0.658			8	121.0	15.1			24	137.25	5.72		

SS	June					August					October				
Factor	DF	SS	MS	F	P	DF	SS	MS	F	P	DF	SS	MS	F	P
Location	3	13.2	4.4	0.38	0.767	3	60.50	20.17	2.93	0.099	3	3.407	1.136	2.12	0.124
Treatment	1	0.6	0.6	.05	0.830	1	4.00	4.00	0.58	0.467	1	0.423	0.423	0.79	0.383
Interaction	3	12.7	4.2	0.37	0.777	3	171.50	57.17	8.32	0.008 **	3	3.724	1.241	2.32	0.101
Error	8	91.5	11.4			8	55.00	6.88			24	12.842	0.535		

Photographic Quadrats

Multivariate Analysis

Only images that contained a significant area of the substrate cobbles were used for analysis. This led to a reduction in the number of replicates at some sites on some dates. Although this has caused a reduction in the power of the multivariate statistics' ability to detect change, they are able to deal with the resulting unbalanced design. The relationship between the species assemblages of the three sites is shown in Figure 6-4. There was clear separation between the three sites. This separation between the sites is confirmed by the results from a one-way ANOSIM which showed a highly significant difference between the sites (R=0.382, P<0.001).

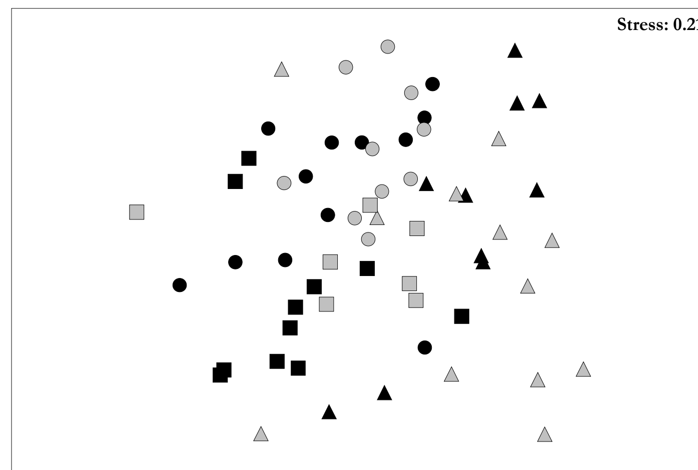


Figure 6-4 MDS plot for species assemblages at the three sites prior to the start of the experiment: ●RG ▲SS ■SL, ■Control ■ Experimental

The nMDS plots for the species matrices generated from the photoquadrats for each site and at each sampling date are presented in Figure 6-6. The crossed experimental designs and relatively high stress values make interpretation of patterns within the plots difficult.

The June plots for SS and RG show a clear mixing of experimental and control areas. By August there is a visible distinction between the treatment groups for the Sea Life site. This separation becomes more evident in the October plots. The results of the crossed ANOSIM test can be seen in Table 6-4. This procedure produces a test statistic R and a related percentage significant level. The R value is generated from the average ranked similarity within, subtracted from average dissimilarity between, replicates from different treatments. This is done separately for each location and averaged to give a measure of community difference (R), removing the effect of location.

Using randomisation/permutations testing a significance level for the differences in R is generated. This is based on the number of random permutations that resulted in a greater difference than the observed. In this case 10,000 permutations were run for each test.

Prior to any experimental manipulation in June although the sites were quantitatively different to each other, at the 5% level there was no significant difference between the control and experimental plots within each site. At two of the sites (SL and SS) there was a significant difference between locations within sites. By August there was a significant difference between the control and experimental plots at the SS and SL sites. For these two sites this significant difference was maintained throughout the rest of the experiment. At the RG site there was no significant difference between the control and the experimental plots at any point during the experiment.

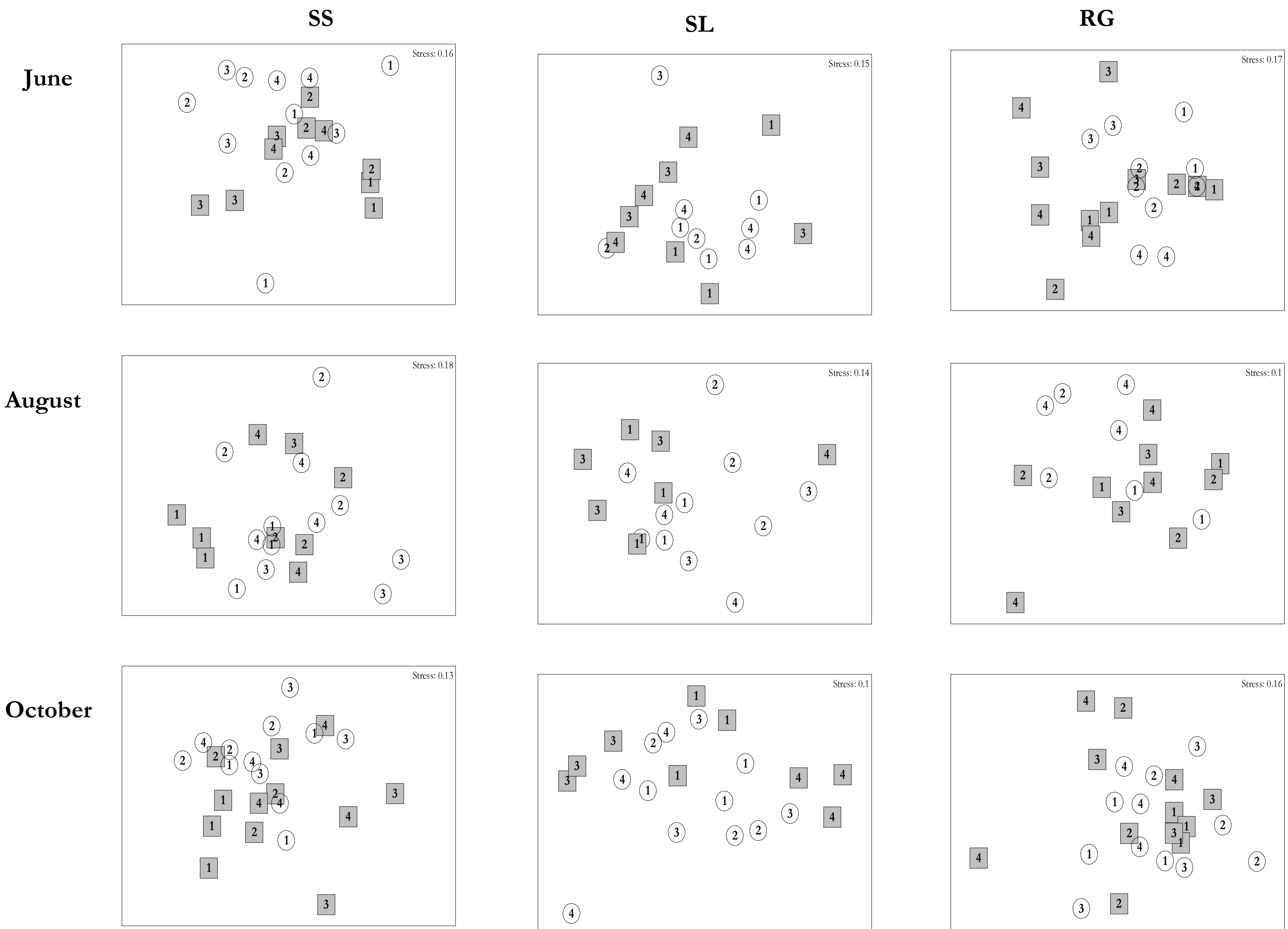


Figure 6-5 Two dimensional MDS of encrusting biota for each site and sampling date. Squares, Control; Circles, Removal. One to four are locations within each site.

