The roles of the cockle *Cerastoderma edule* L. on ecosystem functioning: cockle comings and goings

Thesis submitted in accordance with the requirements of the University of Liverpool
for the degree of Doctor in Philosophy by Christopher Peter Cesar
September 2009

ABSTRACT

There is increasing interest regarding the impacts of human activities on the functioning of marine systems. A primary driver of change to marine systems is through the impacts of fishing. Biomass-dominant target species have the potential to mediate a number of ecosystem functions, either directly or indirectly, through the influences that taxa have on ecological processes and/or other biotic or abiotic components of the system. This thesis investigates the roles of the cockle, *Cerastoderma edule* on ecosystem functioning within intertidal sedimentary systems.

A series of investigations revealed that cockles have the potential to mediate benthic primary productivity through their roles in the recycling of nutrients and effects of sediment structure and have impacts upon assemblage biomass and functional diversity. However, the roles of cockles on other aspects of ecological functioning were less apparent.

An investigation also assessed the suitability of the use of assigning taxa to functional groups when assessing functional diversity. Taxa were shown to have the potential to change their feeding activity following disturbance, with evidence suggesting a change to benthic-pelagic coupling. This change however, would not have been observed with investigations of functional traits alone and thus supports the use of direct measures of functions to support functional trait diversity measures.

It is imperative for ecological investigations to consider long-term changes to population dynamics. However, particularly in marine systems, such data are generally lacking. This thesis presents a novel approach, using information from sea fisheries reports to gain a semi-quantitative 30-year data set on cockle landings within Morecambe Bay, north-west England. This technique revealed evidence of temporal changes to the functional processes at a large scale.

This thesis provides evidence for cockles acting as key contributors to ecological functioning. The functional role of cockles is tempered by the high degree of redundancy within these assemblages, highlighting a number of issues relating to the use of field studies and encouraging a move towards increased use of traditional ecology in future studies.

ACKNOWLEDGEMENTS

First and foremost, I thank Professor Chris Frid for his supervision of this project and for all his assistance, support and advice. I fully appreciate his patience and his staid reluctance to hand back thoroughly-shredded early drafts of written sections. I also thank the University of Liverpool for their funding of this project.

I appreciate the efforts and assistance of Les Connor in the laboratory and for his wry sarcasm. Thanks also to Doctors Conrad Chapman and Stan van den Berg at the Department of Earth and Ocean Sciences for their interest in the project and for allowing me to spend time working with them, and for having the patience to answer my questions regarding electrochemistry. I also thank Professor George Wolff at DEOS for providing me with equipment and information for the measuring of nutrient fluxes and for taking the time to discuss stable isotope analyses. Thank you too to Doctor Julie Bremner for her constructive comments.

I massively appreciate and owe a huge debt of thanks to all those who came to help me in the field, braving treacherous muddy sand, beating winds and rain and hauling weighty mud up scenic but almost impassable terrain: Keith Hatton, Rebecca Ahmed, Dave Cavanagh, Sarah Cavanagh, Sarah Fowke, Gregor Govan, Karen Hall, Tom Heyes, Diane Jones, Tom Laughland, Hazel Lewis, Elaine Mayers, Jess Mettam, Katherine Nisbet, Laura Nuttall, Ravi Pandalaneni, Odette Paramor, Leonie Robinson, Valerie Samedi, Delores Sellars, Matt Spencer, Heidi Tillin, Tony Tollitt, Alex Winrow-Giffin, Carl Wright and the rangers and dog based at Wirral Country Park. I am greatly indebted to Bill Cook and Bob Houghton from North West & North Wales Sea Fisheries Committee, whose quad bike handling skills made an almost impossible route into Morecambe Bay merely perilous. I also thank Bob and Bill, along with Mark Prime at the University of Hull for their helpfulness, time and effort in the retrieval and electronic-rendering of information from the SFC's records.

On a personal note, I thank my office mates and the Marine Tea fraternity not already mentioned for idle and inane banter, their (often conflicting) advice and, most-importantly, tea, cake and biscuits: Lucy Abram, Mhari Alexander, Ian Goodhead, Jon Green, Cheryl Knowland, Rob Marrs, Katherine Nisbet, Catherine Scott, Angela Sims and Jack Thomson.

Outside of the office, I really appreciate the friendship, chit-chat and fun quiz-times I've had with: Brian O'Hare, Clare Gabbott, Rich & Ange Midgely, Stephen O'Hare, Kat Smith and Dom Stockton. Cheers also to my brother Andrew for a lifetime of nonsensical banter.

I owe a massive debt to my parents. Without their love, support and encouragement, I would never have gotten to this stage: I am truly grateful.

Last, but certainly not least, I thank Hayley Smith, for too many reasons to list. I thank you for all your love and for your unconditional support and understanding. Thank you.

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CHAPTER 1: ECOSYSTEM FUNCTIONING

1.1 Introduction

There is an increasing drive to develop our understanding as to how anthropogenic activities affect the functioning of ecosystems, with conservation activities increasingly focused on maintaining ecosystem goods and services (E.C. 2007). The concept of ecosystem functioning may be defined in a number of ways, including the biodiversity and evenness of assemblages, the interactions between organisms within a system and the physical environment and processes within a system (*e.g.* nutrient cycling and hydrodynamic processes, Jax 2005). However it is defined, the functioning of an ecosystem is fundamentally affected or mediated by the organisms living within the system (Naeem *et al.* 2002) and hence, functioning is potentially impacted by any human activities which affect the biota of a system.

Ecosystem functions include processes and interactions ranging from those between individual organisms, to small sub-groups, to entire communities (Christensen *et al.* 1996; Naeem *et al.* 2002). Given the large numbers of interactions that potentially contribute to ecosystem functioning, it is perhaps not surprising that a large number of methods and metrics have been used as proxy measures of ecosystem functioning (Lawton 1994; Hooper *et al.* 2005; Duffy 2006). A commonly-utilised proxy is the biological diversity of ecosystems, using this as an expression of the overall productivity and functional vigour of the system. The theoretical basis of this approach is that increased biodiversity is reflected by increased ecosystem functioning (*e.g.* primary productivity, Hector *et al.* 1999). This approach and the interpretation of some key studies in this field have made the validity of this approach a heavily-debated issue within ecology (Kaiser 2000). As well as biodiversity-based approaches, investigations of the functional roles that species play have become increasingly used. This approach categorises taxa into functional groups or guilds that share ecologically-important behavioural or physiological traits or phenotypes and functioning is indexed by investigating the prevalence and distribution of the different functional groups within systems. A further development in this area has been through the application of multivariate statistical approaches, allowing the consideration of multiple functional groups simultaneously, *i.e.* investigating the prevalence within assemblages of a number of functionally-important biological traits.

This section explores the theoretical framework of these approaches and discusses some of the advantages and limitations of each. Research into biodiversity-ecosystem functioning (BEF) has progressed much in recent years and these recent advances are also discussed.

1.2 Taxonomic approaches

Analysis of the diversity of species within ecosystems has been one of the most heavily-investigated aspects of ecology (see Hooper *et al.* 2005) and the concept of using some measure of biodiversity (including species richness, diversity, rarity, evenness) as a measure of the health (or functioning) of ecosystems has been explored extensively. Typically, increased species diversity is reflected by some ‘improvement’ to the delivery of one or more of the ecosystem functions under investigation (*e.g.* Hector *et al.* 1999; Jonsson & Malmqvist 2000; Zak *et al.* 2003). There has been a great deal of controversy regarding this approach however, and much debate has arisen amongst ecologists as to the interpretation of a number of key studies in this field and the transferability of findings from synthetic to natural systems (*e.g.* Grime 1997; Huston 1997; Naeem *et al.* 1999; Kaiser 2000; Wardle *et al.* 2000). However, recent studies have gone some way to resolving these issues. Two meta-analyses on over 100 experiments have found that having more species within an assemblage results in a greater productivity and a more efficient exploitation of system resources (Balvanera *et al.* 2006; Cardinale *et al.* 2006).

A number of hypotheses have been developed to explain the relationship of species diversity to ecosystem functioning. The facilitation hypothesis (Loreau & Hector 2001) argues that some species have positive impacts on the ecological role(s) carried out by other species and thus with an increasing number of species, ecosystem functioning is also enhanced. Cardinale *et al.* (2002), for example, found that diverse insect assemblages increased the topographical complexity of stream benthic habitats and improved the feeding success of individuals within the habitat. Under the niche differentiation hypothesis, species richness reflects the resource-exploitation of the system and the more species that are present within the system, the more efficiently the resources are exploited and the more productive the system is (Tilman *et al.* 1997a; Loreau & Hector 2001). These hypotheses both predict that having more species present within an ecosystem will enhance the functioning of the ecosystem and they are generally grouped under the complementarity hypothesis.

A large proportion of the studies into the biodiversity effects on ecosystem functioning have defined biodiversity as species richness (*e.g.* Naeem & Li 1997; Loreau & Hector 2001; Bell *et al.* 2005). However, a highly sophisticated experimental study by Wittebolle *et al.* (2009) investigating microbial assemblages in >1200 microcosms found that the evenness of taxa within a system is also highly important with regards to ecosystem function. This investigation showed that the functioning of highly uneven assemblages, dominated by few taxa were much less resilient to an environmental stressor (salt stress) than more-evenly populated assemblages.

While a large body of literature has used assemblage taxonomy-based approaches to assess ecosystem functioning, these methods do have some practical and theoretical limitations. Primarily, it is difficult to compare assemblages that are separated across substantial geographic ranges. The limited geographic ranges of taxa, coupled with the heterogeneity of superficially-similar habitat types over even small scales (*e.g.* Vroom *et al.* 2005), make taxonomy-based comparisons between different assemblages difficult. A more relevant limitation in terms of assessments of ecosystem functioning is that such data are limited to what is essentially nominal information regarding the identities of taxa within an assemblage. This provides ecologists with little information as to how these species are interacting, feeding, excreting, egesting or in some way contributing to the actual functional processes within the system. There is an increasing body of literature suggesting that it is not the richness or the evenness of taxa themselves present within an assemblage that mediates ecosystem functioning, but rather, it is the richness or evenness of the functional roles that taxa play that is important to the functioning of an ecosystem (McGill *et al.* 2006; Green *et al.* 2008).

1.3 Functional group approaches

An alternative to using strictly taxonomic approaches to measure ecosystem functioning is to group species together that have common effects on, or contribution to, specific functional processes. The theoretical assumption of this approach is the argument that there is a degree of redundancy among species in terms of the extent to which they contribute to ecosystem functioning. This means that individual species may be substituted with other species that carry out the same functional role with minimal impact to ecosystem functioning (Walker 1992). It is these ‘functional groups’ of species that are of greater importance to ecosystem functioning than the identity of individual species and as long as the functional diversity is maintained within a system, then the delivery of ecosystem functions is maintained (Diaz & Cabido 2001).

The functional group approach has been utilised across a diverse range of habitats including terrestrial (Kahmen *et al.* 2006), freshwater (Devin *et al.* 2005) and marine systems (Michaud *et al.* 2005). Within these systems, a number of different functional groups have been used as the basis of classification, grouping taxa by, for example: organism morphology (Voigt *et al.* 2007; Wardle *et al.* 2008), trophic group (Bremner *et al.* 2003a; Scherer-Lorenzen 2008), method of bioturbation (Mermillod-Blondin *et al.* 2004), life history (Powell 2007) and reproductive methods (Siepel 1994).

A primary advantage of this method over taxonomy-based approaches is that it allows geographically-disparate assemblages to be compared (*e.g.* Bell 2007) where taxonomy measures would be inappropriate. Additionally, this method can provide some indication as to the differences in the underlying functional processes between study assemblages which are difficult to determine using taxonomic approaches alone. For example, increased functional diversity within a system would, under the niche differentiation hypotheses, enhance the resource exploitation of the system (Tilman *et al.* 1997b; Loreau & Hector 2001).

