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BIODIVERSITY AND ECOSYSTEM FUNCTIONING: EXPERIMENTAL TESTS USING ROCKPOOLS AS A MODEL SYSTEM

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by

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Copyright Statement

BIODIVERSITY AND ECOSYSTEM FUNCTIONING: EXPERIMENTAL TESTS USING ROCKPOOLS AS A MODEL SYSTEM

John N Griffin

How anthropogenic changes to species composition and diversity are likely to affect the properties of the ecosystems of which they are an integral part, and by extension the goods and services humans derive from them, is a key question in ecology. Despite over a decade of vigorous empirical research and theoretical developments, there remain many unknowns. Using intertidal rockpools and laboratory marine mesocosms, I used a variety of approaches to address several of these relatively poorly studied issues. In particular, the work presented here focused on the relative roles of species composition and richness, as well as the extent to which such effects are context-dependent.

The first study (Chapter II) takes advantage of a successional gradient of macroalgal species composition and diversity resulting from the periodic addition of artificial rockpools to a coastal defense structure. The results show that the focal ecosystem properties (macroalgal biomass and productivity) were largely determined by species composition (and functional traits). Macroalgal species evenness, but not diversity, peaked at intermediate stages during the chronosequence, but no measure of diversity had a detectable influence on primary productivity. The results confirm the prediction that effects of species diversity will be outweighed by compositional changes during succession.

I used an experimental approach in Chapters III to V, manipulating the composition and richness of intertidal molluscan grazers (Chapters III and V) and intertidal predatory crabs (Chapter IV) and measuring their effects on prey assemblages as focal ecosystem processes. In a 13-month field experiment (Chapter III) I found that effects on the composition and functioning of developing rockpool communities were determined by grazer composition, not the number of species. Laboratory mesocosm experiments show that the influence of species richness on ecosystem processes can be context-dependent. The effect of resource partitioning (of the multi-species prey assemblage) among predators was only detectable at high predator densities where competitive interactions between individual predators were magnified. A factorial experiment using the rate of algal consumption by molluscan grazers as a response variable, provides the first empirical test of the prediction that the balance between species richness and identity effects can be determined by the degree of spatial heterogeneity (Chapter V). Species identity had strong effects on homogeneous substrates, with the identity of the best-performing species dependent on the substrate. The strengths and limitations of the predominantly small-scale experimental approach employed here are discussed (Chapter VI).

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Author's Declaration

At no time during the registration for the degree of Doctor of Philosophy has the author been registered for any other University award without prior agreement of the Graduate Committee.

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Relevant scientific seminars and conferences were regularly attended at which work was often presented. I have reviewed manuscripts for *Functional Ecology*, *Journal of Animal Ecology*, *Applied Ecology* and *Ecology*. Chapter IV has been published in Ecology. Additionally, I have prepared a review chapter for a forthcoming edited book and contributed to several other synthetic book chapters (see below).

Publications:

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Duffy, J.E., Emmerson, M., Srivastava, D., **Griffin, J.N.**, Sankaran, M. Forecasting decline in ecosystem services under realistic scenarios of extinction *In* The Disentangled Bank: Human impacts on biodiversity and ecosystem functioning. Edited by Naeem , S., Loreau, M., and Bunker, D. Oxford University Press. *In submission*

Noël L, **Griffin J.N**, Moschella P.S, Jenkins S.R, Thompson R.C, Hawkins S.J. Changes in diversity of intertidal assemblages during succession and associated consequences for ecosystem function. *In* The Ecology of Marine Hard substrate communities.. Springer-Verlag. *In press*

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GENERAL INTRODUCTION

CHAPTER I

1.1 INTRODUCTION

Humans depend on the functioning of natural and managed ecosystems for economic prosperity (Constanza et al. 1997, Balmford et al. 2002), well-being (Wilson 1992), and indeed our very survival. The functioning of ecosystems is driven in a large part by living organisms, and further, interactions among them and with their environment (Chapin et al. 2000). We are, however, inadvertently changing the composition and diversity of these organisms (Pimm et al. 1995, Lotze et al. 2006, Worm et al. 2006, Byrnes et al. 2007). The question of how such changes are likely to affect ecosystem functioning has been met with an explosion of research over the last 15 years (reviewed by Loreau et al. 2001, Hooper et al. 2005, Balvanera et al. 2006, Cardinale et al. 2006). Great progress has been made, but there remain many uncertainties, and we are undoubtedly a long way from being able to predict the consequences of biodiversity changes in any given natural system. My work, presented in this thesis, aims to plug some of the gaps in our understanding. Specifically, using tractable rocky shore organisms and ecosystems, it addresses issues relating to the mechanistic basis of diversity effects, as well as the extent to which such effects are context-dependent.

1.2 THE HISTORICAL CONTEXT

Although so-called 'biodiversity-ecosystem functioning' (BEF) research is widely thought of as a child of modern ecology, it has deep historical roots. Indeed, in *The Origin of Species*, reviewing perhaps the first ecological experiment (Hector and Hooper 2002), Darwin (1859) noted that '*It has been experimentally proved that if a plot of ground be sown with one species of grass, and a similar plot be sown with several distinct genera of grasses, a greater number of plants and a greater weight of dry herbage can thus be raised*'. Who knows, though, when this question was first posed? How the number, or

range, of plant varieties cultivated together influences yield may have been pondered since the dawn of agriculture.

BEF research certainly has firm roots in the 20th century, during which time the theoretical and methodological basis of contemporary research was developed, and the effect of diversity on various ecosystem properties was regularly re-visited. Important concepts regarding the coexistence of species were developed and tested during the early-mid 20th century. Gause (1934) proposed the principal of competitive exclusion, which stated that two competing species cannot coexist on a single resource. This theory was supported by experiments with a range of model systems (single celled protozoa: Gause 1934; flour beetles: Park 1948; fruit flies: Merrell 1951; *Daphnia*: Frank et al. 1957). Further evidence for the competitive exclusion principle came from field observations of differences in patterns of resource use between similar species (Lack 1944, 1945), which were apparently reflected in subtle morphological differences related to resource acquisition (Darwin 1859, Lack 1947, Brian 1957; but see Strong et al. 1979, Connell 1980 for criticisms of this interpretation).

Such interspecific 'niche differentiation' would later underpin a mechanistic interpretation of species richness effects within the BEF framework (see section 1.3). Before this however, as early as the 1950s, Elton (1958) proposed that diverse natural communities would be more resistant to invasion by virtue of a more complete use of available resources. Also based on the theory on niche differentiation, Harper (1967) reasoned that complex ecosystems would use environmental resources more efficiently than simple ones. Meanwhile, agricultural research found some benefits of mixing crop variety mixtures (reviewed by Trenbath 1974).

Despite several precursors of the modern BEF research agenda discussed above, research was firmly focused on the factors and mechanisms *maintaining* species diversity,

rather than the *effect* of diversity. The apparent failure of the competitive exclusion principle to explain high species diversity in systems with limited resource heterogeneity promoted Hutchinson (1961) to propose that constantly changing environmental conditions may prevent the establishment of competitive equilibrium, allowing coexistence of a large number of species on a single resource. During the 1970s and early 1980s the role of non-equilibrium processes in maintaining species diversity was increasingly recognized. Spearheaded by work on rocky shore ecosystems, the role of grazing (Lubchencho 1978) predation (Paine 1966) and physical disturbance (Sousa 1979, Dethier 1984) were all identified as mortality-causing agents that can prevent competitive exclusion, thereby maintaining species coexistence and diversity. The mechanisms maintaining species diversity remain a key issue in ecology today (e.g. Chesson 2000, Kelly 2008) and have recognized implications for the BEF relationship (Mouquet et al. 2002, Pacala and Tilman 2002, Loreau et al. 2004).

During the 1980s and early 1990s the widespread and accelerating impact of humans on natural ecosystems was becoming increasingly evident. In fact, the possibility that humans may be on the verge of precipitating a mass extinction event had even been raised (Wilson 1992, Pimm 1995). Concern that such biodiversity loss may pose a threat to the functioning of ecosystems, prompted a thought (and experiment)-provoking meeting of ecologists in 1993 with the aim of evaluating existing knowledge, and perhaps more importantly, reviewing hypothetical links between biodiversity and ecosystem functioning (Schultze and Mooney 1993). The form of the relationship between species richness (or other measures of biodiversity) and ecosystem functions emerged as a critical issue. Numerous hypothetical relationships have been proposed (Naeem 2002), but 3 alternative models capture the range of possibilities.

Under the 'rivet popping' model (Ehrlich and Ehrlich 1981; Fig. 1.1), loss of any species results in an equal reduction in the ecosystem function, resulting in a linear relationship between species richness and function. Alternatively, ecosystems may be relatively tolerant to species loss; species remaining may functionally compensate for extinct species, in other words, some species are 'redundant' (Walker 1992, Lawton and Brown 1993; Fig. 1.1). At one extreme, a single species could maintain the processes, but perhaps a more realistic version of this model proposes that certain, similar species, are redundant (Wilson 1992). A third, so-called 'idiosyncratic' model (Fig. 1.1) proposes that there is no simple relationship; instead, strong interactions between species and their environment create highly context-dependent effects of species loss (Lawton 1994). These alternative models provided a useful framework for early empirical studies; they served to 'articulate the hypothesis' (Naeem 2002).

Ecologists seem to have realised that a universal trajectory is unlikely to exist given the variability among species and contexts (Schlapfer and Schmid 1999), shifting focus to the mechanistic basis of any BEF relationship. If we understand mechanisms, there is a possibility that we can explain and predict BEF relationships in disparate systems and contexts.

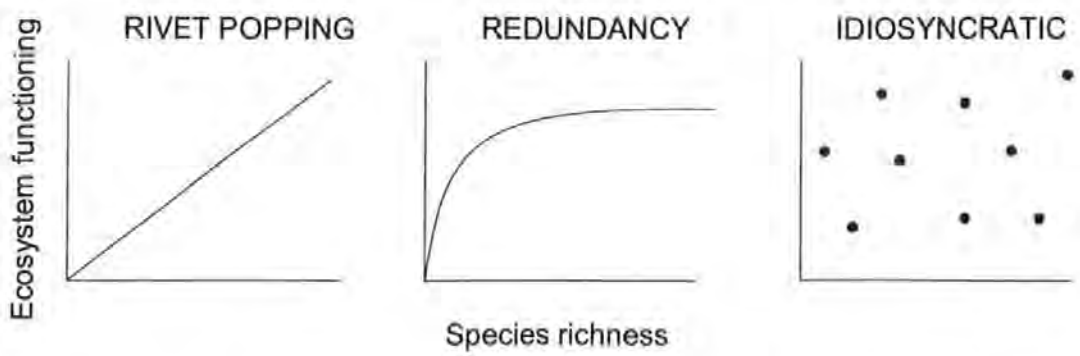


Figure 1.1. The main alternative hypothetical relationships between species richness and ecosystem functioning (see text).

1.3 MECHANISTIC LINKS BETWEEN BIODIVERSITY AND ECOSYSTEM FUNCTIONING

The mechanisms underpinning BEF relationships can be divided into 2 categories: i) species identity, and ii) species complementarity effects. Species identity effects are nothing new to ecologists. It has long been known that species vary in the efficiency at which they mediate particular ecosystem processes. This interspecific variability can impact on the BEF relationship for the simple fact that increasing richness increases the probability that particularly efficient species will be included. The most productive species may out-compete others, and in long-term studies eventually dominate the species mixture (Huston 1997). This could generate a perceived effect of species richness, as the diverse mixture (dominated by the most productive species) would out-perform lower diversity treatments, lacking productive species. However, those species that are productive in monoculture do not necessarily perform well in species mixtures, where interspecific competition plays an important role. Loreau and Hector (2001) later coined the term 'selection effect', which may be positive (as in the case of Huston's 'sampling effect') or negative, depending on the correlation between species' monoculture and polyculture performances (Fig. 1.2). Calculation of such 'selection effects' are restricted to those studies where species-specific performances can be ascertained in polyculture – generally making them applicable only to studies of biomass accumulation. In studies where biomass is maintained and other processes measured (i.e. short-term studies *sensu* Petchey 2003), researchers often stick to the term 'species identity' (O'Connor and Crowe 2005, Bruno et al. 2005) to refer to the effects of the presence (or loss) of particular species.

Species complementarity effects are often thought of as 'true diversity effects' or effects of 'diversity *per se*' (Loreau 1998, Loreau et al. 2001). 'Complementarity' describes

the phenomenon of increased performance in multiple co-occurring species due to interactions among them, and can arise through several different mechanisms (Fig. 1.2). Under niche partitioning, species differ in the use of limiting resources, reducing interspecific – relative to intraspecific – competition, as well as increasing the total spectrum of resources exploited (e.g. Fridley 2001). This mechanism depends on differences between species in patterns of resource-use that appear widespread in nature (e.g. Schoener 1974). Species do not necessarily have to differ in *what* resource they consume, but can also partition resources through time, which can be a result of interspecific differences in responses to environmental conditions (Yachi and Loreau 1999).

It is becoming increasingly recognised that functional diversity (the range of traits relating to the resource-use), as opposed to species richness *per se*, is the facet of biodiversity linked to ecosystem functioning via niche partitioning (Hooper and Vitousek 1998, Diaz and Cabido 2001). Even so, species richness and functional diversity are inevitably correlated to some degree, and often, it seems, quite tightly (Petchey and Gaston 2002). Interspecific facilitation (through habitat amelioration, resource provision etc.) is also thought of as a mechanism based on diversity *per se*, since it depends on positive interactions between multiple species (see Bertness and Calloway 1994, Bruno et al. 2003). Species complementarity effects are not completely independent of probabilistic 'sampling' effects, however: including more species increases the probability of including strong facilitators or combinations of species with marked niche partitioning (Loreau et al. 2001).

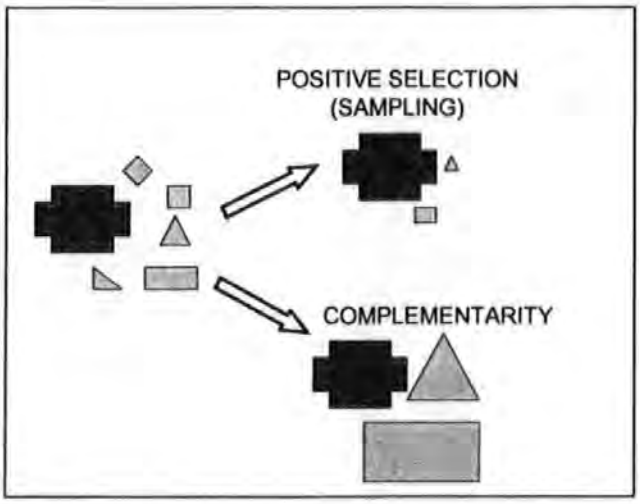


Figure 1.2. A schematic representation of the positive selection and complementarity effects. Shapes represent different species, with the size of shapes proportional to productivity. The arrows represent random assembly of species from a regional pool. Under the sampling effect, productive species in monoculture also perform best in polyculture, to the expense of others. Where species complementarity takes place, species increase in productivity relative to their respective monoculture rates.

1.4 DETECTING EFFECTS OF BIODIVERSITY: OVERYIELDING

The effect of increasing species richness depends on the net outcome of selection (either positive [sampling effect] or negative) and species complementarity (Loreau and Hector 2001). The magnitude (and importance) of this net effect is typically assessed by the presence – and degree – of overyielding (e.g. Hector et al. 2002). The term 'overyielding' originates from agricultural research, but is commonly used to assess the magnitude of species richness effects and also to make inferences regarding underlying mechanisms; it simply means enhanced production, but in the BEF context can apply to enhanced performance with respect to any ecosystem process.

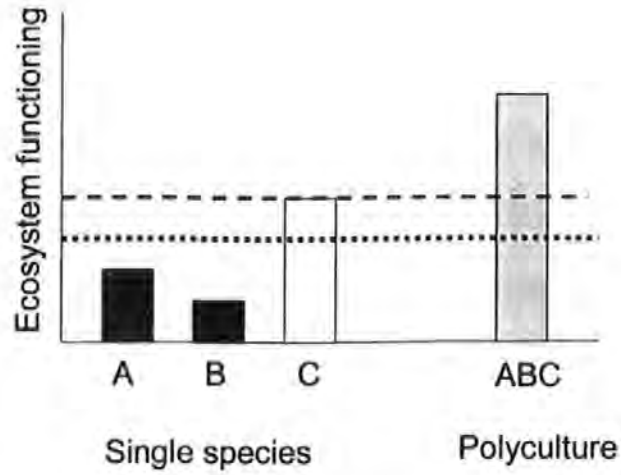


Figure 1.3. The 2 types of overyielding in a simple BEF experiment. The finely dashed line represents the average monoculture, which corresponds to the expected rate of functioning in a substitutive experimental design; the longer-dashed line is the best-performing monoculture. In this example, the polyculture exceeds both the mean and best-performing single species i.e. it shows non-transgressive and transgressive overyielding, respectively.

Non-transgressive overyielding occurs when a species mixture out-performs the mean of its component monocultures; in other words, it performs better than expected. Given the possibility that sampling effects can drive this form of diversity effect in long-term studies, transgressive overyielding, where the species mixture out-performs even the best monoculture, has been put-forward as a conservative 'acid test' of species complementarity (Loreau 1998).

1.5 EMPIRICAL EVIDENCE

The first experimental tests of the BEF relationship were published in the mid-1990s. Using synthesised indoor (Naeem et al. 1994) and field (Tilman et al. 1996) temperate plant communities, a positive relationship between biodiversity and the accumulation of biomass (productivity) was demonstrated. These pioneering studies were heavily criticised for confounding richness with other factors. The increased probability of including highly productive species in diverse mixtures (the sampling effect) became a

particularly controversial issue (Huston 1997, Wardle 1999, Kaiser 2000; see *Mechanistic Links*). Experiments subsequently became more rigorous in design and interpretation, with possible effects of 'sampling' explicitly accounted for (e.g. Duffy et al. 2003), or statistically isolated (following Loreau and Hector 2001). The vigorous period of empirical work during the late 1990s and early 2000s included an ever-expanding range of systems and processes. From leaf-shredding by aquatic invertebrates (Jonsson and Malmquist 2000), to nutrient flux from the marine benthos (e.g. Emmerson et al. 2001), each study provided a new twist on the interpretation of the BEF relationship. Effects of diversity within a single trophic level formed the bulk of empirical studies (and still does; Balvanera et al. 2006). Indeed, this was logical given the complexity of multi-trophic systems – the old adage 'learn to walk before you can run' certainly applied here.

Nevertheless, incorporating species interactions in more realistic multi-trophic food webs became a top priority for empirical research – not least because species in higher trophic levels tend to be more vulnerable to extinction (see e.g. Duffy 2002). Experiments on the effect of grazer diversity were conducted (Duffy et al. 2001, Duffy et al. 2003, Gamfeldt et al. 2005, O'Connor and Crowe 2005), demonstrating that positive effects of species richness can extend to higher trophic levels. Researchers even started to manipulate predator richness within the context of BEF research (Bruno and O'Connor 2005). Generalities in terms of predator richness effects have been elusive; cascading effects (Bruno and O'Connor 2005, Duffy et al. 2005), intraguild predation (Finke and Denno 2004), indirect interactions and apparently idiosyncratic effects of predator interactions appear to be the source of a large amount of variability among studies (Cardinale and Bruno 2008). Understanding the complex effects of extinction in food webs remains one of the biggest challenges in BEF research (Duffy et al. 2007). Lessons from a long-history of multi-predator research (reviewed by Sih et al. 1998), as well as food web theory

(Emmerson et al. 2004), may help to answer some of the many unknowns. However, there will likely be no substitute for large-scale experimental and observational studies.

Despite considerable variability in ecosystem functioning within levels of species richness, and among studies in terms of the effect of species richness (Hooper et al. 2005), some important generalities have been garnered from the >100 empirical studies that have examined the BEF relationship through controlled experiments, analysed by Cardinale et al. (2006). They found a generally positive, but saturating, effects of species richness on ecosystem processes that was consistent across trophic levels. Importantly, however, they found that the most diverse species mixture exceeded the mean – but not the best-performing – monoculture. They interpreted this as most parsimonious with the widespread existence of sampling effects.

A more detailed analysis, including 44 grassland studies in which selection and complementarity effects could be statistically partitioned, revealed that selection effects are commonly (43% of studies) negative, and almost invariably weak (Cardinale et al. 2007). Complementarity effects, on the other hand, were positive and grew stronger through time, perhaps owing to temporal niche partitioning. This study suggests that the conclusion of Cardinale et al. (2006) may be inaccurate; the absence of transgressive overyielding, whilst an 'acid test' of complementarity, does not say much about the underlying mechanisms. After a decade of debate, it has emerged that, at least within primary producers, species complementarity may be a valid explanation for apparently widespread effects of species richness on ecosystem functioning.

1.6 BEF RESEARCH IN MARINE SYSTEMS

The use of marine ecosystems to explore the BEF relationship was initially delayed relative to the pioneering work in grasslands during the mid-1990s. Giller et al. (2004)

cautioned that fundamental differences between aquatic and terrestrial systems (in terms of organism/population – environment interactions) preclude the direct extrapolation of BEF findings from terrestrial ecosystems to the aquatic realm. The results of BEF experiments in marine systems to date (reviewed by Stachowitz et al. 2007) have been interpreted within the general framework of BEF research. Marine ecologists have been less concerned with repeating - and comparing their results to - earlier work of terrestrial ecologists, and more with driving the whole field of BEF research forward in general. In particular, marine ecologists have led the field in multi-trophic BEF experiments (e.g. Duffy et al. 2003), taking advantage of the tractable multi-trophic communities present in shallow coastal waters.

Although there have been no formal comparisons between marine and terrestrial BEF effects, several experiments that measured the accumulation of seaweed biomass in a shallow marine system found very similar results to terrestrial grasslands – weak negative selection combined with complementarity, resulting in a weak positive net effect of diversity (Bruno et al. 2005, 2006). While biodiversity effects on small spatial scales typical of BEF experiments may be comparable across terrestrial and marine systems, the differences between systems may well become apparent when biodiversity effects are scaled-up; greater connectivity between habitat patches in marine systems, in terms of both organisms and resources, may greatly affect the BEF relationship at larger scales (Hawkins 2004). Scaling-up of BEF relationships remains an important challenge, especially in highly interconnected marine meta-communities (Raffaelli 2006).

1.7 THIS THESIS

The research I have presented in this thesis covers a range of issues relating to the question of how biodiversity affects ecosystem processes. The chapters address discrete

questions and are distinct in themselves, but are nonetheless united by several common threads. Firstly, broadly-speaking, I used the same system throughout the work: the rocky shore. The rocky shore has long been a mainstay of pioneering ecological research (e.g. Connell 1961). Secondly, all of the studies address, either directly or indirectly, the relative roles of species identity and richness.

Tractable, multi-trophic communities and rapid growth and turnover of organisms render the rocky shore an ideal system in which to employ an experimental approach to explore the effects of biodiversity on ecosystem functioning. I chose to use intertidal rockpools as replicate units in field experiments because they form delineated 'natural mesocosms'; whilst they are subject to natural processes and environmental conditions, the organisms within pools can be linked to the processes they mediate (Nielsen 2001, Bracken and Nielsen 2004, Martins et al. 2007).

The studies presented in chapters II and III both used the relatively homogeneous set of rockpools on Plymouth Breakwater. The homogeneity of these pools provided a rare opportunity to examine functional effects of biodiversity in a field setting without the complications of considering variability in the environment. These chapters have another thing in common: they both relate to ecosystem development (succession). I took advantage of the fact that the rockpools are of known age and hosted natural communities representing a progression of stages throughout succession (Chapter II). This 14-year chronosequence, or space-for-time substitution, allowed me to address some key BEF questions in the context of succession. I described changes in composition, diversity and functioning through time, and also explored links between these variables.

Observations on nearby rocky shores, as well as literature searches, led me to the question of how consumers affect macroalgal succession. Numerous past experiments have shown that consumers can have strong effects on macroalgal succession, but very few have

separated the roles of different consumer species, let alone considered how the richness of consumer species may influence succession. The rockpools on the breakwater proved ideal for the attachment of cages, which enabled small, mobile consumers to be manipulated. I was thus able to experimentally test how consumer identity and richness affect algal succession and the associated ecosystem process of gross primary productivity (Chapter III).

Chapters IV and V explore aspects of the context-dependency of species identity and richness effects on ecosystem processes. The complexity that multiple 'contexts' adds to the experimental designs, as well as the experimental control required to tease-apart interactive effects, limited these tests to laboratory mesocosms. Chapter IV tests how the richness and identity of common intertidal crabs influences the overall rate of prey capture. Furthermore, it explores how the total density of crabs modifies these effects (Griffin et al. 2008). Chapter V tests a key theoretical prediction: that the importance of species richness will increase with environmental heterogeneity. In this case, environmental heterogeneity was represented in space, by the topological complexity of the substrate upon which molluscs graze for algae on the rocky shore.

Findings from the experiments are discussed in detail in the respective chapters. The General Discussion (Chapter VI) highlights some of the limitations of the work presented, as well a drawing attention to some exciting new avenues in biodiversity-ecosystem functioning research in general.

LINKS BETWEEN COMMUNITY AND FUNCTIONAL PROPERTIES DURING SUCCESSION IN INTERTIDAL ROCKPOOLS

CHAPTER II

ABSTRACT

Understanding the link between species and ecosystem-level processes has recently emerged as a major research priority. We took advantage of a long-term chronosequence of standardised rockpools, created through the periodic maintenance of a breakwater, to examine how community properties (species diversity, biomass and composition) and a fundamental ecosystem processes (gross primary productivity, measured as oxygen flux rates), vary through primary succession. We also explored the ability of a relevant functional trait (specific thallus area) and measures of species diversity to explain this variation. Our results show that changes in assemblage composition during ecosystem development influence the mass-specific rate, but have no consistent effect on the area-specific rate, of our selected ecosystem process (gross primary productivity). Furthermore, we provide evidence to support the biomass ratio hypothesis, showing that a rapid decline in mass specific productivity can be predicted from a species-level functional trait.

Algal species evenness, but not diversity, peaked at intermediate stages during the chronosequence, but no measure of diversity had a detectable influence on primary productivity. In fact, both species diversity and evenness were negatively correlated with algal biomass. This suggests that during succession the commonly reported positive relationship between biodiversity and total biomass can be reversed. The role of a high biomass, dominant species later in succession in driving the observed negative diversity/evenness - biomass relationship is discussed.

KEYWORDS: Ecosystem functioning; ecosystem process; primary productivity; GPP; chronosequence; functional trait; rock pool; tide pool; succession;

2.1 INTRODUCTION

Human activities are both directly and indirectly changing the species composition, species diversity and functioning of ecosystems (Chapin et al. 2000). Elucidating the links between species and ecosystem-level properties is the key to understanding the functional consequences of such change, and has thus emerged as a major research priority in modern ecology (Lavorel and Garnier 2002, Hooper et al. 2005).

Much of our understanding of the functional consequences of biodiversity is based on small-scale, tightly-controlled, experimental work (Cardinale et al. 2006). While this approach is invaluable in the elucidation of causal and mechanistic links, the extent to which conclusions from such work applies in relation to naturally occurring gradients of species diversity and composition has seldom been tested (but see Tylianakis et al. 2008). Succession, or ecosystem development, occurs through time following disturbance or the creation of new substrata, and is a pervasive phenomenon in natural systems. During succession, species composition, diversity and biomass all typically change (e.g. Connell and Slatyer 1977) providing an opportunity to study links between these community properties and ecosystem functioning (Vile et al. 2006).

Species diversity has been shown to change through succession in many cases, mediated by the balance between colonisation and extinction. The intermediate disturbance hypothesis postulates that diversity will be maximal at intermediate rates, or intensities, of disturbance, when both competitively superior and opportunistic species co-exist (Grime 1973, Connell 1978). Applied to succession within a patch, this conceptual model implies a peak at an intermediate state (Sousa 1979). However, species richness has also commonly been observed to increase logistically to a steady state across several successional sequences (Odum 1969, Peet 1978, Whittaker et al. 1989).

Changes in species diversity may have consequences for ecosystem-level functional properties. A large body of experiments has demonstrated that species richness can enhance the magnitude of aggregate ecosystem properties such as productivity and nutrient flux (reviewed by Hooper et al. 2005, Balvanera et al. 2006, Cardinale et al. 2006). Although the inclusion of particular species can have large effects on the focal ecosystem property (Cardinale et al. 2006), it is becoming increasingly recognised that species complementarity, as mediated by mechanisms such as resource partitioning (Bracken and Stachowitz 2006, Griffin et al. 2008) and facilitation (e.g. Cardinale et al. 2002), may also have an important influence on ecosystem properties.

Species composition may also change markedly during succession, with consequences for primary productivity, standing biomass and other ecosystem properties. Initially, species able to pre-empt and dominate abundant resources through rapid colonisation and growth are successful. Such species typically have a high proportion of photosynthetic tissue relative to structural tissue, and exhibit correspondingly high mass-specific rates of photosynthesis. Conversely, those competitively superior species that become dominant later in succession invest in structural material and defences to conserve internal supplies, as reflected in a reduced proportion of photosynthetic tissue and lower mass-specific rates of photosynthesis (Kira and Shidei 1967, Odum 1969). Meanwhile, total biomass typically accumulates rapidly early in succession before reaching a steady-state as costs of maintenance equilibrate gross primary productivity (Odum 1969).

The gradient of species-specific photosynthetic rates through succession can be described through relevant functional traits (Vile et al. 2006, Garnier et al. 2004). Functional traits typically summarise key physiological or morphological characteristics, providing a mechanistic link to the mediation of ecosystem processes (Lavorel et al. 1997, Poorter and Bongers 2006). For example, specific leaf area (SLA) describes the light-

capturing leaf area relative to biomass, and has been shown to relate closely to species-specific growth rate in plants (Poorter and Remkes 1990, Poorter and Evans 1998). By extension, such functional traits potentially provide a predictive link between species composition and ecosystem functioning. It has been proposed that species' functional trait values can be scaled to predict ecosystem processes if weighted by their relative biomass in a community. This 'biomass ratio hypothesis' (Grime, 1998) implicitly assumes that mechanisms such as resource partitioning and facilitation produce effects on ecosystem functioning that are negligible compared to those resulting from the functional traits of component species. The prediction remains largely unexplored, having been tested in only a single system to date (abandoned vineyards: Garnier et al. 2004, Vile et al. 2006), and requires further testing in a broad range of systems and contexts before its generality can be assessed.

In order to study community and ecosystem-level changes through the typically long periods required for ecosystem development in terrestrial systems, ecologists have used chronosequences (space-for-time substitutions) to infer temporal dynamics (e.g. Lichter 1998). The use of chronosequences involves the implicit assumption that community and ecosystem changes across sites are representative of the development of any one site within the sequence (Pickett 1989). Whilst of undoubted value, chronosequences require careful interpretation owing to confounding environmental factors that often vary across sites (Pickett 1989). Additionally, this problem may be compounded by an association between site age and location if the chronosequence is created, for example, by a retreating glacier (e.g. Fastie 1995).