Table 6-4 Results of crossed ANOSIM test for the three experimental sites. The test is comparing differences in R value between treatments (control/removal) and within site locations. Values are based on the Bray-Curtis similarity matrix using square rooted abundance

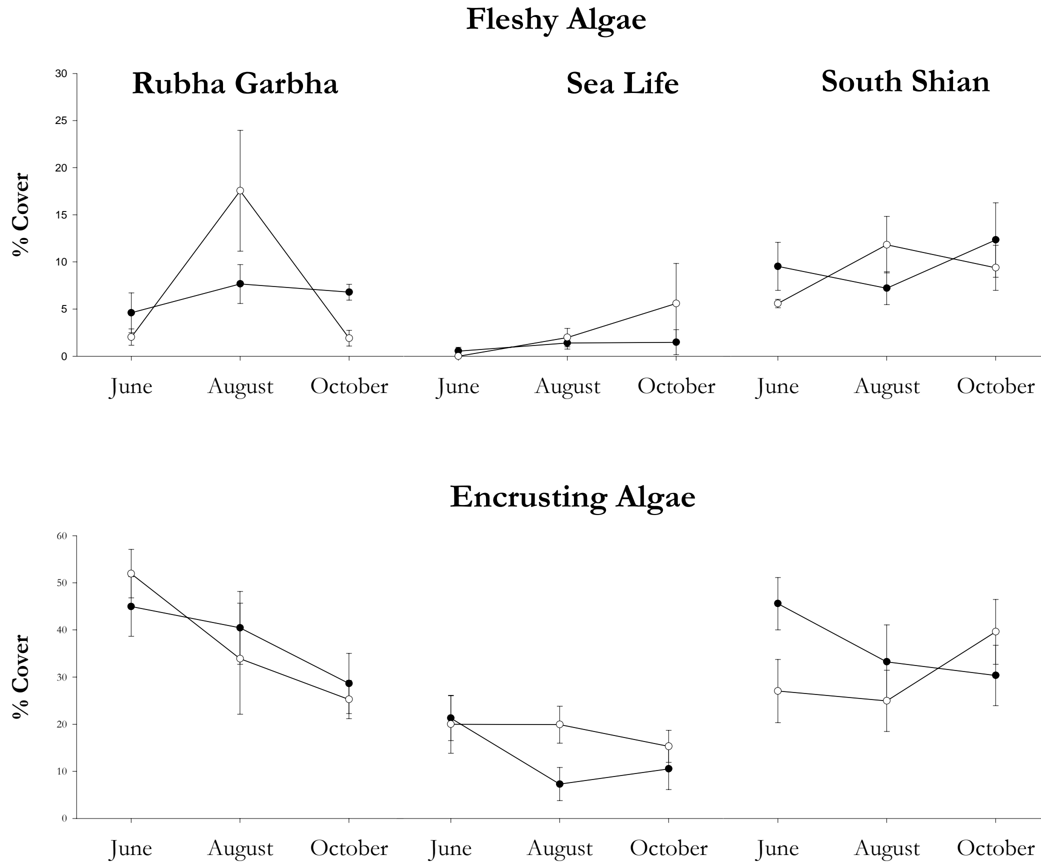
	Treatment		Location	
	R statistic	Significance (%)	R statistic	Significance (%)
		RG		
June	0.207	5.6	0.229	5.8
Aug.	-0.019	51.0	0.05	62.1
October.	0.194	5.4	0.119	12.6
		SL		
June	0.319	5.3	0.233	4.2*
Aug	0.435	2.5 *	0.262	3.8*
Oct	0.728	0.2 *	0.398	0.4 *
		SS		
June	0.228	8	0.318	4.2*
Aug	0.334	4.3 *	0.367	0.4*
Oct	0.25	4.3 *	0.219	2.1*

The R values generated from the ANOSIM test can be used as a measure of community divergence (Clarke 1993). At the Sea Life site there was a progressive increase in the R value over the period of the experiment, representing a continued divergence in the community structure between the control and experimental areas. The SS site shows an increase in the R value between June and August, followed by reduction between August and October. At the RG site there was a huge decrease in the R-value between June and August. This very marked decrease was then followed by a return to a similar level of the R value by October to that prior to the start of the experiment.

Univariate Analysis

In conjunction with the multivariate analysis of species assemblages, univariate analysis was carried out on individual components of the species assemblages. These components were: encrusting algae as an amalgamation of the functional groups of *Lithophyllum* and *Hildenbrandia* species and fleshy algae which was a conglomeration of the Chlorophyceae, Phaeophyceae and Rhodophyceae functional groups. The three other elements were sediment, *Pomatoceros* and Spirorbid worms.

Figure 6-7 . Percent coverage of fleshy and encrusting algae at the three sites, data are grouped across all locations within sites. ● Control:○ Experiment



At all three sites there was an increase in fleshy algae from June to August for the experimental sites, and at all three sites the rate of this increase is greater than for the control (see Figure 6-7). From August the percentage cover of fleshy algae in the experimental plot fell to below that of the control at two of the sites, while at the SL site the percentage cover relative to the control increased.

Encrusting algae at the RG site showed a consistently decreasing trend for both the control and the experimental plots. At SS and SL sites there were no clear trends. At the SS site both the control and experimental showed a decrease in encrusting algae between June and August; the control then continues to decrease while the experimental plots increase. At the SL site, there was a reduction of encrusting algae between June and August for the

control, while the experimental area shows a small increase. This pattern is reversed between August and October.

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Table 6-5 Effect of treatment on the percentage cover of fleshy algae at the three sites, over three sampling periods.

Source	df	June				df	August				df	October			
		SS	Adj MS	F	P		SS	Adj. MS	F	P		SS	Adj. MS	F	P
Treatment	1	1.73	1.17	0.65	0.45	1	5.26	5.00	1.20	0.32	1	3.11	3.11	0.85	0.39
Location (Treatment)	6	10.86	1.81	1.35	0.30	5	21.08	4.22	1.40	0.30	6	21.91	3.65	1.23	0.34
Error	14	18.83	1.35			10	30.15	3.02			16	47.53	2.97		
Total	21	31.42				16	56.48				23	72.55			

Fleshy Algae RG

Source	df	June				df	August				df	October			
		SS	Adj. MS	F	P		SS	Adj. MS	F	P		SS	Adj. MS	F	P
Treatment	1	8.04	8.43	2.66	0.15	1	8.04	8.43	2.66	0.15	1	0.40	0.40	0.07	0.79
Treatment (location)	6	19.31	3.22	2.20	0.11	6	19.31	3.22	2.20	0.11	6	33.14	5.52	1.29	0.31
Error	14	20.46	1.46			14	20.46	1.46			16	68.54	4.28		
Total	21	47.801				21	47.80				23	102.08			

Fleshy Algae SS

Source	df	June				df	August				df	October			
		SS	Adj. MS	F	P		SS	Adj. MS	F	P		SS	Adj. MS	F	P
Treatment	1	1.24	0.88	1.01	0.34	1	0.01	0.09	0.01	0.92	1	0.11	0.11	0.31	0.60
Treatment (location)	5	3.93	0.79	0.61	0.70	5	3.45	3.447	0.53	0.75	5	1.75	0.35	0.46	0.80
Error	11	14.24	14.24			11	14.27	14.27			14	10.72	0.77		
Total	17	19.41				17	17.72				20				