The assumption however that species may be assigned to categories unambiguously and that species within a functional group contribute equally to ecosystem functioning may not necessarily be the case. Many studies have shown that some species contribute to a greater degree than others, with some taxa potentially acting as ‘key’ contributors to ecosystem function (*e.g.* Chalcraft & Resetarits 2003; Lyons *et al.* 2005; Coll *et al.* 2007). The inherent complexity of ecosystems may also be problematic when utilising a functional group approach. Current knowledge of the workings and functioning of many natural systems is still rather limited and it is often difficult to predict *a priori* what the impacts of some change (generally anthropogenic in nature, *e.g.* pollution, overexploitation, non-indigenous species invasion or an extinction event) will be on ecosystem functioning. This is a primary reason for many ecologists to restrict their experimental manipulations of systems to meso- and microcosm-based studies containing few species within a few functional groups (*e.g.* Widdicombe *et al.* 2004; Michaud *et al.* 2005; Bruno *et al.* 2008). This approach confers a high degree of control to the investigator and allows specific hypotheses to be addressed and developed. The approach does impose some substantial restrictions however, in that the experimental assemblages used are ‘non-natural’. For this reason, the findings of such studies are generally difficult to transfer to natural communities or ecosystems. Additionally, under this approach, it is impractical to measure aspects of more than only a limited number of functions. Sampling experimental plots or containers for a large number of functions (*e.g.* nutrient analyses and sediment organic matter content and primary production, *etc.*) is costly, time-consuming and increases the risk of introducing experimental artefacts into the data.

1.4 Multiple functional group approaches

The delivery of functions within an ecosystem is essentially a by-product of the different methods that taxa have developed in order to survive and reproduce. Different feeding strategies, for example, predation, deposit feeding, filter feeding *etc.*, can influence elemental cycling within a system (*i.e.* through the import or export of materials). It is therefore the taxa within ecosystems that drive the functioning of those systems. However, the identity of these taxa alone tells us nothing about how exactly they contribute to the functioning of the system. Grouping taxa into functional groups or guilds allows us to infer what effects changes in the abundance or diversity of species will have on the delivery of the one or two functions used to define the guild.

Recent international agreements have urged shifts towards whole-ecosystem-based approaches to management (E.C. 2007). This means that the investigation of only a limited number of functions is insufficient. Instead, ecologists must seek new ways of integrating the complex and multivariate array of ecosystem components and processes. A concept that is integral to any new approach is that, rather than looking at a large number of ecosystem functions individually, it should be able to explore many functions at the same time. This is because the ecological niche that a taxon occupies can conceptually be considered as a point within an *n*-dimensional hypervolume, with each dimension representing a single ecosystem function (*sensu* Hutchinson 1957). The exploitation of resources by species within an ecosystem is dependent on the local availability of that resource as well as the number of species competing for that resource. Niche breadth (or niche width) is an important component of this concept. That is, opportunistic taxa are able to utilise a larger range of resources (typically food resources), *i.e* a broader niche width, than those taxa with specialised, narrow niche widths (MacArthur 1972).

One method of considering multiple ecosystem processes or functions is through the use of Biological Traits Analyses (BTA), investigating the ecological and life-history strategies (or traits) that taxa employ within ecosystems and hence indicating the ecological niches occupied within the system. BTA does not measure ecosystem functioning *per se*; only direct measures of ecosystem properties (*e.g.* primary productivity, nutrient fluxes *etc.*) are able to do this. However, this approach does offer an indication as to the prevalence of a suite of functionally-important biological traits within a system and provides a wider scope than other function-based approaches and thus may be used as a proxy for the functioning of a whole assemblage (see also Bremner 2008). BTA was initially developed in lentic systems for investigations of the habitat templet theory (Southwood 1977; Statzner *et al.* 1994) and has since been used in freshwater (Statzner *et al.* 2001), terrestrial (Ribera *et al.* 2001; Hausner *et al.* 2003) and, increasingly, marine systems (Bremner *et al.* 2003a; 2005; 2006a; Tillin *et al.* 2006).

Within BTA, taxa within study assemblages are scored according to their affinity to sub-categories (or modalities) of each biological trait to be investigated. These modalities cover the potential range of strategies that the taxa might employ. For example, when investigating intertidal soft-sediment macrofauna, the trait ‘feeding mode’ may be divided into the modalities: predator, scavenger, deposit feeder, suspension feeder or grazer. Information for the biological traits of taxa ideally comes from published, peer-reviewed literature; however other information sources may also be utilised (*e.g.* secondary literature, reports and grey literature, expert knowledge, *etc.*). Due to the fact that uncertainty may exist for the trait preferences of some taxa or that taxa may show affinity for more than one modality within a trait, *e.g.* adopting different feeding modes depending on environmental conditions or food availability (Fauchald & Jumars 1979), a fuzzy coding approach is often used to score taxa (Chevenet *et al.* 1994). This approach allows scores to be distributed across more than one modality. BTA may utilise any measure to enumerate the prevalence of taxa within an assemblage (*e.g.* presence/absence, taxon abundance or biomass data); this quantification data is used to provide a weighting to the trait coding data and this weighted information is examined using ordination techniques (see Bremner *et al.* 2003a).

The type of biological traits chosen for BTA will influence the outcome of the analysis and should be those traits which are important contributors to ecosystem functions (Frid *et al.* 2008). If specific ecological questions are being asked from an anthropocentric perspective, such as the effects of fishing on ecosystem properties (*e.g.* Morato *et al.* 2006; de Juan *et al.* 2007; Pranovi *et al.* 2008), then the traits used may be selected accordingly. However, the number of traits investigated will also influence the outcome of the analysis. The number of traits is closely linked to the ability of BTA to describe the relationships between assemblages, with more traits explored providing more informative insights in to the functioning of study systems (Bremner *et al.* 2006a).

An advantage of BTA over other approaches is that it potentially uses a larger number of variables than functional group analyses. This means that BTA has the potential to provide information as to the functional structure of assemblages that would be difficult to predict *a priori* and so would likely be overlooked under more data-limited functional group analyses. Additionally, the use of BTA on manipulative experimental or time series data can reveal more subtle or idiosyncratic changes across a wider range of traits and allows the generation of new, or the refinement of existing, hypotheses as to the functioning of systems.

Despite these advantages, some precautions are required with the use of BTA. Firstly, there is as yet no general consensus regarding the scoring systems used to enumerate the affinity of taxa to the various traits, in terms of how many modalities traits are sub-divided into. This is not a problem within individual studies (provided that authors are consistent with their own data), but does mean that caution must be taken when comparing between different studies. The approach is also data-intensive, requiring the collection and analysis of large volumes of literature. Although a substantial body of literature exists for traits such as the feeding preferences, reproductive strategies and living locations of taxa, other potentially key functional traits are less well-covered in the literature, particularly for less common taxa. In such cases, it is often possible to find information at the Genus or Family level and the use of fuzzy coding allows more than one modality to be expressed if different taxa within a Genus or Family adopt different strategies.

A potentially more-important shortcoming of BTA, particularly with manipulative experimental data, is in the assignment of taxa to the expression of specific traits. This shortcoming however, is also a problem inherent within other functional group approaches in which taxa are categorised into distinct trait categories. This method makes it impossible, in situations where taxa are able to adopt more than one strategy, to detect the actual, realised behaviour of organisms. If an event causes the behaviour and hence trait expression of taxa to change, then this will also affect the functional roles carried out by taxa within the study system (see Chapter 4 for further investigation into this problem).

1.5 Tidal flat systems and the cockle *Cerastoderma edule* L.

Much work has been dedicated to investigations of the roles that species play in the functioning of ecosystems. Much of this work however, has been based within the terrestrial, lotic or lentic ecosystems (Tilman 1999; Vaughn & Hakenkamp 2001; Schindler & Gessner 2009). Relatively little work has been carried out in the marine environment, though interest in the field is increasing and recent studies based within the marine environment are proving valuable in the field of biodiversity and ecosystem functioning (Duffy in press; Figure 1.1).

A substantial proportion of human habitations are located in coastal regions (Small & Nicholls 2003). As a result, coastal zones are often subjected to considerable anthropogenic impacts. Coastal systems also represent areas of substantial productivity, and as a result, these zones are associated with many large-scale fishing grounds (*e.g.* Frid *et al.* 2000b; Freire *et al.* 2002; Tulp *et al.* 2008). These regions therefore, are of ecological importance as well as great economic and sociological interest. Within coastal regions, benthic habitats are heavily involved in the cycling of energy, materials and nutrients (Marcus & Boero 1998). As well as being productive habitats themselves, organisms within the benthos also have the potential to mediate ecological processes and productivity throughout the water column and hence influence coastal productivity (Prins & Smaal 1994). This makes benthic habitats of great ecological interest and many studies within the field of biodiversity-ecosystem functioning have been carried out in these habitats (*e.g.* Waldbusser *et al.* 2004; Forster *et al.* 2006; Ieno *et al.* 2006). From a practical point of view, these are easily-accessible habitats and tend to be species-poor compared to subtidal habitats (Sanders 1968; Beukema 1991; Frid *et al.* 2009a), meaning that experimental manipulations of species or functional groups can be carried out with relative ease.

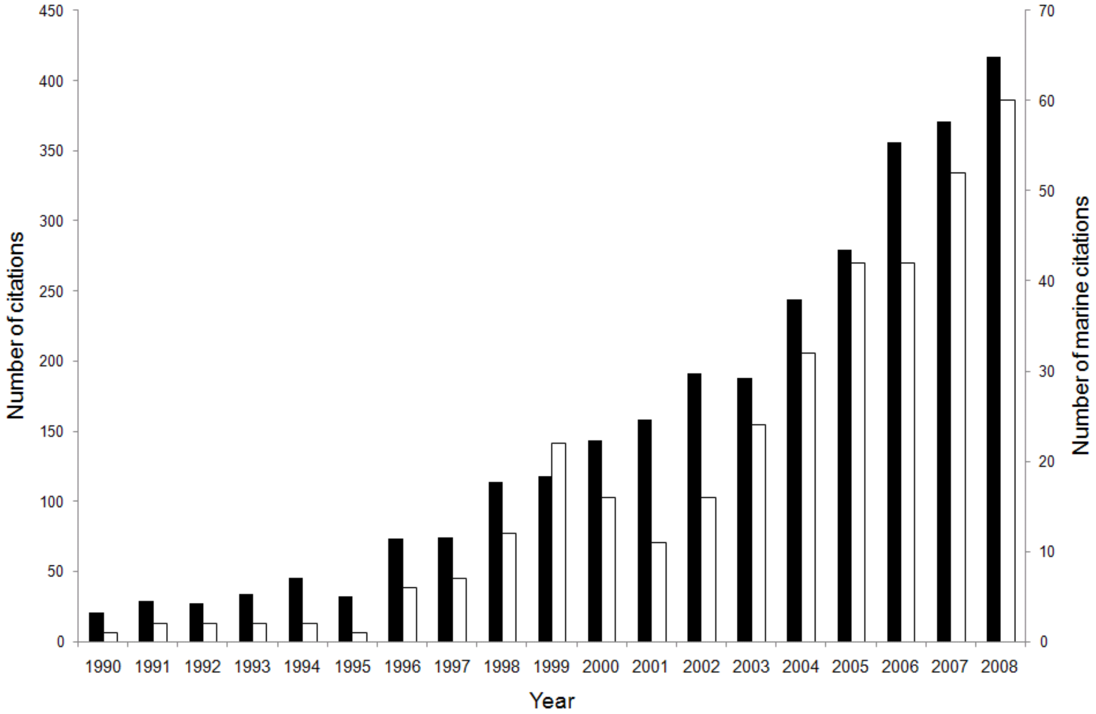


Figure 1.1. The increasing interest in the field of ecosystem functioning in recent years, showing the number of citations that include the terms 'ecosystem functioning' (black) and 'ecosystem functioning' + 'marine' (white) (note different scales). Literature searches were carried out using Scopus.