We utilised a unique intertidal rock pool chronosequence, which represented a snap-shot of primary succession over a 14-year period. These pools were artificially created as part of a sea defence scheme on Plymouth Breakwater (Appendix A) from a standard

mould and materials, and were subject to uniform environmental conditions, meeting the assumptions for interpreting chronosequences. The pools hosted rich and abundant seaweed assemblages, together with populations of three species of patellid limpets. Intertidal seaweed assemblages have proven to be excellent testing grounds for theories of successional mechanisms (Turner 1983, Farrell 1991, McCook and Chapman 1992) owing to their rapid dynamics relative to terrestrial systems. However, the extent to which community and ecosystem properties are linked has never been explored, partly due to the difficulty of quantifying ecosystem-level processes in open marine systems. Net primary productivity cannot be accurately quantified from measurements of accumulated biomass, as in closed systems, as a substantial proportion of fixed carbon is exported to neighbouring systems via detritus or transferred trophically (see Raffaelli and Hawkins 1996 for review). To measure a key ecosystem process mediated by seaweed, we used a well-tested field technique to isolate the oxygen production rate *in situ* (instantaneous gross primary productivity). This was possible because, during periods of emersion, rock pools are effectively self-contained natural mesocosms, providing an opportunity to quantify both community composition and corresponding ecosystem functioning (Nielsen 2001, Bracken and Nielsen 2004, Bracken and Stachowitz 2006). Our aim was to link macroalgal species, community and ecosystem-level properties within the context of ecosystem development (succession). Specifically, we tested the following hypotheses: (i) the biomass-specific rate of gross primary productivity will decline during succession, reflected by similar decreases in our selected functional trait aggregated at the community level; (ii) seaweed species diversity will comply to the intermediate disturbance hypothesis; and (iii) such species and community-level changes (hypotheses i and ii) will be related to ecosystem-level properties.

2.2 MATERIALS AND METHODS

2.2.1 Site and experimental design

The rock pool chronosequence was on Plymouth breakwater (completed in 1841, approximately 1.5km long and 0.1km wide), a large coastal defence structure situated at the mouth of Plymouth sound, south-west UK. Large concrete blocks (2.5m high, 2.4m x 4.8m on the upper surface), marked with the year in which they were constructed, have been periodically added to the wave exposed southern side to protect the main structure from wave damage (Appendix A). These blocks all have two depressions in their upper surface which were incorporated to facilitate positioning of the blocks. These effectively created two artificial rock pools (Appendix A), separated by approximately 1.5m. These pools vary minimally in size and shape (mean ± SD: depth = 0.31 ± .04 m; total rock surface area = 0.81± 0.09 m^2; volume = 54.48 ± 7.63 l). The pools are subject to natural colonisation, hosting communities that resemble those of natural rock pools in the area, with dominant macroalgal assemblages and patellid limpets (Griffin J, personal observation). The orientation and positioning of the blocks rendered the pools subject to similar wave exposure and tidal height (c. 3m above Chart Datum). Two blocks from the following six age classes, in years since establishment, were selected for study: 1, 2, 5, 7, 11, and 14. Blocks of the same year class are interspersed, ensuring spatial independence. We quantified the community composition and corresponding metabolism (functioning) of both pools, and then averaged across the pools, hence blocks formed the independent replicates (n = 12).

2.2.2 Community composition and diversity

To measure changes in community composition through the 14-year chronosequence, we combined percent cover and biomass data. Encrusting coralline algae

(*Phymatolithon* and *Lithophyllum spp.*) could not be removed from the underlying rock; hence their abundance was quantified as a mean percentage cover within six haphazardly placed 20cm x 20cm quadrats. All other biota were removed from the pools, sorted to species-level (where possible), washed in distilled water and oven-dried at 60 degrees to constant weight (deWreede, 1985). We removed biota from pools within three days of final metabolism measurements.

To separate the contribution of the relatively heavy calcium carbonate skeleton of *Corallina officinalis* (a dominant species in mature pools) to overall algal biomass, we first calculated the mean % contribution of calcium carbonate to the mass of *Corallina*. Specifically, we dissolved skeletons of 10 x ~ 30 g samples of *Corallina* taken from unused pools at the study site in weak acid for 24 hours, followed by measurement of calcite free biomass (Carpenter 1986). This produced a proportional calcium carbonate biomass value of (mean ± SD) 80.47 ± 3 %. We used this value to calculate the calcite-free biomass of *Corallina*. We then derived 2 alternative measures of overall biomass of the algal assemblage: i) total algal biomass, and ii) calcite-free algal biomass.

We used 3 alternative measures of biodiversity based on dry masses (thus excluding crustose species): species richness (the number of species), species diversity (Shannon index H'), and species evenness (Pielou's index J'). These were calculated according to both total algal biomasses and calcite-free algal biomasses.

2.2.3 Community metabolism (functioning)

For each pool we gained estimates of instantaneous community respiration (ICR) and net community productivity (INCP) by measuring rates of oxygen exchange between biota and the discrete body of water (the pool) in both light and artificially darkened conditions. These measurements enabled the calculation of instantaneous gross primary

productivity (IGPP), our ecosystem processes of interest in this study. We focused our analyses on this measure as it excludes community respiration, and thus the confounding effect of animal biomass on the link between macroalgae and ecosystem processes. Measurements were made on two days (separated by 14 days) and compared for consistency, before averaging the values for each pool. The method employed here was developed by Kinsey (1985) for use in open aquatic systems and has previously been successfully applied to rock pool communities (Nielsen, 2001; Martins et al. 2007). We measured the concentration of oxygen in each rock pool (HQ20 Hach Portable LDO™, Loveland USA) before and after an hour-long dark period (community respiration), and finally after a period of re-exposure to natural light (including both photosynthesis and community respiration) (see Nielsen 2001). We created artificial darkness by covering the pools with opaque black polythene coated with a reflective white synthetic cloth to prevent warming of the pools. We adjusted the duration of the light period (30 – 59 minutes) based on initial observations to equilibrate the increase in DO concentration (approximately 2 mg O_2) between pools with varying rates of DO flux, and according to contrasting irradiance on the two days. The water was thoroughly mixed prior to each measurement to average within pool variation. Measurements were made between 10 am and 1 pm on both days. The intensity of photosynthetically active radiation (PAR), measured every 30 minutes (LI-COR-250, LI-COR™, Lincoln, Nebraska, USA), was relatively consistent within days, but varied between days, averaging (±S.E.) 226.5 ± 19.9 μmol on day 1, and 1568.2 ± 28.7 μmol on day 2 (n=6 on both days). Initial water temperature was very similar among pools on the same day, but varied substantially between days (day 1: 10.45 ± 0.04 °C, day 2: 14.7 ± 0.17 °C). Furthermore, water temperature varied minimally during the dark period (day 1: 0.02 ± 0.03 °C, day 2: -0.13 ± 0.13 °C) and during the following light period (day 1: -0.07 ± 0.38 °C, day 2: 1.04 ± 0.10 °C).

We corrected rates of oxygen exchange for diffusion at the water-air interface by applying a diffusion constant (K=0.32 g m^{-2} hr^{-1}) calculated for shallow (<1m) sheltered water with very limited wave action (see Kinsey 1985 for correction methodology). To ensure that diffusion remained minimal (<10% of change in concentration) initial oxygen readings were made within two hours of pool emersion, the dark period always preceded the light period, and measurements were only made when wind conditions were less than 10 kmh^{-1}. Standardised measures of INCP (light period) and ICP (dark period) were calculated (Martins et al. 2007):

$$(\Delta[O] \times V)/X \qquad (1)$$

Where $\Delta[O]$ = change in oxygen concentration per unit time, V = pool volume, and X = one of 3 alternative measures: area of substratum, total algal biomass or calcite-free algal biomass. Measured values of NCP and CR were used to calculate GPP from the following fundamental equation:

$$GPP = NCP - CR \qquad (2)$$

2.2.4 Functional Properties of component species

We adapted the established functional effect trait 'specific leaf area' commonly used in plants (e.g. Cornelissen et al. 2003), to form an analogous measure for macroalgae. Because in many macroalgal species there is no clear distinction between photosynthesising and structural areas, we re-defined this trait to include the complete macroalgal thallus. We measured the specific thallus area (STA, m^2 kg^{-1} dry mass) from digital images (UTHSCTA *Image Tool* San Antonio, TX, USA) of 12 specimens of each of 8 of the most abundant

species recorded in the chronosequence (Appendix B). These species accounted for a large proportion of total algal biomass (93.91 ± 1.74%) in the study pools.

To test whether a functional trait of component macroalgal species can be scaled to the ecosystem level (the biomass ratio hypothesis), aggregated values of specific thallus area (STA_{agg}) were calculated using data from 8 of the most abundant species as (Garnier et al. 2004):

$$STA_{agg} = \sum_{i=1}^{n} P_i \times STA_i \qquad (3)$$

where P_i is the relative contribution of species i to the biomass of the community, n is the number of abundant species considered, and STA_i the value of specific thallus area of species i.

2.2.5 Analysis

To distil variability among pools in terms of algal community composition and relative abundances, we performed principal component analysis on untransformed calcite-free algal biomass data in PRIMER-6 (PRIMER Ltd. Plymouth, UK). The % cover of crustose coralline algae were standardised to *Corallina* biomass to give it an equal weighting as this other primary-space occupying calcified species. This analysis identified the species that were most important in distinguishing between pools, and also facilitated use of these orthogonal descriptors (principal components) in further analyses linking community composition and ecosystem properties.

To describe changes in algal and limpet biomass through the chronosequence the fit of alternative simple models were assessed based on adjusted R^2 values. These model fits are intended to be purely descriptive and are not testing any specific hypotheses.

To test the intermediate disturbance hypothesis in relation to both algal species richness and evenness (according to total and calcite-free biomass), we tested the fit of unimodal polynomial regressions to changes in these metrics across the chronosequence. The effect of pool age on ecosystem functioning and community properties was tested using linear regression analyses, with pool age log-transformed in those cases where log-fits were identified. We formally tested the biomass ratio hypothesis by performing linear regression analyses with 2 measures of aggregated community specific thallus area (i) according to total algal biomasses, and ii) based on calcite-free algal biomass) as predictors of observed gross primary productivity, respectively normalised. We also added the total percentage cover of crustose coralline algae to these regression models to test whether the unexplained variability was related to abundance of this algal group (the STA of which could not be obtained).

We performed Pearson's correlation analyses to assess the relationships between community properties (diversity, evenness, aggregated STA, composition [PCs], biomass) and ecosystem 'functional' properties (biomass, mass and area-specific gross primary productivity). The large number of tests this exploratory analysis entailed produces a non-trivial possibility of committing several type I errors (wrongly rejecting the null hypothesis). We opted not to correct for such multiple tests using the Bonferroni correction or the less conservative procedure of Benjamini and Hochberg (1995) because this would greatly inflate the probability of committing type II errors (Gotelli and Ellison 2004, Moran 2003, Nakagawa 2004). Instead, significant correlations were interpreted cautiously with the caveat that further work is required to confirm these preliminary findings.

2.3 RESULTS

2.3.1 Community composition and diversity

The identity of dominant species changed through the chronosequence as rock pool communities matured (Fig. 2.1; see Appendix B for abundances of all species recorded). Sheet-like algae (*Ulva spp.)* were most abundant in the first two years, but declined throughout successive year classes. In contrast, the robust turf-forming coralline alga *Corallina officinalis* was absent from pools within the first year, increasing in abundance rapidly thereafter until it dominated the biomass of later-successional pools. The main canopy-forming brown alga in this system, *Himanthalia elongata*, exhibited fluctuating abundance between successive year classes in the middle of the chronosequence, but remained consistently abundant in the older pools.

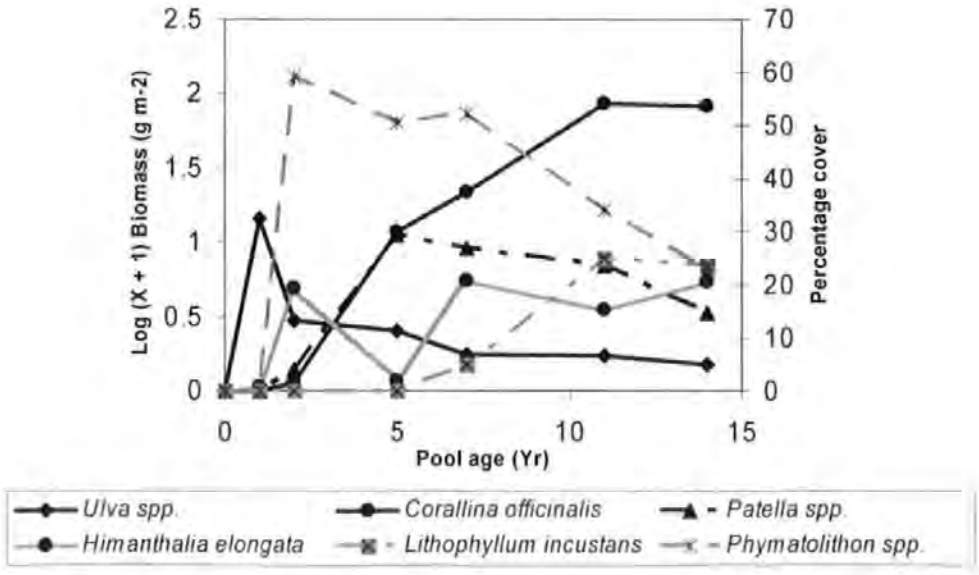


Figure 2.1. Community development as illustrated by changes in the mean abundance of dominant species with increasing pool age (n = 2 for each pool age; see Appendix B for abundances of minor species). Crustose coralline species (*Phymatolithon spp.* and *Lithophyllum incrustans*) were measured as a percentage cover of primary space, shown on the secondary axis. Dry masses of other species were log transformed to compress the data for presentation purposes only

Table 2.1. The species-specific contributions (Eigenvectors) to the first three principal components. Only those species with a >0.1 value in relation to any of the components is included. The highest contributor (whether positive or negative) to each component is indicated in bold.

	Principal components		
Species	**1**	**2**	**3**
Corallina officinalis	**-0.924**	0.373	-0.005
Ulva spp.	0.344	**0.741**	-0.193
Crustose coralline	-0.145	-0.471	-0.193
Himanthalia elongata	-0.077	-0.268	**0.505**
Laminaria digitata	-0.005	-0.133	0.433

The first three principal components (PCs) captured a high proportion (91.8%; PC1 = 65.7%, PC2 = 19.6%, PC3 = 6.5%) of variance among pools in the composition and relative abundances of algae. PC1 strongly, and negatively, relates to the biomass of *Corallina* (Table 2.1); PC2 most strongly relates to the biomass of *Ulva spp.*, and is also negatively related to the abundance of crustose coralline algae; PC3 is both negatively related to the abundance of crustose coralline algae and positively related to the biomass of the 2 brown canopy-forming species *Himanthalia elongata* and *Laminaria digitata* (Table 2.1). Ordination according to the first 2 PCs generally clustered pools of the same age (with the exception of year 11 pools; Fig. 2.2). The relatively consistent movement of pools with increasing pool age from a high to low PC1 score (Fig. 2.2) reflects the increasing biomass of *Corallina*. The initially high level according to PC1 (in year 1 pools) reflects the high biomass of *Ulva* and low abundance of crustose coralline algae (Fig. 2.2). The immediate decrease can be attributed to both a reduction in *Ulva* biomass and a increase in the abundance of crustose algae. The subsequent movement towards higher PC2 scores

(Fig. 2.2) shows the reduction in crustose coralline algae, which directly competes for primary space with *Corallina* (PC1).

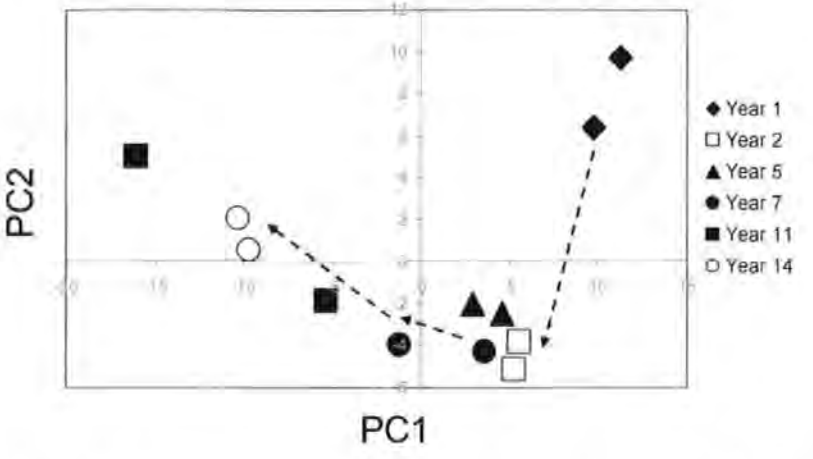


Figure 2.2. Ordination of pools across the chronosequence according to the first two principal components. Arrows indicate direction of increasing pool age. See Table 2.1 for loadings of principal component axes.

Note that the ordination according to the first 2 principal components produced a classic 'horseshoe' effect arising from the unimodal distribution of several of the species across the chronosequence (Fig. 2.1). The ordination of pools presented in Fig. 2.2 should thus be interpreted cautiously. The use of PCA was necessary however, to allow use of loadings scores in correlation analyses.

Total algal biomass rose exponentially during succession (Fig. 2.3). Calcite-free algal biomass also increased with pool age, but only linearly (Fig. 2.3). The negative correlations between PC1, but not PC2 or PC3, and both the total and calcite-free biomass of algae (Table 2.2), indicate that *Corallina* (see Table 1 PC scores) was largely responsible for these patterns (Fig. 2.3). Limpets were absent from – or occurred at very low abundance in – year 1 and 2 pools. The biomass of limpets then rapidly increased, peaking in pools aged between five and seven years. In year 14 pools, limpet biomass was very low (Fig. 2.3). This pattern was best described by a unimodal, quadratic regression (Fig. 2.3).

Algal species diversity (H') did not fit a quadratic regression against pool age when calculated using either total or calcite-free algal biomass. Species evenness (J') complied to a quadratic regression when calculated with calcite-free algal biomass, peaking in year 5 pools and declining thereafter (Fig. 2.4); however, a quadratic regression did not fit evenness when calculated with total algal biomass (Fig. 2.4). Species richness reached a plateau after just 2 years, and did not conform to a quadratic regression (Fig. 2.5).

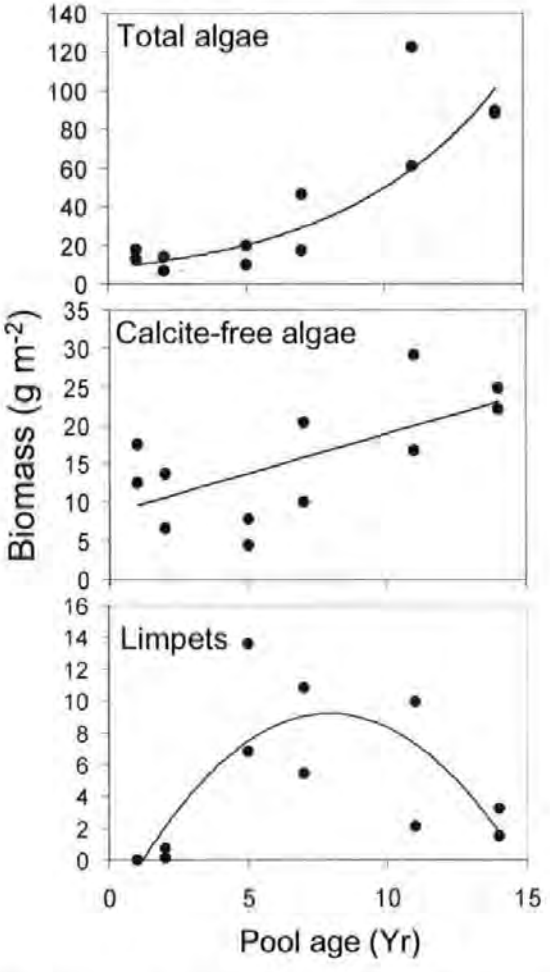


Figure 2.3. Changes in biomass during community development: the exponential increase in total algal biomass with pool age (top panel; r^2=0.779; Y = 8.3631 e $^{0.1782X}$); the linear increase in calcite-free total algal biomass (middle panel; r^2 = 0.426; Y = 1.0322 X + 8.6181); the mid-successional peak in total limpet biomass, conforming to a second order polynomial equation (r^2 = 0.6123; Y = - 0.2041 X^2 + 3.2279 X – 3.5223). n = 12 for all response variables.

Species evenness and diversity were strongly negatively correlated with both calcite-free and total algal biomass (Table 2.2). Species richness was unrelated to either measure of algal biomass, however (Table 2.2).

2.3.2 Community metabolism and aggregated properties

Measures of gross primary productivity on the two separate replicate days produced a consistent ranking of pools (per unit area: Spearman's rank $r_s = 0.649$, $P = 0.023$, $n = 12$), confirming the ability of field measures to reliably detect differences in ecosystem functioning between pools. Furthermore, this validated averaging productivity values for each pool to give an single 'index' of productivity generated over days with contrasting light conditions. Observed community and pool-age effects on productivity are thus more likely to be general.

Gross primary productivity did not change consistently with pool age when normalised to calcite-free algal biomass (Fig. 2.6). In fact, with the exception of the large variability between year 11 pools, this measure of specific productivity was relatively consistent throughout ecosystem development (Fig 2.6). This measure of productivity was negatively related to the calcite-free biomass of algae (Table 2.2). Gross primary productivity normalised to total algal biomass, on the other hand, declined logarithmically with pool age (Fig. 2.6). This measure of productivity was strongly negatively correlated with total algal biomass (as intuitive given the increasing biomass and steady/idiosyncratic rate of prod per unit of area) and most strongly correlated to PC1 (the inverse of *Corallina* abundance), showing that species composition effects on the amount of inactive material (calcite) underpins the logarithmic decline.

Table 2.2. Pearson's correlation analyses between community properties and higher-level ecosystem properties. Species richness/diversity/evenness is based on calcite-free measures. STA = specific thallus area; PC = Principal Components, used to describe the algal community (methods, Table 2). Significant correlations are indicated in bold.

	Algal Biomass				Gross primary productivity					
	Total		Calcite-free		Area-specific		Total biomass-specific		Calcite-free biomass-specific	
	r	p	r	p	r	p	r	p	r	p
Species richness	0.163	0.613	0.01	0.976	-0.12	0.709	-0.33	0.295	-0.042	0.897
Species diversity	-0.684	**0.014**	-0.729	**0.007**	-0.281	0.375	0.528	0.078	0.423	0.171
Species evenness	-0.77	**0.003**	-0.793	**0.002**	-0.283	0.373	0.622	**0.031**	0.474	0.119
Total biomass	NA	NA	0.892	**<0.001**	0.218	0.496	-0.868	**<0.001**	-0.551	0.063
Calcite-free biomass	0.892	**<0.001**	NA	NA	0.441	0.151	-0.655	**0.021**	-0.659	**0.02**
STA (total biomass)	-0.575	**0.05**	-0.295	0.352	-0.084	0.796	0.756	**0.004**	-0.032	0.921
STA (calcite-free)	-0.542	0.069	-0.37	0.237	-0.208	0.516	0.582	**0.047**	0.041	0.899
PC 1	-0.957	**<0.001**	-0.753	**0.005**	-0.128	0.692	0.911	**<0.001**	0.417	0.178
PC 2	0.27	0.396	0.434	0.158	0.112	0.729	-0.025	0.938	-0.446	0.146
PC 3	0.322	0.308	0.531	0.076	0.386	0.216	-0.184	0.567	-0.533	0.075

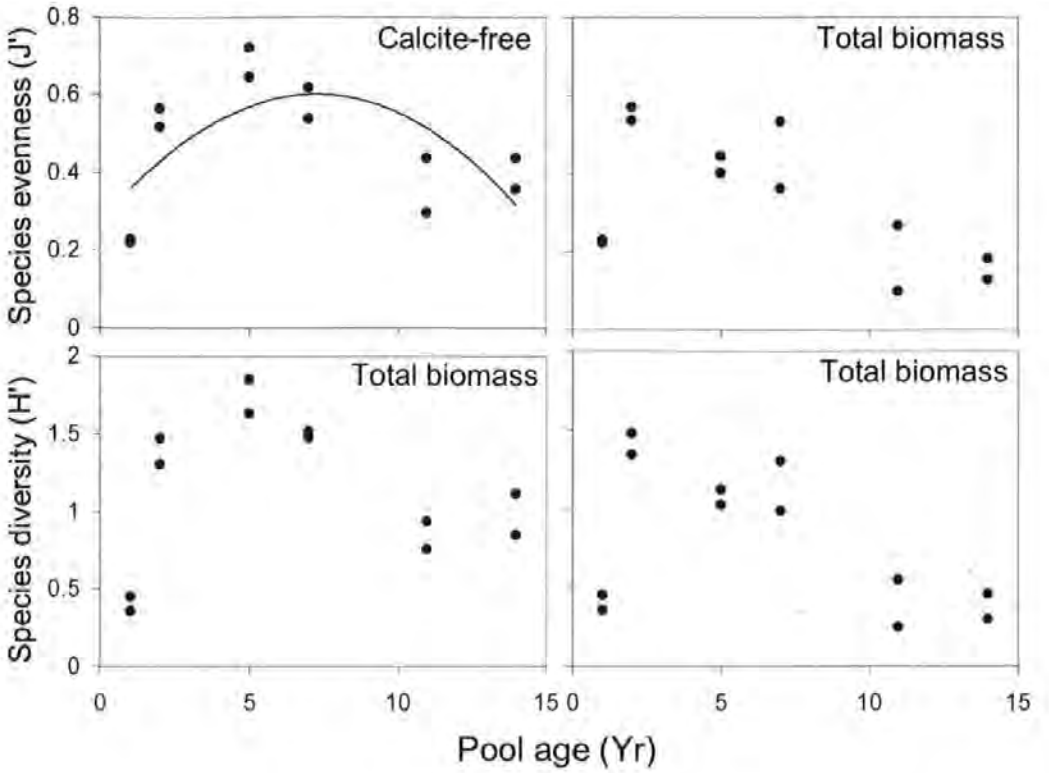


Figure 2.4. Tests of the intermediate disturbance hypothesis based on both total and calcite-free algal biomasses changes across the chronosequence. With calcite-free algal biomasses, the response of species evenness (J', top left panel; $Y = -0.0062 X^2 + 0.0902 X + 0.2758$; $r^2 = 0.502$, $P = 0.043$) but not the diversity (H', $r^2 = 0.452$, $P = 0.067$) was significantly described by a second order quadratic regression. With total algal biomasses, the response of neither species evenness (J', top right panel; $r^2 = 0.446$, $P = 0.070$) nor diversity (H', bottom right panel; $r^2 = 0.439$, $P = 0.074$) were significantly described by second order quadratic regressions. $n = 12$ for all response variables.

Normalised to surface area, productivity varied relatively little between pools of the same year class, but varied idiosyncratically among year-classes across the chronosequence (Fig. 2.6). Community composition (PC 1-3), species diversity (H'), evenness (J'), richness and biomass were all unrelated to this pattern (Table 2.1).

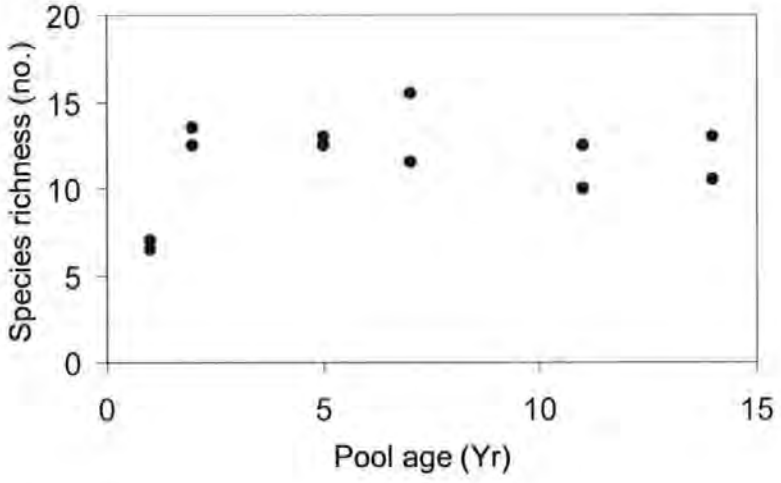


Figure 2.5. A test of the intermediate disturbance hypothesis based on species richness across the chronosequence. Species richness did not conform to a second order quadratic regression (r^2 = 0.003, P = 0.858). The number of species was lower in the year 1 pools, but reached a plateau after 2 years. n = 12.

Logarithmic models tightly fitted the decline in community specific thallus area with increasing pool age, calculated using both calcite-free biomass and total algal biomass (Fig. 2.7). Community specific thallus area, calculated as according to total and calcite-free algal biomass respectively, proved to be a good predictor of biomass specific productivity normalised to total algal biomass, but not to calcite-free algal biomass (Fig. 2.8). Both of these models were substantially improved by the addition of the % cover of crustose coralline algae. The regression between total-mass specific productivity and community aggregated STA became tighter, and remained significant (GPP = -0.0150 + 0.000801 crustose + 0.00297 STA_{agg}; crustose P = 0.024, STA_{agg} P = 0.001; overall model fit, r^2 = 0.77, P<0.001). The regression between calcite-free mass specific productivity and community aggregated STA, however, narrowly failed to reach statistical significance at an alpha = 0.05 level (r^2 = 0.448, P = 0.069).