Fleshy AlgaeSL

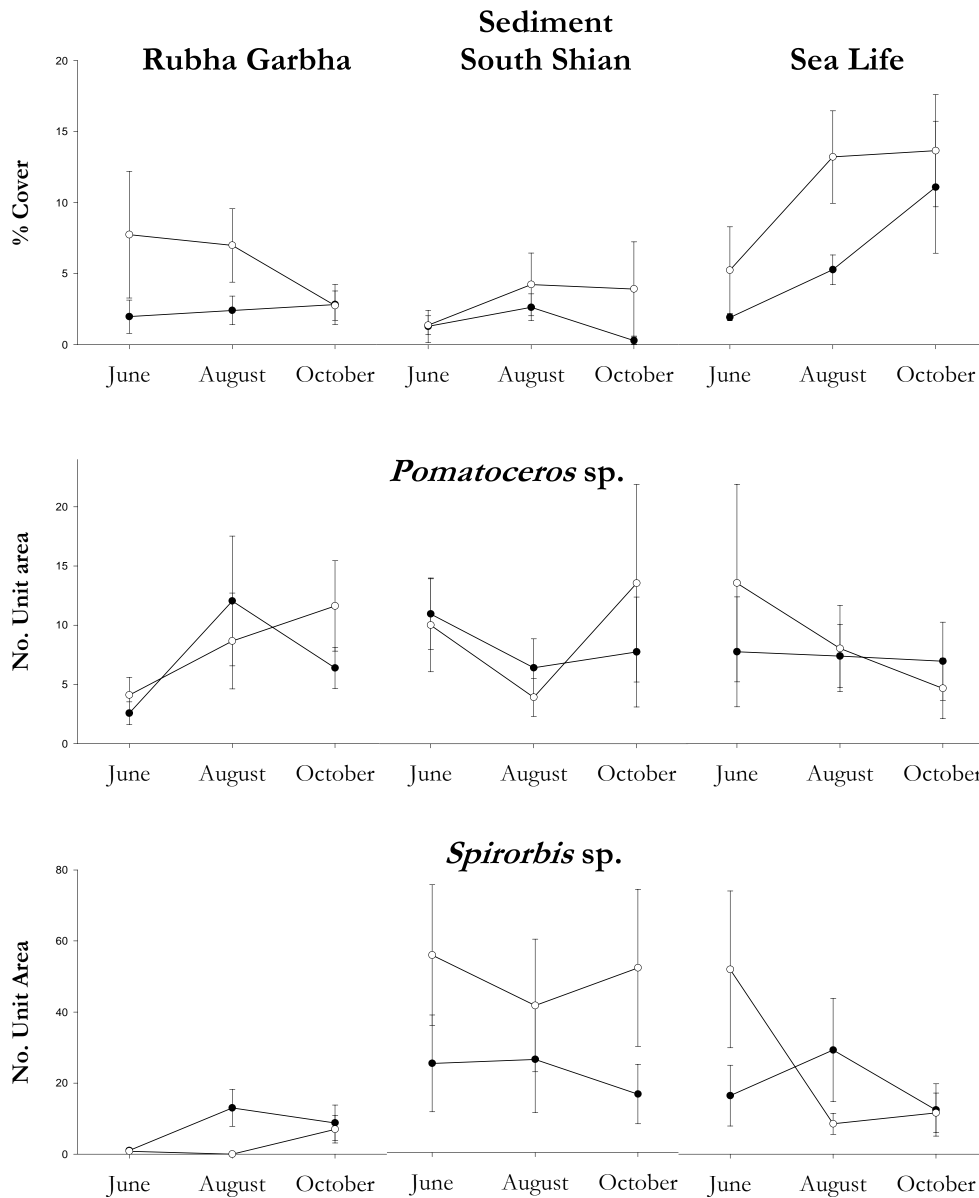

Figure 6-8 Percent coverage of sediment and the number of *Pomatoceros* and *Spirorbis* worms at the three sites, data are grouped across all locations within sites. ● Control ○Experimental

Each site was analysed separately using a general linear model ANOVA with location being nested within treatment (Table 6-5).There was no significant difference in the percentage cover of fleshy algae found between treatments or locations at any sampling events at any of the sites. The percentage of the substrate covered in sediment in the experimental plots at the RG site decreased over the period of the experiment, while that of the control plots

increased. At the other two sites sediment showed a rapid increase in the experimental plots relative to the control between June and August (Figure 6-8).

The pattern for the spirorbid worms showed a more consistent pattern of response over the three sites. In all the experimental sites there was a reduction in number per unit area between June and August, followed by an increase between August and October. In the control plots there was a contrasting pattern of increase between June and August followed by a reduction between August and October.

DISCUSSION

In order to try and understand the role that grazing plays in structuring benthic communities, it has been a common technique to manipulate the density of the grazer or grazers, and to study the impact that this manipulation has on the species assemblages in those areas (Jones & Kain 1965, Ebert 1977, Menge 1978). In those studies examining the grazing impacts of sea urchins, one of two methods are commonly used to maintain these experimental manipulations. Cages or barriers have been used in a number of studies (Paine & Vadas 1969, Ebert 1977, Dean et al. 1988). However as a result of a number of problems associated with cages, both logistical and experimental, a number of more recent studies have opted to regulate urchin densities by regularly revisiting the sites and manually re-establishing the experimental conditions (Andrew & Underwood 1993, Benedetti-Cecchi & Cinelli 1995, Bulleri et al. 1999, Villouta et al. 2001).

Throughout this study the experimental plots were revisited on as regular a basis as the tidal cycle would allow and the urchins were removed from the experimental plots. Periodical estimates of the urchin population in the control and experimental plots revealed that during the four months in which the experiment ran, only one site was significantly different between the two treatments. The RG site showed a significant difference prior to the experiment and at the August and September sampling. On the final sample date there was no significant difference at any of the sites.

These data suggest that the basic experimental condition of reduced urchin grazing in the experimental plots relative to the control had not been established. However as the plots were cleared on a fortnightly basis, for a period the urchin density within the experimental plots was close to zero. Furthermore, after the experiment was started, only on one occasion was the number of urchins in the control greater than the experiment. It can be suggested that there was an overall reduction in the grazing pressure on the experimental

plots relative to that of the control, although the magnitude and longevity of the reduction cannot be estimated.

Multivariate analysis revealed a complex pattern of putative response to the effects of reduced urchin grazing pressure. At one of the three sites (SL) there was a marked divergence between control and experimental plots. At another (RG) there was no significant impact of the removal of urchins although this was the only site to record significant reduction in urchin populations between the control and the experimental plots. The third site (SS) showed a divergence between the control and experimental followed by a slight convergence. As well as differences at the level of the site, inter-site variation in the form of variation between locations within a site was demonstrated at the two sites that showed a significant change following the removal of urchins.

It has been suggested that it may be easier to understand the multivariate response of the whole community by examining the role of individual components of the assemblages (Anderson & Underwood 1997). At both sites where a significant difference in the multivariate structure was recorded there was a marked increase in the proportion of substrate covered in sediment between the control and the experimental plots. The removal of gastropod grazers from a sheltered intertidal estuary also led to an increase in sediment (Anderson & Underwood 1997). There was a similar increase in sedimentation following the removal of the sea urchin *Parechinus angulosus* (Day & Branch 2002) from subtidal reefs in South Africa. The authors tentatively attributed the poor survival of juveniles of the abalone *Haliotis midae* in areas where the urchins had been removed to the build up of sediment. The mechanism by which urchins reduce sediment may be two fold, either indirectly through their locomotion moving sediment or directly through their grazing activity, where the sediments may be ingested directly or as result of grazing on other material.