Manipulative investigations tend to focus on biomass-dominant taxa (*e.g.* Beadman *et al.* 2004). It is the biomass-dominant taxa that represent a substantial proportion of ecosystem resources, in terms of organic carbon and energy. Bivalve molluscs such as the cockle *Cerastoderma edule* L., often dominate faunal assemblage biomasses (*e.g.* Ivell 1981a; Attril 1998). As largely-sedentary organisms, bivalves can be incorporated into manipulative experiments with relative ease in investigations of ecological functioning (*e.g.* Rossi *et al.* 2008). Due to their biomass-dominant status, cockles have the potential to play key roles in the functioning of coastal systems.

1.6 Thesis overview

The general aim of this thesis was to investigate the functional roles that cockles play within intertidal assemblages in the north-west UK and how changes to the abundance of cockles may affect this functioning. In order to be able to investigate how an ecosystem is affected by any disturbance, some knowledge of the ecological dynamics of the system is required. To this end, Chapter 2 describes a baseline investigation describing how assemblages within three temperate shores change over time. This chapter investigated seasonal variation in the biological aspects of these assemblages (through univariate and multivariate analyses) as well as how the potential delivery of macrofauna-mediated ecological functions can change, through the use of Biological Traits Analysis.

A primary concern for ecosystem managers is the potential for human activities to degrade the functioning of ecosystems. A major source of anthropogenic disturbance is through fishing activity. Fisheries tend to target biomass-dominant taxa which, due to the large proportion of organic resources (*i.e.* organic carbon and energy) they represent, are important contributors and/or mediators of ecosystem function. Therefore, the removal through fishing of such species may influence the functioning of these ecosystems. Chapter 3 describes an experiment in which sediments at two shores in north-west England were experimentally-fished and adult cockles removed, using the methods employed by commercial cockle-harvesters. The sediment properties (surface chlorophyll, sediment organic matter and sediment granulometry) were monitored over four months, at the end of which faunal assemblages were examined and impacts to faunal composition and the functional structure were ascertained.

An initial aim of Chapter 3 was to develop a technique for the accurate measurement of nutrient fluxes *in situ*. This technique was based on a novel microelectrode technique within a purpose-built micro-benthic chamber developed within the Department of Earth and Ocean Sciences at the University of Liverpool (see Chapman & Van Den Berg 2005). This would have allowed an important ecosystem function (the availability of nutrients) to be measured over a very short timescale (a scale of minutes-to-hours rather than the hours-to-days necessary when using larger benthic chambers, An & Joye 2001; Chapman & van den Berg 2005) and allow repeated measurements from experimental plots with minimal disturbance to the benthos. This therefore would reduce the potential for the introduction of experimental artefacts. However, development of this technique did not progress at sufficient pace for the technique to be utilised in the current study, in that the micro-electrodes were only sensitive to nitrate concentrations in excess of 25 mg l^{-1}. Nitrate levels at this concentration are more comparable to the maximum acceptable levels of 50 mg l^{-1} as set by the EC Nitrates Directive (EC 1991) than the values of 0.01-0.02 mg l^{-1} recorded during preliminary investigations within the surface waters of the Dee estuary where a study site for this investigation was located. However, these techniques show considerable promise and when methodologies become refined, for example through the use of various combinations of electroplating and the addition of reactive chemicals to the micro-benthic chamber itself (van den Berg, University of Liverpool, *pers. comm.*), would represent a valuable tool for the monitoring of ecosystem functioning *in situ* with little disturbance to assemblages.

Much recent work into the assessment of the ecological functioning within systems has involved investigations of the functional roles that taxa play within assemblages, grouping taxa by the functional traits they express. However, a concern with this approach is the potential for taxa to express more than one trait and hence vary in their delivery of ecological functions. This is explored in Chapter 4, where the potential for changes to the feeding behaviour of taxa following disturbance is investigated. Taxa from experimentally raked and non-raked plots were compared, examining the ratios of stable isotopes of carbon and nitrogen as proxies of feeding behaviour.

Fishing directly affects the densities of the target taxa. In north-west UK, cockle fishing is restricted to hand-gathering, which is likely to occur at a patchy and localised scale (*i.e.* not impacting the entire tidal flat system). The agencies that oversee intertidal fisheries carry out annual cockle stock assessments and use this information to decide upon whether fisheries should be opened to exploitation. However, these agencies differ in what they consider a suitable cockle density at which to allow cockle beds to open to exploitation and little, if any consideration is made as to the effects of different cockle densities on the delivery of ecosystem functions. Chapter 5 describes an experiment in which cockle densities were manipulated in the field and the impacts of different cockle densities on the availability of porewater nutrients was investigated.

An investigation was initially proposed in order to ascertain whether biomass alone is a sufficient method of enumerating organisms when investigating the impact of species on ecosystem function. Taxa are known to show ontogenic differences in how they carry out ecologically-important metabolic processes. For example egestion rates differ per unit body mass in younger than in adult organisms (*e.g.* Normant *et al.* 2004). An experimental investigation was designed therefore to manipulate the abundance of the cockle *Cerastoderma edule* in experimental plots, whilst maintaining total cockle biomass and changes to an ecosystem property (nutrient availability) measured. However, the cockle abundances present at the study site were highly-patchy in their distribution, making the collection of adequate numbers of cockles impractical within the time frame available. This study therefore could not be initiated, though the rationale of this investigation and the proposed methodology are given in the Appendix.

The drivers behind the temporal variability of species within systems are numerous and are likely to differ in their relative strengths and affects on specific taxa and in specific environments. However, the relative strengths of different drivers are difficult to ascertain. This is particularly pertinent when long-term information on populations are lacking. Chapter 6 addresses this by using the fishery-status reports of the North West & North Wales Sea Fisheries Committee (NWNWSFC), which document fishing activity throughout the region. These reports provide direct and indirect indications as to the status of fished stocks. In order to investigate the drivers behind cockle population changes, a thirty year semi-quantitative analysis was carried out using the information within the NWNWSFC annual reports and other long-term data sources to assess how cockle stock vitality relates to numerous other factors.

Chapter 7 discusses the findings of these investigations in a broader scope, considering the roles of *Cerastoderma edule* in the ecological functioning of coastal systems. Discussion is also made as to what ecologists are able to, and should seek to, interpret from experimental findings. This has implications for investigations of the role of organisms in the functioning of ecosystems.

CHAPTER 2: BASELINE INVESTIGATIONS OF THE FAUNAL ASSEMBLAGE AND FUNCTIONAL STRUCTURES OF THREE TIDAL FLATS IN NORTH-WEST ENGLAND

2.1 Introduction

The study of the properties and processes occurring within marine ecosystems and the delivery of goods and services within these systems (termed ecosystem functioning, Naeem *et al.* 2002) is a rapidly-expanding field of ecology. Human activities in marine systems, ranging from pollution and fishing to the introduction of invasive species and climate change, may have substantial impacts on the biodiversity of taxa within these systems (Halpern *et al.* 2008). Changes to the biodiversity within an ecosystem can result in changes to the delivery of ecological functions within these systems (Balvanera *et al.* 2006).

Temperate intertidal habitats are ideal environments to investigate the effects of human activities and changes to biodiversity on ecosystem function. These habitats are an important component of coastal systems, with influences extending to the primary productivity within the pelagos (Cloern 1982). Tidal flats generally tend towards a relatively low biological diversity with fauna generally present at a lower total biomass than within other habitats (*e.g.* Sanders 1968; Beukema 1991; Frid *et al.* 2009b) and this allows species or functional groups to be experimentally manipulated *in situ* with relative ease (*e.g.* Volkenborn & Reise 2007; Rossi *et al.* 2008). Investigations of faunal impacts on ecosystem function within the intertidal generally focus on manipulations to and responses within the macrofaunal community (*e.g.* Bolam *et al.* 2004; Rossi *et al.* 2007).

Macrofauna tend to be dominant contributors to faunal biomass (Fenchel 1978; Gray 1981) and hence represent a substantial store of organic carbon and energy resources within intertidal systems. In addition, macrofauna represent an important trophic link, consuming primary producers and microorganisms as well as oxygenating sediments and providing refugia for micro- and meiofauna within the system and hence going some way to mediate the regeneration of nutrients within coastal systems (Prins & Smaal 1994; Beadman *et al.* 2004). Macrofaunal organisms are also important food-sources for bird and demersal fish species as well as being the targets of fisheries and bait-digging (Drinnan 1957; Pihl 1985; Beukema & Cadée 1996; Masero *et al.* 2008). Therefore, as well as being important contributors and mediators of ecosystem goods and services, macrofauna are also important with regards to the socio-economic and aesthetic components of intertidal systems (Beaumont *et al.* 2007).

Much theory and experimental evidence suggests that the maintaining of biological assemblages is essential in order to preserve the delivery of ecological functions, either through the maintaining of species diversity or the diversity of functional traits (*e.g.* Naeem *et al.* 1994; Tilman 1999; Cardinale *et al.* 2006). To this end, intertidal macrofaunal assemblages have been used in numerous investigations into the effects of human activity on ecosystem functioning (Defeo *et al.* 2009) and studies on the marine benthos have aided much of the recent developments in the field of biodiversity and ecosystem function (BEF) (Gamfeldt & Hillebrand 2008; Duffy in press). However, despite the growing body of work, it is often the case that little or no preparatory investigations are carried out in order to establish ecological baselines, in terms of how the identities and diversity of taxa present, faunal biomass or the identities and diversity of functional traits expressed by taxa within a study system differ and vary over spatial and temporal scales.

There is a high degree of inherent variability and fluctuation of conditions within temperate tidal flats. Salinity and temperature, and exposure to irradiation and desiccation all change over hourly timescales with the ebb and flow of tides. These factors can influence the suitability of different habitats within an ecosystem to different taxa and explains the observed variations in the composition of assemblages within temperate tidal flats (Dyer *et al.* 2000). These factors also show seasonal variability and as conditions change between seasons, taxa respond, for example by reproducing at times when food availability is likely to be at its peak or environmental conditions more amenable to juvenile development. Not all taxa however, respond to conditions at the same time or in the same manner. Some taxa for example reproduce at specific times of the year, for example with spawning events triggered by increased water temperatures (Seed & Brown 1977). Other taxa meanwhile, have no strict reproductive triggers and hence are able to reproduce throughout the year (*e.g.* Anger *et al.* 1986). This means therefore, that the relative taxonomic compositions within assemblages are likely to change over both spatial and temporal scales. Without any knowledge of this variability however, there is a potential for any anthropogenic and/or experimental changes occurring within assemblages to be masked. Baseline monitoring programs therefore, are essential in order to provide site-specific ecologically-relevant knowledge of study assemblages. The aim of the present study was to establish a biological baseline for three intertidal sedimentary sites within north-west England over a period of 27 months, with assemblages sampled in spring and autumn. This investigation provides baseline knowledge of local ecology prior to the commencement of investigations into the impacts of cockle fishery impacts on ecosystem function.

Cockle beds at Thurstaston, within the Dee estuary (Figure 2.1) have been relatively unfished over recent years, with fishing occurring only once since 2001 (DEFRA 2008). West Kirby, also within the Dee estuary, has been fished more-intensively than Thurstaston, with cockle beds fished between 2001-2003, taking ca. 1000 tonnes per fishing season over a timescale of 1-5 weeks (Bryan Jones, Environment Agency, pers. comm.; DEFRA 2008). Beds at Warton Sands, within Morecambe Bay had been the most intensively fished up until 2004, with up to 300 cocklers present on each low tide, working the lucrative Warton Sands cockle grounds for the estimated 5000 tonne harvestable stock they contain (Bill Cook, North West & North Wales Sea Fisheries Committee, pers. comm.). The current study therefore, can provide some information as to the biological and functional structure of macrofaunal communities subjected to differing fishing intensities. However, any findings must be interpreted with some caution as within the scope of the current study there can be no true replication of fishing intensities. That is, the information gained will inform as to the ecology of three shores that have been fished at different intensities, however only one shore at each intensity 'treatment' was sampled and all replicate plots sampled within each shore (*i.e.* sampled within each 'treatment') must therefore be treated as pseudoreplication (Hurlbert 1984). Despite this however, this study does provide an opportunity to investigate an ecological 'experiment' at the scale of the habitat, much beyond the scale of most ecological investigations (*sensu* Beukema *et al.* 1998) and therefore can still potentially provide information on the impacts of cockle fisheries on ecosystem function that would be financially impractical, labour-intensive and/or ethically ambiguous to obtain with a thoroughly-designed experiment.