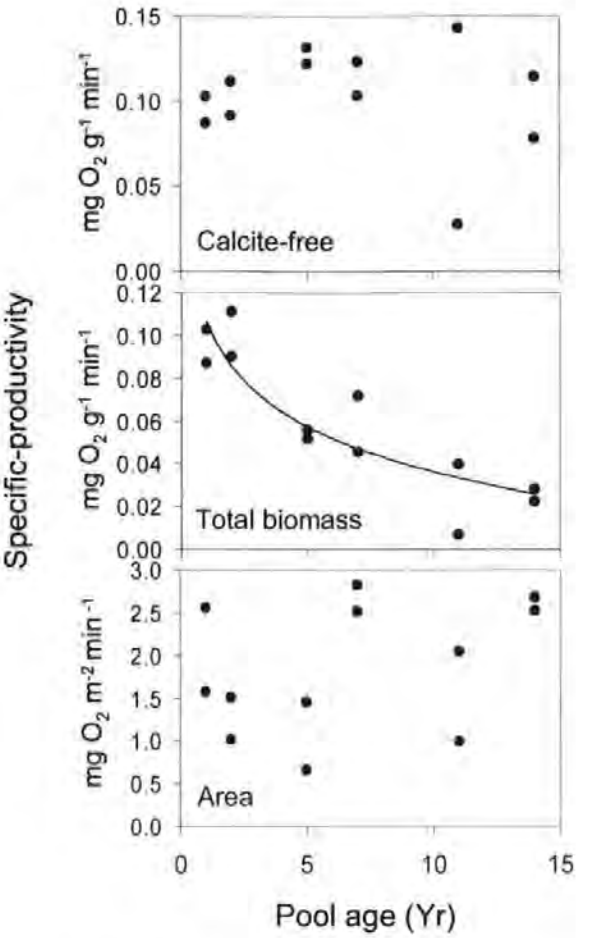


Figure 2.6. The relationship between pool age and gross primary productivity. Productivity exhibited no consistent pattern when normalised to either calcite-free biomass (top panel) or area (bottom panel). Normalised to total algal biomass (middle panel), however, productivity declined logarithmically with increasing pool age (r^2 = 0.795, P <0.001; Y = - 0.0337 Ln X + 0.0889). n = 12 for all response variables.

2.4 DISCUSSION

2.4.1 Summary of main findings

Succession has commonly been described in terms of changes in species composition and diversity, but the links between such changes and aggregated ecosystem properties have seldom been examined. Our results show that compositional changes during ecosystem development only influence gross primary productivity when normalised to total algal biomass, including the calcite skeleton of the dominant species, *Corallina*. Our data

also show that the rapid decline in total-mass specific productivity can be predicted from a species-level functional trait – specific thallus area, providing evidence to support the biomass ratio hypothesis.

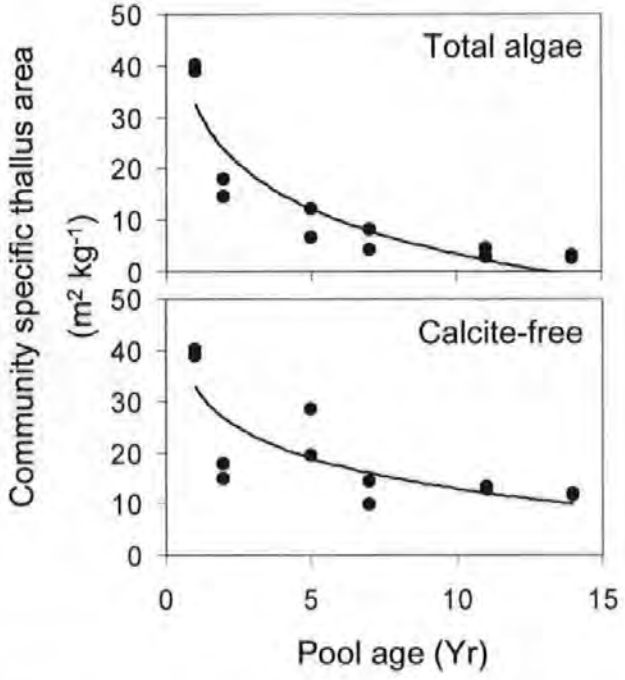


Figure 2.7. Relationships between pool age and specific thallus area aggregated at the community level. Community specific thallus area declined logarithmically with pool age when based on total algal biomasses (top panel; $r^2 = 0.847$, $P < 0.001$; - 12.639 Ln X +32.571) as well as on calcite-free algal biomasses (bottom panel; $r^2 = 0.633$, $P = 0.002$; Y = - 8.658.1 Ln X + 32.861). $n = 12$ for all response variables.

2.4.2 Changes in productivity

Total-mass specific productivity declined most rapidly early in succession (Fig. 2.6), which is consistent with a previous study of abandoned vineyards, despite the use of a different measure of mass-specific productivity (aboveground net primary productivity, Vile et al. 2006). The more general effect of a decline in the efficiency of energy flow during succession, following an early peak, has long been hypothesised (Kira and Shidei

1967, Odum 1969) and supported by empirical evidence in terrestrial systems (Mellinger and McNaughton 1975, Lichter 1998).

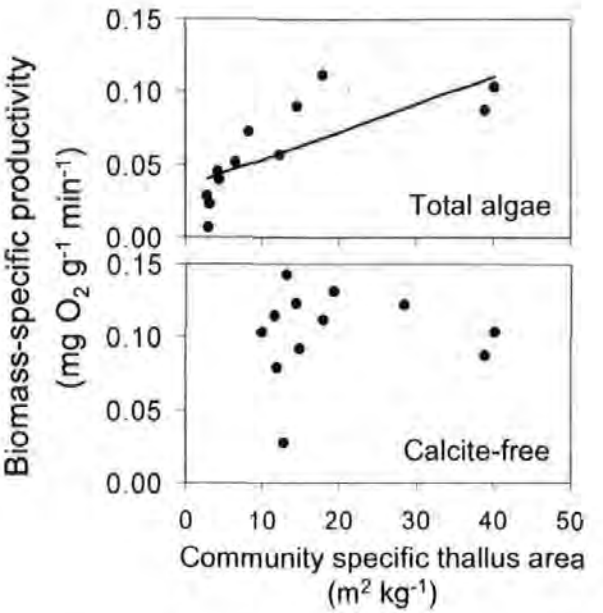


Figure 2.8. Relationships between community specific thallus area and biomass-specific productivity. Both variables were calculated based on total algal biomass (top panel) and calcite-free algal biomass. Linear regression analyses show that community specific thallus area can predict specific productivity based on total algal biomass ($Y = 0.0019 X 0.0348$; $r^2 = 0.572$, $P = 0.007$) but not calcite-free algal biomass ($r^2 = 0.002$, $P = 0.899$).

Our results highlight the importance of considering structural tissue in understanding this effect. The tight relationship between total mass-specific productivity and the first principal component (Table 2.2), which is itself closely related to the abundance of *Corallina* (Table 1.2), shows that the decline in this ecosystem process could be largely attributed to an increase in the abundance of *Corallina*; more specifically, its calcite skeleton. The calcite skeleton of *Corallina* provides an important defence against herbivory (Steneck and Dethier 1994), but is photosynthetically inactive and forms a large proportion (~80%, see *Methods*) of the mass of this species. The importance of calcite in

driving the observed pattern is further supported by the relatively consistent rate of calcite-free biomass specific productivity across the chronosequence (Fig. 2.6).

The large and consistent reduction in total-mass specific productivity was not apparent in area-specific productivity, which varied idiosyncratically during succession. Despite having the lowest rate of total-mass specific productivity, the oldest pools exhibited rates of area-specific productivity at least as great as the youngest (Fig. 2.6). This implies that the greater standing stock of macroalgae in mature pools (Fig. 2.3) compensates for reduced rates *per unit of* total biomass. Area-specific productivity was not, however, related to any individual property of the algal community (Table 2.2). A possible explanation for this is that community properties had interactive effects on this measure of productivity. The inability of biomass-specific functional traits to be scaled to predict total ecosystem functioning (per unit area) has been reported elsewhere (Garnier et al. 2004; Vile et al. 2006). Further study, with a much greater number of replicate pools, or plots, would be required to untangle these potentially complex effects.

2.4.3 The community aggregated functional trait

Changes in the composition and relative abundances of species through succession was also reflected in the functional trait pioneered for this study, specific thallus area. The community aggregated value of this trait declined logarithmically, whether based on total or calcite-free algal biomasses. This indicates that the thallus of dominant seaweed species increased in mass per unit of surface area during succession, reflecting an increase in investment in structural tissue, relative to light-capturing thallus area. Again, this finding is consistent with the chronosequence studied by Vile et al. (2006) in which specific leaf area declined logarithmically with field age (Garnier et al. 2004). Together, our findings suggest that, despite differences between taxa and more general differences between marine and

terrestrial systems (e.g. Giller et al. 2004), common patterns of succession and analogous changes in functional traits can be observed.

In support of the biomass ratio hypothesis, community aggregated specific thallus area proved to be a significant predictor of a measure of specific-productivity (Fig. 2.8). However, this only applied to total-mass specific productivity; calcite-free specific thallus area could not linearly predict calcite-free biomass-specific productivity. The fact that the multiple regression model could explain >40 % of the variance in calcite-free specific productivity suggests that a greater number of replicate pools may have revealed a statistically significant regression model. The prediction of calcite-free specific productivity was inevitably more sensitive than total-mass specific productivity to variability in the efficacy of predictors, because differences among pools in specific-productivity were less marked in the absence of calcite (Fig. 2.6). Our analysis also highlights the importance of considering crustose species in the prediction of photosynthesis in this system. More generally, we suggest that, in lieu of data on a relevant functional trait for all abundant species in a system, knowledge simply of the abundance of the unknown species(s) can help to improve trait-based predictions.

2.4.4 Changes in diversity

Although empirical tests of the intermediate disturbance hypothesis (IDH) in a range of systems have often refuted the hypothesis (Mackey and Currie 2001), the strongest support has come from studies of marine communities on hard substrata (Sousa 1979; Jara et al. 2006; Svensson et al. 2007) where competition for space can lead to exclusion of inferior competitors. Our study shows that conclusions can depend on the measure of species 'diversity' used. In this study, species evenness (J'), and only when based on calcite-free algal biomasses, complied with our strict test of the IDH (Fig. 2.4). (Species evenness was dictated by the abundance of calcified *Corallina* when total biomass was

considered, causing it to peak after just 2 years). Notably, species richness displayed a pattern more consistent with the logistic model (Peet 1978, Whittaker et al. 1989); while richness was much lower in the year-1 pools (6-7 species), it rapidly reached a plateau at ~14 species after only 2 years. Dominance of *Corallina* thus reduced species evenness later in succession, but did not eliminate an increasing number of species; rather, species were able to persist, likely as epiphytes on *Corallina* or canopy-forming species (Noël 2008). These species were thus resistant to competitive exclusion since they were able to exploit secondary, rather than primary, space.

A focused period of research has demonstrated that species and functional diversity can have a positive effect on the magnitude of ecosystem processes (Cardinale et al. 2006). The links between species diversity and ecosystem properties during succession has, however, seldom been considered (but see Weis et al. 2007 for a microcosm example). Here, we did not detect a link between any measure of seaweed diversity and our focal ecosystem process, gross primary productivity. The fact that total-mass specific productivity can be predicted according to the biomass ratio hypothesis, but is not related to diversity alone, suggests that changes in composition (and associated changes in functional traits) during succession have a greater effect than diversity. Strong effects of species composition are common in manipulative biodiversity experiments (Hooper et al. 2005), and can be expected to be especially marked during succession, where the strategies of dominant species change from rapid growth to conservation of internal supplies (Huston and Smith 1987; reflected in mass-specific monoculture rates of photosynthesis, Littler and Littler 1980). Our findings thus support the view that the functional traits of component species, rather then diversity, are the principal drivers of function at the ecosystem level (Wardle et al. 1997).

Notably, our analyses revealed that species evenness and diversity were both negatively correlated with algal biomass (both total and calcite-free; Table 2.2). These negative correlations provide a rare counter-example to studies showing a positive relationship between species diversity and ecosystem functioning (including biomass standing stock, e.g. Hector et al. 1999). In our system, species diversity/evenness and biomass were likely reduced and elevated, respectively, by the increasing dominance of *Corallina.* Dominance of a species with a large effect on a particular ecosystem process is known as the positive selection effect in biodiversity-function studies (Loreau and Hector 2001). Our study suggests that during later stages of succession, the operation of an analogous effect can concurrently reduce species evenness/diversity and increase biomass, producing a negative relationship between evenness/diversity and biomass.

2.4.5 Concluding remarks

Functional traits have been forwarded as a foundation for the development of a predictive link between species and ecosystem functioning (Lavorel and Garnier 2002, Naeem and Wright 2003). Whilst much of the emphasis of research to date has been on terrestrial species (especially grassland species), our results have shown that analogous traits can be used in marine macroalgae to predict the efficiency of a key ecosystem process. Future work should build on our attempt to integrate the use of functional traits and measures of the diversity of species in the prediction of ecosystem functioning. The development, and testing, of traits that predict area-specific rates of ecosystem processes should be a particular focus of future empirical work.

2.5 APPENDICES

2.5.1 APPENDIX A. Images of the study site and rockpools

Figure 2.9. A sub-set of the dated blocks that are periodically added to the seaward side of Plymouth Breakwater.

Figure 2.10. An example of a rockpool of known age used in this study. Pools were located on the upper surface of blocks.

2.5.2 APPENDIX B. Table 2.3. Mean abundances (±SD) of macroalgal taxa across the chronosequence (g dry weight m^{-2} of rock substrate). *Corallina* was measured including its calcite skeleton which comprises ~ 80 % of its total mass.

Pool age	**1**		**2**		**5**		**7**		**11**		**14**	
Species	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Dumontia contorta	0	0	0	0	0	0	0	0	0	0	0.005521	0.011041
Porphyra spp.	0.0073	0.0130	0.0012	0.0023	0.0426	0.0463	0.0215	0.0270	0.0729	0.1458	0.0108	0.0146
Scyosiphon lomentaria	0.0012	0.0024	0.0027	0.0021	0.0634	0.0533	0.0064	0.0102	0.0297	0.0352	0.0236	0.0285
Leahesia	0.0012	0.0024	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Ulva spp.	13.3534	2.7500	1.9606	0.5557	1.5785	0.7479	0.7886	0.7846	0.7409	0.5936	0.5162	0.7771
Apoglossum rusticum	0.0000	0.0000	0.1438	0.0977	0.0034	0.0045	0.0412	0.0453	0.0343	0.0609	0.0218	0.0290
Boergeseniella sp.	0.0469	0.0937	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Callophyllis lactinata	0.0000	0.0000	0.0292	0.0558	0.0000	0.0000	0.0440	0.0879	0.0346	0.0691	0.0114	0.0228
Ceramium spp.	0.0643	0.0868	0.0751	0.0950	0.1101	0.0617	0.0454	0.0307	0.1164	0.1016	0.0445	0.0468
Cladophora spp.	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0384	0.0290	0.0050	0.0100	0.0321	0.0468
Gelidium crinale	0.0000	0.0000	0.0162	0.0295	0.1890	0.1328	0.4266	0.1751	0.3608	0.2720	0.2668	0.2656
Gelidium latifolium	0.0000	0.0000	0.0000	0.0000	0.0252	0.0395	0.0066	0.0083	0.0000	0.0000	0.0000	0.0000
Gastroclonium ovatum	0.0000	0.0000	0.0832	0.0607	0.3716	0.3132	0.0254	0.0347	0.1030	0.1252	0.4631	0.1454
Lomentaria articulata	0.0000	0.0000	0.0748	0.0983	0.0056	0.0066	0.1466	0.2204	0.0568	0.0636	0.0527	0.1054
Lomentaria clavellosa	0.0036	0.0072	0.0000	0.0000	0.0072	0.0094	0.0022	0.0025	0.0343	0.0549	0.0011	0.0022
Osmundia pinnitifida	0.0036	0.0072	0.0805	0.0540	0.4815	0.4198	0.0173	0.0315	0.0101	0.0105	0.1711	0.2675
Polysiphonia brodiei	0.6582	1.0867	0.7997	0.9846	0.3856	0.3939	0.1266	0.1190	0.4108	0.3751	0.1193	0.1290
Himanthalia elongata	0.0709	0.1355	3.9865	3.7432	0.1789	0.1751	4.4956	2.7177	2.4998	2.4864	4.3947	1.9127
Laminaria spp.	0.7574	0.8718	2.7128	2.0062	0.2435	0.3598	4.5103	2.8347	1.2992	1.9553	0.5548	0.6485
Sargassum muticum	0.0084	0.0168	0.0337	0.0674	0.0000	0.0000	0.0000	0.0000	0.0108	0.0216	0.0000	0.0000
Palmaria palmata	0.0814	0.0987	0.1595	0.2717	0.2875	0.3509	0.3385	0.0858	0.0000	0.0000	0.0331	0.0466
Chondrus crispus	0.0000	0.0000	0.0006	0.0011	0.0000	0.0000	0.0000	0.0000	0.0108	0.0216	0.0000	0.0000
Codium fragile	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0010	0.0020	0.0144	0.0287
Mastocarpus stellatus	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.4394	0.8789
Corallina elongata	0.0000	0.0000	0.1458	0.2631	10.8069	5.6943	20.6190	13.6579	85.5288	47.8282	81.4866	8.5188

SPECIES COMPOSITION, NOT RICHNESS, DETERMINES CONSUMER EFFECTS ON ROCKPOOL COMMUNITY STRUCTURE AND FUNCTIONING

CHAPTER III

ABSTRACT

A key challenge in research linking biodiversity and ecosystem functioning is to incorporate the trophic interactions that characterise natural systems. Relative to primary producers, research on the effects of consumer species richness and identity (composition) has been both delayed and less prolific. There is a particular shortage of studies investigating such effects in the context of ecosystem development (or succession). We therefore manipulated the richness and composition of 3 species of molluscan grazers added to rock pools denuded of existing biota, creating all possible species combinations in a nested, substitutive design.

After the 13-month field experiment our results show that the identity, not the richness, of consumers determined effects on the developing community. Consistent with previous studies, the presence of grazers generally shifted dominance from sheet-like ephemerals to grazer-resistant crustose and turf-forming coralline algae. However, the limpet *Patella ulyssiponensis* suppressed the abundance of even the most resistant macroalgal groups, reducing total biomass. Animal biomass was also lower in the *Patella* treatment, suggesting that a strong effect of grazer identity can extend to indirect effects on non-prey species. *Patella* maintained the highest biomass in experimental pools, matching field observations, and helping to explain its dominant effect. Despite marked effects on macroalgal composition and biomass, the rate of primary productivity as determined by oxygen flux was largely unaffected by grazer composition or richness, suggesting compensatory enhanced productivity of heavily grazed macroalgae, and highlighting the importance of separating standing stock and ecosystem processes in future studies. Possible explanations for the general lack of strong species complementarity effects in this study are discussed.

KEYWORDS: Biodiversity, ecosystem development, ecosystem functioning, grazing, herbivory, primary productivity, succession, species identity, species richness.

3.1. INTRODUCTION

Over the last two decades the effects of biodiversity on ecosystem functioning has emerged as a fundamental research theme (reviewed by Loreau et al. 2001, Hooper et al. 2005, Balvanera et al. 2006, Cardinale et al. 2006). In light of rapid and pervasive human alterations to biodiversity (Pimm et al. 1995, Worm et al. 2006, Byrnes et al. 2007), answering this question has important implications for the management of ecosystem services (Kremen et al. 2005). Early seminal work focused on the effect of plant richness on primary production in temperate grassland plots (e.g. Tilman et al. 1996). Increasingly however, both theoretical (e.g. Thèbault and Loreau 2003) and empirical work (reviewed by Duffy et al. 2007) has aimed to incorporate the trophic interactions that characterise natural systems – shifting emphasis towards the often complex effects of species diversity in multi-trophic food webs. Elucidating the relative roles of species richness and identity (composition) remains a priority for empirical research, and is a common thread that unites both early and recent work. The answer to this question also has important implications for conservation (Srivastava and Velland 2005) - informing us whether we should focus on conserving particular species or species richness *per se*.

Species richness can enhance ecosystem processes through complementarity – a class of mechanisms including resource partitioning and facilitation (Cardinale et al. 2002). By definition, the action of such mechanisms requires the presence of phenotypic diversity – typically represented by multiple species. Where interspecific resource partitioning occurs, for example, increasing species richness will allow a greater proportion of the resource spectrum to be utilised, potentially enhancing an ecosystem process above that of the single best-performing species (e.g. Tilman and Lehman 2002, Råberg and Kautsky 2007, Griffin et al. 2008). In contrast, the sampling effect (Huston 1997), or positive selection effect

(Loreau 2000), results from the increased probability of including a particular species (or multiple species) in diverse mixtures that has a dominant influence on resource acquisition and ecosystem functioning. As the sampling effect depends on particular species, it is a manifestation of a species 'identity' effect.

Empirical studies in a range of systems have, in general, shown that the magnitude of ecosystem processes increases with species richness (reviewed by Hooper et al. 2005, Balvanera et al. 2006, Cardinale et al. 2006). A recent synthesis has demonstrated that in temperate grassland studies the effect is underpinned by complementarity (Cardinale et al. 2007). This suggests that species richness is more important than the identity of species included in grassland communities. However, whether this applies at higher trophic levels, or with respect to other ecosystem processes, is not yet clear.

Relative to primary producers, research on the effects of consumer species richness on ecosystem processes has been both delayed and less prolific (Balvanera et al. 2006). An understanding of the relative roles of consumer richness and identity is nevertheless urgently required, particularly given that consumers tend to be more extinction-prone than primary producers (e.g. Duffy 2003). Moreover, primary consumers play a critical role in many ecosystems particularly in the marine environment, strongly affecting primary producer biomass, diversity and composition, with potentially large impacts on ecosystem functioning (e.g. McNaughton 1985, Hughes 1994; Paine 2002, Worm et al. 2002; Mumby et al. 2007). Studies that have explicitly manipulated the diversity and identity of primary consumers have reached varying conclusions regarding their impacts; whereas several field experiments found that species identity dominated consumer effects on ecosystem functioning (Duffy et al. 2001, O'Connor and Crowe 2005), further experimental field (Duffy et al. 2003) and laboratory microcosm (Naeem and Li 1997, Gamfeldt et al. 2005) studies found marked effects of consumer species richness. Given this variability, further

experiments that explicitly distinguish between consumer identity and richness effects are required before general conclusions, analogous to those emerging from primary producer studies (Cardinale 2007), can be reached.

Herbivores are expected to have particularly marked effects on the recruitment and initial growth of primary producers, as the early life-history stages of primary producers typically lack the mechanisms that protect them from predation as adults (Santelices 1990, Carson and Root 1999). During the early stages of community development, following the loss of a pre-existing community or the creation of new habitat, herbivores may thus have strong effects on community composition (Hawkins 1981, Lubchencho 1983, Belliveau and Paul 2002, Lotze et al. 2001), diversity (Lubchencho 1978), and biomass (e.g. Hixon and Brostoff 1996). Disturbances are typically patchy (Dethier et al. 1984), allowing mobile herbivores to migrate into a recently disturbed area from surrounding, unaffected, habitat (Hartnoll and Hawkins 1985, Burrows and Hawkins 1998). Several studies have demonstrated species-specific 'identity' effects of herbivores on recruitment in marine systems (Carpenter 1986, Parker et al. 1993), even when con-generic species were considered (Moore et al. 2007). However to date, with the exception of a single study in a seagrass system (France and Duffy 2006), the effect of consumer species richness on recruitment and ecosystem processes during early succession has not been established.

Our study system utilised relatively homogeneous man-made intertidal pools naturally dominated by diverse erect assemblages of macroalgae. Macroalgal assemblages are an integral part of the functioning of coastal ecosystems throughout the expansive areas in which occur; macroalgae are at the base of coastal food webs, provide essential habitat and nursery areas for numerous associated plants and animals, and link marine carbon and nutrient cycles (Smith 1981, Duggins et al. 1989, Duarte 1995, Worm et al. 2000). Pioneering (Jones 1946) and recent (Jenkins et al. 2005, Paine 2002, Moore et al. 2007)

field research has shown that consumers as a group can play an important role in controlling macroalgal communities on rocky shores. A notable experiment tested the effect of consumer identity and richness on mature macroalgal assemblages in intertidal pools in Ireland (O'Connor and Crowe 2005), but the study presented here represents the first test on the recruitment phase in this system. We explicitly tested the effects of consumer richness and identity on the development of an early-successional community and an associated fundamental ecosystem process – gross primary productivity. Specifically, we quantified effects on community structure through differences in primary producer and animal species composition, relative abundances and biodiversity. The aspects of ecosystem functioning we focused on were: i) the accumulation of primary producer (macroalgae) and non-manipulated animal biomass, and ii) the area-specific and mass-specific rates of gross primary productivity.

3.2. MATERIALS AND METHODS

3.2.1. Study Site

We conducted this experiment in relatively homogeneous intertidal rock pools situated on the seaward side of a large coastal defence structure - Plymouth breakwater (completed in 1841, approximately 1.5km long and 0.1km wide), UK. Two pools, separated by approximately 1.5m, are located on the upper surfaces of each of numerous large concrete blocks (2.5m high, 2.4m x 4.8m on their upper surface). Compared to natural rock pools, the dimensions of the pools varied minimally (mean ± SD: depth = 0.31 ± 0.04m; total rock surface area = 0.806 ± 0.088m^2; volume = 54.48 ± 7.63l). They have vertical sides, making area and volume calculations simple (see below). The pools are subject to natural colonization, and hosted communities that resemble those of natural rock pools in the region, with dominant ,macroalgal assemblages. The orientation and

positioning of the blocks render the pools subject to similar wave exposure (moderate to high) and tidal height (C. 3m above Chart Datum). Additionally, the physical homogeneity of the pools themselves (size, rugosity and substrate material) created an opportunity to isolate the role of consumers in a relatively controlled setting whilst maintaining exposure of assemblages to the natural marine environment.

3.2.2. Experimental design and establishment

The total area of substrate and volume of each pool was calculated from digital images (Image J™; substrate area: [perimeter x depth] + pool surface area; volume: pool surface area x depth). With entire pools forming the replicate units, we manipulated both the richness and composition of three consumer species added to the pools: the orange-footed limpet *Patella ulyssiponensis*, the topshell *Gibbula umbilicalis* and the periwinkle *Littorina littorea* (as O'Connor and Crowe 2005); hereafter referred to by their generic names in full or as an initial (i.e. P, G, or L). We selected these species because they are the most abundant primary consumers in mid-shore rock pools at local sites (Griffin, J. *unpublished data*), and thus most likely to have strong influences on ecosystem processes (Grime 1998). We incorporated a range of sizes within each species, including approximately the standard deviation (SD) around the mean size recorded in field surveys. Individuals outside this range were excluded because small individuals could escape from the cages, which were chosen to also allow passage of light and propagules. The ranges of sizes were (maximum shell length): *Littorina* 14-18 mm, *Gibbula* 12-14 mm, and *Patella* 25-40 mm.

We employed a substitutive design, such that initial total consumer density (≈14 g shell-free dry mass m^{-2}) was equalized across treatments varying in richness, requiring a reduction in the density of component species with increasing richness. The substitutive

design makes the assumption that after local extinction of species those remaining compensate for their loss by increasing in numbers or biomass, i.e. show density compensation (e.g. Griffin et al. 2008). Since 'extinct' species are replaced by individuals of those remaining, intraspecific and interspecific interactions are directly compared (Griffen 2006, Jolliffe 2000). In grazer treatments, initial biomass was always equally divided among species present (i.e. 14g m^{-2}/number of species), allowing attribution of treatment effects independent of initial relative abundances. Surveys of local shores showed that all species had overlapping densities across pools, although mean density varied among species. Total biomasses used in this experiment were within the range found in the field survey (Griffin, J. *unpublished data*). We included all species in monoculture (G, L, P), all 3 possible 2-species combinations (GL, GP, LP) and a treatment containing all 3 species (GLP). Species composition was nested within richness levels, since both 1 – and 2 – species levels consisted of multiple treatments with unique species compositions (Jonsson and Malmquist 2001, O'Connor and Crowe 2005, Wojdak 2005). Additionally, grazer-free caged (CC) and un-caged controls (UC) were established for comparison to test for the effect of cages on response variables (O'Connor and Crowe 2005). The grazer-free caged treatment also formed an important control to establish the extent of grazer effects *per se*.

Prior to the addition of grazers, all animals (including existing grazers) were manually removed from the pools. After emptying the pools of water, we used a large propane burner to subsequently thoroughly clear all remaining macro-biota from the pools, ensuring that all remnants of the pre-existing communities (including highly resistant coralline forms) were visibly removed. Cages (8mm stainless steel welded wire mesh) were then constructed over pools to maintain experimental treatments by preventing grazer dispersal and immigration of large-bodied grazers.

Grazers were collected from local shores, carefully transplanted to a Perspex substrate, and maintained in flowing seawater for no more than 3 days prior to transplantation to the study pools. 2 weeks after the initial establishment of treatments, we checked the abundance of grazers and added individuals to maintain equal densities to compensate for transplant-induced mortality (primarily of *Patella*). Secondary additions were largely successful. Visual estimates of density at the mid-point in the experiment (6 months) showed that all grazers had suffered considerable, but variable, rates of mortality. This could have been caused by resource limitation early in the experiment. *Patella* was able to maintain a higher biomass than the other 2 species. In line with a standard substitutive experiment, we aimed to re-equilibrate interspecific densities. We thus added appropriate numbers of *Gibbula* and *Littorina* to reach a biomass equal to the mean of *Patella* (in treatments with an equal number of species). In order to avoid promoting possible negative density-dependent effects, we did not keep 'topping-up' treatments throughout the remainder of the experiment. Instead, we allowed grazer densities to reach a natural 'equilibrium' and interpreted results in light of these final densities.

Each treatment had 4 replicates, giving a total of 36 pools studied. Treatments were randomly assigned to pools, resulting in interspersed replicates. The manipulations were fully established in July 2006 and ran for a total of 13 months until late August 2007. The cages were thoroughly cleaned every 3-4 months throughout the experiment in order to prevent the excessive build-up of ephemeral algae, which could shade the underlying pools.

3.2.3. Measurement and calculation of response variables

All response variables were measured upon termination of the experiment. We gained estimates of instantaneous gross primary productivity through the well-tested technique of measuring rates of oxygen flux between biota and the discrete body of water

(the pool) in both light and artificially darkened conditions (see Nielsen et al. 2001, Martins et al. 2007, Chapter II) for detailed descriptions of this technique applied to rock pools). We measured the concentration of oxygen in each rock pool (HQ20 Hach Portable LDO^{TM}, Loveland USA) before and after an hour-long dark period (community respiration), and finally after a period of re-exposure to natural light (including both photosynthesis and community respiration). Gross primary productivity was calculated by simply compensating net oxygen flux under light conditions with oxygen consumption under darkened conditions (Nielsen et al. 2001). Measurements were made in each pool on 3 replicate days under consistently bright, sunny conditions (August 23-25 2007), before averaging the values for each pool. We corrected rates of oxygen exchange for diffusion at the water-air interface by applying a diffusion constant (K=0.32 g m^{-2} hr^{-1}) calculated for shallow (<1m) sheltered water with very limited wave action (see Kinsey 1985 for correction methodology). We standardized measures of gross primary productivity to both the total surface area of the pool (area-specific GPP; e.g. Martins et al. 2007) and the mass of macroalgae (biomass-specific GPP; e.g. Littler and Littler 1980, Chapter II).