The only other clear univariate trend over all the sites was the response of the spirorbid worms. *Psammechinus miliaris* has been previously reported to prey on spirorbid worms (Kelly & Cook 2001). A number of previous studies have shown that the presence of grazers can increase the level of recruitment of spirorbid worms (Keuskamp 2004). This current study would seem to support this relationship. At all sites between June and August there was a positive relationship between the number of spirorbid worms and levels of urchin grazing. It is possible that this difference between the control and experiment was due to differential survival of recruits. The fact that there is then a subsequent negative relationship between the level of urchin grazing and the number of worms suggests that urchin grazing may impact at another life stage post recruitment. It is feasible that the presence of urchins facilitates a higher level of recruitment by increasing free space or reducing sediment but confers greater post settlement mortality through grazing. This difference in response of different life stages to sea urchin grazing has been reported in a number of different organisms including corals (Sammarco 1980) and limpets (Fletcher 1987).

The spatial variation in the impacts of grazing observed in this study can have several sources. Of particular importance in this study may be the variation in grazing pressure between sites. As the results have shown, components of the species assemblages have a differential response to the variation in grazing pressure. This response was similar to that described by Benedetti-Cecchi et al (Benedetti-Cecchi et al. 1998) who reported that individual components of community structure respond differently to a range of grazing pressures. Filamentous algae responded to a partial removal of urchins while fleshy algae only responded to a total removal. Although it was not possible to control the degree of grazing, it is almost certain that the sites were subject to different grazing pressures. This may account at least for some of the observed spatial heterogeneity within the current

study. Other important factors that could have contributed include the physical differences between the sites. Even though all sites were chosen to be as qualitatively as similar as possible, the nMDS plot shows that each site had a different community composition prior to the start of the experiment. As well as differences between the sites there was also a clear difference between locations within sites at two of the sites. These differences may be an effect of environmental gradients such as exposure or freshwater input. If the communities vary between locations and sites before the experiment, then it can be expected that any successional changes that occur as a result of the urchin removals will also change between location and site. Only at the RG site was there no significant difference between locations, or through treatment effects. This site is subject to a high level of fresh water run off from adjacent fields (Kelly pers comms.). This additional stressor may have had a greater impact on the benthic community structure than the partial removal of the sea urchin population.

This study has highlighted the difficulties in designing and implementing experimental study into the impacts of urchin grazing on natural community structure. The difficulties came principally from maintaining the experimental conditions by hand removing the urchins. This method was chosen to remove experimental artefacts associated with cages (Hulberg & Oliver 1980, Schmidt & Warner 1984, Stocker 1986). However when studying a species which is as mobile and numerous as *P. miliaris*, hand removal of urchins to maintain experimental conditions is difficult especially when coupled with a limited access to the sites due to their low position on the shore. This difficulty was compounded further by the natural variability between sites prior to the experimental set up. It could be expected that this natural variability in existing community structure would lead to greater variability in the results of removal of urchins further confusing the subsequent analysis. For these reasons any conclusions drawn from this experiment can at best be regarded as tentative, and as a pilot study for further investigations. With these provisos the study has suggested that the grazing of *P. miliaris* can have a structuring effects on the intertidal

community assemblages of a Scottish sea loch. It has been demonstrated that the impacts of urchin grazing varies at a range of spatial scales, and if a general model of the impacts of urchin grazing is to be constructed then this spatial heterogeneity needs to be accounted for.

Chapter 7

IMPACTS OF ECHINOID GRAZING ON BENTHIC INVERTEBRATE RECRUITMENT

INTRODUCTION

The factors that regulate the community structure of a group of organisms making up the biological component of an ecosystem are diverse (Taylor 1935). One such synecological driver which has long been recognized in playing an important role is predation. The impact that predation could have on a single species was shown relatively early (Woodworth 1908); however the development of these ideas to include the impact of predation on communities, as opposed to single species came later. Many early studies were concerned with the effects of grazing (a form of predation) on grass-land and forest communities. These investigations showed that grazing could alter both the composition and biomass of the target community. Townsend (1928), for example, showed that if grazers were excluded from an area of upland grassland, then it readily returned to a diverse forest community through a number of successional communities. In the marine environment, Newcombe (1935) enclosed mussels (*Mytilus*) in experimental cages, observed population changes in the Bay of Fundy (Nova Scotia) and showed these to be governed not by abiotic factors but by the predatory action of sea urchins and starfish (Newcombe 1935). In a translocation experiment at Loch Ine (Ireland), it was shown that the effects of predation varied with shore characteristics, with predation having a greater role in structuring sheltered compared to open coast shores (Kitching et al. 1959).

In a landmark study, Paine (1966) found that fencing out starfish predators led to dramatic changes in the fauna of rocky intertidal shores, resulting in decreased diversity. He speculated that local species diversity was related to the number of predators and their

ability to prevent a single species from monopolizing a limiting resource. A similar conclusion was reached by Menge (1976) in that predation was a primary mechanism for maintaining the community structure and species richness (diversity) on all but very exposed coasts. The importance of abiotic and biotic factors in mediating the action of predators had been suggested in earlier work but was specifically tested by Menge. He found that the feeding rate, and as such the predatory impact, was directly related to the relationship between the intrinsic properties of the predator as well as the physical environment.

When trying to determine the impacts of predation on community diversity, it is crucial to define vague terms, such as diversity, and how they will be measured. Paine used what he called a 'local diversity', which was defined as the number of species in the sample. A similar definition was used in a more complex tripartite definition of diversity suggested by Whittaker (1960). This structure has become the basic building frame for measuring diversity. It consists of: Alpha diversity being defined as the number of species within a stand, community or sample; Beta diversity defined as the degree of community differentiation or change in community composition in relationship to a complex gradient or pattern of environments; and Gamma diversity as the species diversity of a range of environments. This framework shows that diversity is intrinsically linked to the spatial scale at which it is measured. These definitions are obviously dependent on what constitutes a sample, a community or an environment, and as such illustrates the principle that all diversity measurements are context specific and can only ever be comparative and never absolute.

The confusion as to appropriate scales at which to measure Beta diversity is confounded by the range of indices and measures that have been developed to quantify or measure it (Koleff et al. 2003). Part of this diversity of diversity measures is as a result of bifurcation

in definition of Beta diversity. The terms Beta diversity and species turn-over have been used interchangeably but in fact species turnover relates specifically to the rate of change of species along a predefined environmental gradient (Vellend 2001). In contrast, Beta diversity is the difference in species composition between local or regional assemblages. The latter definition is the one used in the current study, and the metric used will be a pair-wise comparison using similarity coefficients (Magurran 1988).

A number of studies have shown that predation can alter the alpha diversity of the community (Vance 1979, Witman 1985). However there is a lack of empirical evidence on how predation acts on the other elements of diversity. There has also been a recognized deficit in the studies addressing Beta diversity in the marine environment (Thompson et al. 1996, Gray 2000). In the current study the role that sea urchin predation has upon the settlers on new substrata was examined, as was the way that these effects vary over spatial scales. Two main hypotheses were constructed: firstly the predatory action of urchins will significantly and drastically reduce the biomass of the community that develops on the new substratum, and secondly, sea urchin predation will alter the diversity of the community at a range of spatial scales.

METHODS

Field sites

The study was conducted in Loch Creran using the same sites as described in Chapter 1, namely South Shian (SS), Sealife (SL) and Rubha Garbh (RG)

Experimental Design

Settlement panels were fixed within cages according to one of three experimental treatments: Treatment 1, Closed cages without *P. miliaris* inside (these cages excluded all grazers >5mm from the panels); Treatment 2 Closed cages with *P. miliaris* inside (eight *P. miliaris* were placed in each Treatment 2 cage, approximating to a density of 32 urchins m^2); Treatment 3 Open cages (these were identical to the cages of Treatment 1 and 2 except they had a number of holes in them to allow natural densities of predators to enter the cages). Cages of all treatment types were deployed at three sites within Loch Creran. Two cages for each treatment were secured to the soft sediment at a depth of approximately 2-3m. The cages were put in place in January 2005 and the settlement panels were sampled six months later.