The baseline information gained provided information as to the dominant macrofaunal taxa in terms of both taxon abundance and biomass at the three shores. In addition, the species richness, biological diversity and evenness of these assemblages were also measured in order to provide a broad range of ecological information on the study systems. Analyses were made of the prevalence of ecologically-important biological traits within these assemblages in order to map the distribution of the life-history and ecological strategies of taxa that govern or mediate a range of ecological functions. All of the measured ecological facets were compared between study shores as well as between seasons. This baseline both provides valuable information as to how the functioning of ecosystems changes spatially and temporally and allows manipulative investigations at these sites to be carried out and interpreted with greater confidence, providing an indication of the variability inherent within the study systems.

2.2 Methods

2.2.1 Site description

Sampling was carried out on three shores in north-west England, UK. Thurstaston and West Kirby are located within the Dee estuary and Warton Sands within Morecambe Bay; both estuaries are macrotidal and open into the eastern Irish Sea (Figure 2.1). The Dee estuary is an accretionary tide-dominated system, with mean spring tidal ranges at the mouth of the estuary at West Kirby in the region of 7-8 metres (Marker 1967; Moore *et al.* 2009). Morecambe Bay is the largest expanse of intertidal sand and mudflats in the UK. It is a highly dynamic system, with migratory sandbanks and channels and mean spring tidal ranges across the bay in the region of 8 metres (Mason *et al.* 1999). The Centre for Environment, Fisheries and Aquaculture Science (Cefas, www.cefas.co.uk) carries out long-term monitoring of sea surface temperatures at Heysham on the south side of the mouth of Morecambe Bay and shows a mean surface temperature of 9.2 ± 2.9 °C in spring and 11.5 ± 3.1 °C for autumn. Faunal assemblages at all three sites have in the past been subject to commercial fishing and bait-digging activity, including a fishery based on hand-gathering cockles. For at least 24 months prior to the commencement of this investigation however, cockling activity at all three sites had been prohibited (Bill Cook, North West & North Wales Sea Fisheries Committee, pers. comm.).

Sampling was carried out over 27 months, with sampling occurring approximately every six months, in spring and autumn (though with one sampling event occurring in summer 2006 at West Kirby) from March 2006 to May 2008 in order to describe the faunal diversity and community structure, the prevalence of functionally-important biological traits and physical aspects of the sediment and how these vary between seasons.

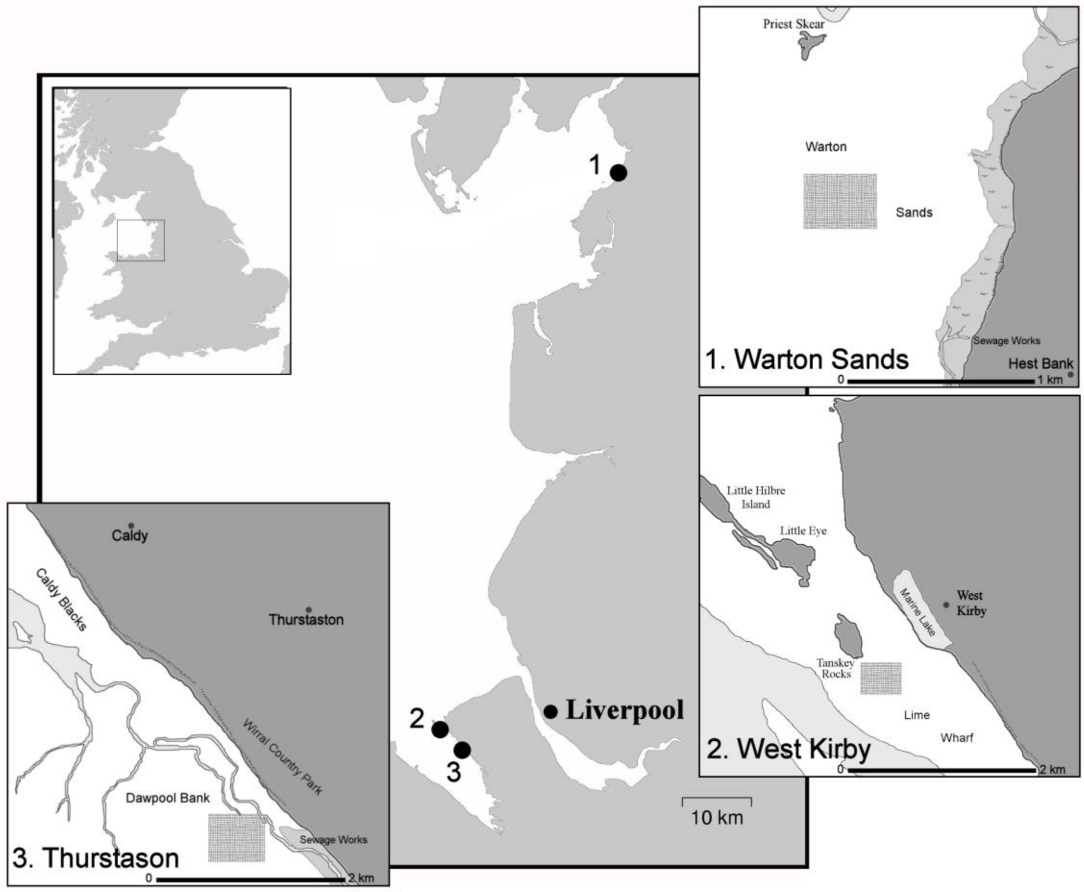


Figure 2.1. Locations of the three intertidal sedimentary cockle beds investigated in North-West England. Hatched areas indicate areas sampled in the current study.

2.2.2 Benthic infaunal analyses: univariate and multivariate analyses

On each sampling occasion, five sediment cores (PVC core, internal diameter 110 mm, equating to a surface area of ~0.01 m^2 x 200 mm depth) were extracted from three randomly-chosen locations from mid-tide level on each shore (n = 15 for each shore), with sampled locations separated by at least 40 metres. Samples were washed over a 500 µm mesh and the residue fixed in 4% formaldehyde solution. Macrofauna were identified to the lowest taxonomic level practical, counted and biomass ascertained by wet weight (±0.0001 g). Bivalve biomass was ascertained with shells retained. Taxa abundance and biomass data for each of the five cores from each of the three locations were summed and values expressed as abundance/ biomass m^{-2}. The three locations were treated as replicates. Total abundance/biomass and number of taxa were calculated for each sample. Pielou's evenness, Shannon-Wiener and Hill's N_2 indices were calculated on the taxa abundance data. Pielou's evenness is an index of how evenly distributed organism abundances are across taxa and is defined between 0 and 1, with 1 representing a perfect evenness (*i.e.* all taxa are equally-represented) and increasing dominance by fewer taxa as values approach zero (Pielou 1966). Shannon-Wiener index (H') is a measure of the information entropy of an assemblage and incorporates both the abundance and the heterogeneity (*i.e.* evenness) of taxa. Hill's N_2 index is a measure of the effective number of species within a sample and is more sensitive to dominant taxa than the Shannon-Wiener index (Hill, 1973; Soetaert & Heip 1990). Univariate indices were compared between shores and seasons using Kruskal-Wallis analyses within the R version 2.9.0 software package. Mann-Whitney U tests were carried out *a posteriori* for all possible pairwise perturbations when significant differences (at $\alpha = 0.05$) were obtained, using Holm's sequential Bonferroni-adjustment to control for multiple tests (Holm 1979).

Multivariate analyses were used to compare community abundances between shores and sampling times using analysis of similarities (ANOSIM, Clarke 1993) based on Bray-Curtis similarities. When significant differences existed between assemblages ($\alpha = 0.05$), pairwise comparisons were used to identify which of the study sites or seasons differed from each other and were further analysed using the SIMPER procedure to ascertain the contribution of individual taxa to the pattern in Bray-Curtis dissimilarity (Clarke & Warwick 1994; Clarke & Gorley 2006). Calculation of the univariate diversity indices and multivariate analyses were carried out using the PRIMER (v.6.1.6) software package (Clarke & Gorley 2006).

2.2.3 Biological traits analysis

To investigate the distribution of biological traits within the study assemblages, individual taxa were scored against fifteen biological traits that reflect the morphological, behavioural and physiological strategies/requirements of the taxa. The selected traits are all of potential importance to the mediation of ecological functions within marine systems, for example, the reproductive methods of taxa may influence the resilience of assemblage following disturbance as well as being of importance to taxa feeding from the pelagos (Bremner *et al.* 2006b, Frid *et al.* 2008). Each trait was divided into a number of modalities (or sub-categories) that covered the potential range of methods by which a taxon may express that trait (Table 2.1). A fuzzy-coding approach was used to score the affinity of each taxon to all of the modalities within each trait (Chevenet *et al.* 1994). Where possible, trait information was obtained from peer-reviewed literature; alternatively, grey literature was consulted. Where trait information could not be found (in <4% of cases), zero scores were used for each modality within the trait so as not to influence the analysis (Chevenet *et al.* 1994). Fuzzy scores were standardised so that within each trait, modality scores summed to one.

To compare the distribution of traits between assemblages, a weighting was applied to the taxa-trait table. Faunal biomass was selected as an appropriate factor by which to weight the trait scores as biomass can be regarded as a more accurate measure of the apportionment of ecosystem resources (such as energy, organic carbon *etc.*) than abundance (*e.g.* Chiarucci *et al.* 1999). The taxa-trait table was weighted by multiplying the trait scores for each taxon by the biomass of the taxon present within each sample. The resulting scores for each trait modality were summed for each sample, resulting in a table containing the biomass-weighted prevalence of each trait within the study assemblage. This information was ordinated using fuzzy correspondence analysis (FCA, Chevenet *et al.* 1994) in the ade4 (version 1.4) package for R (version 2.9.0, CRAN 1999; Dray & Dufour 2007).

2.2.4 Sediment granulometry

On each sampling occasion, three sediment cores (internal diameter 30 x 100 mm) were extracted at each site. Sediments were dried (at 60°C to constant weight), weighed (±0.01 g), soaked overnight in sodium hexametaphosphate solution to aid dispersion of clay particles and washed over a 63 µm sieve. After re-drying, retained sands (>63 µm) were weighed and sediment grain-size fractions separated within a Wentworth-scale sieve stack as described by Eleftheriou & McIntyre (2005). Sediment granulometries were compared using percentage silt content as inferred by subtraction; values were √-transformed prior to analysis with general linear model analysis of variance.

Table 2.1. Biological traits and modalities used in the fuzzy coding of taxa for biological traits analysis (BTA) to describe functional diversity (adapted from Bremner *et al* 2006b; Frid *et al.* 2008). Codes refer to Figures 2.6 and 3.3.