Following measurement of oxygen flux rates, the abundances of all taxa within each pool was ascertained. Firstly, we estimated the percentage cover of encrusting coralline algae and bare rock within 4 replicate 400cm^2 quadrats (Dethier 1984). Secondly, we collected all erect macroalgae and fauna from all pools. We then sorted these in the laboratory to the finest taxonomic level possible (mostly to species), dried all these taxa in the oven at 60°C for 3 days, and reweighed them (deWreede 1985).

We calculated the total mass of macroalgae in each pool (per m^{-2} of pool substrate) by summing the masses of component species. We similarly calculated the total mass of animals that were not manipulated (hereafter 'animal biomass'). Additionally, we separately calculated both the species-specific and total mass of manipulated grazers.

Macroalgal richness (number) and diversity (Shannon's H' index, see e.g. Magurran 2004), and animal richness were all also calculated as univariate response variables.

The relative tolerance of macroalgal taxa to consumption is likely to be related to their functional morphology (Steneck and Dethier 1994). Effects of consumption are thus likely to be most evident when macroalgal taxa are grouped according to morphology and/or functional traits. Macroalgal taxa were therefore further divided into morpho-functional groups (i.e. crustose coralline, foliose, canopy, sheet-like and turf-forming), according to known functional attributes of the species (categories adapted from Littler and Littler 1981, Arenas et al. 2006). Additionally, substrate devoid of visible macroalgal/sessile invertebrate cover was included as a pseudo morpho-functional grouping ('bare rock'), to allow the incorporation of this ecosystem state into analyses of community composition.

3.2.4. Analysis

We used analysis of variance (ANOVA) and permuted multivariate analysis of variance (PERMANOVA, Anderson 2001) to examine univariate and multivariate effects of grazer manipulations, respectively. Consistent with the experimental design, both of these analyses were nested, with consumer species composition nested within richness levels (e.g. Wojdak 2005). This allowed the effects of both consumer richness and identity/composition (within richness levels) to be assessed. Where significant effects were identified in the main analysis, appropriate post-hoc pairwise comparisons were performed. Where post-hoc tests failed to identity a significant contrast at the 5% alpha level, the effect was tentatively credited to the contrast with the lowest *P*-value.

For the ecosystem functioning response variables, we used planned comparisons to test whether the 3-species mixture was significantly different from the mean monoculture

(non-transgressive overyielding, e.g. Fridley 2001). As an additional guide to identity the presence and magnitude of grazer mixture effects, we calculated the expected magnitude of ecosystem functioning response variables in multi-species treatments from the mean component monoculture (Jonsson and Malmquist 2003). We then qualitatively compared the observed magnitude of ecosystem functioning against the expected within each multi-species treatment.

To assess the effect of grazing *per se*, we included the grazer-free caged treatment (CC; as a richness = 0 level) for planned comparisons against both the mean of all grazer treatments, and against the full-complement of grazers (3-species treatment) for all univariate response variables (except for the biomass of manipulated grazers).

Univariate analyses were validated through Levene's test for heterogeneous variances, multivariate analysis through the PERMDISP2 (Anderson et al. 2006) procedure, which tests for heterogeneity in multivariate dispersion between treatments. In the univariate analyses, non-manipulated total animal biomass (hereafter 'animal biomass') and 'animal richness' were log-transformed to achieve homogeneity of variances. Transformations failed to produce homogeneous variances in 'animal diversity (H')', thus this response variable was excluded from analyses. In the multivariate analyses, PERMDISP2 identified heterogeneous multivariate dispersal among treatments for animal taxa, which standard transformations could not remove. We therefore performed PERMANOVA on untransformed data, but interpreted the results cautiously, as significant effects could be produced through differences between groups in terms of multivariate dispersal. To allow % measurements (for 'bare rock' and 'crustose coralline algae') to be comparable to dry-mass measurements of other macroalgal morpho-functional groups, all data were normalized prior to PERMANOVA analysis. Where PERMANOVA with pairwise comparisons identified significant between group differences in composition (of

macroalgal taxa, morpho-functional groups, or animal taxa), the 'Similarity Percentages' (SIMPER) procedure was used to identify those taxa/groups underlying the difference (Clarke and Warwick 2001). Multivariate analyses were performed on Bray Curtis dissimilarity measures, and permuted 999 times. Univariate analyses were performed in SPPS (SPPS inc, Chicago), multivariate analyses in PRIMER (Clarke and Warwick 2001).

To check whether the cages used to contain the manipulated grazers significantly affected response variables, we conducted both univariate and multivariate comparisons detailed above between caged (CC) and uncaged (UC) control treatments.

3.3. RESULTS

A single replicate from each of the following treatments was excluded from the experiment owing to damage to cages following winter storms: L, G and CC. The results thus pertain to the remaining replicates. Comparisons between caged and uncaged grazer-free controls indicated that cages did not have a significant effect on any of the univariate or multivariate responses ($P>0.1$ in all cases). Although grazer treatments were initially equal in total grazer biomass and approximately equilibrated at the mid-point in the experiment (see *Methods*), the biomass of grazers in all treatments had declined, to varying degrees, by the end of the 13 month experiment (Fig. 3.1). The final biomass of grazers in multi-species treatments were very similar to that expected from species-specific final biomasses in monoculture (dotted lines, Fig. 3.1).

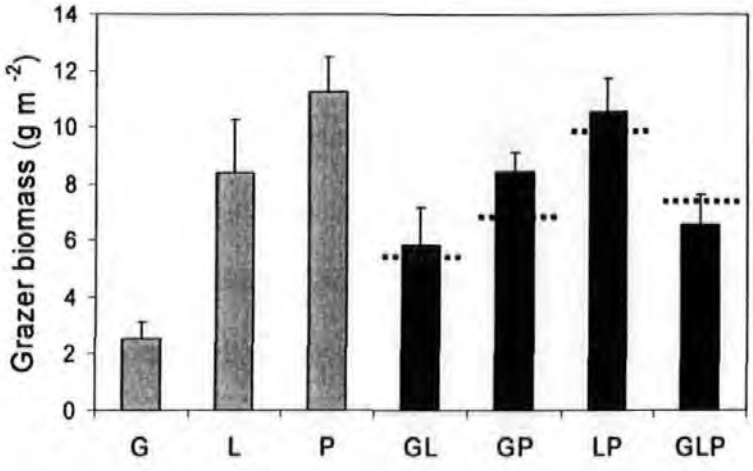


Figure 3.1. The mean (+SE) final biomass (shell-free) of manipulated grazers according to treatment. Single species treatments (light grey): G = *Gibbula*, L = *Littorina*, P = *Patella*; 2-species treatments (dark grey): GL = *Gibbula* + *Littorina*, GP = *Gibbula* + *Patella*, LP = *Littorina* + *Patella*; 3-species treatment (black): GLP = *Gibbula*, *Littorina* and *Patella*. Starting biomass was ~14g m^{-2} in each treatment; the biomass of grazers thus fell in all treatments. Dashed lines indicate the biomass expected in multiple species treatments based on the mean of component monocultures.

Table 3.1. Nested ANOVAs on accumulated biomass/productivity response variables. Animal biomass (unmanipulated species) was log-transformed (see *Methods*). Treatment codes as in Fig. 3.1. Bold numbers denote significant effects (at an alpha = 0.05 level). Only the significant (or marginally non-significant) Tukey HSD results are reported. See Fig. 3.2.

		Animal Biomass			**Macroalgal biomass**			**Area-specific GPP**			**Mass-specific GPP**		
Source of variation	**df**	**MS**	***F***	***P***	**MS**	***F***	***P***	**MS**	***F***	***P***	**MS**	***F***	***P***
Richness	3	0.07	0.269	0.846	946.5	2.549	0.185	0.159	0.036	0.99	0.01	7.82	**0.024**
Composition (richness)	4	0.266	3.078	**0.038**	367.7	0.756	0.566	4.542	3.19	**0.034**	0.001	0.246	0.909
Residual	21	0.087			486.7			1.424			0.005		
Post-hoc (Tukey HSD)		G vs. P ($P = 0.011$); L vs. P ($P = 0.018$)						GL vs. GP ($P = 0.094$)			2 vs. 3 ($P = 0.069$)		

Table 3.2. Results of PERMANOVA testing for treatment effects on the composition of macroalgae and non-manipulated animals. MF = morpho-functional group (see *Methods*). Treatment codes as in Fig. 3.1. The significance levels of pairwise comparisons are indicated in those cases where the main analysis indicates significant effects. R denotes richness levels in pairwise comparisons.

	Macroalgal taxa				Macroalgal MF groups				Animal taxa			
Source of variation	**df**	**MS**	***F***	***P***	**df**	**MS**	***F***	***P***	**df**	***MS***	***F***	***P***
Richness	3	2753.8	1.639	**0.0234**	3	1774.7	2.1863	**0.0418**	3	3429.7	1.5528	**0.0379**
Composition (richness)	4	2361.9	1.406	0.0592	4	2013.6	2.48018	**0.0227**	4	3092.9	1.4003	0.0736
Residual	21				21	811.7			21			
Comparisons	**t**	***P***			**t**	***P***		**t**	***P***		**t**	***P***
Among R					***Among R***		***Within R***		***Among R***			
0 vs. 1	1.41	**0.0255**			*0 vs. 1*	2.11	**0.0198**	*G vs. L*	0.7	0.6005	*0 vs. 1*	1.63	**0.0143**
0 vs. 2	1.31	0.0864			*0 vs. 2*	1.72	**0.0234**	*G vs. P*	1.6	0.1185	*0 vs. 2*	1.06	0.3337
0 vs. 3	1.56	0.0532			*0 vs. 3*	2.33	0.0631	*L vs. P*	2.2	**0.0317**	*0 vs. 3*	1.29	0.2001
1 vs. 2	1.34	0.0768			*1 vs. 2*	1.11	0.2914	*GL vs. GP*	2.2	**0.0323**	*1 vs. 2*	1.45	**0.0464**
1 vs. 3	1.14	0.1953			*1 vs. 3*	0.67	0.6351	*GL vs. LP*	1.6	0.0892	*1 vs. 3*	0.82	0.6709
2 vs. 3	1.04	0.3818			*2 vs. 3*	1.22	0.2293	*GP vs. LP*	1.1	0.2691	*2 vs. 3*	1.11	0.2924

3.2.1. Macroalgal biomass

The presence of grazers produced a relatively small effect on macroalgal biomass, as shown by the comparison between pooled grazer treatments and the caged control (CC) (Fig. 3.2A, $F_{1,28} = 0.515$, $P = 0.479$). There was no evidence of a grazer richness effect at the 2-species level, with macroalgal biomass actually slightly exceeding that expected from single-species treatments (Fig. 3.2A). There was also no evidence of 'overyielding' at the highest grazer richness level - GLP did not differ from the mean monoculture (planned comparison, $F_{1,13} = 1.147$, $P = 0.305$). The overall analysis confirmed that neither grazer richness nor composition affected macroalgal biomass (Table 3.1). Notably however, a qualitative comparison indicates that with all 3 species present (GLP), macroalgal biomass was reduced to a level comparable to that of the best-performing monoculture (P) and lower than that predicted from single-species effects (Fig 3.2A). Indeed, both P and GLP treatments reduced macroalgal biomass to a mean level approximately 50% lower than the caged control; although these effects were not detected statistically, in part due to among-replicate variability (Fig. 3.2A).

3.3.2. Animal biomass

Across all the treatments, grazers did not affect animal biomass, on average, compared to the grazer-free controls ($F_{1,28} = 0.742$, $P = 0.396$). GLP did not differ from caged controls ($F_{1,6} = 0.656$, $P = 0.455$), or the mean monoculture ($F_{1,13} = 0.042$, $P = 0.84$). The biomass of animals was not affected by grazer richness (Fig. 3.2B, Table 1). However, grazer species composition had a striking effect on this response variable within the single species level; specifically, compared to the P treatment, animal biomass was over 4 times greater in both the G and L treatments (Fig. 3.2B). This effect was conspicuously absent

when these species were combined (GL), as the animal biomass was far lower than expected.

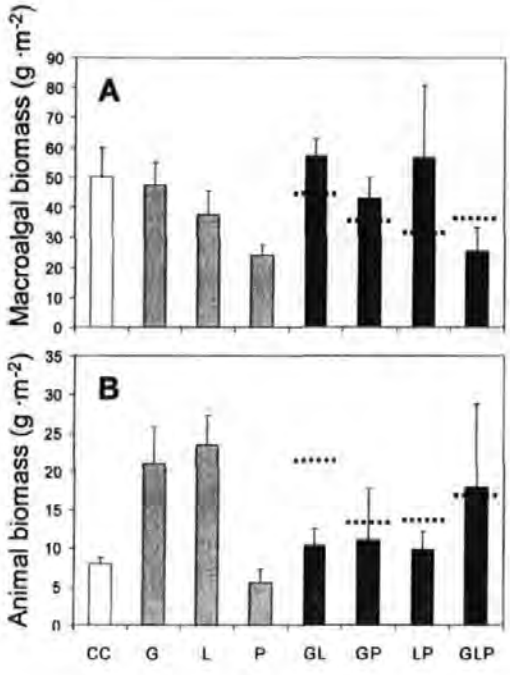


Figure 3.2. Treatment means (+SE) of accumulated macroalgal biomass (A) and accumulated non-manipulated animal biomass (B). There was no detectable effect of treatment on macroalgal biomass (A). Species composition affected mean animal biomass (B), which varied significantly between treatments at the single-species level (G vs. P, and L vs. P). White bar = caged control (CC); all other treatment codes as in Figure 3.1. Dashed lines indicate the expected level of each response variable in multiple species treatments based on the mean of component monocultures.

3.3.3. Macroalgal composition

The composition of the macroalgal assemblage, both in terms of taxa and morpho-functional (MF) groups, was affected by the presence of grazers (i.e. richness 0 (CC) vs. 1, 2 and 3 grazer species). At the level of macroalgal taxa, the largest difference was between the caged control (CC) and the 1-species level, although marginally non-significant

differences were also evident between CC and the 2 – and 3 – species levels (Table 3.2). A considerable proportion of these differences could be attributed to the higher abundance of *Ulva* spp. within grazer-free (CC) pools (Table 3). The kelps *Laminaria digitata* and *Sacchoriza polyschides*, as well as the red alga *Palmaria palmata* were also more abundant within the caged controls (Table 3.3). Certain taxa were, however, in greater abundance in grazer treatments, i.e. *Corallina* spp., *Ceramium* spp. and *Himanthalia elongata* (Table 3.3). In terms of macroalgal MF groups, the effect of grazer presence was also strongest between caged-control and 1-species grazer treatments, but the 2-species treatments also had a highly significant effect (Table 3.2). The 3-species effect was weaker, but remained marginally non-significant. The difference between caged-control and grazer treatments was largely driven by the reduced abundance of sheet-like macroalgal forms, and the greater cover of bare-rock within grazer treatments (Table 3.4). Reduced abundance of canopy-forming and foliose algae on the one hand, and increased abundance of turf-forming and crustose coralline forms on the other, also contributed substantially to the differences (Table 3.4). With grazers present, no differences occurred among richness levels (i.e. 1, 2 or 3 species,) in terms of either macroalgal taxa, or MF group composition (Table 3.2).

Grazer species composition affected macroalgal composition at the level of MF group, but not taxa (Table 3.2). Within the 1-species grazer level, L and P differed in effects on macroalgal MF groups. P modified the composition of primary rock cover, reducing the cover of crustose coralline algae, with a correspondingly higher cover of bare rock than the L treatment. Additionally, foliose and canopy groups were both lower in the P treatment (Table 3.4). The different effects of P and L on primary rock cover and foliose algae in monoculture were largely maintained when these species were combined with G, explaining the compositional effect (GL vs. GP) at the 2-species level. Turf-forming and

sheet-like groups were also more abundant in the GL treatment; whereas canopy algae were slightly lower (Table 3.4).

Table 3.3. Results of SIMPER analysis identifying the contributions of macroalgal taxa to significant differences between caged control (CC) and single species treatments (see Table 2). Treatment codes as in Fig. 1. The average abundances (Ave.) are indicated, in addition to the percentage contribution to the overall difference between the treatments (%). Only taxa contributing >5% are included. In descending order of % contribution to treatment difference.

	CC	1 sp.	
	Ave.	Ave.	%
Ulva spp.	16	4.1	27.4
Laminaria digitata	4.7	3.4	9.8
Palmaria palmata	4.2	1	8.6
Saccorhiza polyschides	3.6	1.9	8
Corallina spp.	0.9	3.2	6.7
Ceramium spp.	2	3.3	6.4
Himanthalia elongata	2.3	3.5	6.5

Table 3.4. Results of SIMPER analysis identifying the contributions of macroalgal morpho-functional groups to significant differences between pairs of treatments (see Table 2). Treatment codes as in Fig. 1. The average abundances (Ave.) in each pair of contrasts is indicated, in addition to the percentage contribution to the overall difference between the treatments (%). Only groups contributing >5% are included. The top two contributors to each comparison are marked in bold.

	L	**P**		**GL**	**GP**		**CC**	**1 sp.**		**CC**	**2 spp.**	
	Ave.	**Ave.**	**%**	**Ave.**	**Ave.**	**%**	**Ave.**	**Ave.**	**%**	**Ave.**	**Ave.**	**%**
Bare rock	39.95	80.3	**45.34**	23.61	58	**33.15**	**27.92**	59.36	**36.73**	27.9	43.53	**22.76**
Crustose coralline	21.67	1.59	**23.76**	13.22	9.18	13.2	3.9	10.88	10.1	3.92	13.95	13.38
Foliose	21.51	4.88	18.68	22.01	6.24	**15.9**	15.08	11.61	11.92	15.1	11.02	11.49
Canopy	8.19	5.48	5.4	13.68	14.6	12.5	19.94	8.9	13.02	19.9	10.53	15.94
Sheet-like				10.76	3.7	7.6	**25.82**	3.95	**20.85**	25.8	7.03	**19.84**
Turf-forming				15.94	2.69	12.75				1.24	11.28	10.76

Table 3.5. Results of SIMPER analysis identifying the contributions of animal taxa to significant differences between caged control (CC) and single species treatments, and between single species and 2-species treatments (see Table 3.2). Treatment codes as in Fig. 1. The average abundances (Ave.) are indicated, in addition to the percentage contribution to the overall difference between the treatments (%). Only taxa contributing >5% are included. The top two contributors to each comparison are marked in bold.

	CC	1 sp.		1sp.	2 spp.	
	Ave.	Ave	%	Ave.	Ave	%
Psammechinus miliaris	0	3.39	**21.73**	3.39	0.02	**18.85**
Mytilus edulis	0.51	2.55	**16.17**	2.55	0.8	
Gobiusculus flavescens	1.54	0.56	10.3			
Polyplacophora spp.	0.29	1.59	9.64	1.59	0.14	8.09
Lipophrys pholis	1.78	1.03	9.53	1.03	1.79	10.38
Gibbula cineraria	0	1.29	6.35	1.29	1.2	10.25
Idotea granulosa	0.66	0.41	5.83			
Carcinus maenas				2.55	0.8	**12.67**

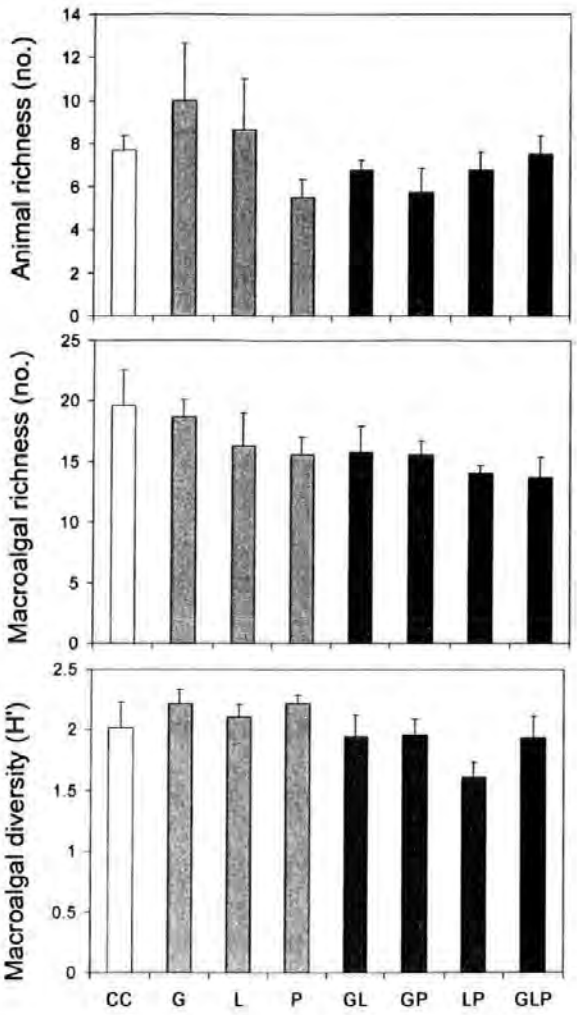


Figure 3.3. Treatment means (+SE) of non-manipulated animal richness (A), macroalgae richness (B) and macroalgae diversity (C). There were no effects of treatment on any of these response variables (Table 3.2). Treatment codes as in Figure 3.1.

Table 3.6. Nested ANOVAs on species richness/diversity response variables. Animal diversity was omitted from analyses as the assumptions of ANOVA could not be met (see *Methods*). A = animal, MA = macroalgae. There were no significant differences according to treatment (see Fig. 3.3).

		MA richness			MA diversity (H')			A richness (Log)		
Source of variation	**df**	**MS**	**F**	**P**	**MS**	**F**	**P**	**MS**	**F**	**P**
Richness	3	25.281	4.037	0.094	0.219	2.603	0.182	0.012	0.38	0.774
Composition (richness)	4	6.233	0.533	0.713	0.084	1.028	0.416	0.033	1.617	0.207
Residual	21	11.69			0.082			0.02		

3.3.4. Animal composition

The composition of animals was affected by the richness of manipulated grazers in complex ways. Differences occurred between both grazer-free controls and 1-species treatments, as well as between 1-species and 2-species treatments (Table 3.2). Compared to the grazer-free control, the single species of grazer increased the biomass of the urchin *Psammechinus miliaris* and the mussel *Mytilus edulis*, whilst decreasing the abundance of 2 species of fish: the shanny *Lipophrys pholis* and two-spot goby *Gobiusculus flavescens*. Increasing richness from 1 – to 2 – species, caused an opposite effect on 3 of these taxa, reducing urchins and mussels, whilst increasing the abundance of the shanny species. Additionally, chitons Polyplacophora spp. and the grey topshell *Gibbula cineraria* were both lower in two species treatments (Table 3.5). It must be noted however, that treatment effects on animal composition must be interpreted cautiously due to heterogeneity of multivariate dispersion (see *Methods*).

3.3.5. Animal and macroalgal richness/diversity

Grazer treatment did not produce any clear differences in macroalgal richness or diversity (H') or animal richness (Table 3.6, Fig. 3.3). There was, however, a marginally non-significant potential effect of grazer species richness on macroalgal richness (P = 0.094). Mean macroalgal species richness declined gradually, though consistently, from zero grazers, through 1, 2 and all 3 grazer species (Fig. 3.3).

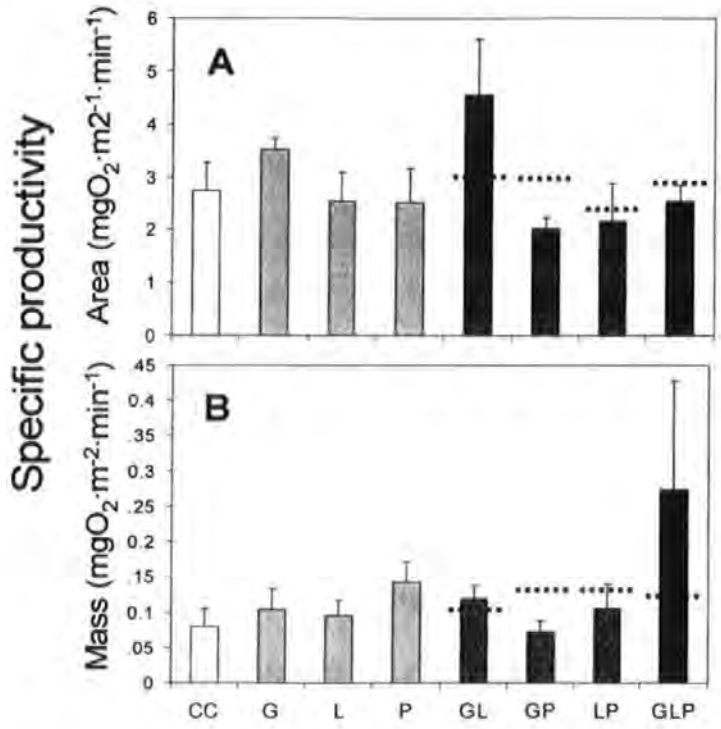


Figure 3.4. Treatment means (+SE) of area-specific (A) and mass-specific GPP (B). A significant composition effect in area-specific GPP (A), can be attributed to the marginally non-significant difference between GL and GP treatments. Mass-specific GPP varied significantly with grazer richness, and marginally non-significantly between 2 species and 3 species treatments. See Table 3.1 for ANOVAs and post-hoc tests. White bar = caged control (CC); all other Treatment codes as in Figure 3.1. Dashed lines indicate the expected level of each response variable in multiple species treatments based on the mean of component monocultures.

3.3.6. Gross primary productivity (GPP)

Area-specific GPP varied according to grazer species composition, but in this case the largest between-group difference occurred within the 2-species level (GL vs. GP, Tukey hsd = marginally non-significant, $P = 0.094$, Fig.2A, Table 3.1). GP exhibited a lower rate of GPP than expected based on monoculture effects. There was no such compositional effect for biomass-specific GPP, although richness affected this measure of ecosystem functioning. The source of this difference was identified as the contrast between 2 – and 3 – species treatments (Tukey hsd = marginally non-significant, $P = 0.069$, Table 3.1). There was a high level of variability within the GLP treatment however (Fig. 3.2B), precluding strong conclusions regarding this treatment effect.

3.4. DISCUSSION

Our results show that the effects of consumers on the structure and functioning of developing rock pool communities were largely determined by the identity of the species present, not richness. Treatments containing the limpet *Patella ulyssiponensis* had the largest effects on both the composition, and the standing stock, of the macroalgal prey. Contrary to the typical result of species richness manipulations (Cardinale et al. 2006), no ecosystem-level response variable showed evidence of overyielding when all 3 grazers were present.

Our findings must be interpreted in light of changes in grazer biomass that occurred during the experiment. Total grazer biomass had fallen during the experiment in all treatments, indicating that grazer mortality exceeded growth. There were marked differences between species in terms of final biomass (Fig. 3.1), despite equilibration of biomasses at the mid-point in the experiment. The relatively low density of G at the single

species level (Fig. 3.1, Table 3.1) was the most striking difference (Fig. 3.1). This implies that the carrying capacity for G may have been lower than the other 2 species. Although in field surveys G reached the high densities established at the start of the experiment, the mean field density on local shores is far lower for G compared to both P and L (Griffin, J. *unpublished data*), mirroring findings from the experiment. The concordance between field and experimental data supports the hypothesis that, in terms of biomass, G cannot compensate for the loss of the other 2 species (P and L). Food limitation of G provides a possible explanation, given that G has the smallest mean body size of all the grazers used here and relative metabolic demand is greater in smaller-bodied organisms (West et al. 1997). In this experiment, as in natural pools, species-specific grazing effects cannot be separated from biomass effects. This should not be considered as a confounding effect; rather, biomass should be considered as a characteristic of each species that in part may determine their impacts.

In line with previous work (e.g. Farrell 1991, Lotze et al. 2001, Belliveau and Paul 2002) the presence of grazers generally accelerated successional turnover of macroalgal taxa and morpho-functional groups, shifting dominance from sheet-like ephemerals to grazer-resistant crustose and turf-forming coralline algae (Table 3.4). The fact that these shifts in species composition generally acted to buffer primary producer biomass against grazer impacts is consistent with a microcosm study (Norberg 2000), and shows that grazer effects may not be detectable at the ecosystem level, despite changes in prey composition. There was, however, an exception. Our results show that *Patella* can negatively affect even the most grazer-resistant of prey species, resulting in large areas of bare rock in place of the crustose coralline algae that dominated other grazer treatments (Table 3.4). This explains the relatively low total macroalgal biomass in the P monoculture (Fig. 3.2A). The strong effect of P on these response variables was likely related to a combination of high biomass

(Fig. 3.1) and the deep rasping foraging strategy of this species (e.g. Steneck and Walting 1982, Hawkins and Hartnoll 1983), which may remove even the most robust of macroalgal propagules and germlings. Our study adds a successional perspective to the study of O'Connor and Crowe (2005), and shows that *Patella* is indeed a key grazer in the rocky intertidal system studied irrespective of whether mature or developing prey assemblages are considered. This species-specific effect of grazers on prey composition and biomass urges caution in the functional grouping of grazers in experiments (Paine 2002, Worm 2002) and models (e.g. Mumby et al. 2007).

The elevated animal biomass in both G and L monocultures, compared to grazer-free controls (Fig. 3.2DB), suggests that a shift in the composition of the algal community had a beneficial effect on recruitment of animals, perhaps due to reduced competition for space (Duffy 2003). In contrast, the P monoculture had no such effect. Although P reduces competition for space by consuming macroalgae, it may reduce invertebrate colonization by both a 'bulldozer' effect, and direct propagule consumption. Analogously, intense urchin grazing on coral reefs can lead to overgrazing and inhibition of coral recruitment (e.g. Bellwood et al. 2004), even though moderate grazing pressure typically enhances coral recruitment (Mumby et al. 2007).

In the multi-species treatments, animal biomass and composition responded unpredictably, possibly due to the propagation of indirect effects of grazer composition and richness. This highlights the complexities involved in predicting the direct and cascading impacts of species loss in multi-trophic systems (Downing and Leibold 2002, Duffy et al. 2007).

It must be noted that in addition to these marked effects of P, there were instances of possible species complementarity effects. Firstly, there was a large effect of grazing on macroalgal biomass (exceeding expected and comparable to the P monoculture) evident in

the GLP treatment, but not in the 2-species combinations (Fig. 3.2A). Secondly, animal biomass was lower when L and G were combined than predicted from their effects in monoculture (Fig. 3.2B). Elucidation of the mechanistic basis of these apparently idiosyncratic effects is beyond the scope of this study. Nevertheless, these results indicate that certain ecosystem properties may not be predicted from knowledge of species' independent effects, instead requiring a detailed understanding of species interactions (see also Jonsson and Malmquist 2003).