Cage design

The cage design used was the result of several trial runs; problems encountered included sea urchins burrowing under cages, squeezing through the mesh, and the cages failing to remain anchored on the bottom. The final cage design was constructed from black plastic with 5mm mesh size, and was 100 mm high and 500 x 500 mm in area and was enclosed on all six sides. The cages were attached to the sea bed using plastic pegs, one at each corner. The settlement panels were black acrylic with a matte textured finish on the downward facing aspect. The panels measured 150 by 150 mm, of which a centre area of 100 by 100 mm was marked. The panels were attached onto the cage roof using cable ties

at each corner. There were four panels in every cage. The cages of Treatment 3 had two holes (50mmx50mm) cut in each side and the top.

Settlement Panel Sampling

The panels were quantified using high resolution digital images. The plates were photographed in the field. From the images individual organisms were identified and delineated manually. Bryozoan and *Spirorbis* species were identified in the field and samples returned to the laboratory for microscopic verification The area of each was determined using the image analysis package Image J. This was then used to calculate the total percentage area for each organism; the total percent cover for all organisms, and the species richness (number of species) for each panel.

Statistical Analysis

One of the cages was lost from one of the sites (SL) during a storm. To retain a fully balanced design this site was removed from the analysis. The total percentage cover (by biota) was compared amongst treatments and sites using a three factor ANOVA, with treatment being fixed and orthogonal to site, which was random. Cages with two levels was nested within site and treatment. Prior to analysis, Cochran's test for homogeneity of variance was performed, and it was found that no transformation was necessary. The alpha diversity was calculated as the total number of species per panel. Difference in alpha diversity between treatments and sites was tested using a two way non-parametric ANOVA as the data were not continuous. For this analysis the data from each cage within treatments were pooled. In order to examine impact patterns of predation on Beta diversity at a range of spatial scales, two similarity indices were used. Such techniques enabled comparison of all panels within a single treatment at a range of spatial scale (centimetres, meters, and kilometres). For example, at the centimetre scale all panels within a single cage were compared to each other, resulting in six values. This was done for all cages within the

treatment and then averaged. For the comparison at meters, each panel within one cage was compared to every other panel in the replicate cage at that site; for kilometres, every panel within a treatment was compared to every other panel in the same treatment but at another site. The two similarity indices used were Jaccard's and Sorenson's, both of which use presence/absence data.

Jaccard $C_J = j/(a+b-j)$

Sorenson $C_S = 2j/(a+b)$

Where j=number of species found in both sites and a=the number of species in sample A with b the number of species in sample B (a decrease in the value of the indices indicates an increase in Beta diversity)

Multivariate analysis was conducted to compare differences between species assemblages for treatment and location. Species abundance (percentage cover) matrices were converted to similarity matrices using Bray Curtis similarity coefficient after the data were double square-root transformed. The Bray Curtis similarity coefficient is an adaptation of Sorenson index modified to take account of abundance or biomass of each species in the sample (Magurran 1988). Non-metric multidimensional scaling (nMDS) plots were used to display the data and visually assess underlying trends. This technique arranges the data point (the settlement panel) such that similar panels are grouped close together. The process is repeated until the plot with the lowest stress (the best fit to the data) is selected. Twenty-five iterations were used to ensure that a true representation was achieved and not a local minimum. To test for the discrete grouping of the data, a permutation based hypothesis testing framework was used (Analysis of Similarity – ANOSIM). This generates a percentage probability that the pattern in the data occurs at random.

RESULTS

A total of 15 species from 5 phyla were found to be growing on the settlement panels after six months (Table 7-1).

Table 7-1 Species found on settlement panels after six months submersion

Annelida
Sabella sp
Spirorbis sp1
Spirorbis corallinae
Spirorbis tridentatus
Pomatoceros sp.
Crustacea
*Balanus balanu*s
Balanus crenatus
Elminius modestus
Mollusca
Amomiidae
Bryozoa
Celleporella hylina
Electra sp
Plagioecia patina
Ascidiacea
Ascidiella aspersa
Ciona intestinalis

There was a consistent and significant difference in the percentage coverage of biota on the settlement panels between the sites and treatments (Table 7-2). The percentage cover was consistently higher at the SS site compared to RG (Figure 7-1). Post hoc SNK testing showed that there were significant differences between all three treatments with Treatment 1 (mean cover 23.52%) having the highest values, followed by Treatment 3 (3.90%) and then Treatment 2 (0.64%).

Table 7-2 Analysis of variance of the percentage cover recorded for each settlement panel for each treatment and site

Source	Degrees of freedom	Sum of Squares	Mean Squares	f ratio	p value
Site	1	19.58	19.58	36.70	0.001
Treatment	2	113.49	56.74	47.48	0.020
Cage(Site.Treatment)	6	3.20	0.53	1.32	0.280
Site.Treatment	2	2.39	1.20	2.24	0.188
Residual	36	14.58	0.41		
Total	47	153.24			

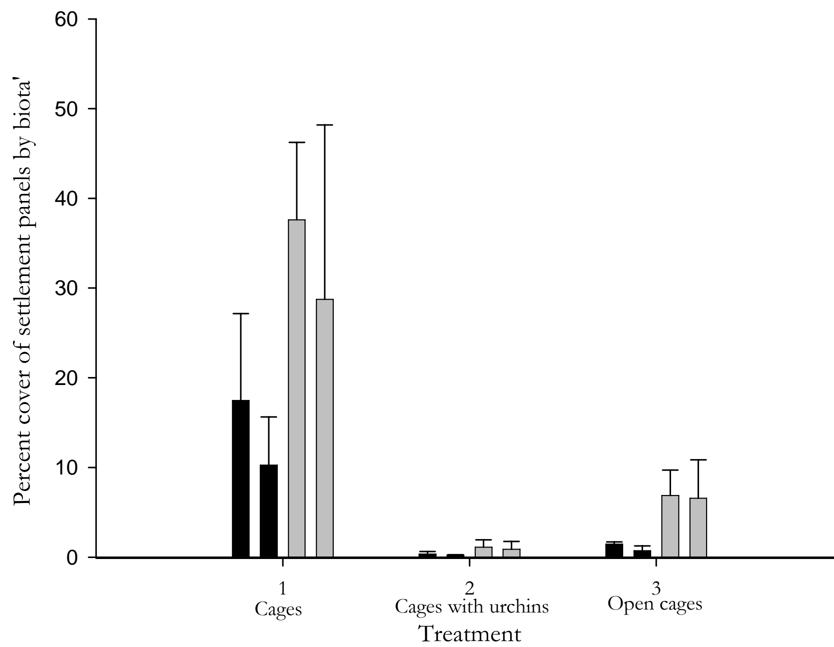

Figure 7-1 The mean percentage cover for all biota for each treatment, location and cage (■ RG, ■SS). Treatment 1-closed cages without *P. miliaris*, Treatment 2-closed cages including *P. miliaris*, Treatment 3-open cages

There was no significant variation from the nested factors of cages indicating that the response was consistent between cages, and as such showed no significant variation at the scale of meters.