Trait	Modality	Code
Adult mobility / dispersal potential (distance per year)	None	D0
	<10 m	D1
	10-100 m	D2
	>100 m	D3
Age at sexual maturity (years)	0.08-0.5	M1
	0.5-1	M2
	1-2	M3
	>2	M4
Body Flexibility	None (<10°)	F0
	Moderate (10-45°)	Fm
	High (>45°)	Fh
Fecundity (eggs released per reproductive event)	10s	F1
	100s	F2
	1000s+	F3
Food type	Phytobenthos	Ph
	Benthic Invertebrates	Bi
	Carrion	Cr
	Benthic POM	Bp
	Plankton	Pl
	Pelagic POM	Pp
Living habit	Tube	Tu
	Burrow	Bu
	Epizoic	Ep
	Free	Fr
Living location	Pelagic	Pe
	Surface	Su
	Interface	In
	Shallow (0-20 cm)	Sh
	Deep (>20cm)	Dp
Longevity (years)	<0.5	L1
	0.5-1	L2
	1-02	L3
	2-5	L4
	>5	L5

Trait	Modality	Code
Maximum size (mm)	0-50	S1
	50-100	S2
	100+	S3
Movement method	None	Nm
	Swim	Sm
	Crawl	Cw
	Burrow	Br
Propagule dispersal	None	N
	Planktonic (0.04 y)	P1
	Plank (0.04-0.08 y)	P2
	Plank (0.08-0.5 y)	P3
	Benthic crawl	Bc
	Raft	Rf
Reproductive frequency	Continuous	Co
	1 event per year	Y1
	2+ per year	Y2
	Less than annual	Yx
Reproductive method	Asexual	Ax
	Sexual (spawn)	Ss
	Sexual (ovigerous)	So
	Sexual (direct)	Sd
Resource capture	Deposit	De
	Suspension: active	Sa
	Suspension: passive	Sp
	Opportunist/Scavenger	Sc
	Grazer	Gr
	Predator	Pr
Tissue components	Calcareous	Ca
	Fleshy	Fl

2.3 Results

2.3.1 Benthic macrofaunal assemblages

Macrofaunal abundances at all three shores were dominated by the algivorous gastropod *Hydrobia ulvae* (Pennant) and the spionid polychaete *Pygospio elegans* Claparède (Table 2.2). Total (*i.e.* across-species) abundances of fauna differed significantly between shores (Kruskal-Wallis H = 16.941, df = 2, $p < 0.001$) and each of the three shores differed significantly from the other two (Holm's sequential Bonferroni-adjusted pairwise comparisons significant at $p = 0.050$). Thurstaston supported the greatest total abundance of macrofauna (1713.3 ± 4489.6 individuals m^{-2}), followed by Warton Sands and West Kirby (1139.9 ± 1878.4 and 242.4 ± 794.0 individuals m^{-2} respectively; Figure 2.2). The number of taxa identified also differed between shores (Kruskal-Wallis H = 16.900, df = 2, $p < 0.001$) with each shore differing significantly from the other two (Holm's sequential Bonferroni-adjusted pairwise comparisons significant at $p = 0.050$). In total, 43 macrofaunal taxa were identified across all three shores, with 33 taxa identified at Thurstaston, 30 at West Kirby and 18 at Warton Sands. Neither the number of taxa, nor the number of individuals present differed significantly between seasons (Kruskal-Wallis H = 3.580, df = 2, $p = 0.167$ and H = 4.433, df = 2, $p = 0.109$ for number of taxa and number of individuals respectively).

Faunal biomasses at all shores were dominated by the cockle *Cerastoderma edule* (Table 2.2). Despite the differences in faunal abundance, no significant differences were observed in the total faunal biomass of taxa at each shore (Kruskal-Wallis H = 3.508, df = 2, $p = 0.173$), with a mean faunal biomass of 548.19 ± 94.93 g m^{-2}. Differences in biomass values however, were observed between seasons (Kruskal-Wallis H = 9.378, df = 2, $p = 0.009$), with samples taken in autumn having significantly higher faunal biomasses (525.80 ± 358.89 g m^{-2}) than those taken in spring (186.20 ± 136.10 g m^{-2}; Holm's sequential Bonferroni-adjusted pairwise comparisons significant at $p = 0.050$).

2.3.1.1 Univariate analyses

Evenness of assemblages differed between the three shores (Kruskal-Wallis H = 8.118, df = 2, p = 0.017), with Thurstaston showing a significantly-greater degree of evenness (0.536 ± 0.112) than Warton Sands (0.345 ± 0.154; Holm's sequential Bonferroni-adjusted pairwise comparisons significant at p = 0.050; Figure 2.3). No significant seasonal differences were observed in community evenness (Kruskal-Wallis H = 3.562, df = 2, p = 0.168).

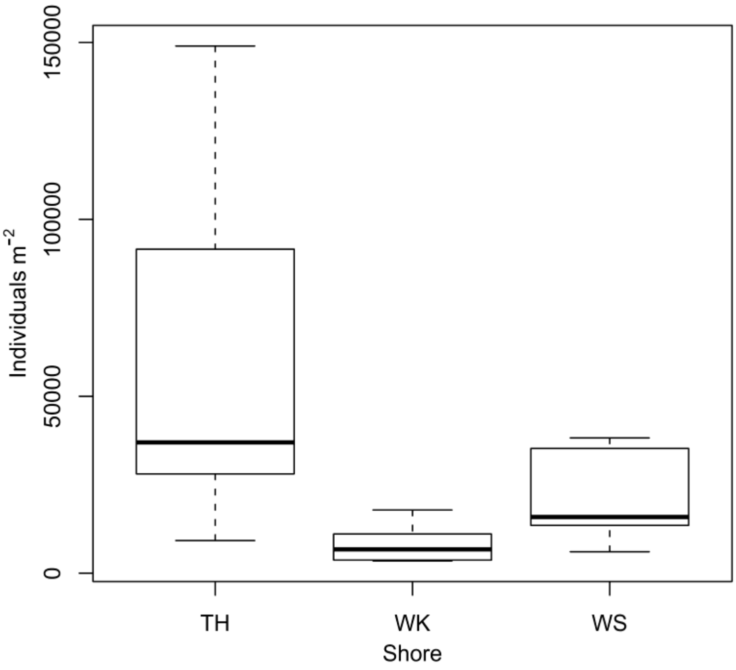


Figure 2.2. Total abundance of individual macrofaunal organisms within assemblages sampled at three shores in north-west England: Thurstaston (TH), West Kirby (WK) and Warton Sands (WS).

Mean values for Shannon's diversity index differed significantly across different shores (Kruskal-Wallis H = 11.548, df = 2, p = 0.003), with index values from Warton Sands (0.788 ± 0.381) lower than those from Thurstaston (1.527 ± 0.363) and West Kirby (1.310 ± 0.373; Holm's sequential Bonferroni-adjusted pairwise comparisons significant at p = 0.050; Figure 2.3). These values did not differ between seasons (Kruskal-Wallis H = 4.865, df = 2, p = 0.088).

Table 2.2. Taxa contributing the most to macrofaunal abundance and biomass at three shores in north-west England; *a)* Thurstaston, *b)* West Kirby and *c)* Warton Sands. These taxa represent 99.8% of macrofaunal biomass at Thurstaston, 99.1% at West Kirby and 99.6% at Warton Sands.

a)

Taxon	Abundance ± S.D. (individuals m^{-2})	Biomass ± S.D. (g m^{-2})
Hydrobia ulvae	18429.1 ± 10932.2	1.8867 ± 1.4806
Pygospio elegans	13736.4 ± 20455.2	0.3665 ± 0.5086
Streblospio shrubsolii	7743.6 ± 13358.7	0.1948 ± 0.3360
Tubificoides benedii	6212.7 ± 3539.1	0.1904 ± 0.0951
Macoma balthica	1781.8 ± 1443.7	3.1150 ± 1.7156
Corophium arenarium	1770.9 ± 5295.5	0.1061 ± 0.3282
Eteone cf. *longa*	1332.7 ± 1432.5	0.0580 ± 0.0554
Polydora cornuta	1283.6 ± 2389.8	0.0275 ± 0.0519
Cerastoderma edule	1083.6 ± 1097.2	13.9082 ± 13.4647
Scrobicularia plana	869.1 ± 1027.7	4.9809 ± 3.9644
Mya arenarium	585.5 ± 663.5	0.9360 ± 1.9742
Hediste diversicolor	312.7 ± 341.7	0.5596 ± 0.5778
Nephtys hombergii	63.6 ± 88.0	0.1399 ± 0.1954

b)

Taxon	Abundance ± S.D. (individuals m^{-2})	Biomass ± S.D. (g m^{-2})
H. ulvae	4138.0 ± 3939.4	0.9611 ± 0.8191
P. elegans	1288.0 ± 2009.8	0.0544 ± 0.0890
Bathyporeia sarsi	748.0 ± 798.2	0.0508 ± 0.0517
C. edule	332.0 ± 415.8	10.0965 ± 10.1737
M. balthica	216.0 ± 127.1	1.3152 ± 0.8909
Spio martinensis	142.0 ± 185.1	0.0021 ± 0.0026
N. hombergii	136 ± 89.8	0.2737 ± 0.1738
E. cf. *longa*	30.0 ± 23.6	0.0025 ± 0.0024
Mytilus edulis	24.0 ± 37.5	0.1614 ± 0.5101
Angulus tenuis	10.0 ± 17.0	0.1251 ± 0.2095
Scrobicularia plana	2.0 ± 6.3	0.5881 ± 1.8598

c)

Taxon	Abundance ± S.D. (individuals m^{-2})	Biomass ± S.D. (g m^{-2})
H. ulvae	13766.7 ± 5298.4	1.4216 ± 0.5715
P. elegans	4711.1 ± 6442.1	0.1103 ± 0.1437
C. edule	753.3 ± 717.3	8.9740 ± 13.8863
S. martinensis	426.7 ± 611.8	0.0078 ± 0.0105
M. balthica	426.7 ± 218.6	2.1832 ± 0.9862
Capitella	177.8 ± 263.1	0.0011 ± 0.0025
N. hombergii	100.0 ± 36.1	0.2874 ± 0.1180
A. tenuis	28.9 ± 44.8	0.0851 ± 0.1698
H. diversicolor	6.7 ± 20.0	0.0764 ± 0.2292

Hill's N_2 values also differed significantly between shores (Kruskal-Wallis H = 10.413, df = 2, p = 0.006), with Thurstaston having higher N_2 index values (3.495 ± 1.238) than Warton Sands (1.802 ± 0.655; Holm's sequential Bonferroni-adjusted pairwise comparisons significant at p = 0.050; Figure 2.3). Hill's N_2 values however, did not differ between seasons (Kruskal-Wallis H = 3.759, df = 2, p = 0.153).

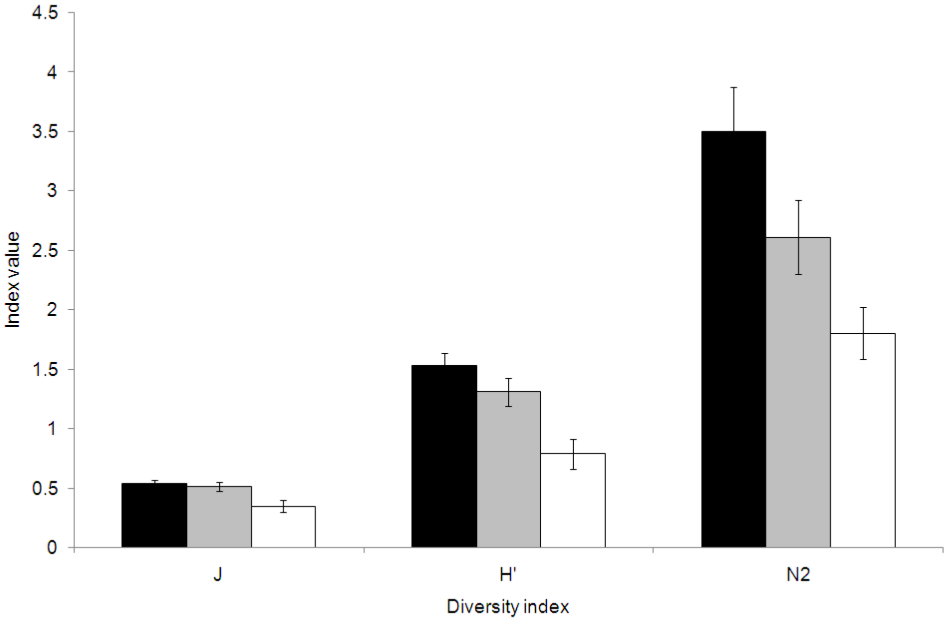


Figure 2.3. Diversity index values from assemblages based at three shores in North West England: Thurstaston (black), West Kirby (grey) and Warton Sands (white). Data represent mean values ± S.E. for evenness of assemblages (J), Shannon diversity (H') and Hill's N_2 (N2).