Previous work has found marked effects of grazing on macroalgal richness and diversity (Lubchencho 1978, Worm 2002). Here however, with the exception of a weak grazer richness effect on macroalgal richness (Table 3.6, Fig. 3.3B), measures of macroalgal and animal biodiversity were relatively insensitive to grazer presence, composition or richness (Table 3.6, Fig. 3.3). Impacts on species richness and diversity may be limited by escapes from grazing in rock crevices (Lubchencho 1983), associational defenses achieved by growth on defended species (e.g. Pfister and Hay 1988) or the shells of *Patella* (Griffin, J. *Personal observation*). The marginally non-significant, but consistent, decline in macroalgal species richness with grazer richness (Table 3.6, Fig. 3.3B) suggests synergistic grazer impacts through complementarity (Duffy 2002), and represents the only consistent effect of grazer richness found in this study. Further, more statistically powerful, experiments would be required before this weak effect can be confirmed.

The differences among treatments in the composition and total biomass of macroalgae were generally dampened in terms of a key process exhibited by the macroalgae i.e. area-specific gross primary productivity (GPP; Fig. 3.2A). The exception was the difference between treatments within the 2-species level – with GL exceeding GP, likely reflecting the different effects of these grazer combinations on the macroalgal

assemblage; the second leading to overgrazing and a high coverage of bare rock (Table 3.4). Although only a weak effect, P grazing enhanced biomass-specific GPP above that of the other grazer treatments (Fig. 3.2B), explaining the dampened effect of P on GPP compared to its strong effect on macroalgal biomass. Mean biomass-specific GPP was far higher in the GLP treatment than 2 species treatments and the expected level (Fig. 3.2B), suggesting that grazer richness may be important in the stimulation of GPP.

Complementarity of grazer species effects on macroalgal species may have stimulated productivity through reduced self-shading (Carpenter et al. 1986), localized nutrient input (Bracken and Neilson 2004) and/or reduced thickness of non-photosynthetic tissue (Littler et al. 1995, Wai and Williams 2005) in a greater proportion of the assemblage, such that energy uptake (photosynthesis) and transfer (grazing) were simultaneously enhanced, whilst standing stock was relatively unaffected. Grazer stimulation of primary productivity would render changes in the standing stock of primary producers an incomplete index of resource consumption and grazer effects on primary producers. A further implication is that caution is required in the interpretation of standing stock as an indication of the rate of primary productivity (but see Paine 2002).

Counter to theory (Holt and Loreau 2001, Thébault and Loreau 2003), and several consumer diversity experiments (Duffy 2003, Gamfeldt et al. 2005, Matthiessen et al. 2007) our results imply that species richness effects are relatively unimportant in determining the effectiveness of grazing in the rock pools studied. The context-dependency of the effect of species complementarity offers a possible explanation. For example, the benefits of resource partitioning may be dependent on the importance - and magnitude of - competitive release (Griffin et al. 2008). In our experiment, both P and L maintained a high proportion of biomass in monoculture, suggesting that competition within these dominant species was relatively weak, possibly precluding an effect of resource partitioning on grazer biomass.

Another important consideration is that, both within and among pools, the conditions were relatively homogeneous, which may have prevented interspecific complementarity from being realized in terms of resource use partitioning (Cardinale et al. 2004, Griffin et al. 2008).

Strong effects of species identity/composition, as documented here, have been a common feature of biodiversity-ecosystem functioning research in studies ranging through primary producers (e.g. Bruno et al. 2005) consumers (e.g. Duffy et al. 2001) and predators (e.g. Straub and Snyder 2006). The obvious implication is that to understand and predict ecosystem functioning we need to primarily consider *which* species are present (or lost). However, over larger temporal and spatial scales, species complementarity, and thus diversity, may well become important (Cardinale et al. 2004, Cardinale et al. 2007). In relation to our study, experiments including the full range of habitat types and environmental contexts over which the focal species coexist, and over multi-generational time periods, would be required before it could be concluded that the ecosystem functions measured here could be maintained by the single dominant species, *Patella ulyssiponensis*.

PREDATOR DIVERSITY AND ECOSYSTEM FUNCTIONING: DENSITY MODIFIES THE EFFECT OF RESOURCE PARTITIONING

CHAPTER IV

ABSTRACT

The link between biodiversity and ecosystem functioning is now well established, but the challenge remains to develop a mechanistic understanding of observed effects. Predator-prey interactions provide an opportunity to examine the role of resource partitioning, thought to be a principal mediator of biodiversity-function relationships. To date, interactions between multiple predators and their prey have typically been investigated in simplified agricultural systems with limited scope for resource partitioning. Thus there remains a dearth of studies examining the functional consequences of predator richness in diverse food webs. Here, we manipulated a species-rich intertidal food web, crossing predator diversity with total predator density, to simultaneously examine the independent and interactive effects of diversity and density on the efficiency of secondary resource capture. The effect of predator diversity was only detectable at high predator densities where competitive interactions between individual predators were magnified; the rate of resource capture within the species mixture more than doubled that of the best-performing single species. Direct observation of species-specific resource use in monoculture, as quantified by patterns of prey consumption, provided clear evidence that species occupied distinct functional niches, suggesting a mechanistic explanation of the observed diversity effect.

KEYWORDS: Resource partitioning; predator; biodiversity; ecosystem functioning; trophic interactions; BEF; food web; density;

4.1 INTRODUCTION

Concerted empirical research over the last decade has shown that species richness can enhance the magnitude of ecosystem functioning in a range of systems (Hooper et al. 2005, Balvanera et al. 2006, Cardinale et al. 2006). Despite this emerging paradigm, the ability to predict the functional consequences of species loss in a given system remains elusive because the strength of species diversity effects has proven highly variable across studies (Balvanera et al. 2006). To transform biodiversity-ecosystem functioning research into a predictive science a clear mechanistic understanding of observed effects is required (Yachi and Loreau, 2007).

Although early seminal research on the effect of biodiversity on ecosystem functioning focused on temperate grassland communities (Naeem et al. 1994 Tilman et al. 1996), recognition that species in higher trophic levels are more vulnerable to extinction (Petchey et al. 1999, Duffy 2002) has recently shifted attention onto multi-trophic systems (e.g. Duffy et al. 2007). Despite the obvious differences between primary producers and mobile, behaviourally complex predators, theory predicts that both sampling and complementarity effects operate to enhance the magnitude of resource capture, irrespective of trophic level (Thébault and Loreau 2003, Ives et al. 2005). The sampling (Huston 1997) – or positive selection (Loreau 2000) – effect occurs when species with extreme trait values dominate resource acquisition and are more likely to be included in diverse species mixtures by chance. In this case, the functioning of the diverse mixture will not exceed that of the best monoculture. In contrast, where niche complementarity is evident, species differing in resource requirements exploit a wider spectrum of resources and experience reduced interspecific competition, thus potentially enhancing the rate of ecosystem processes beyond that of the best-performing single species (Trenbath 1974, Loreau and Hector 2001). Direct evidence of resource partitioning is rare despite recognition of its

importance in mediating species coexistence (Chesson 1991). Instead, functional complementarity is often inferred from diversity effects that exceed those that can be explained by the sampling effect alone (Loreau and Hector 2001, Duffy et al. 2003). In contrast to primary producers, however, the resource use patterns of predators can be readily assessed, potentially enabling tractable studies of the functional consequences of diversity mediated through niche partitioning (Ives et al., 2005).

A rich history of experiments examining trophic interactions between multiple predators and their prey has identified the complexities of predicting aggregate predator effects on prey consumption (e.g. Losey and Denno 1998, Finke and Denno, 2004, Siddon and Witman 2004). Such work has become increasingly integrated with research on the link between biodiversity and ecosystem functioning through experiments that maintain total predator density across gradients of species richness (e.g. Wilby et al. 2005, Straub and Snyder 2006). Due to the strong focus on agricultural pest control, previous studies have typically included very low levels of prey heterogeneity (but see Bruno and O'Connor 2005, Byrnes et al. 2006), thereby limiting the potential for resource partitioning among predators. The role of predator diversity within diverse natural food webs thus remains largely unknown.

The density of organisms within natural communities is determined not only by resource availability, but by a suite of factors that may act to maintain density below the carrying capacity of the environment. For example physical and biological disturbance (e.g. Sousa 1984), recruitment limitation (e.g. Connolly and Roughgarden 1998) and top down control (including human exploitation) (e.g. Castilla 1999) are all known to influence density independently of local resource availability. Assuming constant resource availability, the density of a species will determine the strength of intraspecific and interspecific competition, which can in turn influence the effect of species diversity on

ecosystem functioning (Cardinale et al. 2004, Weis et al. 2007). Despite the critical role that competition may play in linking diversity and ecosystem functioning, the potential role of density in modifying diversity effects mediated by niche partitioning has yet to be tested empirically.

The efficiency of resource capture by consumers, as measured by the rate of prey consumption, represents an important ecosystem process within complex food webs (Duffy 2002) and allows investigations of multi-trophic interactions within the context of theory linking biodiversity and ecosystem functioning. We conducted a factorial mesocosm experiment to test the hypothesis that predator density modifies the effect of predator species richness on the efficiency of trophic energy transfer between diverse intertidal prey assemblages and three species of predatory crabs. Our results show that clear effects of diversity only occurred at high predator density. Furthermore, our results suggest that predator species exhibited marked differences in the patterns of resource use, representing functional complementarity through resource partitioning.

4.2 METHODS

We manipulated the diversity and density of three common intertidal predatory crabs, *Cancer pagurus*, *Necora puber* and *Carcinus maenas*, in outdoor mesocosms, 0.6m x 0.4m (area) x 0.2m (depth), which were subject to a constant flow of fresh filtered seawater at the Marine Biological Association Laboratory in Plymouth, UK. The predator species are abundant and have overlapping distributions within rocky intertidal habitats on the south west coast of the UK. Furthermore, these predators have similar body sizes, enabling the isolation of species identity and diversity effects from those of body size, which can have a strong influence on trophic interactions (Jennings et al. 2001).

We employed a substitutive design, frequently used in biodiversity-ecosystem functioning studies (e.g. Polley et al. 2003, Bruno et al. 2005) including those examining predator diversity effects (Siddon and Witman 2004, Byrnes et al. 2005, Snyder et al. 2006), such that overall density was maintained across a gradient of diversity. We crossed a substitutive gradient of predator diversity with total overall density, allowing the independent and interactive effects of diversity and density to be examined simultaneously (Benedetti-Cecchi 2004). Four different predator assemblages were constructed, three with each species in monoculture and one with all three species combined. Density consisted of two levels: low with three, and high with six predator individuals in total. The high diversity predator community thus contained one and two individuals of each of the three species at low and high density, respectively. These densities are commonly observed in the intertidal at small spatial scales, where crabs aggregate in areas of suitable refugia. We defined a narrow size range for each species to ensure approximately equal masses (52 ± 2g wet biomass) of individuals within and among species. Predator-free controls were included to confirm that all reductions in prey were due to consumption. Each treatment was replicated 6 times in total, but limited space to house mesocosms restricted our experiment to a randomized, temporally blocked design, such that two replicates of each of nine treatments were included in three temporal blocks. The start of each block was separated by two weeks to enable collection of animals during spring tides.

Predators were collected from under boulders within the intertidal zone at Looe, Cornwall, UK. To exclude effects of satiation at the time of collection, crabs were starved for 4 days prior to experiments in large holding tanks with continuous flowing seawater containing numerous refuges to limit potentially harmful agonistic interactions. Each experimental mesocosm contained a 3cm layer of coarse sand and gravel upon which rocks of a range of sizes were arranged to re-create a topographically complex, refugium-rich

environment mimicking rocky shores (Appendix G). Habitat-forming materials were collected from local shores and rinsed with fresh water to remove macro-biota before introduction to mesocosms. We covered each mesocosm with a tightly fitting fine nylon mesh to ensure animals were unable to escape. The experiment was conducted between January and March 2007.

Initial prey composition and abundance did not vary among mesocosms (approximately 8.71g dry weight in total, Appendix B). Five species of prey were used, all naturally co-occurring and collected from the intertidal zone of local rocky shores. Barnacles (*Chthamalus* spp.) could not be transplanted into mesocosms individually, and were therefore transplanted in dense aggregations attached to pieces of rock. Individuals were counted before adding the rock pieces to the mesocosms. For all other prey species we included six individuals within each of two size classes (large and small) to incorporate an element of resource heterogeneity occurring in natural prey populations. Mussels *Mytilus edulis*, periwinkles *Littorina littorea*, topshells *Gibbula umbilicalis*, and limpets *Patella ulyssiponensis*, were all added to the mesocosms three days before the start of each experiment (see Appendix A).

We used the number and identity of prey absent from each mesocosm upon termination of the experiment to calculate consumption rates for each replicate predator assemblage. We calculated the consumption rate (shell-free dry biomass per day) of each prey type separately to elucidate patterns of prey consumption, and summed these to derive the total rate of prey consumption (Appendix C). We also derived per capita rates to facilitate direct comparisons across density levels. Based on the consumption of each prey type in monoculture, we calculated expected patterns in polyculture and derived proportional deviations from expected (Appendix C). A relatively short experimental duration (five days) was chosen so that extinction of prey types did not occur within

mesocosms, which could prompt prey-switching, blurring initial resource use differences between species and potentially weakening the diversity effect (Ives et al. 2005).

Non-transgressive overyielding occurs when the magnitude of an ecosystem process in a diverse mixture exceeds that of the average monoculture value of component species (Fridley 2001). To test for this form of diversity effect, and for the potential interactive effect of total density upon it, we performed a two-way analysis of variance (ANOVA), followed by *a priori* planned contrasts between the average single-species and the polyculture treatment at both densities. Inclusion of all three species in monocultures facilitated a test of transgressive overyielding, which identifies effects of diversity that could not be produced by the dominance of any single component species. To test for this effect we performed *a priori* planned contrasts between the best performing monoculture and the polyculture (Bruno et al. 2005, Duffy et al. 2005) at both low and high densities separately. We conducted a permutated multivariate ANOVA (PERMANOVA, Anderson 2001) to test for differences in the composition and relative abundances of prey types consumed by the three predator species independently and in combination. This provided a test of resource partitioning among predator species in terms of the multivariate prey base.

4.3 RESULTS

We recovered all prey items from predator free controls upon termination of the experiment, confirming that all prey losses were due to predator consumption rather than other causes of mortality or escapes. All crabs survived the experiment uninjured, showing no evidence of physically harmful agonistic interactions or intra-guild predation. There were no significant differences among temporal blocks or interactions between block and experimental treatments (Diversity x Block $F_{6,24}$ =0.82, P= 0.57; Density x Block $F_{2,24}$ =

0.66, P=0.53; Block $F_{2,24}$=2.5, P=0.11), hence these data were pooled for subsequent analysis.

The monocultures of the three predator species captured similar prey quantities within density levels (Fig 4.1, Appendix D). When species were combined in the polyculture there was significantly greater consumption compared to the pooled single species treatments (P=0.003, Appendix E). This diversity effect was, however, dependent upon predator density, with the polyculture out-performing the mean (non-transgressive overyielding) and the maximum (transgressive overyielding) monoculture among the high density treatments only. Specifically, at high density the predator mixture more than doubled the rate of prey consumption, exceeding the average monoculture by 144% ($F_{1,20}$=10.64, P=0.004) and the best-performing monoculture by 134% ($F_{1,11}$=6.02, P=0.029).

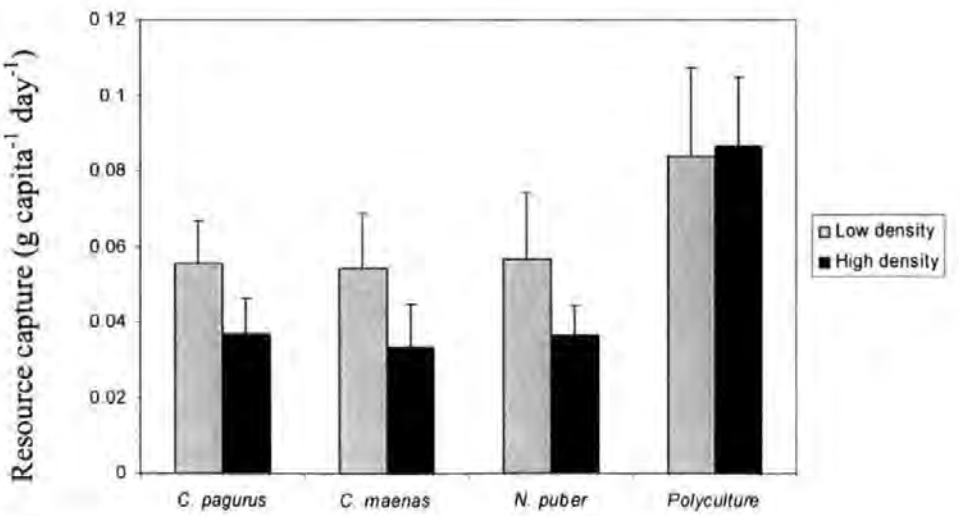


Figure 4.1. Mean (± SE) aggregate *per capita* resource capture rate for all monocultures and the three species mixture at both low and high densities (PC_{Total}, see Appendix C for calculation) (n=6). Significant differences occurred between monocultures and the mixture across high – but not low – density treatments. See text and Appendix E for ANOVA results.

At low density the effect of diversity was far weaker, with the aggregate rate of resource capture of the polyculture only 52% ($F_{1,20}$=2.11, P=0.16) above the average monoculture and 48% ($F_{1,11}$=0.90, P=0.364) above the highest single species treatment (Fig 4.1., Appendix E). The differential effects of diversity between low and high density comparisons can be understood by comparing the effect of density in monocultures and the polyculture. A doubling of monoculture density only marginally elevated total resource capture (Appendix D), resulting in a density-dependent suppression of per capita resource capture efficiency within respective monocultures (marginally significant at an α level of 0.1, $F_{1,30}$=3.03, P=0.09). In the polyculture however, increasing density led to a large increase in total resource capture (Appendix D); density thus had no effect on per capita rates within the polyculture (Fig. 4.2, P=0.93)

All three predator species in monoculture differed markedly in patterns of prey consumption (Fig. 4.2). Whilst all predators demonstrated an ability to consume every prey species, clear and consistent differences between species in the patterns of trophic interactions were evident, with particular prey types most heavily consumed by specific predators (Fig. 4.2). PERMANOVA, with *post-hoc* pair-wise comparisons, confirmed that all the predators exhibited distinct patterns of resource use ($F_{3,40}$ = 3.98, P< 0.001, Appendix F). Moreover, although density significantly affects overall patterns of prey consumption ($F_{1,40}$ = 4.14, P = 0.01), species-specific differences were robust to changes in total density (interaction $F_{3,40}$ = 0.42, P = 0.95).

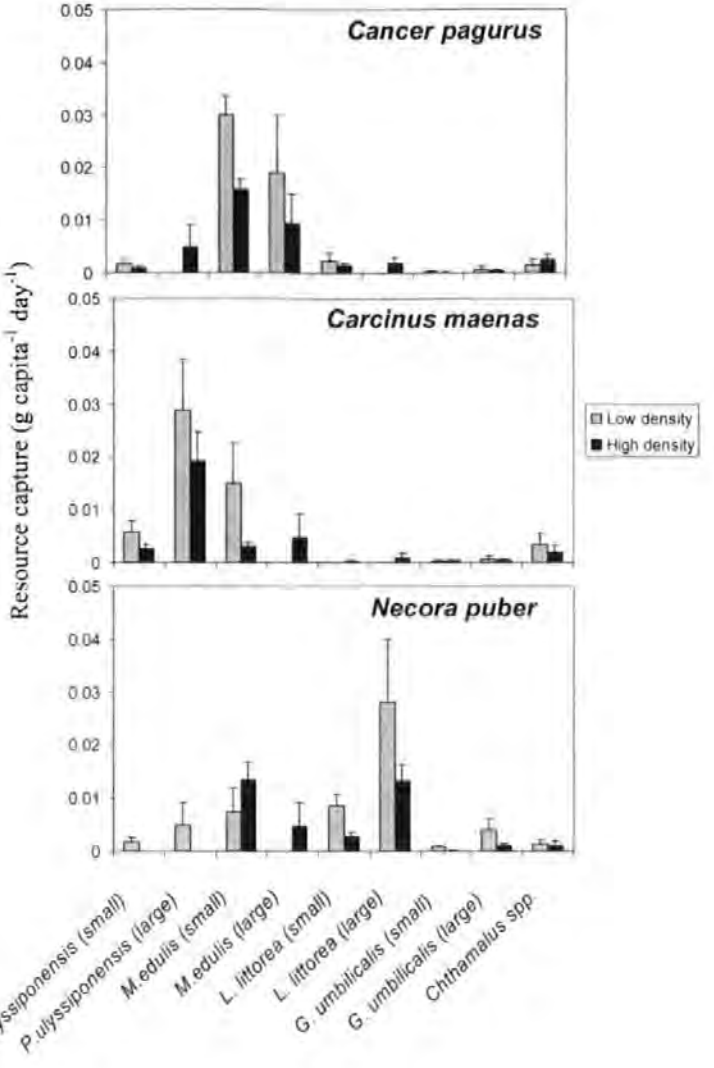


Figure 4.2. Mean (± SE) observed rates of consumption (resource capture) of each prey type for individual predator species (PC_{ij}, see Appendix C for calculation) at two predator densities (n=6). Predators exhibited significantly different patterns of prey consumption (Appendix F).

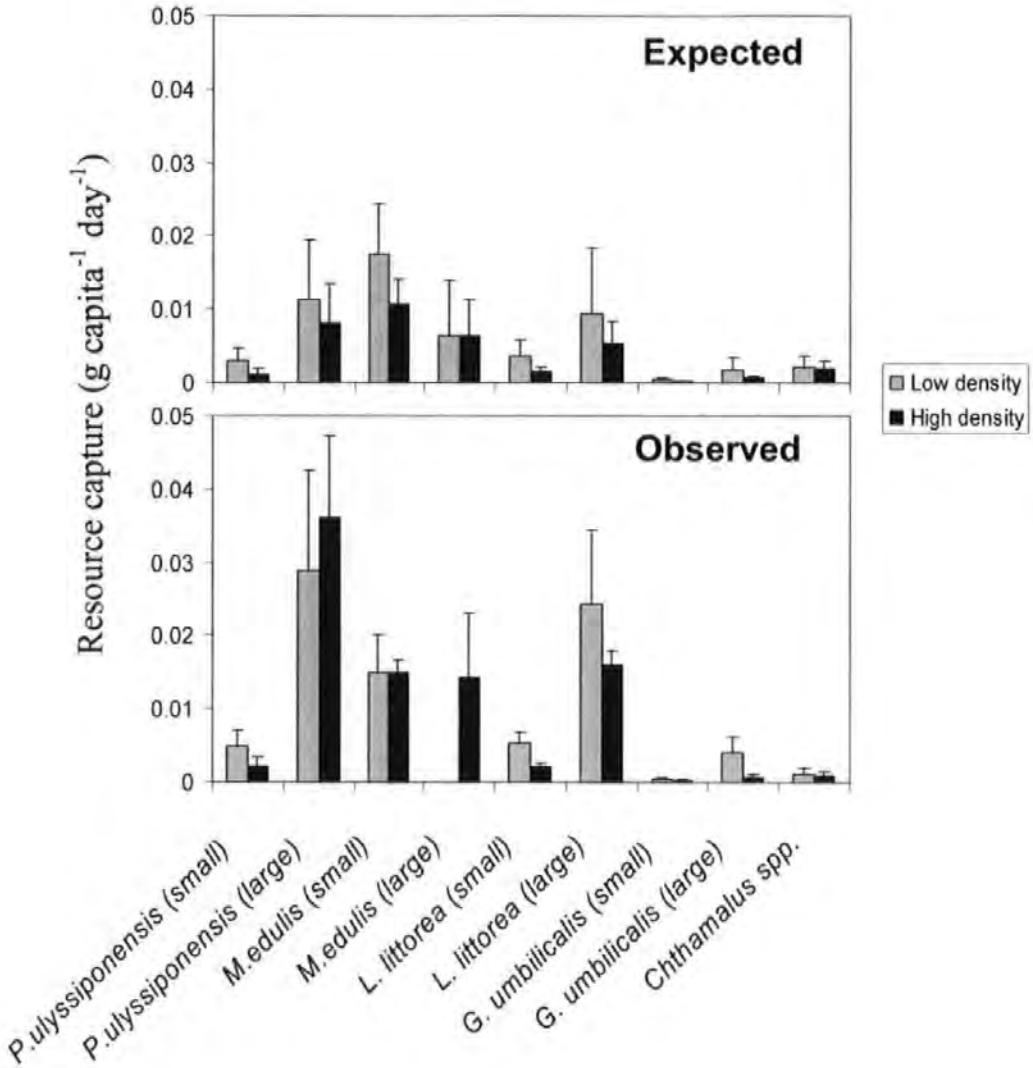


Figure 4.3. Mean (± SE) expected (Pe_{ij}) and observed (PC_{ij}) rates of resource capture for the diverse three-species predator mixture at both high and low density. See Appendix C for calculation of variables, and Appendix G for proportional deviations from expected rates

Calculations based on the resource use patterns of single predator species predict a relatively even distribution of prey consumption rates in the multi-species treatments (Fig. 4.3). The observed pattern of trophic interactions was consistent with this prediction (Fig. 4.3). Deviations from this expected pattern indicated which resources were exploited to a greater degree at high predator diversity (Appendix G). In particular, the consumption of both size classes of *L. littorea* and *P. ulyssiponensis* far exceeds the null expectation at both densities (Fig. 4.3, Appendix G), consistent with the hypothesis that resource partitioning can enhance the total rate of resource capture by component species.

3.4 DISCUSSION

Our results show that predator species richness can have a strong effect on the efficiency of resource capture within a model intertidal food web. By crossing diversity treatments with total density, we tested the role of density in modifying the species richness effect, revealing that the loss of diversity leads to a more dramatic drop in ecosystem process rate when predators occur at high density. Furthermore, detailed analysis of species-specific trophic interactions demonstrated that the three predators occupy distinct fundamental functional niches (*sensu* Rosenfeld 2002), providing a possible mechanistic explanation of the observed effects.

Our finding that the diversity effect was strongest, and only detectable, at high density (Fig. 4.1) is not surprising considering the critical role played by competition in the manifestation of the niche partitioning effect. Our analysis identified density-dependent depression of per capita resource capture in single species treatments (at a 0.1 α level), but no such effect occurred in the polyculture (Fig 4.1.). This strongly suggests that the strength of negative interactions is less between heterospecifics than conspecifics. Where negative intraspecific interactions are weak or absent in single species contexts, which may

be the case at low density in our experiment, individuals gain less – or no – advantage from occurring within a mixed community of consumers (Wilby et al. 2005). The apparent relaxation of negative interactions with increasing species richness thus only produced a clear effect of diversity at high predator density where negative interactions between conspecifics within monocultures were strongest.

An ability to predict the consequences of species loss requires an understanding of the factors that modify diversity effects (Cardinale et al. 2000). Our experiment demonstrates that density should be considered in future attempts to understand the functional consequences of biodiversity within both observational and experimental studies. For example, high disturbance systems, and the early successional communities that typify them (e.g. Martins et al. 2007), may not be resource limited, potentially precluding diversity effects (Weis et al. 2007). On the other hand, management activities, such as the provision of protected areas, reduce direct and indirect human impacts on populations, typically increasing the abundance and diversity of predators (e.g. Babcock et al. 2007). The outcome of our experimental diversity manipulation implies that as the density of such predators increases, so too may the value of diversity for ecosystem functioning.

The sampling or positive selection effect (Huston 1997, Loreau 2000), has been credited as the principal mechanism leading to positive diversity effects in both biodiversity-ecosystem functioning (Cardinale et al. 2006) and natural enemy studies (Denoth et al. 2002, Straub and Snyder 2006). However, both theory (Cardinale et al. 2004, Loreau 2004) and experiments (Dimitrakopoulos and Schmid 2004, Gamfeldt et al. 2005, Snyder et al. 2006) suggest that the limited resource heterogeneity typically provided in such studies may preclude resource partitioning, instead highlighting strong effects of particular species – those most suited to the specific conditions, or prey type, provided. We aimed to incorporate the range of prey types, in terms of sizes and species identity,

available in the natural environment, thus providing realistic conditions with a high potential for resource partitioning. Indeed, all predators exhibited distinct resource use patterns in monoculture (Fig. 4.2). We could not provide incontrovertible evidence of resource partitioning, as our experimental set-up precluded measurement of species-specific resource use within the diverse treatment. However, the increased consumption of a broad spectrum of prey types in the polyculture (Fig. 4.3), representing dietary preferences of all three predators, suggests that all species maintained niche differences and benefited from resource partitioning. Moreover, theory and past experiments support the supposition that distinct resource use patterns observed in monoculture would likely have been maintained, or even exaggerated, in the presence of interspecific competition (Odum and Barrett 2005). Our experimental findings thus strongly suggest that resource partitioning can have a marked effect on ecosystem functioning, providing a rare empirical example (but see Bracken and Stachowitz 2006, Kahmen et al. 2006) of a phenomenon that has long been regarded as a key link between species richness and the magnitude of ecosystem functioning (Naeem et al. 1994, Loreau et al. 2001). Although the evidence indicates that all species maintained niche differences in polyculture, the consumption of some prey types deviated disproportionally from expected values (Fig. 4.3, Appendix G). This suggests that individuals of certain predator species may have benefited to a greater degree from occurring within a mixed assemblage, enhancing the consumption of their preferred prey types relative to others'. Further insight into the role of predator diversity could be achieved by documenting the division of resource acquisition rates among species within mixtures. This would provide a more direct test of resource partitioning and facilitate application of statistical techniques to distinguish between complementarity and selection effects (Loreau and Hector 2001, Fox 2005).

The appropriate experimental design to test the effect of species richness on ecosystem functioning continues to be debated (Balvanera 2006, Weis et al. 2007), primarily because species richness cannot be manipulated without a concurrent change in either total density or the density of component species. To clarify interpretation of experiments, the specific questions addressed by alternative designs need to be clearly defined. The substitutive design, used here, equalizes total density across single and multiple species treatments, testing whether, given density-compensation, local extinction of species will influence the magnitude of an ecosystem process. In effect, species which are lost through local extinction are experimentally replaced with individuals of those remaining, thus intraspecific and interspecific interactions are directly compared (Jolliffe 2000, Griffen 2006). Where resource partitioning occurs, as in this experiment, replacing heterospecifics with conspecifics will reduce the breadth of resources exploited, increasing competition and depressing the rate of resource acquisition. We thus suggest that the substitutive design is an effective approach to detect the functional consequences of resource partitioning.

Our experiment identifies the potentially strong effect of resource partitioning among predators in nature, but is only the first step. Species richness remains the most commonly used measure of diversity in experimental tests. However, resource partitioning results from species' differential resource use, and is thus an effect of functional diversity (*sensu* Petchey and Gaston 2002) as opposed to species richness *per se*. The field might thus increase its predictive capacity by shifting the emphasis of biodiversity-function research from species – to functional – diversity. Multiple predator-prey systems such as the one described here, provide an ideal opportunity to test the theoretical prediction that ecosystem processes will increase with functional diversity independently of species richness (Petchey and Gaston 2006). Furthermore, in order to gauge the possible cascading

effects of predator extinction we need to couple theory with longer-term and larger-scale manipulative and observational studies that incorporate predator and prey dynamics, adaptive morphological and behavioural prey responses, as well as indirect interactions. Future experiments should also represent natural systems as we have, incorporating environmental and resource heterogeneity that allows both stable coexistence and the enhancement of ecosystem processes through niche partitioning.