The alpha diversity, as defined by the species richness of a single panel, was highest for Treatment 1 at the RG site with a total of 10 species recorded. There was a significant treatment effect, but no site or interaction effects were found (Table 7-3). On average Treatment 1 had the highest alpha diversity (Figure 7-2), and treatment 2 the lowest

Source	Sum of squares (SS)	Mean squares (MS)	SS/MS_{Total}	Degrees of Freedom	Cumulative Chi Square	p value
Site	792.19	-	0.096	1	0.873	0.127
Treatment	5442.78	-	0.328	2	1.000	<0.001
Interaction	980.60	-	0.059	2	0.922	0.078
Error	1799.88	-		42		
Total	9015.50	8288.91		47		

Table 7-3 Two way non parametric ANOVA for the species richness at each site and treatment

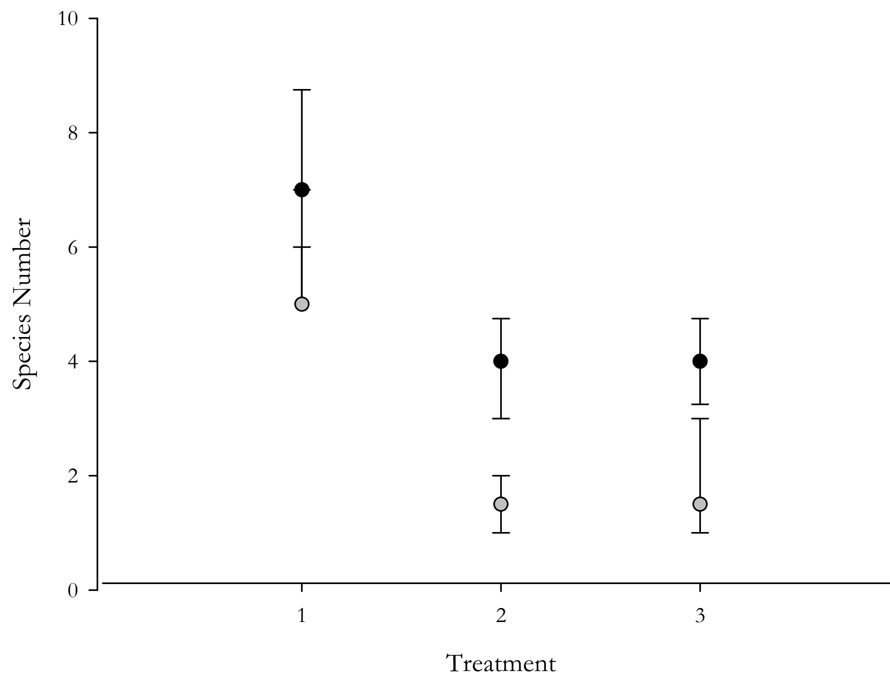

Figure 7-2 Median alpha diversity with treatment and site. Error bars show inter-quartile range **(● RG, ●.SS) Treatment 1-closed cages without *P. miliaris*, Treatment 2-closed cages including *P. miliaris*, Treatment 3-open cages**

Figure 7-3 Non metric multidimensional scaling plot of the double square rooted species composition for each treatment and site, based on Bray-Curtis similarity index. ■ RG, ■ SS, ■ Treatment 1, ▼ Treatment 2, ● Treatment 3

Multivariate analysis of the species composition of the settlement panels revealed a clear segregation between the sites and treatments (Figure 7-3). This difference was confirmed by a crossed ANOSIM, which showed there was a significant difference in the species composition across treatments when averaged across sites ($r=0.699$, $p<0.01$). Post-hoc

pair-wise comparisons showed that there were significant differences between all treatments (Treatment 1,2 r=0.913 p<0.01; Treatment 1,3 r=0.978 p<0.01; Treatment 2,3 r=0.235 p<0.01).

Beta diversity varied between the treatments consistently with the two indices used, but only at the largest spatial scale of the study. There was no difference in levels of Beta diversity, when measured using the pair-wise comparisons between the treatments at a scale of centimeters, or meters. However, when the comparison between the treatments was made at the scale of kilometers there was a significant difference between the treatments (Table 7-4 &Table 7-5). For Treatment 1 (all predators excluded from cage), there was no reduction in value of the indices between the spatial scales. For Treatments 2 and 3, there was a dramatic reduction in the indices between meters and kilometers. For both indices the reduction in similarity was less for Treatment 3 than for 2 (Figure 7-4 & Figure 7-5). A reduction in the similarity indices is analogous to an increase in Beta diversity. As such there was clear and significant increase in the Beta diversity of communities developing on panels that were subject to predation compared to those protected from predation, when measured at the scale of kilometers.

Table 7-4 Spatial variation in the pair wise Jaccard's similarity indices for each treatment

Source	Degrees of Freedom	Sum of Squares	Mean Squares	f	p	
		Centimeters				
Treatment	2	184.54	92.27	0.35	0.70	
Residual	69	18059.59	261.73			
Total	71	18244.14				
		Meters				
Treatment	2	914.69	457.35	1.40	0.25	
Residual	93	30350.87	326.35			
Total	95	31265.56				
		Kilometers				
Treatment	2	12205.94	6102.97	44.35	<0.001	***
Residual	189	26064.04	137.91			
Total	191	38269.98				

Table 7-5 Variation in the pair wise Sorenson similarity indices for each treatment

Source	Degrees of Freedom	Sum of Squares	Mean Squares	f	p	
		Centimeters				
Treatment	2	114.47	57.32	0.31	0.73	
Residual	69	12561.49	182.05			
Total	71	12675.96				
		Meters				
Treatment	2	241.99	120.99	0.32	0.73	
Residual	93	35564.93	382.42			
Total	95	35806.92				
		Kilometers				
Treatment	2	6781.11	6781.11	47.07	<0.001	***
Residual	189	144.05	144.05			
Total	191	40787.86				

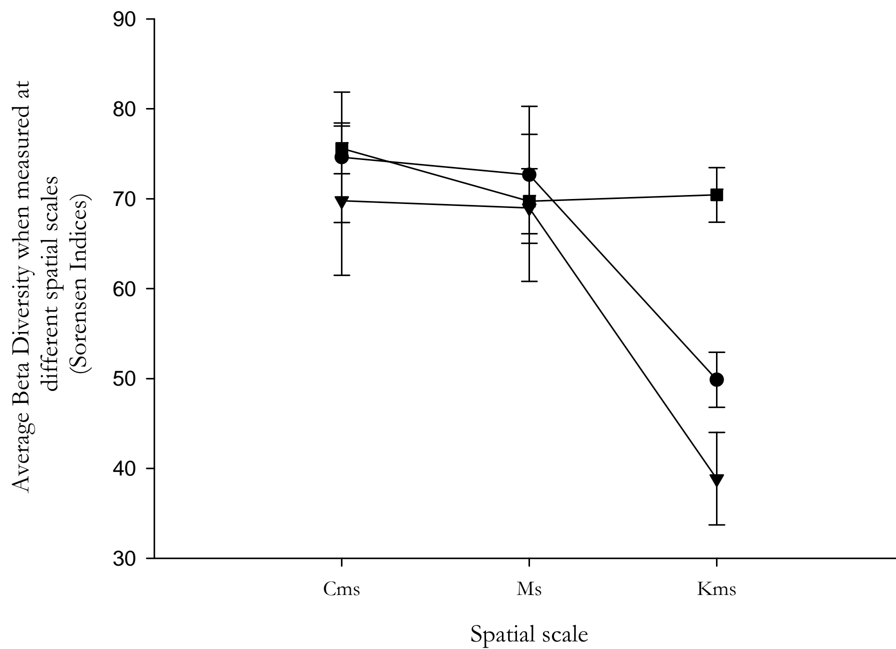

Figure 7-4 The relationship between Beta diversity and the spatial scale at which it is measured ■ Treatment 1, ▼ Treatment 2, ● Treatment 3.