2.3.1.2 Multivariate analyses

Significant differences were observed in assemblage compositions between study shores (R = 0.440, p <0.001, Figure 2.4), with all three shores differing significantly from the other two (pairwise interactions significant at p = 0.01). The taxa contributing the most to these differences are shown in Table 2.3. Assemblage compositions also differed between sampling seasons (R = 0.143, p = 0.037), with mean dissimilarities between autumn and spring of 64.51%; the taxa contributing most to these differences are shown in Table 2.4.

Table 2.3. Results of the calculation of similarity percentages (SIMPER) on assemblages from three study shores. Mean dissimilarities between shores were: Thurstaston & Warton Sands = 59.77%, Thurstaston & West Kirby = 77.72% and Warton Sands & West Kirby = 62.18%.

Shore	Warton Sands	West Kirby
Thurstaston	1) *Hydrobia ulvae* (-17.11%)	1) *Hydrobia ulvae* (-26.00%)
	2) *Pygospio elegans* (-13.39%)	2) *Pygospio elegans* (-13.42%)
	3) *Tubificoides benedii* (-9.37%)	3) *Tubificoides benedii* (-12.24%)
	4) *Streblospio shrubsolii* (-5.31%)	4) *Streblospio shrubsolii* (-5.81%)
	5) *Corophium arenarium* (-4.00%)	5) *Corophium arenarium* (-5.45%)
	6) *Macoma balthica* (-2.10%)	6) *Macoma balthica* (-3.07%)
	7) *Eteone longa* (-0.89%)	7) *Bathyporeia sarsi* (+2.11%)
	8) *Cerastoderma edule* (-1.15%)	8) *Eteone longa* (-1.80%)
		9) *Cerastoderma edule* (-1.43%)
West Kirby	1) *Hydrobia ulvae* (+38.02%)	
	2) *Pygospio elegans* (+13.35%)	
	3) *Bathyporeia sarsi* (-3.51%)	
	4) *Cerastoderma edule* (+2.42%)	

Note: For each comparison, taxa are ranked by the relative contribution made to the multivariate dissimilarities between assemblages (cumulative cut-off point at 90% dissimilarity). Values in parentheses indicate the difference in abundance of each taxon (column minus row abundance) as a percentage of the column abundance. For example, mean *Hydrobia ulvae* counts at Warton Sands were 17.11% less than those at Thurstaston and *Pygospio elegans* were on average 13.35% more abundant at Warton Sands than at West Kirby.

Table 2.4. Results of the calculation of similarity percentages (SIMPER) comparing macrofaunal assemblages sampled seasonally from three study shores over 27 months. Average dissimilarities between spring and autumn were 64.51%.

Species	Difference (Spring and Autumn)
Hydrobia ulvae	-25.61%
Pygospio elegans	-13.88%
Tubificoides benedii	-5.77%
Corophium arenarium	+3.65%
Streblospio shrubsolii	-3.22%
Bathyporeia sarsi	+2.5%
Cerastoderma edule	-2.11%
Macoma balthica	-1.91%

Notes: For each comparison, taxa are ranked by the relative contribution made to the multivariate dissimilarities between assemblages (cumulative cut-off point at 90% dissimilarity). Percentile values indicate the difference in abundance of each taxon between spring and autumn. For example, mean *Hydrobia ulvae* abundances in spring were 25.61% less than those at in autumn.

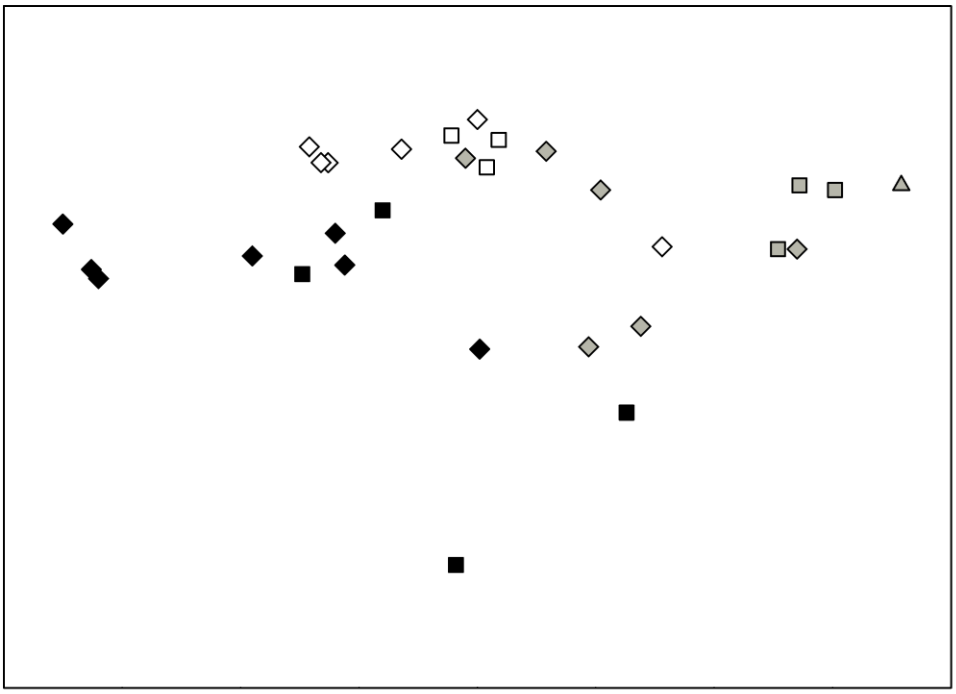


Figure 2.4. Non-metric multidimensional scaling ordination on Bray-Curtis similarities between macrofaunal assemblages from three study shores: Thurstaston (black), West Kirby (grey) and Warton Sands (white). Season of sampling is indicated by symbol shape: square = spring, triangle = summer, diamond = autumn. MDS Stress = 0.08.

2.3.2 Biological Traits Analysis

The distribution of biomass-weighted biological traits within all assemblages were dominated by traits common to bivalve species, such as high fecundity (representing a proportion of 0.986 ± 0.012 of taxa biomass) broadcast-spawning (0.904 ± 0.017) and active suspension and deposit feeding (0.756 ± 0.062 and 0.216 ± 0.062 respectively). The first two axes of the fuzzy correspondence analysis (FCA) explained ~70% of variance within the data, with ~43% across axis 1 (FCA1) and ~27% on FCA2 (Table 2.5). The majority of the variance on FCA1 was related to traits relating to the dispersal of taxa (*i.e.* adult dispersal and propagule dispersal traits) and the longevity and feeding behaviour (*i.e.* resource capture and food type) of taxa (Table 2.5).

Shores differed significantly on FCA2 (Kruskal-Wallis, H = 19.579, df = 2, p <0.001), but not on FCA1 (H = 1.003, p = 0.606). Assemblages at Thurstaston tended towards positive values on FCA2, with West Kirby and Warton Sands tending towards negative values (Figure 2.5). Thurstaston housed a greater biomass of taxa living deeper within the sediment as well as those with limited mobility and those feeding on benthic particulate matter (Figure 2.5, 2.6). As the trait information is weighted by the biomass of taxa expressing a particular trait, this pattern is likely to be associated with the relatively high biomass of the deep-dwelling bivalve *Scrobicularia plana* at Thurstaston, relative to West Kirby and Warton Sands. Seasons differed significantly on FCA1 ((Kruskal-Wallis, H = 7.722, df = 2, p <0.021), but not on FCA2 (Kruskal-Wallis, H = 3.340, df = 2, p <0.188), with autumn assemblages tending towards positive values on FCA1 and showing a greater biomass of longer-lived, sedentary, active suspension-feeders than those sampled in spring (Figure 2.5, 2.6). Therefore, the greatest proportion of difference between samples (*i.e.* that explained on FCA1) is related to seasonal differences in trait compositions and less related to study shore (explained on FCA2).

Table 2.5. Relative inertia and correlation ratios of biological traits on the first two axes of the fuzzy correspondence analysis. Correlation ratios represent the proportion of variance explained by the different traits across each axis.

	Axis 1	Axis 2
Relative inertia (%)	0.425	0.267
Correlation ratio		
Adult dispersal	0.222	0.111
Body flexibility	0.136	0.002
Food type	0.118	0.107
Living location	0.074	0.166
Longevity	0.146	0.074
Max size	0.023	0.161
Propagule dispersal	0.216	0.062
Resource capture	0.166	0.03

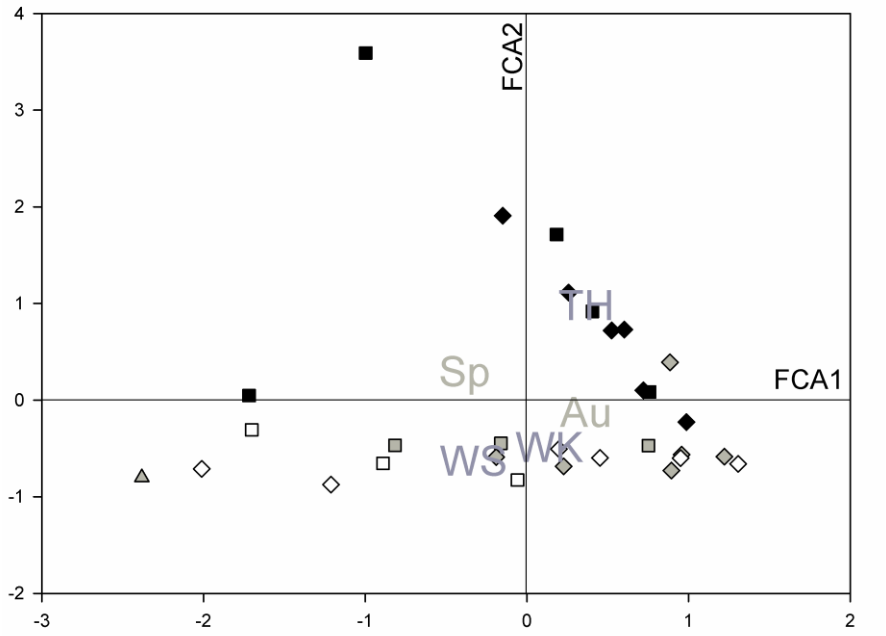


Figure 2.5. Ordination of the first two axes of the FCA for the three experimental assemblages. FCA1 explained approximately 43% of variance in the data and FCA2 explained approximately 27%. Study shore is indicated by symbol shading: black = Thurstaston, grey = West Kirby, white = Warton Sands. Sampling season is indicated by symbol shapes: square = spring, triangle = summer, diamond = autumn. Text in grey identifies the position of the centroids for the each shore (TH = Thurstaston, WK = West Kirby, WS = Warton Sands) and the sampling season (Au = autumn, Sp = spring).

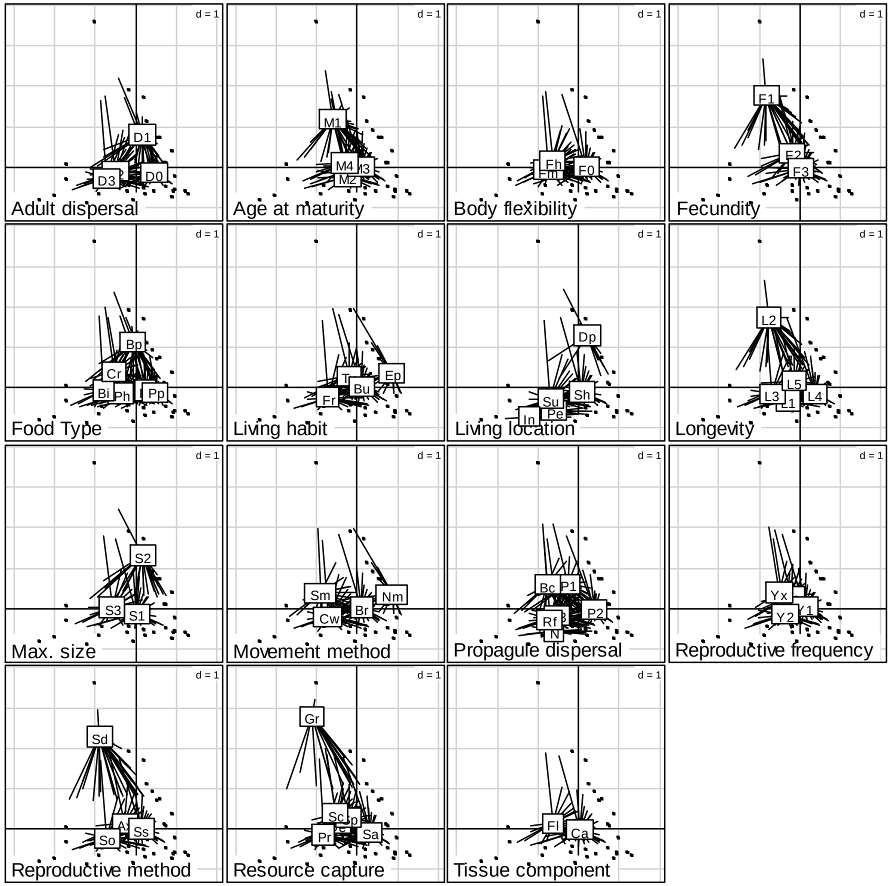


Figure 2.6. Ordination of biomass-weighted biological trait categories from the first two axes of the FCA, allowing interpretation of the traits responsible for the differences between assemblages. Points represent the FCA coordinates for each experimental plot (as in Figure 2.5) and label locations represent the centroid for each trait modality and lines link the plots to the modalities. Modality labels refer to Table 2.1.