To increase the relevance and applicability of biodiversity-ecosystem functioning research we must develop a capacity to predict the consequences of extinction based on measurable properties of both natural and managed ecosystems. We show here that a fundamental property of communities, the density of consumers in relation to their resources, can modify the effect of diversity as mediated by resource partitioning. By explicitly examining mechanistic links between diversity and ecosystem functioning we can develop a predictive capacity, enabling us to quantify the expected loss of ecosystem functioning under scenarios of global and local environmental change.

4.5 APPENDICES

4.5.1 APPENDIX A. Description of prey assemblage.

Table 4.1 Mean size and dry shell-free biomass of all prey types available to all replicate predator assemblages at the beginning of the experiment is shown.

Prey species	*P.ulyssiponensis*	*P.ulyssiponensis*	*M.edulis*	*M.edulis*	*L. littorea*	*L. littorea*	*G. umbilicalis*	*G. umbilicalis*	*Chthamalus spp.*
Size class	Small	Large	Small	Large	Small	Large	Small	Large	
Mean longest axis (mm)	19.72	37.24	33.628	59.68	11.576	17.52	3.58	18.13	4.005
Mean mass (g)	0.06	0.347	0.108	0.684	0.034	0.135	0.007	0.048	0.0002
Initial density	6	6	6	6	6	6	6	6	800-1000
Total mass (g)	0.36	2.082	0.648	4.104	0.204	0.81	0.042	0.288	0.16 - 0.2

4.5.2 APPENDIX B

Calculation of resource capture efficiency

Size-specific shell-free dry-mass measurements of representative samples of all prey species were used to derive the mean consumption rate of each separate prey type by each replicate predator assemblage (C_{ij}) calculated as:

$$C_{ij} = \frac{D_j - D_{ij}}{t}$$

Where D_j is the initial density of prey j (equal to predator-free controls upon experiment termination); D_{ij} is the density of prey j at the end of the experiment in the presence of predator assemblage i; and t the duration of the experiment in days.

For each replicate predator assemblage, we summed consumption rates of individual prey types to calculate the total daily rate of resource capture (C_{Total}):

$$C_{total} = \sum_{i=1}^{n} C_{ij}$$

To aid comparisons of resource-capture efficiency across levels of total predator density, we also derived per capita consumption rates for each prey type (PC_{ij}) and total resource capture (PC_{Total}):

$$PC_{ij} = \frac{C_{ij}}{D_i}$$

$$PC_{Total} = \frac{C_{Total}}{D_i}$$

Where D_i is the density of predators (number of individuals) in each replicate predator assemblage.

In order to deduce effects of diversity on the composition and abundance of individual prey types consumed in polyculture, we calculated expected prey-specific per capita consumption (Pe_{ij}) based on density-specific monoculture effects (PC_{ij}) as:

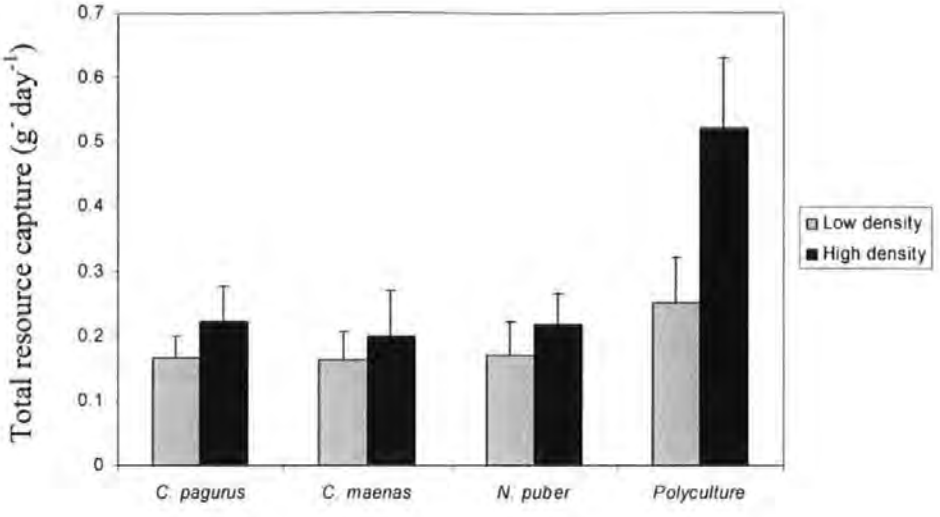
$$Pe_{ij} = \frac{\sum_{j=1}^{n} PC_{ij}}{n}$$

Where PC_{ij} is the observed per capita consumption of prey type i by predator j in monoculture (at either low or high density), and n is the number of predator species. Standard errors for expected means were calculated from mean monoculture values for each species, such that $n = 3$. We calculated proportional deviations from expected multi-predator effects on each individual prey type as:

[observed (PC_{ij}) - expected (Pe_{ij})/ expected (Pe_{ij})] x 100

4.5.3 APPENDIX C Total resource capture within all treatments.

Figure 4.4 Mean (± SE) aggregate total resource capture rate for all monocultures and the three species mixture at both low and high densities (C_{Total}, see Appendix B for calculation) (n=6).



4.5.4 APPENDIX D. Analysis of predator diversity and density effects

Table 4.2. Two way ANOVA of effects of crab diversity and density on overall consumption rate and targeted asymmetrical contrasts of the effect of the average monoculture versus the polyculture for data pooled across density levels and separately for low and high density.

Source	SS	df	MS	F	P
Density	0.0328	1	0.0328	1.48	0.23
Diversity	0.2223	3	0.074	3.33	0.03
Monocultures alone	0.0026	2	0.0013	0.08	0.92
Average monoculture vs polyculture	**0.2198**	**1**	**0.2198**	**9.88**	**0.003**
Density x Diversity	0.0164	3	0.0055	0.25	0.86
Residual	0.8895	40	0.0222		
Low density					
Diversity	0.0592	3	0.0197	0.71	0.56
Monocultures alone	0.0003	2	0.0002	0.01	0.99
Average monoculture vs. polyculture	**0.0589**	**1**	**0.0589**	**2.11**	**0.16**
Residual	0.5585	20	0.0279		
High density					
Diversity	0.1796	3	0.0599	3.62	0.03
Monocultures alone	0.0029	2	0.0014	0.12	0.88
Average monoculture vs polyculture	**0.1767**	**1**	**0.1767**	**10.64**	**0.004**
Residual	0.3311	20	0.0166		

4.5.5 APPENDIX E. Analysis of species-specific resource consumption

Table 4.3. Permutated analysis of variance (PERMANOVA) on Bray-Curtis similarities derived from square-root transformed interaction strengths. Comparisons show *a posteriori* pair-wise tests between individual treatments.

Source of variation	df	MS	F	P
Species	3	7852.8	3.98	<0.001
Density	1	3434.8	4.14	0.01
Species x Density	3	830.56	0.42	0.95
Residual	40			

Comparisons	t	P
C. pagurus vs. *C. maenas*	2.4688	0.0006
C. pagurus vs. *N. puber*	2.1046	0.0034
C. maenas vs. *N. puber*	2.1374	0.0018
C. pagurus vs. polyculture	2.3389	0.0022
C. maenas vs. polyculture	1.8507	0.0124
N. puber vs. polyculture	0.9066	0.5192

4.5.6 APPENDIX F. Comparison of prey consumption patterns between monocultures and the polyculture

Table 4.4. Observed and expected consumption of all prey types at both high and low predator density. Proportional deviation from expected (%) is also shown (see Appendix A for calculation of these values)

Prey species	*P.ulyssiponensis*	*P.ulyssiponensis*	*M.edulis*	*M.edulis*	*L. littorea*	*L. littorea*	*G. umbilicalis*	*G. umbilicalis*	*Chthamalus* spp.
Prey size	Small	Large	Small	Large	Small	Large	Small	Large	
Expected (low density)	0.00305	0.0112	0.0175	0.00633	0.00364	0.00938	0.000442	0.00175	0.00211
Expected (high density)	0.00152	0.009	0.0107	0.0143	0.00158	0.00531	0.000187	0.000549	0.00216
Observed (low density)	0.005	0.0289	0.015	0	0.00523	0.0244	0.000408	0.00395	0.00115
Observed (high density)	0.00208	0.0361	0.015	0.0143	0.00214	0.0131	0.000255	0.000659	0.000958
Deviation (low density)	0.00194	0.0176	-0.0025	-0.00633	0.00158	0.015	-0.000034	0.00219	-0.00097
Deviation (high density)	0.000555	0.0265	0.00425	0	0.000555	0.00782	0.0000681	0.0001094	-0.0012
% Deviation (low density)	63.636	157.142	-14.285	-100	43.478	160	-7.692	125	-45.669
% Deviation (high density)	36.363	275	39.534	0	35	147.0588	36.363	20	-55.769

4.5.7 APPENDIX G

Figure 4.5 Image of a mesocosm used in the experiment

Fine grey mesh was tightly fitted over the surface to prevent prey escape. Mesocosms measured 0.6m x 0.4m (area) x 0.2m (depth).

SPATIAL HETEROGENEITY INCREASES THE IMPORTANCE OF SPECIES RICHNESS FOR AN ECOSYSTEM PROCESS

CHAPTER V

ABSTRACT

The role of biodiversity in mediating ecosystem processes has been the subject of focused theoretical and empirical attention over the last several decades. Theory predicts that the balance between species richness and identity effects will critically depend on the degree of environmental heterogeneity, which dictates the extent to which differences between species in patterns of resource use can be expressed. We conducted a mesocosm experiment to explicitly test this hypothesis. We manipulated the richness and identity of intertidal molluscan grazers, as well as the spatial heterogeneity of the substrate upon which they grazed algae, measuring algal consumption as our focal ecosystem process. The grazer treatments consisted of three monocultures and a single polyculture including all three species; heterogeneity was represented as the proportion of topographically complex and flat substrate. Species identity had strong effects on homogeneous substrates, with the identity of the best-performing species dependent on the substrate. On the heterogeneous substrate, suitable conditions for all three species were represented, allowing the expression of spatial complementarity of resource use and the enhancement of total algal consumption. The implications of our findings for the design and interpretation of biodiversity-ecosystem functioning studies are discussed.

KEYWORDS: Biodiversity; Complementarity; Consumers; Context-dependency; Ecosystem functioning; ; Grazing; Resource partitioning; Spatial heterogeneity Species identity; Species richness.

5.1. INTRODUCTION

The effect of biodiversity on ecosystem functioning, and by extension ecosystem goods and services, has emerged as a key research priority over the last several decades (e.g. Loreau et al. 2001). The role of species richness has been a particular focus of attention. Synthesis of the resultant large body of empirical studies has uncovered a generally positive, but saturating, relationship between species richness and the magnitude of various ecosystem functions (Cardinale et al. 2006; see also Hooper et al. 2005, Balvanera et al. 2006). Importantly however, within these experiments the presence of particular species typically has an effect comparable to – or even greater than – that of species richness (Cardinale et al. 2006). Understanding the factors that dictate the relative strength of particular species and species richness *per se* is the key to developing an ability to predict the effects of species extinction (or gain) on ecosystem functioning in any given system or context.

Interspecific niche partitioning, or complementarity, is considered one of the principal mechanisms underpinning the effect of species richness on ecosystem processes (e.g. Loreau 1998, Duffy 2002). Niche complementarity can occur through the exploitation of different resources (e.g. Bracken and Stachowitz 2006, Kahmen et al. 2006), as well as spatial (Albrecht and Gotelli 2001) or temporal (Yachi and Loreau 1999) differences in the use of the same resource. Increasing species richness results in a more complete use of the available spectrum of resources when niche complementarity is evident (Tilman 1997, Loreau 2000), potentially enhancing the aggregate rate of resource uptake (Trenbath 1974, Yachi and Loreau 1999).

Crucially, the expression of interspecific complementarity may depend on the occurrence of a heterogeneous environment or resource space (Cardinale et al. 2004). Under homogeneous conditions a single species, that which is best-suited to the specific

environment or resource, may dominate the acquisition of resources (e.g. Straub and Snyder 2006, Råberg and Kautsky 2007). Intuitively, the identity of the dominant species can switch depending on the conditions (Yachi and Loreau 1999, Cardinale et al. 2004, Gamfeldt et al. 2005), and simultaneously considering multiple conditions may render multiple species important for the aggregate use of resources (Råberg and Kautsky 2007). The degree of heterogeneity may thus dictate the relative strength of species identity and niche complementarity effects (Tilman and Lehman 2002, Cardinale et al. 2004). Indeed, it is a common and ubiquitous pattern that environments are patchy with consequences for both species composition and productivity (Kane and Poulson 1976, McQuiad and Dower 1990, Downes et al. 1998), and environmental heterogeneity in space and time are thought to be critical in the mediation of long-term species coexistence (Hutchinson 1961, Chesson 1991). Spatial heterogeneity has yet, however, to be explicitly and experimentally considered in the biodiversity-ecosystem functioning framework (Cardinale et al. 2004, Dyson et al. 2007).

Molluscan grazers are important consumers of primary producers in both freshwater (Steinman 1996) and marine (Hillebrand 2002) environments and have the potential to control both the productivity and standing stock of algae (Hawkins and Hartnoll 1983). Furthermore, grazer distributions are often strongly influenced by habitat topology (Downes et al. 1998). On rocky shores, the degree of substrate complexity is often highly variable over small spatial scales as a result of the formation and type of rock, as well as the growth of barnacles, bivalves and encrusting coralline algae. Grazing molluscs must negotiate this substrate to access their algal prey (Hawkins 1981, Lubchencho 1983). In a mesocosm study, we tested the hypothesis that increasing environmental heterogeneity increases the importance of grazer richness for the rate of algal consumption. Environmental heterogeneity was represented as the relative proportion of flat and

topographically complex substrates. By virtue of differences in body size and shape, as well as foraging strategy, we predicted that three species of molluscan grazers would vary in their foraging efficiency depending on the type of substrate, generating strong species identity effects on homogeneous substrates. We further hypothesized that an effect of richness would emerge under heterogeneous conditions, when niche complementarity among multiple species could be expressed.

5.2. MATERIALS AND METHODS

5.2.1. Experimental design and set-up

We manipulated the richness and identity of three molluscan grazers (the limpet *Patella vulgata* Linnaeus, the periwinkle *Littorina saxatilis* Olivi, and the topshell *Gibbula umbilicalis* da Costa; hereafter referred to by their generic names only), as well as the form and heterogeneity of the physical substrate on which they grazed. The grazer treatments consisted of three separate single species treatments (all possible monocultures), and a mixture of all three species (the polyculture) (Fig. 5.1). The physical substrate consisted of three treatments: flat, rough and heterogeneous (Fig. 5.1). We employed a fully-factorial experimental design (Fig. 5.1), allowing us to examine both the independent and interactive effects of the two orthogonal factors: *i*) grazer identity/richness and *ii*) substrate form/heterogeneity. All treatments were replicated five times. We conducted the experiment during May 2008 within the seawater flow-through facility at the Marine Biological Association Laboratory in Plymouth, United Kingdom.

We modified the substitutive design in order to manipulate grazer richness and identity within the boundaries of naturally occurring densities. Under the substitutive approach, the biomass (or number) of organisms is typically equalized across treatments varying in species identity and richness. In our system, however, the grazer species vary

>100 fold in mean individual biomass (shell-free dry mass) and at the population level do not occur at equivalent standing biomasses; the larger bodied species maintain a much larger standing stock (*Patella*> *Gibbula*> *Littorina*). Equilibrating densities according to either biomass or number of individuals was thus inappropriate here. We therefore chose species-specific monoculture densities within the range typically observed on local shores. Consistent with a standard substitutive design, the density of each species in the polyculture was equal to its density in monoculture divided by the number of species in the polyculture (*i.e.* three in this case). This resulted in a total polyculture density equal to the mean monoculture (Table 5.1), which is also consistent with a standard substitutive approach.

The three species used here are all widespread, abundant, and coexisting inhabitants of rocky shores in the south west of the United Kingdom. Grazers were collected by hand from local rocky shores during tidal emersion. *Patella* were carefully removed from the rock and immediately transferred to a Perspex^{TM} sheet to allow re-attachment and to limit physical stress. *Gibbula* and *Littorina* are more robust, and were thus simply placed in buckets. Owing to the relatively small size of the mesocosms, we opted to reduce the mean intraspecific body size of organisms relative to the natural mean size on local shores, whilst maintaining a range of body sizes within each grazer species. Specifically, the size ranges were (maximum shell length): *Patella* = 19 – 43 mm; *Gibbula* = 3 – 7 mm; *Littorina* = 3 – 9 mm). Prior to addition to the mesocosms, grazers were acclimated to experimental conditions within tanks subjected to flowing water for up to 4 days.

We constructed the substrates from untreated pure limestone flooring tiles. We created the 'rough' substrate by fixing (Araldite^{TM} adhesive) small cubes (1.5cm^2), of two lengths (1.5 cm and 3 cm) arranged vertically and interspersed with each other, to the flat substrate. Small gaps (≈ 0.2 cm) were left between the cubes, creating an environment with numerous crevices and only small areas of flat rock surface. The experimental substrates

measured 10 cm x 20 cm. We cut them all in half, splitting the rectangle into two squares, before re-gluing them. This allowed the separation of rough and smooth parts of the heterogeneous substrate (Fig. 5.1) for analysis. For consistency, we applied the same protocol to homogeneous environments.

Manipulation of substrate topology resulted in a total substrate area that varied among the three substrate treatments. The rough substrate contained approximately a three-fold greater surface area than the flat; the heterogeneous substrate two-fold greater. We accounted for this by elevating the total grazer abundance by corresponding amounts, such that for each grazer treatment, grazer abundance per unit area was approximately equilibrated across substrate treatments (Table 5.1).

We initially haphazardly divided substrates among five separate tanks (150 cm^2 area x 10 cm depth), and subsequently rearranged them within and among tanks every two days throughout the experiment to average out possible tank or positional effects among treatments and replicates. Prior to the addition of grazers, the initially bare substrates remained exposed to flowing seawater (and algal propagules within it) for five weeks to allow colonization and growth of epilithic algae.

We sampled five grazer-free control substrates within each of the three substrate treatments both at the beginning of the grazing trial (following the five-week colonization period), and at the end of the trial. These controls allowed comparisons among substrates in terms of the total biomass and composition of the algal assemblages, in addition to the identification of possible changes in algal biomass through the trial. The grazing trial lasted 6 days.

5.2.2. Measurement and calculation of response variables

As the area-specific biomass of algae differed among the substrate types at the start of the experiment (see *Results*), we had to control for this difference to calculate the absolute magnitude of algal consumption. We simply subtracted the final standing algal biomass within each replicate from the mean biomass recorded in controls of the same substrate type (Fig. 5.2; *Results*). This was a valid approach since the variation among control replicates was very low. Additionally, to generate a comparable index of resource consumption across the disparate experimental substrates, we calculated the relative grazing by dividing the difference (D) between standing stocks of control (C) and grazer (G) treatments with the control standing stock:

$$D = \frac{(C - G)}{C}$$

Area-specific algal biomass means for each of the substrate types, as ascertained from the controls, were used to represent control standing stock.

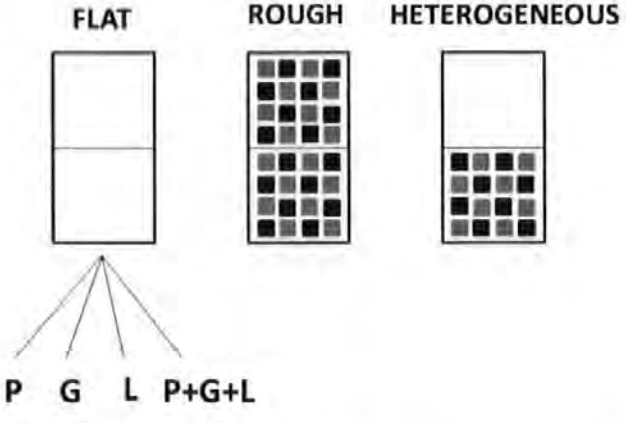


Figure 5.1. A schematic representation of the experimental units and design used here. The 4 grazer treatments (P = *Patella*, G = *Gibbula*, L = *Littorina*, P+G+L = all species combined in polyculture) all occurred under each substrate treatment. Additionally, we included grazer-free controls (not shown) within each substrate type, sampled both before and after the grazing trial. The twelve experimental treatments and six control treatments were all replicated five times.

Table 5.1. The numbers of grazers in each treatment. Note that the mean monoculture density (to the nearest whole individual) is equal to that of the polyculture under the modified substitutive design used here. We accounted for differences in total substrate area between flat, rough and heterogeneous treatments by increasing grazer density a corresponding amount (see *Methods*).

	Monocultures				Polyculture			
	Patella	*Gibbula*	*Littorina*	Mean	*Patella*	*Gibbula*	*Littorina*	Total
Flat	3	15	20	13	1	5	7	13
Rough	9	45	60	38	3	15	20	38
Heterogeneous	6	30	40	25	2	10	13	25

On day three, after the grazers had been given time to move to preferred substrates, we recorded their position (in terms of substrate type) to allow identification of species-specific preferences.

We measured the final biomass of algae within each replicate substrate thorough an established technique involving extraction of chlorophyll a in methanol (see Thompson et al. 1999 for a detailed description). We placed each 10 cm^2 sub-unit of the experimental substrate in 200 ml of methanol (99.8%) for 15 hours to extract chlorophyll a. We then centrifuged (Pico, Sorvall, UK) 3 ml samples of the resultant solution to remove any particulates, before measuring light absorbance at wavelengths of both 665 and 750 nm in a spectrophotometer (CE 2011, Cecil Instruments Ltd, Cambridge, UK). We then calculated total biomass per unit of substrate area from absorbance levels using known relationships (Thompson et al. 1999). Proportional cover of algal types were estimated visually (see Appendix).

5.2.3. Analysis

Consistent with the experimental design, we used 2-way analysis of variance (ANOVA) with grazer richness/identity and substrate identity/heterogeneity as fixed, orthogonal factors. The relative consumption of algae constituted our focal ecosystem-level response variable, because it provided a comparable measure across substrate types. We additionally performed the analysis on the absolute rate of algal consumption as a precaution to ensure that key conclusions based on the relative response were robust. Significant treatment effects were elucidated through post-hoc Tukey HSD tests. ANOVAs were validated through both the visual inspection of plots of residuals and Levene's test. All univariate analyses were performed in SPSS 15.0 (SPSS inc, Chicago). Treatment effects on algal diversity (H'), evenness (J') and composition/ relative abundances were similarly tested using PRIMER-6 (PRIMER-E Ltd. Plymouth, UK) (see Appendix for methods).

We explicitly tested for non-transgressive overyielding of our ecosystem process by comparing the mean, pooled, monoculture with the polyculture using a planned comparison. This form of diversity effect can result from the dominance of highly productive species in long-term studies (*sensu* Petchey 2003). Where biomass remains constant in short-term experiments such as the one presented here, the elevation of the focal ecosystem process above that expected from the monoculture performance of component species measures the net effect of species complementarity (Petchey et al. 2003). We could straightforwardly test for this effect because the expected rate of algal consumption in polyculture is equal to the mean of the component monocultures under the design we employed (Table 5.1). We similarly tested for the presence of transgressive overyielding by comparing the best-performing monoculture with the polyculture. Transgressive overyielding is an 'acid test' of species complementarity in long-term studies (Loreau

1998). In our short-term study, we tested for this effect to gauge whether species richness effects exceeded that of species identity.

5.3. RESULTS

Grazer-free controls showed that substrate treatment affected the accumulated area-specific biomass of algae (Fig. 5.2; flat > heterogeneous > rough; $F_{2,29} = 32.995$, $P<0.001$). These controls also indicated that area-specific algal biomass did not change during the 6-day experiment in the absence of grazers (Fig. 5.2; $F_{1,29} = 0.851$, $P = 0.366$). There were also no overall or temporal differences in the composition/relative abundance of algal types, nor their diversity or evenness (Fig. 5.3) according to substrate treatment.

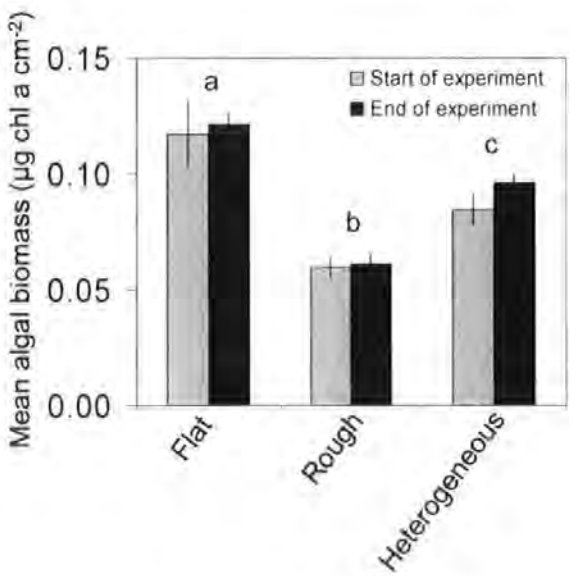


Figure 5.2. Mean algal biomass in controls at both the start and the end of the experiment. Substrate type affected the mean area-specific biomass of algae ($F_{2,29} = 32.995$, $P<0.001$). There was no change during the experiment ($F_{1,29} = 0.851$, $P = 0.366$), nor any interaction between treatment effects and start/finish ($F_{2,29} = 0.265$, $P = 0.770$). Letters indicate results of *post-hoc* Tukey HSD tests.

Overall, the relative rate of algal consumption (D) depended on grazer treatment, with both the *Gibbula* monoculture and the polyculture treatments exceeding the *Patella* and *Littorina* monocultures (Table 5.2, Fig. 5.4). Substrate type also affected the relative rate of algal consumption; the relative rate of grazing was higher on the heterogeneous compared to the flat substrate (Table 5.2, Fig. 5.4). Notably, substrate type modified the effect of grazer treatment (substrate x grazer interaction, Table 5.2, Fig. 5.4).

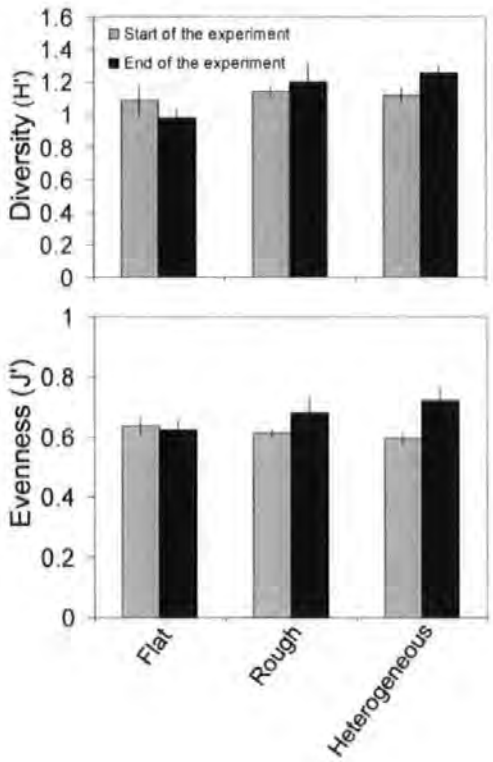


Figure 5.3. The diversity and evenness of algal groups according to substrate type. There was no effect of substrate type on diversity ($F_{2,30}$ = 2.501, P = 0.103) or evenness ($F_{2,30}$ = 2.501, P = 0.103). Moreover, there was no change in these metrics through the experiment (F2, 30 = 0.172, P =0.682, $F_{1,30}$ = 4.093, P = 0.054

Considering intraspecific variability in performance according to substrate, *Gibbula* performed most efficiently on the rough substrate, *Patella* was best-suited to the flat

substrate, whilst *Littorina* performed better on both heterogeneous and rough substrates compared to the flat (Fig. 5.4).

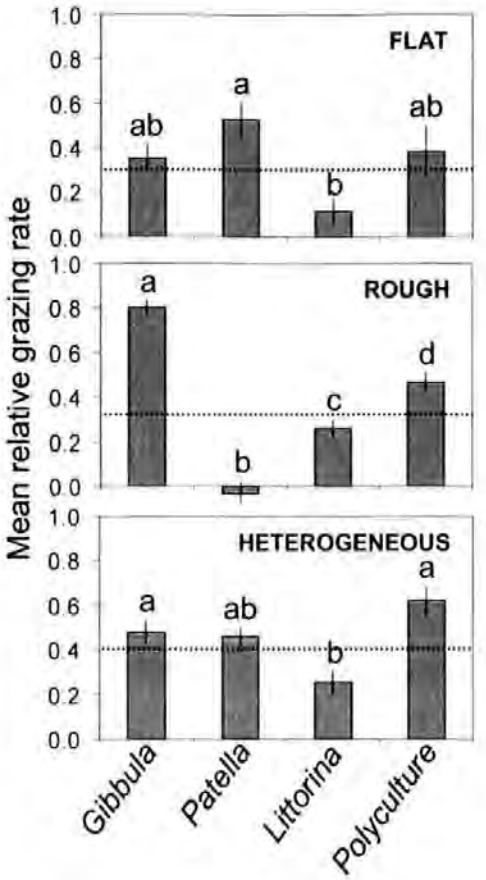


Figure 5.4. The mean (± SE) relative rate of algal consumption according to grazer treatment within substrate types. The dotted horizontal lines indicate the rate expected in the polyculture based on the mean of the monocultures. Both the *Gibbula* and polyculture treatments exhibited the highest rate overall (Table 5.3., *Results*). There was a higher overall relative rate of grazing under heterogeneous compared to flat conditions (Table 5.3., *Results*). Letters indicate group membership from *post-hoc* comparisons performed within each substrate type. Under homogeneous conditions species identity effects dominated: *Patella* maximized the rate on the flat, *Gibbula* on the rough. Notably, the polyculture exceeded the mean monoculture (non-transgressive overyielding) only under heterogeneous conditions (*Results*).

Across all of the grazer treatments, grazing rate was maximised by different individual species depending on the type of homogeneous substrate: *Patella* on the flat substrate, *Gibbula* on the rough (Fig. 5.4). Indeed, there was no evidence of species

richness effects on either of these substrates; the polyculture did not exceed the respective mean monocultures (non-transgressive overyielding; flat: $F_{1,19} = 0.200$, $P = 0.660$; rough = $F_{1,19} = 0.550$, $P = 0.468$). Furthermore, the possibility of transgressive overyielding was ruled out by the fact that the best monoculture exceeded the polyculture (Fig. 5.4). On the heterogeneous substrate the rate of grazing was maximized by the polyculture.