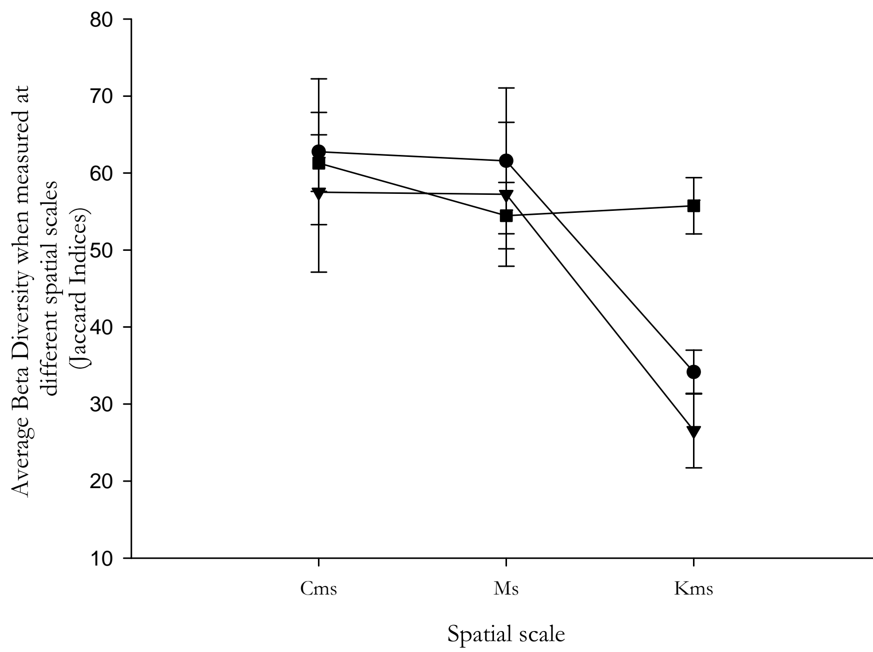

Figure 7-5 The relationship between Beta diversity and the spatial scale at which it is measured ■ Treatment 1, ▼ Treatment 2, ● Treatment 3.

DISCUSSION

Echinoids are well known to show very strong influences on benthic communities through their grazing of algae (Hagen 1983, Tegner & Dayton 1991, Bulleri et al. 1999, Villouta et al. 2001). The current study demonstrated that such effects can be direct on the benthic fauna through grazing on their new recruits and that this can be so intensive that it can overshadow all other predation. Sea urchin grazing can act to reduce both the local (alpha) diversity and the percentage cover of the invertebrate communities on which the urchins prey. Removal of *Centrostephanus coronatus* from subtidal rock reefs south of Los Angeles, California (Vance 1979), reduced both invertebrate diversity and biomass. Intensive grazing by large aggregations of *Strongylocentrotu*s sp. on the New England coast was coincident with a major shift in the dominance of epifauna, which was ascribed to urchins directly denuding the substrata of nearly all encrusting invertebrates (Witman 1985). The current study would both support and explain such community response as the *P. miliaris* grazing drastically reduced the species diversity and the percentage cover of communities that developed on the study settlement panels at both sites. Post recruitment predation has been shown to have a large effect on invertebrate community composition by impacting early life stages of organisms that may develop a size refuge to urchin predation (Turner & Todd 1991, Osman & Whitlatch 2004). It has been demonstrated that *P. miliaris* exhibits prey selection that results in an ontogenic shift in the vulnerability of sessile invertebrates to predation: barnacles smaller than 7.5 mm are preferentially predated upon relative to larger barnacles (Otero & Kelly 2001). It is likely that impacts of sea urchin predation on benthic invertebrate community structure are likely to be most acute on newly settled communities, in which none of the organisms will have had a chance to grow and escape the threat of urchin predation through size refuge.

Vance (1979) argued that sea urchin grazing should act to reduce diversity on a small scale but may act to increase diversity on larger scales. The current study provides a test of this

hypothesis and indeed the pattern shown was consistent with this hypothesis. Spatial dependence has been shown in a number of key factors that drive ecosystem diversity. The effect of spatial scales on competition for space in benthic communities revealed that the variability in competition is largest at 10^5 meters indicating that large scale processes are driving the variability (Barnes & Kuklinski 2004). Disturbance has been described as a primary driver for diversity. A study looking at the impacts of iceberg scouring on benthic communities found that diversity was decreased at the local scale and increased at regional scales (Gutt & Piepenburg 2003). The increase in Beta diversity alongside the reduction in alpha diversity reported in the current study clearly shows that the effect of predation on community structure is scale dependent. The impact of predation on Beta diversity is, however, less well documented than its impact on alpha diversity. This is the first study to show that the effects of predation on Beta diversity are dependent on the scale at which it is measured. Our study shows that predation has different effects on species diversity across cm-km spatial scales measured. This study demonstrated that at the scale of centimetres and meters predation did not significantly alter the diversity of communities. However, at the kilometre scale there was a significant difference between all three treatments. This indicates that panels within cm and meters of each other were similar to those of the same treatment, although there were distinct differences between treatments. That is, at the scale of kilometres assemblages which had been predated developed significantly different community compositions to those kilometres away, whereas those which had been protected from predation were similar and showed the same level of similarity at the scale of kilometres as they did at meters and centimetres. Influences on alpha and Beta diversity have been a relatively recent and important line of investigations on predation. The Beta diversity of tadpoles in savannah areas of the Amazon was affected by predation leading to an increase in species number within a region, while predation had little or no effect on variation in alpha diversity (Azevedo-Ramos et al. 1999). Conversely a

study on the impacts of grazing on grassland showed that grazing increased the alpha diversity but reduced the Beta diversity (Frank 2005).

Investigations into the influence of echinoids on community structure have often used cages or fences to exclude urchins from substrata or settlement panels (Sammarco et al. 1974, Ebert 1977). Rapidly it became apparent that the cages themselves could lead to changes in the community composition of the study area independently or synergistically of the exclusion of the predators/herbivores. Using cages to exclude predatory fish from soft substrata Hulberg and Oliver (1980) concluded that the effect on infaunal communities could not be explained by the presence or absence of predators, and that the presence of cages changed the sediment deposition regime, resulting in a different faunal community developing. Cages may also reduce water movement and light availability through increased density of foulers (Kennelly 1983). In the current study there was a significant difference between Treatments 2 and 3, however the magnitude of difference (as described by the r statistic from the ANOSIM test) was smaller than the differences between Treatment 1and 2, and Treatment 1 and 3. This strongly suggests that the effects from caging artefacts were smaller than the effects from the differential grazing pressure.

The term keystone was coined to describe the phenomenon of a single native species that modifies the appearance and species composition of an environment (Paine 1966). It was originally applied both to the sea star *Pisaster ochraceu*s within the temperate rocky intertidal and the gastropod *Charonia* sp. for its role in regulating the population of the corallivorous sea star *Acanthaster planci*. Since then its initial definition has been broadened to include terms such as keystone herbivores, and keystone habitat architects. A number of regular urchin species are pivotal or even debatably 'keystone' in the habitats in which they occur (Brey 1991, Lessios et al. 2001, Barnes et al. 2002), or are important links in trophic cascades (Wootton 1995, Shears & Babcock 2003, Micheli et al. 2005). The current study

has shown that urchin grazing can have a dramatic impact on invertebrate species composition and its presence leads to radically different communities developing. The invertebrate community structure in areas where *P.miliaris* exist in high densities resembles the community structure of invertebrates that developed on the panels that were caged with urchins, being dominated by *Spirobris* and *Pomatoceros* (polychaetes) worms. The phenomenon of urchin barrens has not previously been described from Scottish waters. However the grazing activities of *P. miliaris* may be causing the formation of intertidal and subtidal urchin barrens. Whether the species can be termed keystone or whether it is useful to do so will require further work to be undertaken, importantly including understanding if there is a trophic level above the urchin that is missing or depleted and as such allowing large populations of *P. miliaris* to dominate the mobile fauna of some Scottish sea lochs

Chapter 8

FINAL OVERVIEW AND DISCUSSION

This study investigated the trophic ecology of *Psammechinus miliaris* in a Scottish sea loch by addressing the dual facets of trophic ecology: the impact on community structure and the flow of energy through ecosystems. Using biochemical proxies for trophic interaction, such as fatty acid composition and stable isotope ratio analysis, attempts were made to describe the diet of natural urchin populations and to place them within a web of trophic interactions and ultimately energy flow. This was combined with manipulative field experiments whose aim was to demonstrate the direct effects of *P. milliaris* grazing on benthic community composition. There is a growing awareness of the importance of integrating these two aspects of trophic ecology and in this discussion I attempt to provide some synthesis between the two and to highlight future research that this may spawn.