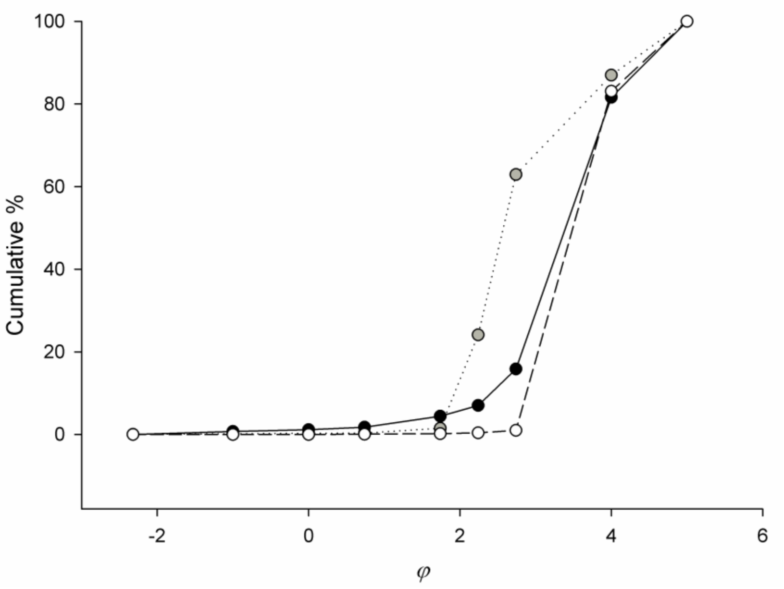


Figure 2.7. Cumulative percentage contribution by mass of different sediment particle diameters at the three investigated shores: Thurstaston (black) and West Kirby (grey) within the Dee estuary and Warton Sands (white) within Morecambe Bay. Sediment grain sizes are expressed in *φ*-notation (Krumbein 1936).

2.3.3 Sediment granulometry

Sediments at Thurstaston and Warton Sands were dominated by very fine sand (63-150 µm, or φ 2.7 – 4) and silt (<63 µm); those at West Kirby were coarser with a greater proportion of sediment retained on the 150 µm mesh size (Figure 2.7). Sediment granulometry, as indexed by silt content, showed significant differences between seasons at the study shores (Shore*Season, $F_{2,47} = 11.913$, $p < 0.001$). Sediment silt contents were higher in autumn at both Thurstaston and West Kirby (Holm's sequential Bonferroni-adjusted pairwise comparisons significant at $p = 0.050$). Silt contents however, were not significantly different between study seasons at Warton Sands (pairwise comparison $p = 0.064$).

2.4 Discussion

This study has shown that three intertidal estuarine shores based in the north-west of England differed significantly in terms of their macrofaunal species diversity and faunal biomass. Differences were also evident in the physical structure of the sediments. Despite these differences however, there were little differences apparent in the functional structure of the assemblages, as investigated through analysis of the distribution of functionally-important biological traits expressed within assemblages.

Seasonal changes in sediment granulometry were observed within the study sites. Such seasonal changes are likely to be related to seasonal fluctuations in wave energy, being higher in winter than summer (Frostick & McCave 1979). In addition, seasonal changes to water temperature can also influence sedimentation with the sedimentation of mud particles particularly sensitive to temperature; this can cause finer sediments to be eroded over winter months, resulting in the coarser granulometries observed in spring (Anderson 1983; Chang *et al.* 2006). However, these observed changes were present only within the two sites located within the Dee estuary, at Thurstaston and West Kirby and not within the Morecambe Bay site at Warton Sands. This may be due to the fact that the Dee estuary shows a greater degree of sedimentary accretion than the area sampled within the south of Morecambe Bay and thus explains why seasonal differences in sediment granulometry were only observed within the Dee estuary (Mason & Garg 2001; Moore *et al.* 2009).

A number of differences in the taxonomic diversity of the study assemblages were observed, in addition to some differences to the functional diversity of these sites. All shores housed a species pool typical of a temperate estuarine intertidal habitat (*e.g.* de Deckere *et al.* 2001; Bolam & Fernandes 2003), with abundances at all three shores dominated by the gastropod *Hydrobia ulvae* and the polychaete *Pygospio elegans*. *Cerastoderma edule* dominated the biomass at all three sites, representing in excess of 50% of the total faunal biomass. Assemblages at Thurstaston within the Dee estuary contained a greater number of individuals across all taxa and also showed a greater diversity of taxa than those at West Kirby and Warton Sands. However, despite assemblages at Thurstaston housing a greater number of species and a greater faunal diversity, significant differences were not observed in the total biomass of fauna between the three assemblages.

Total faunal biomasses did differ by season, with assemblages sampled in autumn housing a greater biomass than those in spring. However, these differences were not reflected by seasonal changes to the total abundances of taxa or the diversity or evenness of assemblages. That is, only the biomass distribution changed significantly between seasons; the total numerical abundances and identities of taxa did not change. This suggests that the observed differences were related either to the seasonal growth of individuals within assemblages, or the replacement of smaller individuals with larger individuals. Similar relationships have been observed elsewhere, for example Soares-Gomes & Fernandes (2005) found no seasonal changes in the density or diversity of bivalve assemblages in Brazil. Additionally, Dittman (1990) found no changes in the overall distribution of taxa between seasons, despite as was observed in the current study, the abundances of some individual taxa changing between seasons.

Assemblages at Thurstaston showed a greater taxonomic diversity and a more even distribution of taxa than the other two assemblages. This greater degree of biodiversity would traditionally be seen as an indicator that ecosystem functioning at Thurstaston is more robust than that at West Kirby or Warton Sands (Walker 1992). Under traditional biodiversity-ecosystem functioning (BEF) theory, increasing species richness is an indicator of a more robust functioning of the ecosystem (see Section 1.1). However, more recent contentions suggest that it is the diversity of ecologically-important biological traits within an assemblage that are more important drivers of ecosystem function than taxonomic diversity *per se* (McGill *et al.* 2006). However, with increasing taxonomic diversity, the likelihood of increasing functional diversity also grows due to the probability of gaining a taxon offering a trait or functional quality not already existent within an assemblage (Loreau 2000). This increase is not necessarily linear; therefore, despite highly significant differences in taxonomic diversity and evenness between the study assemblages, the observed differences in the distribution of biological traits were less apparent. That is, only a relatively low proportion of the variance in the trait-distribution data was explained by the sampling site. Instead, the factor explaining most of the difference in the distribution of traits was related to seasonal differences between assemblages. Such seasonal differences in the functional diversity of assemblages have been observed elsewhere (*e.g.* Bonada *et al.* 2007). The fact that there were evident seasonal differences in the distribution of functional traits and the total biomass of fauna within the benthos, but no significant changes in the taxonomic diversity or total species abundances suggests that it is the growth of a particular species set within the assemblages that is driving the change. The change in trait composition to being dominated by largely sedentary suspension feeders in autumn suggests that this difference is driven by the growth of bivalve taxa over summer months (Seed & Brown 1978).

Despite assemblages at Thurstaston housing a significantly greater degree of biodiversity than those at West Kirby and Warton Sands, no significant differences in assemblage biomass were observed. This suggests that, although the organic material contained within the three assemblages (*i.e.* assemblage biomass) did not differ significantly, there was the potential for the functional diversity of the assemblages to differ (Balvanera *et al.* 2006; Cardinale *et al.* 2006, Section 1.1). However, the minor differences in the distribution of traits between assemblages suggests that, at least in terms of the traits measured, the different assemblages present within the three study shores contain ecologically-similar species. Under the redundancy hypothesis (Walker 1992), the additional species present at Thurstaston would be seen as ‘ecological insurance’ and therefore serve to preserve ecological functioning in situations where the total number of species, or the abundances of dominant species, decline. That is, effects of the removal or severe reduction in the abundance/biomass of one species on ecological functioning will be shielded by the presence of functionally-similar species, hence allowing functional processes within the system to be maintained (Walker 1992; Walker 1995). This suggests that assemblages at Thurstaston are better-able to maintain ecological functioning in the face of introduced disturbances that may reduce species diversity, for example storm events, fishing impacts, pollution *etc.* than those at the less-diverse Warton Sands site. That is, the greater number of taxa at Thurstaston, despite offering no real functional uniqueness, in terms of supplying new biological traits not expressed by other taxa within the assemblage, means that this site shows a greater potential for ecological reliability in the face of species loss (Naeem 1998).

The apparently greater functional redundancy at Thurstaston may however be related more to the number and identity of traits that have been studied. It has been suggested that the competition for resources by benthic macrofauna is low, with the major controls relating to ‘top down’ predation pressure (Reise 1977; Virnstein 1977;

Evans 1983). Low competition between taxa for the available resources suggests that, with an increasing number of traits examined, more taxa will be shown to possess a unique suite of traits and thus a unique niche within the assemblage (Bremner *et al.* 2006a). Therefore, if the relative abundance of a taxon were to change, then there is the likelihood that the functional diversity and delivery of ecological functions would be affected. This supports the analysis of as large a collection of biological and ecological traits as is practical to obtain for investigations into ecological functioning. The ability of different shores to respond to a sudden reduction in the abundance of a biomass-dominant taxon will be explored further in Chapter 3, where experimental fishing for the biomass-dominant cockle *Cerastoderma edule* will be carried out within assemblages at the species-rich Thurstaston and the relatively species-poor Warton Sands.

CHAPTER 3: EFFECTS OF EXPERIMENTAL SMALL-SCALE COCKLE (*CERASTODERMA EDULE* L.) FISHING ON ECOSYSTEM FUNCTION*

*chapter published Cesar & Frid (2009)

3.1 Introduction

There is an increasing drive to consider ecosystem dynamics in terms of ecological functions; the maintaining of these functions being seen as a key factor in sustaining the goods and services provided by ecosystems (*e.g.* E.C. 2007). In littoral systems, these functions include the cycling of organic carbon and nutrients, food provisioning, biological productivity and energy transfer through the system (Christensen *et al.* 1996).

There has been much debate as to the role that species diversity plays for the functioning of ecosystems (*e.g.* Kaiser 2000) and a number of hypotheses have been developed to explain the effects of species on ecosystem function (Chapter 1). Species do not evolve in order to provide ecological functions. Instead, the delivery of functions is a consequence of the methods that taxa have developed in order to survive and reproduce, with different taxa developing different approaches to, for example resource capture and reproduction.