In this case, the performance of the polyculture exceeded that of the mean monoculture ($F_{1,19} = 8.203$, $P = 0.010$) but was not significantly greater than the best-performing single species ($F_{1,19} = 2.591$, $P = 0.146$). Species-specific effects of substrate type, as identified in the monocultures on homogeneous substrates, were also evident under heterogeneous conditions: *Patella* performed best on the flat sector, *Gibbula*, and to a lesser extent *Littorina*, on the rough (Fig. 5.5). In the polyculture under heterogeneous conditions, algal consumption was similar on both the flat and the rough sectors (Fig. 5.5). Absolute rates of algal consumption displayed comparable patterns within substrate types, with non-transgressive overyielding evident only under heterogeneous conditions (Fig. 5.5).

Table 5.2. Results of 2-way ANOVA on the relative grazing rate. *Post-hoc* analyses indicate that the *Gibbula* monoculture and the polyculture treatments exceeded the *Patella* and *Littorina* monocultures when all substrates were considered. The significant substrate x grazer interaction resulted from context-dependent grazer identity and richness effects (Fig 5.5.)

	SS	df	MS	*F*	*P*
Source					
Environment	0.131	2	0.065	3.484	0.039
Grazers	1.078	3	0.359	19.114	<0.001
Env x Grazers	1.565	6	0.261	13.874	<0.001
Error	0.902	48	0.019		
Total	3.676	59			

Treatment effects on the composition/relative abundances were generally weak. Notably, *Gibbula* grazing left a higher proportion of the brown filamentous macroalga *Ectocarpus sp.* than other treatments under both rough and heterogeneous conditions (Appendix). Effects on algal diversity and evenness were also relatively weak and variable. Under rough conditions, *Gibbula* reduced algal diversity relative to *Littorina* grazing, whilst under heterogeneous conditions *Patella* and the polyculture reduced algal evenness relative to *Gibbula* and *Littorina* (Appendix).

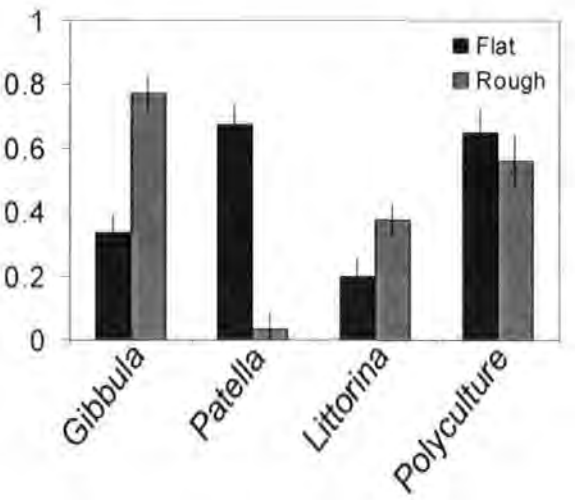


Figure 5.5. The mean (± SE) relative rate of algal consumption depending on substrate sector within the heterogeneous treatment. Different grazer species showed complementarity in terms of the substrate type they grazed most efficiently upon. When combined in polyculture, both flat and rough sectors were exploited to a similar extent.

The three grazers differed markedly in substrate use (Fig. 5.7). *Patella* occurred almost invariably on the flat substrate when it was available (Fig 5.7: 100% on flat [mean ± SE proportion of individuals]; 96.7 ± 3.3% on heterogeneous) but occurred on the sides of the mesocosms where it was unavailable (rough 93.3 ± 6.6%). *Gibbula* was common on both substrates within respective homogeneous treatments (Fig 5.7: on flat 87.8 ±1.9%; on rough

99.5 ± 0.5%), but occurred predominantly on the rough substrate under heterogeneous conditions (Fig. 5.7: 6.7 ± 1.0% on flat; 89.1 ± 1.0% on rough). A larger proportion of *Littorina* were found attached to the mesocosm sides in the flat (76.4 ± 3.4%) compared to the rough (32.1 ± 3.3%), homogeneous treatment. This apparent preference for rough substrate also occurred under heterogeneous conditions (2.9 ± 1.4% on flat; 39.4 ± 4.8% on rough). Species-specific patterns of substrate use were generally very similar in both monoculture and polyculture within substrate types (Fig. 5.7). The one exception to this pattern was the reduced proportion of *Littorina* grazing on the rough homogeneous substrate in the polyculture, where it increased in abundance on the mesocosm sides (Fig. 5.7: 32.1 ± 3.3% in monoculture; 72.7 ± 6.8% in polyculture).

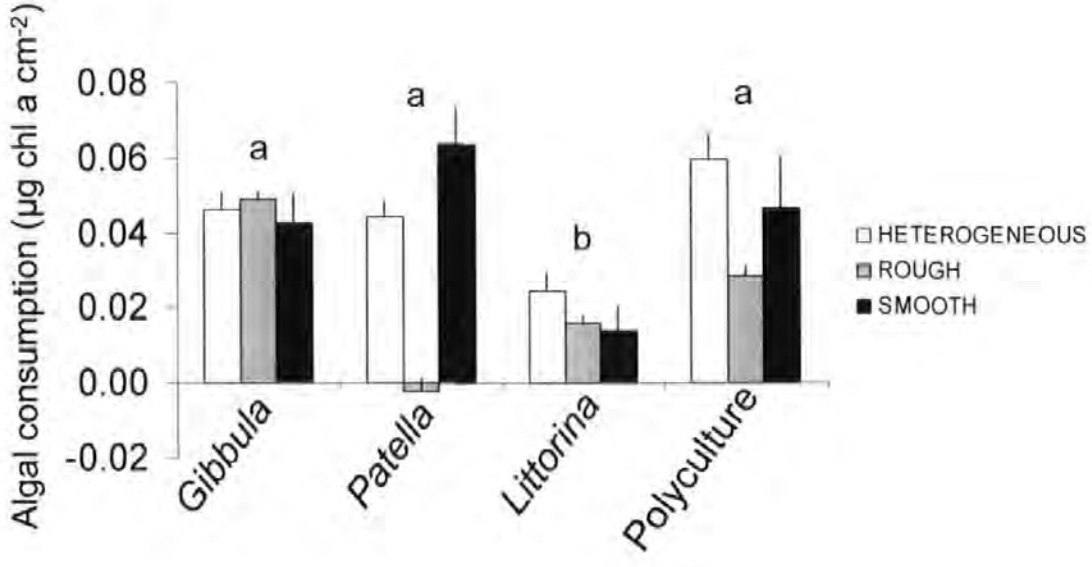


Figure 5.6. Mean (± SE) absolute rates of grazing across all treatments. There were significant effects of grazer treatment and substrate, as well as an interaction between these crossed factors. Letters denote grazer treatment group membership according to *post-hoc* Tukey tests. Tukey tests also indicate that algal consumption was lower under rough, homogeneous, conditions.

Table 5.3. Results of 2-way ANOVA on the absolute grazing level according to the crossed factors: substrate and grazer treatment.

	SS	df	MS	*F*	*P*
Source					
Environment	0.005	2	0.003	12.189	<0.001
Grazers	0.007	3	0.003	11.49	<0.001
Env x Grazers	0.009	6	0.003	6.927	<0.001
Error	0.01	48	<0.001		
Total	0.032	59			

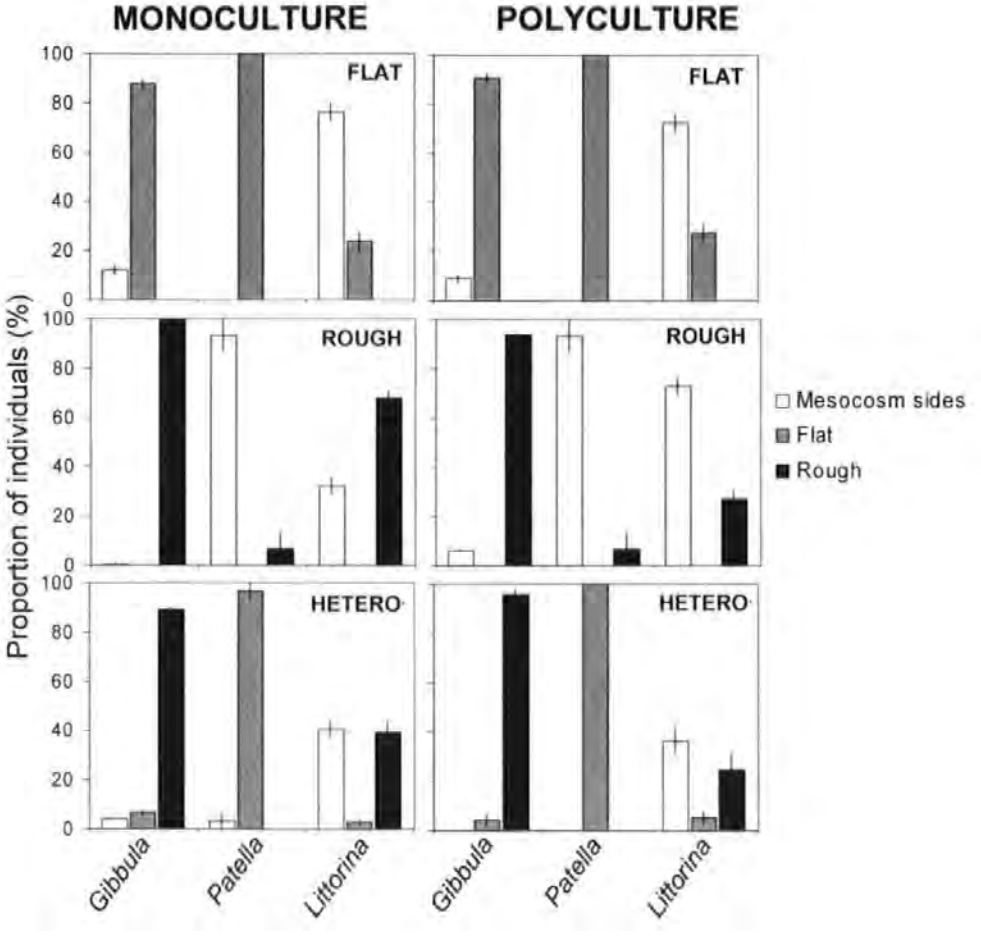


Figure 5.7. The proportion of individuals of each species on different substrate treatments and in monocultures and within the polyculture. Notably, the species exhibited closely resembling patterns of substrate use in both monoculture and polyculture. Data were recorded on day 3, the mid-point of the experiment.

5.4. DISCUSSION

Our results demonstrate clearly that spatial heterogeneity of environmental conditions increases the importance of species richness for an aggregate ecosystem process. Particular species maximized grazing rates within homogeneous environments, with the identity of the dominant species dependent on the substrate. Heterogeneous conditions, on the other hand, allowed the expression of species complementarity, enhancing the ecosystem process above that expected from monoculture rates of component species.

Our findings strongly suggest that spatial niche complementarity underpins the observed interactive effects of environmental heterogeneity and species richness. Grazer species differed in their ability, and tendency, to forage on the algal prey depending on the complexity of the habitat (Fig. 5.4). Under homogeneous conditions, interspecific differences in ability and preference to forage could not be expressed. Instead, the ecosystem process was maximized by the species best-suited to the specific substrate available (Fig. 5.4). Heterogeneous conditions effectively facilitated a match between multiple species and their preferred conditions, allowing species complementarity to be realized. The combination of *Gibbula* and *Patella*, in particular, resulted in a high-level of resource consumption within both rough and flat sectors (Fig. 5.5). In effect, niche complementarity can be viewed as a combination of species identity effects across heterogeneous patches (Cardinale et al. 2004).

The context-dependent effects of grazer richness and identity were likely underpinned by differences in functional morphology among the species, which determined spatial complementarity. The effective rasping foraging strategy of *Patella* (Hawkins and Hartnoll 1983), combined with the fact that it has the largest mean body size and standing stock of the three species (*Methods*, Table 5.1), renders it a key grazer on northern European rocky shores (Jenkins et al. 2001, Moore et al. 2007). The relatively large foot

area and conical shell of *Patella* largely constrained its movements (Fig. 5.7), and thus grazing impact (Fig. 5.4), to areas of flat rock in this experiment. Congruently, this species is most abundant on relatively flat surfaces on local rocky shores (Griffin, 2008). With smaller bodies and more maneuverable, slender shells, both *Littorina* and *Gibbula* were able to access algae within the rough substrates (Fig. 5.4, 5.5); a behavior that is likely adaptive, providing protection from predators and wave-dislodgment under field conditions. Although the mean grazing impact of *Littorina* was relatively low (Fig. 5.4), this species likely increased the spectrum of resources exploited by accessing the algae within crevices that were too small for *Gibbula*. Thus, all three species may have contributed to the observed non-transgressive overyielding within the heterogeneous treatment (Fig. 5.4). Additionally, dietary complementarity within habitat types may have played a subtle role in our experiment. Notably, we found evidence that *Gibbula*, but not the other two species, avoided consuming the brown filamentous macroalga *Ectocarpus sp.* (Appendix). The presence of all three species may thus have increased the spectrum of algal types consumed.

Experiments performed on small spatial scales, using mesocosms or experimental plots, form a substantial contribution to our current understanding of the functional consequences of biodiversity. If such experiments under-represent the environmental and resource heterogeneity that typically characterizes the natural ecosystems they aim to simulate, they run the danger of underestimating the value of species richness for ecosystem functioning (Ives et al. 2005). Indeed, several recent experiments have demonstrated that resource heterogeneity can enhance the effect of species richness (Gamfeldt et al. 2005, Bracken and Stachowitz 2006, Råberg and Kautsky 2008). Adding to these experiments, our results show clearly the importance of spatial heterogeneity. We

suggest that heterogeneity must be carefully considered before extrapolating experimental findings to broader, landscape, contexts.

The hypothesis that species richness will have a greater effect on ecosystem functioning under heterogeneous conditions is supported by theory (Cardinale et al. 2004) and a recent analysis of observational data (Tylianakis et al. 2008). Our factorial experiment provides the first explicit empirical support for this hypothesis, and underlines the importance of niche complementarity in mediating this effect. Environmental heterogeneity in space and time, combined with interspecific niche differentiation are thought to be essential ingredients in the mediation of long-term species coexistence (Hutchinson 1961, Chesson 1991). Just as this combination of factors maintains diversity, it renders it important for ecosystem functioning: diverse niches require diverse species to fill them in order to maximize consumption and other ecosystem processes.

5.5. APPENDIX: Measurement and results of treatment effects on algal composition/relative abundances and diversity

Methods

We recorded the composition of the algal assemblage through visual estimates of % cover of component groups, both at the beginning and the end of the experiment. Grazing treatments produced large, but variable, areas of bare rock by the end of the experiment. In order to provide comparable measures of algal composition/relative abundance, which were independent of variable absolute grazing impacts, we recalculated algal cover as a proportion of the total algae remaining. We then calculated diversity and evenness using the Shannon index (H') and Pielou's index (J'), respectively (see e.g. Magurran 2004). On day three, after the grazers had been given time to move to preferred substrates, we recorded their position (in terms of substrate type) to allow identification of species-specific preferences.

Analysis of Similarity (ANOSIM) procedures, based on Bray-Curtis distances and 999 permutations, were used to test for differences in algal composition/ relative abundances within each substrate type according to grazer treatment based on proportions of algal groups rescaled to total remaining algal cover. Treatment effects were elucidated through pair-wise comparisons and the Similarity Percentages (SIMPER) procedure. Multivariate analyses were performed in PRIMER-6 (PRIMER-E Ltd, Plymouth)

Results. Multivariate analysis of the effects of grazer treatment on algal relative abundance/composition within each substrate type.

Table 5.4. Analysis of similarity (ANOSIM) within substrate types.

	FLAT		ROUGH		HETEROGENEOUS	
	R Statistic	Significance (%)	R Statistic	Significance (%)	R Statistic	Significance (%)
Global test	-0.005	48.9	0.565	**0.1**	0.225	**0.9**
Pairwise comparisons						
Gibbula vs. *Patella*	-0.236	98.4	1	**0.8**	0.7	**0.8**
Gibbula vs. Polyculture	-0.124	93.7	0.888	**0.8**	0.032	31.7
Gibbula vs. *Littorina*	0.032	37.3	1	**0.8**	-0.044	50
Patella vs. Polyculture	-0.204	94.4	0.292	5.6	0.512	**0.8**
Patella vs. *Littorina*	0.136	18.3	0.328	4.8	0.224	7.9
Littorina vs. Polyculture	0.396	**2.4**	0.052	31	-0.076	77.8

Table 5.5. SIMPER analysis on % cover data, showing the only comparisons that were identified as significant in ANOSIM. Only those algal groups contributing over 5% to the difference between treatments are shown in each analysis.

	Ectocarpus sp.	Cyanobacteria A	Cyanobacteria C	Diatoms	*Ulva* sp.
FLAT					
Polyculture	**8.16**	53.34	22.35	**14.48**	
Littorina	**36.55**	48.01	33.44	**5.19**	
Contribution (%)	40.09	20.8	19.3	15.52	
ROUGH					
Gibbula	**96**	**4**	14.67	0	48
Patella	**0.71**	**54.33**	21.48	19.08	0.71
Contribution (%)	37.06	22.28	8.38	8.4	21.52
Gibbula	**96**	4	14.67		**48**
Polyculture	**14.57**	36.29	44.64		**5.71**
Contribution (%)	37.22	16.85	17.46		22.75
Gibbula	**96**	4	14.67		**48**
Littorina	**10.27**	35.99	27.19		**2.35**
Contribution (%)	40.57	17.46	11.72		25.87
HETEROGENEOUS					
Gibbula	**22.46**	**40.94**	27.4	6.72	7.52
Patella	**3.1**	**56.31**	13.97	9.98	1.95
Contribution (%)	27.6	28.35	19.93	8.57	8.05
Polyculture	14.06	**27.94**	**33.89**	9.86	6.76
Patella	3.1	**56.31**	**13.97**	9.98	1.95
Contribution (%)	13.91	36.72	26.61	11.61	6.98

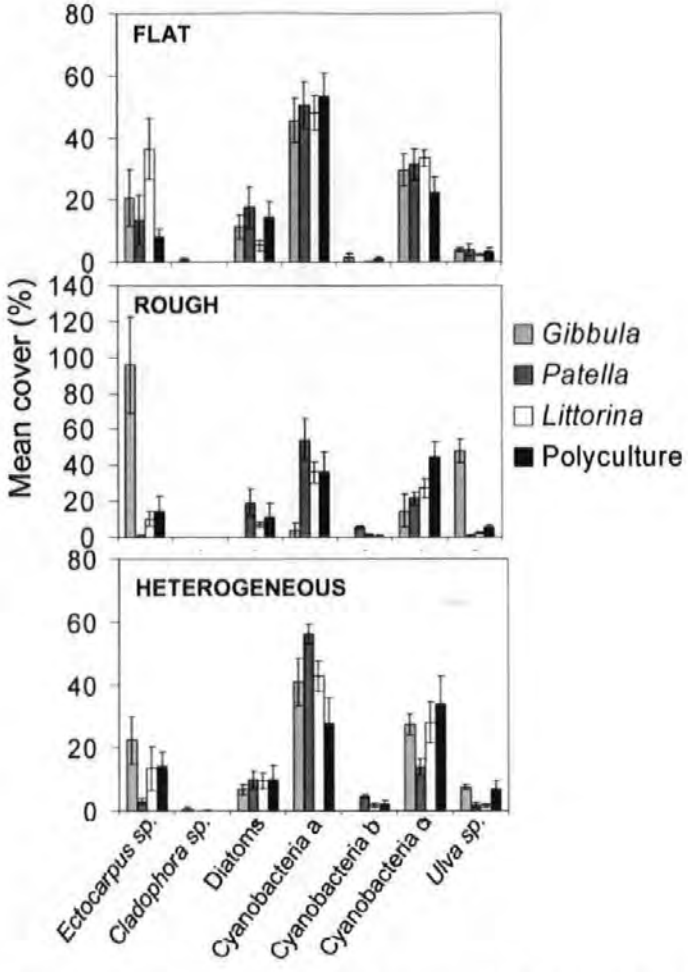


Figure 5.8. The mean cover of algal groups present at the end of the experiment (excluding bare substrate, *i.e.* the % cover refers to the relative abundance of species within the extant algal assemblage). Notably, a greater proportion of brown foliose and *Ulva* algae remained in *Gibbula* monocultures than under the other treatments.

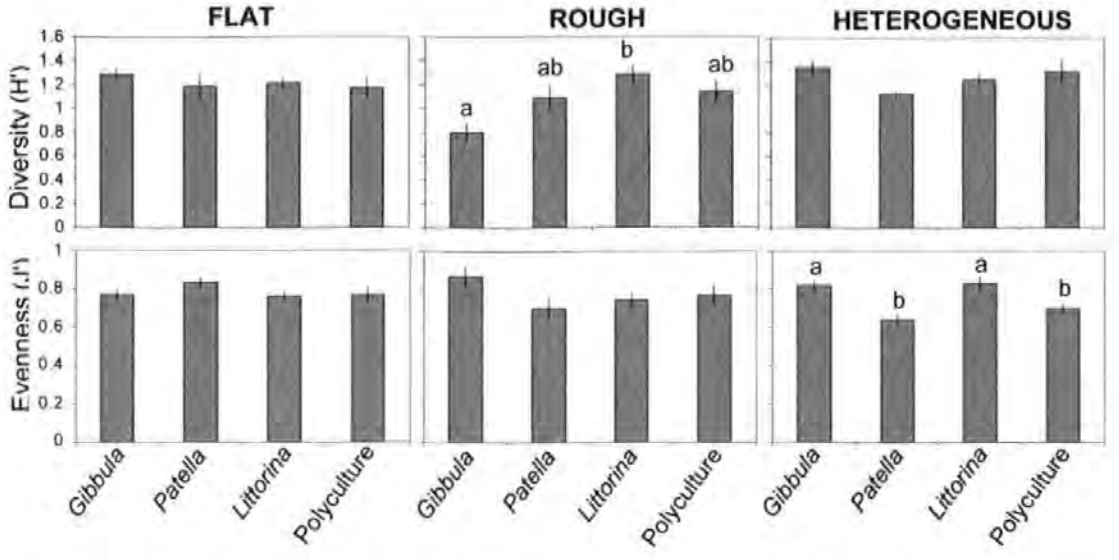


Figure 5.9. The mean (± SE) diversity and evenness of algal groups in grazer and substrate treatments. Significant effects of grazer treatment occurred in only 2 cases: for diversity under rough conditions, and for evenness under heterogeneous conditions. Letters denote differences identified through *post-hoc* Tukey tests.

Table 5.6. Results of the 2-way ANOVA on A) Pielou's evenness (J), and B) Shannon diversity index (H'). See Fig. 1 for presentation of means and *post-hoc* tests.

A.

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	.232(a)	11	.021	2.743	.008
Intercept	35.413	1	35.413	4602.072	.000
tm	.090	3	.030	3.882	.015
substrate	.011	2	.006	.729	.487
tm * substrate	.131	6	.022	2.844	.019
Error	.369	48	.008		
Total	36.014	60			
Corrected Total	.602	59			

B.

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	1.170(a)	11	.106	3.552	.001
Intercept	84.240	1	84.240	2813.842	.000
tm	.129	3	.043	1.439	.243
substrate	.361	2	.181	6.034	.005
tm * substrate	.679	6	.113	3.782	.004
Error	1.437	48	.030		
Total	86.846	60			
Corrected Total	2.607	59			

GENERAL DISCUSSION

CHAPTER VI

6.1. INTRODUCTION

The preceding four chapters documented a suite of studies united by a common theme: the link between biodiversity and ecosystem functioning. Each chapter explored a distinct aspect within this broad theme. As such, individual chapters can be thought of as contributing pieces to four separate puzzles and have thus been linked to existing knowledge within the areas they respectively address (see Discussions in Chapters II to V). Here, I will bring the findings of all the chapters together by discussing the implications of experimental design, particularly the spatio-temporal scale of BEF experiments, with special reference to experiments in this thesis.

6.2. SCALE, CHANGING PERSPECTIVES AND BIODIVERSITY EFFECTS

6.2.1. A different perspective on succession

The absence of a detectable diversity effect when comparing across pools of different successional stages is not surprising given the large variability in the composition and relative abundances of species that themselves exhibit a highly variable rates of productivity (see Littler and Littler 1980) and possess contrasting functional traits (Chapter II). The lack of a diversity effect may depend on the perspective taken, however. In lieu of complete successional sequences varying in species diversity I was unable to test the role of species diversity across the whole of succession. I hypothesize, however, that the heterogeneity represented by the time since a patch has been disturbed (or originally created) may produce an opportunity for complementarity analogous to that observed in Chapter V. If the complete successional sequence is considered, an important effect of macroalgal diversity may well be evident. Different species dominate at different stages in succession, possibly acting to stabilize and enhance the mean rate of productivity throughout succession; in other words, they exhibit temporal complementarity (Yachi and

Loreau 1999). In the absence of *Ulva* spp. or equivalent early colonizers, for example, the rate of productivity and standing biomass in early successional pools would likely be much lower than that observed.

The model of succession (*sensu* Connell and Slatyer 1978) has several important implications for the extent of temporal complementarity among species during succession. Positive effects of early colonizers on later colonizers (facilitation; Clements 1916) promote succession, but this form of facilitation may not produce an effects of diversity across patches – depending on a specific combination of species rather than richness *per se*; where this mechanism is important the loss of early colonizers would perhaps have the greatest impact on ecosystem functioning. Under the 'tolerance' model, interspecific interactions are neutral and observed patterns of succession are the result of differences in species' life-history traits, consistent with a temporal complementarity interpretation. If inhibition of later colonizers is strong (Drury and Nisbet 1973), observed differences in species' abundances during succession (apparent temporal complementarity), may be largely a result of negative interspecific interactions; for example, in the absence of *Ulva*, *Corallina* would be able to achieve a similarly high abundance early in succession. Elucidating the dominant mechanism of succession may well shape our appreciation of how diversity (and temporal complementarity) affects ecosystem functioning throughout succession.

6.2.2. Scale, power and heterogeneity issues within mesocosm experiments

The experimental sophistication that is required to disentangle species richness and identity effects, not to mention elucidate mechanisms, necessitates large numbers of treatments (Allison 1999, Schmid et al. 2002, Benedetti-Cecchi 2004). Given limitation on resources, this can lead to compromised numbers of replicates within treatments.

Variability among replicates within treatments can be reasonably high given noise adding factors such as variability in the performance of individuals within species and subtle environmental differences between experimental plots in the field (e.g. Chapters II and III). Limited replication and the variability inherent in ecological experiments combine to limit the statistical power to detect effects of diversity, which may be weaker than those of species identity (e.g. Chapter III). In retrospect, I would recommend simplifying experimental design (e.g. having just 2 levels of species richness) to maximize statistical power.

Another consequence of the high number of treatments (combined with invariably limited resources) in BEF experiments is the use of small experimental units. Indeed, our current empirical understanding of the relationship between biodiversity and ecosystem functioning is mainly based on small plots in grasslands and aquatic micro/mesocosms (Bulling et al. 2006, Cardinale et al. 2006), and the studies in this thesis are no exception. But how applicable are the findings of the small-scale experiments to broader scales (Levin 1992, Carpenter 1996, but see Lawton 1995)?

Small-scale units present several issues for the interpretation of experiments. First, the behaviour of the organisms may be influenced by both the restricted nature of the container itself, and also conditions within it. This is a possible issue in experiments within this thesis, although pre-cautions were taken to avoid such artifacts wherever possible. For example, numerous rocks were added to mesocosms to create refuges for crabs (Chapter IV) avoiding the magnification of agonistic interactions that often occurs in the absence of refugia (e.g. Finke and Denno 2002).

Another major concern is that findings from small-scale experiments may be highly context-dependent. Conditions are often tightly controlled and homogenized in small-scale experiments (and rightly so – maximizing the chances of detecting effects of

manipulations). There is a downside to this rigorous reductionist approach though. Observed effects may represent context-dependent processes, and thus only apply to the restricted set of conditions included in the experiment (Cardinale et al. 2000). The issues of context-dependency and heterogeneity are inextricably linked. As Chapter V demonstrates, different species are likely to dominate under different conditions (contexts), but will exhibit complementarity, enhancing ecosystem processes, when such conditions are combined (see also Cardinale et al. 2004). The experiment reported in Chapter III employed relatively homogeneous rock pools: they all had flat sides, varied minimally in size, shape, wave exposure and shore height. The conclusion of this experiment, that the patellid limpet had dominant effects on the measured ecosystem properties, needs to be interpreted cautiously. Yes, it applies within these homogeneous habitats, but it may not in natural rockpools, where a matrix of sand, turf-forming and crustose coralline algae, as well as both flat and more rugose rock create a heterogeneous substrate. One option to limit this problem is to incorporate natural environmental and or resource variability in each experimental unit. An example of this approach is provided in Chapter IV, in which particular attention was paid to representing heterogeneity, in this case of the prey resource. This resource heterogeneity allowed subtle interspecific differences in patterns of resource use to be expressed, which may have been critical in the generation of a species richness effect. In sum, experiments need to be interpreted in light of the conditions – and range of conditions – represented within experimental units.

In my opinion, small-scale mesocosm experiments are excellent tools for elucidating mechanistic links between biodiversity and ecosystem functioning (Chapters IV and V). They also provide an opportunity to test the influence of additional factors on the BEF relationship, i.e. the context-dependency (Cardinale et al. 2000, Biles et al. 2003, Chapter IV, V). However, extreme caution should be applied in the direct application of

results from such experiments to larger spatio-temporal scales. In short, mesocosm experiments demonstrate what *can* happen (and possibly *how* it happens), but not that it *does* happen to an equivalent extent in natural systems. Another option, of course, is to embrace natural variability and conduct studies over broad spatial scales, accepting that what is lost in precise experimental control, and perhaps replication, will be gained in realism (Raffaelli 2006).

6.2.3 Complementary approaches to small-scale experiments

Naturally occurring gradients of species diversity and composition provide opportunities to understand the biotic mediation of ecosystem processes at larger scales, albeit with less experimental control (Emmerson and Huxham 2002). Danovaro et al. (2008), for example, found an exponential relationship between both species and functional diversity and ecosystem process across 116 deep sea sites. This raises the possibility that ecosystem processes may be initially rapidly lost with declining biodiversity in this system. Worm et al. (2006) synthesized data from the world's fisheries, showing that fish diversity is related to the resistance of fish stocks to over-exploitation, as well as the speed at which they recover following protection. The chronosequence study (Chapter II) did not involve a direct manipulation (apart from the periodic – and serendipitous – addition of new pools), and as such represents a case in which observed effects can be considered completely representative of nature. The problem, of course, is that causal effects cannot be confidently assigned because multiple variables change concurrently. Such studies are important though, as they help to contextualize and inspire existing and future experimental manipulations, respectively.