This study has shown that urchin grazing is a major structuring influence on benthic communities of a Scottish sea loch both in the intertidal and shallow subtidal environments. The grazing of *P miliaris* reduces biomass and the alpha diversity while increasing the regional diversity of newly settled subtidal invertebrates. The experimental difficulties experienced in chapter six mean that the results must be interpreted with a degree of caution. However, in the intertidal repeated removal of the urchins did lead to the development of significantly different communities amongst existent community structures. Grazing (Black 1976), and specifically sea urchin grazing (Kitching & Ebling 1961, Vance 1979), has been shown at many localities to be a driver of benthic community structure and the findings from the current study support this. However few studies have explicitly addressed the issue of diversity and spatial scale (Azevedo-Ramos et al. 1999), and even fewer have used replicated manipulative experiments (Gray 2000). The current study

has shown the importance of considering spatial scale when constructing models for the role of predation in benthic community composition.

The trophic ecology of *P. miliaris* not only alters the energy transfer through, and within, the system by changing benthic community structure, it is also symptomatic of the primary drivers of the ecosystem in which they exist, and can be used to examine the way these ecosystems function. The results of this study suggest that there are differences in the way in which closely associated habitats, the intertidal and shallow subtidal, function in terms of their trophic ecology. The results also show that there is spatial and temporal heterogeneity in the way that the intertidal habitat functions. Any analysis using both the fatty acid analysis and the stable isotope analysis from the current study is temporally confounded as the studies were conducted in subsequent years. However the studies were similar in geographic extent and are useful as pilot studies. At the SS site the intertidal populations had high levels of filter-feeding invertebrate associated fatty acids and the highest $\delta^{15}N$ values. This is strong evidence that the populations of *P. miliaris* there are eating predominantly animal material, with carbon ultimately derived from pelagic sources. This is in contrast to the intertidal of the RG site, where the fatty acid profiles suggest a green algal diet, and the $\delta^{15}N$ values for the summer are on average the lowest recorded. This supports the hypothesis that they are eating primarily ephemeral green algae; benthically fixed carbon. Comparison of $\delta^{13}C$ values would be ideal for discrimination but these are confounded by the lipid content of the gonad (Deniro & Epstein 1977). Any future study into carbon source of these urchins should, therefore, use tissue other than the gonad. Candidate tissues would be either digestive tract or muscle tissue from the Aristotle's lantern. Of these the digestive tract is also used for nutrient storage (Lawrence et al. 1966) and would have similar associated problems as the gonad Aristotle's lantern muscle has been previously used with other species of urchins (Rodriguez 2003), however for a small species such as *P. miliaris* obtaining enough material would be problematic. Another option

would be to determine the $\delta^{13}C$ of specific fatty acids. This would enable determination of the source of specific fatty acids and so discriminate where the carbon was fixed (Villinski et al. 2004).

The two beach study sites (SS&RG) may look qualitatively similar, but they clearly have major differences in the way in which carbon flows through them. The flow of carbon through ecosystems is one of their principal defining features in terms of functions (Whipple 1999). Spatial separation of consumers and the ultimate autotrophic source of carbon on which they rely is not uncommon in marine environments, and takes two predominant forms: the movement of carbon from terrestrial to marine habitat or vice versa (Heatwole 1971, Bustamante et al. 1995, Bouillon et al. 2004, Connolly et al. 2005); or the movement of carbon between the benthos and pelagos (Hobson et al. 2002, Tyler et al. 2003, Schiel 2004). The current study of the trophic interactions of *P. miliaris*, provides evidence that both these pathways occurred at the SS site. At this site, carbon fixed in the pelagic environment is an important constituent of the diet of intertidal urchins and as such is moving from the wholly marine environment to the semi terrestrial intertidal. At another site (RG) benthic carbon fixed *in situ* (ephemeral green algae) is the predominant form. By investigating the trophic interactions of an omnivorous urchin species it is possible to gain meaningful insights into the ecosystem functioning of their habitats.

The indication that functionally significant levels of omnivory are regulating benthic community composition has interesting theoretical implications. Traditional models of benthic community regulation have focused on top-down (predation mediated) versus bottom-up (productivity driven) (Menge 2000). The development of the top-down model was originally conceptualised by the 'green earth' theory of Hairston et al (1960). This stated that in a four level system (plants, herbivores, predators and detritivores) herbivore abundance was controlled by carnivores, and as such plants were not under functional

control of herbivores and this led to a green earth. This framework is, however, based around a world in which discrete trophic levels exist. In the presence of omnivores, herbivores are subject to predation from the omnivore but also may be subject to competition for food from the omnivore. In this case it is easy to envisage a situation where either the omnivore or the herbivore becomes excluded through competition for a common resource leading to the destabilisation of the food web (Pimm & Lawton 1978). The role of omnivory and the destabilization of food webs has attracted much attention and has been the focus of many modelling studies which have confirmed this tendency (Polis & Holt 1992, Diehl 1993). This runs contrary to observations stating that omnivory is common in the real world (Darnell 1961, Garcia-Arberas & Rallo 2002). There is however evidence that increased levels of productivity can lead to the stabilization of food webs containing omnivores (Polis et al. 1997, Morin 1999). As such, top down control by an omnivore may be limited to situations where bottom up forcing in the form of productivity is high enough to enable the existence of stable food webs that contain omnivory. Such an argument shows how these two driving forces are, in fact, coupled. From this it could be tentatively hypothesised that at the SS site increased benthic productivity supplemented by the input of allochthonous energy from the pelagic environment was supporting and enabling higher levels of omnivory to exist. Examination of the trophic interactions of *P. miliaris* seems to be a good experimental forum for providing empirical evidence to explore the relationship between community regulation, productivity and omnivory.

If omnivory is both a descriptor and a driver of ecosystem function then it is important to understand the mechanisms behind the phenomenon. Omnivory has been described as a mechanism for an individual overcoming the stoichiometric mismatch between primary producer and herbivore (Denno & Fagan 2003). The concept of stoichiometry has been developed to include micro-nutrients, such as essential fatty acids, as well as elemental

nutrients (Anderson & Pond 2000). The current study showed that there was a high degree of heterogeneity in the trophic position of individual *P. miliaris* within a population and between populations and in their fatty acid profiles. There was also significant and consistent variation in the gonadal somatic indices. There were site specific patterns of association of high levels of certain fatty acids (principally 22:6(n-3)), high trophic position and high levels of GSI. Theory states that those animals best meeting their Stoichiometric requirement will benefit from more efficient trophic energy transfer (Hessen & Elser 2005), and for this to be manifested by higher GSI values. Further investigation as to the role of stoichiometric mismatch in regulating both population dynamics and individual foraging behaviour of *P. miliaris* is a potentially valuable line of investigation. Given this heterogeneity, understanding foraging behaviour at the level of the individual becomes crucial to understanding a range of subjects from population ecology to ecosystem functioning. Individual foraging behaviour has been posited as both a cause and consequence of omnivory (Singer & Bernays 2003). Within this study I tried to develop some tools for the identification of individual *P. miliaris* to enable subsequent study of individual urchins' trophic interactions. If future research can address the individual foraging behaviour of urchins with the biochemical requirements that drive it, then it may be possible to integrate the two facets of trophic ecology and to construct a fully integrated model of the trophic ecology of *P. miliaris*, from individual to ecosystem.

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