Combining observational and experimental approaches can help to 'ground-truth' experiments, whilst elucidating mechanisms (e.g. Silliman 2005). This approach is

potentially problematic in BEF research however, because species diversity and composition tend to be highly sensitive to variability in the environment (Huston 1994, Chapter II), which may produce spurious correlations between aspects of biodiversity and ecosystem functioning.

Observations of naturally positive BEF relationships have been reported to cautiously corroborate experimental results (Bruno et al. 2005, Byrnes et al. 2006). Interestingly, my own unpublished field data of grazer abundances on local rocky shores showed an exponential relationship between grazer species richness and standing biomass across replicate quadrats. The fact that an experimental manipulation of grazers (Chapter III) failed to detect species richness effects suggests that the natural pattern on shores is a result of another process (such as grazer aggregation) rather than species complementarity. I expect that many correlations between diversity and ecosystem process in natural systems will be the result of covariates (such as nutrient availability); there really is no substitute for experimental manipulation in determining cause-effect relationships.

Species removals from natural systems may allow rigorous tests of BEF hypotheses free from the limitations of small-scale experiments. Ecologists can exploit situations where biodiversity is being *directly* affected by human activities. Where specific species or functional groups are removed by humans, for example through selective logging or hand-net collection of fish on coral reefs for the aquarium trade, there is opportunity to study effects on ecosystem properties (Wardle 2008). More generally, long-term removal experiments (allowing sufficient time for possible compensation by 'redundant' species) are an under-used tool to understand effects of species and functional diversity (Diaz and Cabido 2001).

6.2.4 Considering temporal scale

The studies in this thesis ranged in duration from 5 days to 13 months (with the exception of the chronosequence, which is a special case as it represented a 14-year period). Temporal scales were chosen based on a combination of practical considerations and the period required to test the questions appropriately. The grazer manipulation (Chapter III) was limited in duration due to the wear of cages and constant maintenance requirements. The short duration in the predator-prey lab experiments (Chapters IV and V) were necessary to avoid the complete consumption of all prey, or particular prey types in any single treatment. This could generate an effect 'ceiling', possibly preventing differences between treatments from being detected.

There are several draw-backs of this short-term approach, however. First, temporal dynamics of species' relative abundances cannot be examined. In lieu of these data, mechanistic interpretation is constrained – partitioning selection and complementarity effects requires species-specific contributions to the focal ecosystem process (Fig. 1.2.), which is most easily obtained within longer-term studies where biomass accumulation is the focal process. Testing the fit of dynamic models to population-level changes through time may also help to elucidate mechanisms (Weis et al. 2007), again requiring longer-term data. Second, the effects observed may be temporally-sensitive. Indeed, the effect of diversity has been shown to increase through time in long-term grassland studies (Cardinale et al. 2007), and the question of whether this is a general phenomenon remains both interesting and important. This may be because of the range of conditions that occur through time under field conditions, which promotes temporal niche differentiation and the enhancement of functioning (Yachi and Loreau 1999; see also 6.2.2) Third, in short-term studies, where biomass-accumulation is negligible, densities are chosen by the researcher,

which can cause the results to be highly sensitive to the experimental design employed (see section 6.2.3).

There is an advantage to short-term BEF experiments, however. The enhancement of an ecosystem process above that expected from a weighted average of the component species effects can be confidently credited to complementarity (Petchey et al. 2003). This is because species identity effects can be accounted-for in the calculation of expected rates (see e.g. Emmerson and Raffaelli 2000). In longer-term studies, non-transgressive overyielding can be generated from positive selection (Fig. 1.2.). Nevertheless, longer-term approaches to testing the hypotheses in chapters III to V would potentially yield greater insight into species interactions and the effects of diversity.

6.3 CONCLUDING REMARKS

Despite focused research since the mid-1990s, there remain many unknowns in the quest to understand the links between biodiversity and ecosystem functioning. Research presented in this thesis sheds light on several previously poorly studied areas. The priority now must be to return to the original motivation of BEF research: to understand the consequences of anthropogenic activities for the functioning of ecosystems, including the goods and services upon which humanity depends. We need to develop ways to scale-up findings from mesocosm experiments to make them more relevant to the provision of ecosystem goods and services. Obviously, researchers should focus efforts on those ecosystem goods and services that are most vital to society (see Kremen et al. 2005), such as pollination (Tylianakis et al. 2006). Embracing patterns and processes on larger scales in 'real world' ecosystems will also be invaluable; removal experiments, studying effects of extinction in natural ecosystems (Wardle 2008) and modeling realistic extinction scenarios (e.g. Bunker et al. 2004) will likely prove to be fruitful avenues of research.

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Reports

PREDATOR DIVERSITY AND ECOSYSTEM FUNCTIONING: DENSITY MODIFIES THE EFFECT OF RESOURCE PARTITIONING

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Abstract. The link between biodiversity and ecosystem functioning is now well established, but the challenge remains to develop a mechanistic understanding of observed effects. Predator–prey interactions provide an opportunity to examine the role of resource partitioning, thought to be a principal mediator of biodiversity–function relationships. To date, interactions between multiple predators and their prey have typically been investigated in simplified agricultural systems with limited scope for resource partitioning. Thus there remains a dearth of studies examining the functional consequences of predator richness in diverse food webs. Here, we manipulated a species-rich intertidal food web, crossing predator diversity with total predator density, to simultaneously examine the independent and interactive effects of diversity and density on the efficiency of secondary resource capture. The effect of predator diversity was only detectable at high predator densities where competitive interactions between individual predators were magnified; the rate of resource capture within the species mixture more than doubled that of the best-performing single species. Direct observation of species-specific resource use in monoculture, as quantified by patterns of prey consumption, provided clear evidence that species occupied distinct functional niches, suggesting a mechanistic explanation of the observed diversity effect.

Key words: BEF; biodiversity; density; ecosystem functioning; food web; predator; resource partitioning; trophic interactions.

INTRODUCTION

Concerted empirical research over the last decade has shown that species richness can enhance the magnitude of ecosystem functioning in a range of systems (Hooper et al. 2005, Balvanera et al. 2006, Cardinale et al. 2006). Despite this emerging paradigm, the ability to predict the functional consequences of species loss in a given system remains elusive because the strength of species diversity effects has proven highly variable across studies (Balvanera et al. 2006). To transform biodiversity–ecosystem functioning research into a predictive science, a clear mechanistic understanding of observed effects is required (Yachi and Loreau 2007).

Although early seminal research on the effect of biodiversity on ecosystem functioning focused on temperate grassland communities (Naeem et al. 1994, Tilman et al. 1996), recognition that species in higher trophic levels are more vulnerable to extinction (Petchey et al. 1999, Duffy 2002) has recently shifted attention onto multi-trophic systems (e.g., Duffy et al. 2007). Despite the obvious differences between primary producers and mobile, behaviorally complex predators, theory predicts that both sampling and complementarity effects operate to enhance the magnitude of resource capture, irrespective of trophic level (Ives et al. 2005). The sampling (Huston 1997), or positive selection (Loreau 2000), effect occurs when species with extreme trait values dominate resource acquisition and are more likely to be included in diverse species mixtures by chance. In this case, the functioning of the diverse mixture will not exceed that of the best monoculture. In contrast, where niche complementarity is evident, species differing in resource requirements exploit a wider spectrum of resources and experience reduced interspecific competition, thus potentially enhancing the rate of ecosystem processes beyond that of the best-performing single species (Trenbath 1974, Loreau and Hector 2001). Direct evidence of resource partitioning is rare despite recognition of its importance in mediating species coexistence (Chesson 1991). Instead, functional complementarity is often inferred from diversity effects that exceed those that can be explained by the sampling effect alone (Loreau and Hector 2001, Duffy et al. 2003). In contrast to primary producers, however, the resource use patterns of predators can be readily assessed, potentially enabling tractable studies of the functional consequences of diversity mediated through niche partitioning (Ives et al. 2005).

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PLATE 1. The crabs (from left) *Necora puber*, *Carcinus maenas*, and *Cancer pagurus* are important coexisting predators in rocky intertidal habitats. Photo credit: P. Moore.

A rich history of experiments examining trophic interactions between multiple predators and their prey has identified the complexities of predicting aggregate predator effects on prey consumption (e.g., Losey and Denno 1998, Finke and Denno 2004, Siddon and Witman 2004). Such work has become increasingly integrated with research on the link between biodiversity and ecosystem functioning through experiments that maintain total predator density across gradients of species richness (e.g., Wilby et al. 2005, Straub and Snyder 2006). Due to the strong focus on agricultural pest control, previous studies have typically included very low levels of prey heterogeneity (but see Bruno and O'Connor 2005, Byrnes et al. 2006), thereby limiting the potential for resource partitioning among predators. The role of predator diversity within diverse natural food webs thus remains largely unknown.

The density of organisms within natural communities is determined not only by resource availability, but by a suite of factors that may act to maintain density below the carrying capacity of the environment. For example physical and biological disturbance (e.g., Sousa 1984), recruitment limitation (e.g., Connolly and Roughgarden 1998), and top down control (including human exploitation) (e.g., Castilla 1999) are all known to influence density independently of local resource availability. Assuming constant resource availability, the density of a species will determine the strength of intraspecific and interspecific competition, which can in turn influence the effect of species diversity on ecosystem functioning (Cardinale et al. 2004, Weis et al. 2007). Despite the critical role that competition may play in linking diversity and ecosystem functioning, the potential role of density in modifying diversity effects mediated by niche partitioning has yet to be tested empirically.

The efficiency of resource capture by consumers, as measured by the rate of prey consumption, represents an important ecosystem process within complex food webs (Duffy 2002) and allows investigations of multi-trophic interactions within the context of theory linking biodiversity and ecosystem functioning. We conducted a factorial mesocosm experiment to test the hypothesis that predator density modifies the effect of predator species richness on the efficiency of trophic energy transfer between diverse intertidal prey assemblages and three species of predatory crabs. Our results show that clear effects of diversity only occurred at high predator density. Furthermore, our results suggest that predator species exhibited marked differences in the patterns of resource use, representing functional complementarity through resource partitioning.

METHODS

We manipulated the diversity and density of three common intertidal predatory crabs, *Cancer pagurus*, *Necora puber*, and *Carcinus maenas* (see Plate 1), in

outdoor mesocosms, 0.6 × 0.4 m in area × 0.2 m depth, which were subject to a constant flow of fresh filtered seawater at the Marine Biological Association Laboratory in Plymouth, United Kingdom. The predator species are abundant and have overlapping distributions within rocky intertidal habitats on the southwest coast of the United Kingdom. Furthermore, these predators have similar body sizes, enabling the isolation of species identity and diversity effects from those of body size, which can have a strong influence on trophic interactions (Jennings et al. 2001).

We employed a substitutive design, frequently used in biodiversity–ecosystem functioning studies (e.g., Polley et al. 2003, Bruno et al. 2005) including those examining predator diversity effects (Siddon and Witman 2004, Byrnes et al. 2005, Snyder et al. 2006), such that overall density was maintained across a gradient of diversity. We crossed a substitutive gradient of predator diversity with total overall density, allowing the independent and interactive effects of diversity and density to be examined simultaneously (Benedetti-Cecchi 2004). Four different predator assemblages were constructed, three with each species in monoculture and one with all three species combined. Density consisted of two levels: low with three, and high with six predator individuals in total. The high diversity predator community thus contained one and two individuals of each of the three species at low and high density, respectively. These densities are commonly observed in the intertidal at small spatial scales, where crabs aggregate in areas of suitable refugia. We defined a narrow size range for each species to ensure approximately equal masses (52 ± 2 g wet biomass) of individuals within and among species. Predator-free controls were included to confirm that all reductions in prey were due to consumption. Each treatment was replicated six times in total, but limited space to house mesocosms restricted our experiment to a randomized, temporally blocked design, such that two replicates of each of nine treatments were included in three temporal blocks. The start of each block was separated by two weeks to enable collection of animals during spring tides.

Predators were collected from under boulders within the intertidal zone at Looe, Cornwall, UK. To exclude effects of satiation at the time of collection, crabs were starved for four days prior to experiments in large holding tanks with continuous flowing seawater containing numerous refuges to limit potentially harmful agonistic interactions. Each experimental mesocosm contained a 3-cm layer of coarse sand and gravel upon which rocks of a range of sizes were arranged to recreate a topographically complex, refugium-rich environment mimicking rocky shores (Appendix A). Habitat-forming materials were collected from local shores and rinsed with fresh water to remove macro-biota before introduction to mesocosms. We covered each mesocosm with a tightly fitting fine nylon mesh to ensure animals were unable to escape. The experiment was conducted between January and March 2007.

Initial prey composition and abundance did not vary among mesocosms (approximately 8.71 g dry mass in total, Appendix B). Five species of prey were used, all naturally co-occurring and collected from the intertidal zone of local rocky shores. Barnacles (*Chthamalus* spp.) could not be transplanted into mesocosms individually, and were therefore transplanted in dense aggregations attached to pieces of rock. Individuals were counted before adding the rock pieces to the mesocosms. For all other prey species we included six individuals within each of two size classes (large and small) to incorporate an element of resource heterogeneity occurring in natural prey populations. Mussels *Mytilus edulis*, periwinkles *Littorina littorea*, topshells *Gibbula umbilicalis*, and limpets *Patella ulyssiponensis*, were all added to the mesocosms three days before the start of each experiment.

We used the number and identity of prey absent from each mesocosm upon termination of the experiment to calculate consumption rates for each replicate predator assemblage. We calculated the consumption rate (shell-free dry biomass per day) of each prey type separately to elucidate patterns of prey consumption, and summed these to derive the total rate of prey consumption (Appendix C). We also derived per capita rates to facilitate direct comparisons across density levels. Based on the consumption of each prey type in monoculture, we calculated expected patterns in polyculture and derived proportional deviations from expected (Appendix C). A relatively short experimental duration (five days) was chosen so that extinction of prey types did not occur within mesocosms, which could prompt prey-switching, blurring initial resource use differences between species and potentially weakening the diversity effect (Ives et al. 2005).

Non-transgressive overyielding occurs when the magnitude of an ecosystem process in a diverse mixture exceeds that of the average monoculture value of component species (Fridley 2001). To test for this form of diversity effect, and for the potential interactive effect of total density upon it, we performed a two-way analysis of variance (ANOVA), followed by a priori planned contrasts between the average single-species and the polyculture treatment at both densities. Inclusion of all three species in monocultures facilitated a test of transgressive overyielding, which identifies effects of diversity that could not be produced by the dominance of any single component species. To test for this effect we performed a priori planned contrasts between the best-performing monoculture and the polyculture (Bruno et al. 2005, Duffy et al. 2005) at both low and high densities separately. We conducted a permutated multivariate ANOVA (PERMANOVA [Anderson 2001]) to test for differences in the composition and relative abundances of prey types consumed by the three predator species independently and in combination.

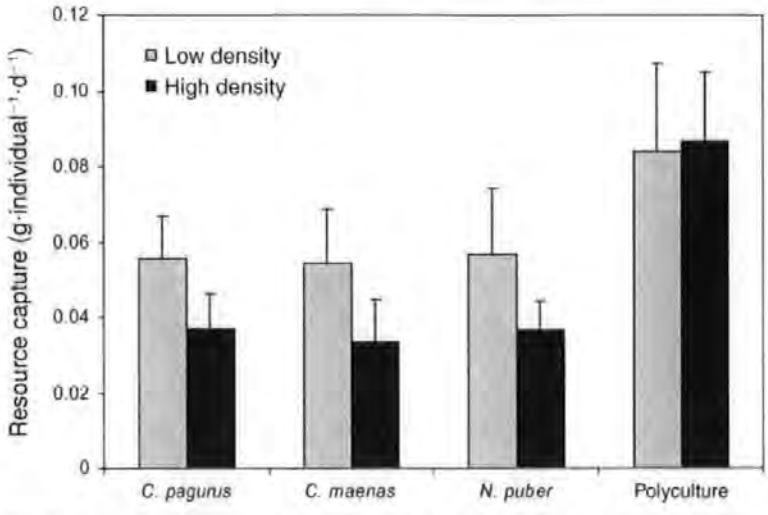

FIG. 1. Aggregate per capita resource capture rate (mean + SE) for all monocultures and the three-species mixture at both low and high densities (PC_{Total}; see Appendix C for calculation; $n = 6$ replicates per treatment) of the crabs *Cancer pagurus*, *Carcinus maenas*, and *Necora puber*. Significant differences occurred between monocultures and the mixture across high-, but not low-, density treatments. See *Results* and Appendix E for ANOVA results.

This provided a test of resource partitioning among predator species in terms of the multivariate prey base.

RESULTS

We recovered all prey items from predator-free controls upon termination of the experiment, confirming that all prey losses were due to predator consumption rather than other causes of mortality or escapes. All crabs survived the experiment uninjured, showing no evidence of physically harmful agonistic interactions or intra-guild predation. There were no significant differences among temporal blocks or interactions between block and experimental treatments (diversity × block, $F_{6,24} = 0.82$, $P = 0.57$; density × block, $F_{2,24} = 0.66$, $P = 0.53$; block, $F_{2,24} = 2.5$, $P = 0.11$), hence these data were pooled for subsequent analysis.

The monocultures of the three predator species captured similar prey quantities within density levels (Fig. 1, Appendix D). When species were combined in the polyculture there was significantly greater consumption compared to the pooled single species treatments ($P = 0.003$, Appendix E). This diversity effect was, however, dependent upon predator density, with the polyculture significantly outperforming the mean (non-transgressive overyielding) and the maximum (transgressive overyielding) monoculture among the high density treatments only. Specifically, at high density the predator mixture more than doubled the rate of prey consumption, exceeding the average monoculture by 144% ($F_{1,20} = 10.64$, $P = 0.004$) and the best-performing monoculture by 134% ($F_{1,11} = 6.02$, $P = 0.029$). At low density, the effect of diversity was far weaker, with the aggregate rate of resource capture of the polyculture only 52% ($F_{1,20} = 2.11$, $P = 0.16$) above the average monoculture and 48% ($F_{1,11} = 0.90$, $P = 0.364$) above the highest single species treatment (Fig. 1, Appendix E). The differential effects of diversity between low and high density comparisons can be understood by comparing the effect of density in monocultures and the polyculture. A doubling of monoculture density only marginally elevated total resource capture (Appendix D), resulting in a density-dependent suppression of per capita resource capture efficiency within respective monocultures (marginally significant at an α level of 0.1, $F_{1,30} = 3.03$, $P = 0.09$). In the polyculture, however, increasing density led to a large increase in total resource capture (Appendix D); density thus had no effect on per capita rates within the polyculture (Fig. 2, $P = 0.93$).

All three predator species in monoculture differed markedly in patterns of prey consumption (Fig. 3). Whilst all predators demonstrated an ability to consume every prey species, clear and consistent differences between species in the patterns of trophic interactions were evident, with particular prey types most heavily consumed by specific predators (Fig. 2). PERMANOVA, with post-hoc pairwise comparisons, confirmed that all the predators exhibited distinct patterns of resource use ($F_{3,40} = 3.98$, $P < 0.001$, Appendix F). Moreover, although density significantly affected overall patterns of prey consumption ($F_{1,40} = 4.14$, $P = 0.01$), species-specific differences were robust to changes in total density (interaction $F_{3,40} = 0.42$, $P = 0.95$).

Calculations based on the resource use patterns of single predator species predicted a relatively even distribution of prey consumption rates in the multispecies treatments (Fig. 3). The observed pattern of trophic interactions was consistent with this prediction (Fig. 3). Deviations from this expected pattern indicated which resources were exploited to a greater degree at high predator diversity (Appendix G). In particular, the

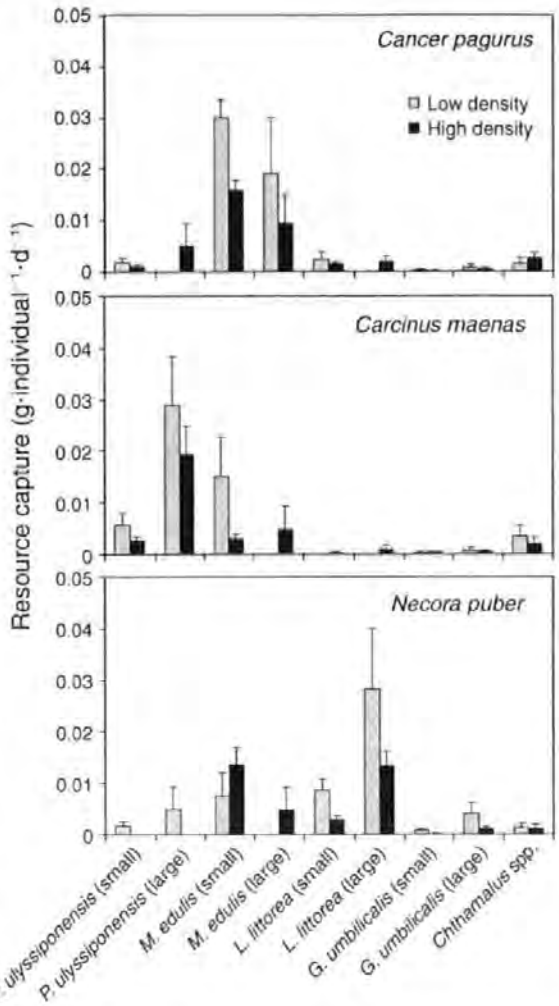

FIG. 2. Observed rates of consumption (resource capture; mean + SE) of each prey type and size for individual predator species (PC_{ij}; see Appendix C for calculation) at two predator densities ($n = 6$ replicates per treatment). Predators exhibited significantly different patterns of prey consumption (Appendix F). Prey are limpets (*Patella ulyssiponensis*), mussels (*Mytilus edulis*), periwinkles (*Littorina littorea*), topshells (*Gibbula umbilicalis*), and barnacles (*Chthamalus* spp.).

consumption of both size classes of *L. littorea* and *P. ulyssiponensis* far exceeded the null expectation at both densities (Fig. 3, Appendix G), consistent with the hypothesis that resource partitioning can enhance the total rate of resource capture by component species.

Discussion

Our results show that predator species richness can have a strong effect on the efficiency of resource capture within a model intertidal food web. By crossing diversity treatments with total density, we tested the role of density in modifying the species richness effect, revealing that the loss of diversity leads to a more dramatic drop in ecosystem process rate when predators occur at high density. Furthermore, detailed analysis of species-specific trophic interactions demonstrated that the three predators occupy distinct fundamental functional niches (sensu Rosenfeld 2002), providing a possible mechanistic explanation of the observed effects.

Our finding that the diversity effect was strongest, and only detectable, at high density (Fig. 1) is not surprising considering the critical role played by competition in the manifestation of the niche partitioning effect. Our analysis identified density-dependent depression of per capita resource capture in single species treatments (at an $\alpha = 0.1$ level), but no such effect occurred in the polyculture (Fig. 1). This strongly suggests that the strength of negative interactions is less between heterospecifics than conspecifics. Where negative intraspecific interactions are weak or absent in single species contexts, which may be the case at low density in our experiment, individuals gain less, or no, advantage from occurring within a mixed community of consumers (Wilby et al. 2005). The apparent relaxation of negative interactions with increasing species richness thus only produced a clear effect of diversity at high predator density where negative interactions between conspecifics within monocultures were strongest.

An ability to predict the consequences of species loss requires an understanding of the factors that modify diversity effects (Cardinale et al. 2000). Our experiment demonstrates that density should be considered in future

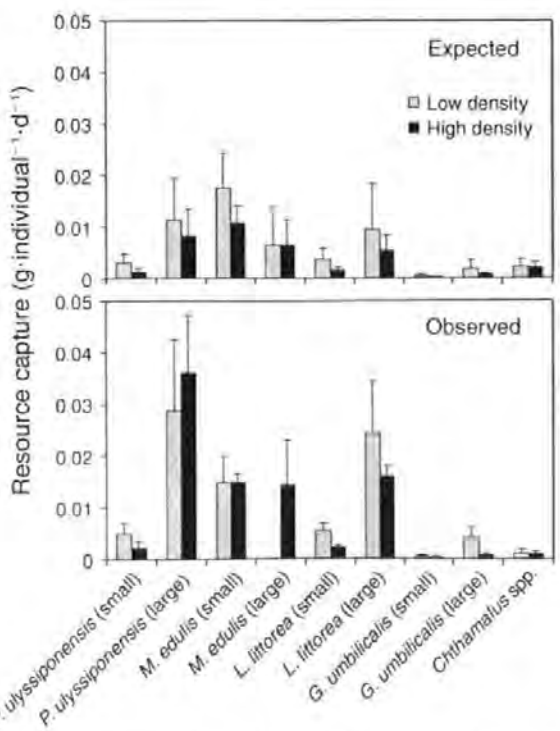

FIG. 3. Expected (Pe_{ij}) and observed (PC_{ij}) rates of resource capture (mean + SE) for the diverse three-species predator mixture at both high and low density. See Appendix C for calculation of variables, and Appendix G for proportional deviations from expected rates. Prey species are as in Fig. 2.

attempts to understand the functional consequences of biodiversity within both observational and experimental studies. For example, high disturbance systems, and the early successional communities that typify them (e.g., Martins et al. 2007), may not be resource limited, potentially precluding diversity effects (Weis et al. 2007). On the other hand, management activities, such as the provision of protected areas, reduce direct and indirect human impacts on populations, typically increasing the abundance and diversity of predators (e.g., Babcock et al. 2007). The outcome of our experimental diversity manipulation implies that as the density of such predators increases, so too may the value of diversity for ecosystem functioning.

The sampling or positive selection effect (Huston 1997, Loreau 2000), has been credited as the principal mechanism leading to positive diversity effects in both biodiversity–ecosystem functioning (Cardinale et al. 2006) and natural enemy studies (Denoth et al. 2002, Straub and Snyder 2006). However, both theory (Cardinale et al. 2004, Loreau 2004) and experiments (Dimitrakopoulos and Schmid 2004, Gamfeldt et al. 2005, Snyder et al. 2006) suggest that the limited resource heterogeneity typically provided in such studies may preclude resource partitioning, instead highlighting strong effects of particular species (those most suited to the specific conditions, or prey type, provided). We aimed to incorporate the range of prey types, in terms of sizes and species identity, available in the natural environment, thus providing realistic conditions with a high potential for resource partitioning. Indeed, all predators exhibited distinct resource use patterns in monoculture (Fig. 2). We could not provide incontrovertible evidence of resource partitioning, as our experimental setup precluded measurement of species-specific resource use within the diverse treatment. However, the increased consumption of a broad spectrum of prey types in the polyculture (Fig. 3), representing dietary preferences of all three predators, suggests that all species maintained niche differences and benefited from resource partitioning. Moreover, theory and past experiments support the supposition that distinct resource use patterns observed in monoculture would likely have been maintained, or even exaggerated, in the presence of interspecific competition (Odum and Barrett 2005). Our experimental findings thus strongly suggest that resource partitioning can have a marked effect on ecosystem functioning, providing a rare empirical example (but see Bracken and Stachowitz 2006, Kahmen et al. 2006) of a phenomenon that has long been regarded as a key link between species richness and the magnitude of ecosystem functioning (Naeem et al. 1994, Loreau et al. 2001). Although the evidence indicates that all species maintained niche differences in polyculture, the consumption of some prey types deviated disproportionally from expected values (Fig. 3, Appendix G). This suggests that individuals of certain predator species may have benefited to a greater degree from occurring within a mixed assemblage, enhancing the consumption of their preferred prey types relative to others'. Further insight into the role of predator diversity could be achieved by documenting the division of resource acquisition rates among species within mixtures. This would provide a more direct test of resource partitioning and facilitate application of statistical techniques to distinguish between complementarity and selection effects (Loreau and Hector 2001, Fox 2005).

The appropriate experimental design to test the effect of species richness on ecosystem functioning continues to be debated (Balvanera et al. 2006, Weis et al. 2007), primarily because species richness cannot be manipulated without a concurrent change in either total density or the density of component species. To clarify interpretation of experiments, the specific questions addressed by alternative designs need to be clearly defined. The substitutive design, used here, equalizes total density across single and multiple species treatments, testing whether, given density-compensation, local extinction of species will influence the magnitude of an ecosystem process. In effect, species which are lost through local extinction are experimentally replaced with individuals of those remaining, thus intraspecific and interspecific interactions are directly compared (Jolliffe 2000, Griffen 2006). Where resource partitioning occurs, as in this experiment, replacing heterospecifics with conspecifics will reduce the breadth of resources exploited, increasing competition and depressing the rate of resource acquisition. We thus suggest that the substitutive design is an effective approach to detect the functional consequences of resource partitioning.

Our experiment identifies the potentially strong effect of resource partitioning among predators in nature, but is only the first step. Species richness remains the most commonly used measure of diversity in experimental tests. However, resource partitioning results from species' differential resource use, and is thus an effect of functional diversity (sensu Petchey and Gaston 2002) as opposed to species richness per se. The field might thus increase its predictive capacity by shifting the emphasis of biodiversity–function research from species diversity to functional diversity. Multiple predator–prey systems such as the one described here, provide an ideal opportunity to test the theoretical prediction that ecosystem processes will increase with functional diversity independently of species richness (Petchey and Gaston 2006). Furthermore, in order to gauge the possible cascading effects of predator extinction we need to couple theory with longer-term and larger-scale manipulative and observational studies that incorporate predator and prey dynamics, adaptive morphological and behavioral prey responses, as well as indirect interactions. Future experiments should also represent natural systems as we have, incorporating environmental and resource heterogeneity that allows both stable

coexistence and the enhancement of ecosystem processes through niche partitioning.

To increase the relevance and applicability of biodiversity–ecosystem functioning research, we must develop a capacity to predict the consequences of extinction based on measurable properties of both natural and managed ecosystems. We show here that a fundamental property of communities, the density of consumers in relation to their resources, can modify the effect of diversity as mediated by resource partitioning. By explicitly examining mechanistic links between diversity and ecosystem functioning we can develop a predictive capacity, enabling us to quantify the expected loss of ecosystem functioning under scenarios of global and local environmental change.

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APPENDIX A

Image of a mesocosm used in the experiment (*Ecological Archives* E089-018-A1).

APPENDIX B

Description of prey assemblage (*Ecological Archives* E089-018-A2).

APPENDIX C

Calculation of resource capture efficiency (*Ecological Archives* E089-018-A3).

APPENDIX D

Total resource capture rate within all treatments (*Ecological Archives* E089-018-A4).

APPENDIX E

Analysis of predator diversity and density effects (*Ecological Archives* E089-018-A5).

APPENDIX F

Analysis of species-specific resource consumption (*Ecological Archives* E089-018-A6).

APPENDIX G

Comparison of prey consumption patterns between monocultures and the polyculture (*Ecological Archives* E089-018-A7).