Species-rich assemblages may be more resilient to species extinction than less-diverse assemblages, providing a greater insurance against the loss of taxa. This concept is an integral part of the 'redundancy hypothesis' (Walker 1992; Naeem 1998), in that many species in the assemblage are able to deliver each ecological function and the localised extinction of one species is compensated for by other taxa carrying out the same functional roles. The identity of taxa removed however, is likely to be of importance, with individual taxa making different contributions to each ecological function. Some taxa make a key contribution to one function while other taxa have very little influence upon it (*sensu* 'keystone species' of Paine 1966; Lavorel & Garnier 2002; Hooper *et al.* 2005). For example, dense aggregations of the filter-feeding mussel *Mytilus edulis* L. have an influence on both the regeneration of nutrients and the primary productivity within coastal systems (Prins & Smaal 1994). *M. edulis* is an efficient filter feeder, removing large amounts of phytoplankton. Dense aggregations also influence near-bed hydrodynamics, causing increased levels of biodeposition and mineralization of biodeposited particles, resulting in increased levels of nutrient regeneration within mussel beds (Prins & Smaal 1994). *Mytilus* aggregates also provide refugia for other fauna, increasing biodiversity and thus influencing energy flow through the system (Ragnarsson & Raffaelli 1999).

The cockle *Cerastoderma edule* L. is common in intertidal assemblages on all British coasts and has been the target of a commercial fishery for over a century (Dare *et al.* 2004). *C. edule* are a major prey item for birds (Drinnan, 1957) and demersal fish (Pihl 1985) foraging on intertidal flats and are active filter-feeders, consuming planktonic flora and fauna and re-suspended microphytobenthos (Kamermans 1993; Kamermans 1994; Sauriau & Kang 2000). At certain times of year a large proportion of the diet of *C. edule* can be the planktonic juvenile stages of benthic organisms, including *C. edule* itself (Flach 1996). Bivalves eject undigested particulate material as mucous-coated pseudofaeces, which can alter the resuspension characteristics of the benthic boundary layer and add to the organic matter content of surface sediments, acting as a nutrient source for primary producers and a food source for surface deposit-feeding fauna (Hempel 1957; Swanberg 1991; Giles & Pilditch 2006). *C. edule* therefore has the potential to impact upon the structuring of intertidal assemblages and be of critical importance in the delivery of ecological functions in these systems, either directly (*e.g.* impacts on energy flow through the removal of settling juveniles) or indirectly (*e.g.* providing a food source to detritivores via the biodeposition of pseudofaeces). Dense beds of *C. edule* occur in situations where the faunal assemblage is species-diverse and productive, but also where very few taxa are present (*e.g.* Ivell 1981b; Attrill 1998). This raises the possibility that these systems deliver quite different ranges and quantities of ecological functions, or that the role of *C. edule* varies in response to the nature of the assemblage. The removal of *C. edule* by a fishery would, presumably, alter the dynamics and ecological functioning of these systems, with the possibility that the response of the more diverse assemblages would differ from that of species-poor assemblages.

The large size of the organism and the availability of harvesting techniques provide an opportunity for the manipulation of cockle diversity within both species-rich and species-poor assemblages. This allows *in situ* field investigations to be carried out: investigating the impact of an experimentally-reduced population of a potentially key species on ecological functioning. To date, manipulative experimental investigations of the impacts of altered assemblage composition on ecological functioning have principally been limited to laboratory-based microcosm studies (*e.g.* Michaud *et al.* 2006; Norling *et al.* 2007). Although such studies confer a high degree of control, allowing specific ecological questions to be approached, by their nature such designs remove a large number of unknown variables that would be present within natural systems. Microcosm experiments generally include only a limited number of taxa and hence only a limited representation of the complexity inherent within 'real' systems (Duffy *et al.* 2001; Bulling *et al.* 2008; Olsgard *et al.* 2008). The outcomes of such studies therefore, are generally less able to be applied to natural assemblages and ecosystems. Therefore, although *in situ* approaches introduce a greater number of unmeasured variables into experimental systems (and hence limit the degree to which the causality behind any responses can be assigned) they allow inference to a greater extent as to what the response of a 'natural' assemblage may be.

Traditional methods of assessing ecosystem functioning are based upon the direct or proxy measurements of a limited number of ecological functions, such as chlorophyll concentration, sediment organic matter content and sediment granulometry (Thrush *et al.* 2006; Sundbäck *et al.* 2007). This approach alone is somewhat limited however, in that it provides only an incomplete view of the functioning of the system as a whole. That is, the responses of one function to a particular effect may be negligible and the observation of a limited number of functions may mean that more subtle changes to ecological functioning, i.e. those difficult to predict *a priori*, are missed. Biological Traits Analysis (BTA) is a complimentary approach to these 'direct' methods. BTA was developed primarily for lotic systems (Charvet *et al.* 1998) and has been increasingly applied to marine systems (Frid *et al.* 2000a; Bremner *et al.* 2003a; Tillin *et al.* 2006; Kenchington *et al.* 2007).

The delivery of ecological functions is, by definition, a consequence of the life history, ecological and physiological strategies that taxa within a system have evolved. Different strategies employed by species affect different ecological functions**.** For example, the living habit employed by a taxon (e.g. being a surface dweller, tube-dweller, active burrower *etc.*) has an influence on the depth of sediment into which oxygen-rich water can penetrate, thus influencing the regeneration of nutrients and the circulation of toxins within the benthos (Aller 1983; Ziebis *et al.* 1996). Mapping the occurrence of these functionally-important traits within a system may therefore be used as a proxy for the analysis of ecological functions.

Species may share a number of traits in common; hence BTA allows a comparison between systems where the finite biogeographic ranges of species makes the interpretation of taxa-based approaches difficult (Charvet *et al.* 2000). Changes to the prevalence of biological traits within a system can provide an insight as to how the ecological functioning of the assemblage has been affected by an external stressor.

The aim of the current study was to test the redundancy hypothesis *in situ* within two contrasting estuarine intertidal assemblages. The following questions were asked: Is the delivery of ecological functions affected by the removal of a potentially keystone species? Does the biodiversity of the affected assemblage affect the extent to which it is disturbed? To facilitate this, *C. edule* was experimentally removed from the benthos and ecological functioning assessed through measurements of surface chlorophyll as a proxy for microphytobenthic primary production, organic matter within the sediment as a proxy for benthic community metabolism and sediment granulometry as a measure of changes to the physical habitat. BTA was carried out on faunal assemblages to assess changes to the functional structure of the faunal communities.

3.2 Methods

3.2.1 Site description and experimental design

Experimental manipulations were carried at Thurstaston within the Dee estuary and Warton Sands within Morecambe Bay. Full descriptions of the macrofaunal communities and sediment characteristics of both shores are detailed in Chapter 2.

At each shore, four replicate 2 × 2 m plots were established for each of three treatments:

1. Fished – surface sediment was hand-raked to a depth of ~10 cm and passed through a 6 mm square mesh. All *C. edule* retained on the mesh were removed and all other organisms were returned to the plot
2. Procedural control – surface sediment was raked as above and all organisms returned to the plot
3. Undisturbed control

The 12 plots on each shore were arranged in a randomised block design, with plot locations marked by a 30 cm plastic peg at each corner of the plot. In order to minimise interactions between plots, each was located at least 5 m from other plots.

The experiment was initiated in June 2007 and plots were sampled every four weeks until September 2007. To minimise the risk of creating artifacts from the repeated sampling of plots, each plot was split into four sub-plots (each representing an area of 1 m^2). At each sampling event, samples were removed from random locations within the central 50 × 50 cm (to avoid edge effects) of only one randomly-selected sub-plot; each sub-plot was sampled once only during the course of the experiment.

Every four weeks, four surface chlorophyll *a* samples were taken using plastic cores (internal diameter 20 mm) pushed into the sediment to a depth of 15 mm. Samples were wrapped in foil and stored in a cool box until return to the laboratory. Prior to analysis, chlorophyll *a* samples were stored at -80°C. Sediment granulometry and organic matter content were ascertained from the removal of one sediment sample from each plot (PVC core, internal diameter 30 × 100 mm). On the first and final sampling occasions, four sediment core samples were taken to investigate the faunal assemblage composition at each plot (PVC core, internal diameter 110 mm × 200 mm depth). These samples were washed over a 500 µm sieve and the residue fixed in 4% formaldehyde.

3.2.2 Infaunal assessment

Macrofauna were identified to the lowest taxonomic level practical and biomass ascertained by wet weight (±0.0001 g). Biomass was chosen as the faunal ‘abundance metric’ in this investigation as biomass is likely to better represent the distribution of resources within the system than species abundance measures (*e.g.* Chiarucci *et al.* 1999). A high abundance of very small organisms are not likely to partition as great a proportion of ecosystem resources (particularly organic carbon) as fewer, much larger taxa occupying a greater biovolume than their low abundance alone would suggest. This is particularly important when assessing the ecological roles being played by taxa, rather than biodiversity *per se* and this approach is supported by other literature (*e.g.* Wilson 1991; Chiarucci *et al.* 1999; McGill *et al.* 2007). Comparisons of macrofaunal communities were carried out using permutational analysis of variance (PERMANOVA, Anderson 2001) based on Bray-

Curtis dissimilarities and probability values calculated from 4999 permutations (see Manly 1997) using the FORTRAN program PERMANOVA (Anderson 2005). When assemblages significantly differed between factors (Shore, Treatment and Time) ($\alpha < 0.05$), *a posteriori* pair-wise comparisons were carried out using PERMANOVA and the significantly-different factors investigated using the SIMPER procedure in the PRIMER v.6.1.6 software package (Clarke & Warwick 1994; Clarke & Gorley 2006), to identify the contribution of individual taxa to differences between factors.

3.2.3 Biological traits analysis

To investigate the distribution of biological traits within the species assemblages, taxa were scored against biological traits reflecting the life-history, morphological and ecological strategies of taxa in order to provide a proxy analysis of a number of ecological functions. The traits selected for analysis were those given in Table 2.1. The steps taken to carry out the biological traits analyses are detailed in Section 2.2.3.

3.2.4 Sediment properties and chlorophyll a concentration

Changes to sediment granulometry were ascertained from the 30 × 100 mm sediment cores using the methods described in Section 2.2.4. Loss on ignition (LOI) was analysed as a proxy for the organic content of sediments. Samples of ca. 10 g oven-dried (at 60°C) sediment were ground with mortar and pestle to even consistency. Homogenised samples were weighed (±0.0001 g) before and after ignition at 500°C for 5 hours and percentage organic matter content inferred by subtraction.

Surface chlorophyll *a* concentrations were taken as an index of microphytobenthic biomass (MacIntyre *et al.* 1996). Surface sediment samples were agitated for 15 minutes in 90% acetone solution and centrifuged for 15 minutes at 2500 rpm. Chlorophyll *a* concentration in the supernatant was determined by spectrophotometry, following Lorenzen (1967) and expressed as mass of chlorophyll *a* per unit mass of dry sediment (µg Chl*a* g^{-1}).

3.2.5 Statistical analyses

Statistical analyses were carried out for each of the sediment properties using linear mixed models (LMM) with the first order auto-regressive (AR1) covariance structure fitted according to the criteria of Wang & Goonewardene (2004), with Time as a repeated variable and experimental Treatment (*i.e.* 'Hand-raked' and 'Hand-raked & cockles removed') and Shore as fixed factors. LMM were selected as the assumption of sphericity *i.e.* homogenous variance across treatment levels, as required in general linear models (GLM), was unlikely to be satisfied (Rowell & Walters 1976). Additionally, unlike GLM, LMM is equally efficient for both balanced and unbalanced designs and hence allows for a degree of missing data and allows for the fact that due to tidal variations, sampling times were not exactly four weeks apart (Wang & Goonewardene 2004; Spilke *et al.* 2005). Percentile data (loss on ignition and silt content) were √-transformed prior to analysis; for clarity however, untransformed values are presented. LMM analyses were carried out in SPSS v15.

3.3 Results

3.3.1 Infaunal assemblages

Analyses revealed significant differences in *C. edule* biomass between the factors Treatment (LMM, F = 9.79, p = 0.001) and Time (F = 7.13, p = 0.013), but no significant differences within the interactions between factors. Plots subjected to the treatment Fishing had a mean *C. edule* biomass of 89.15 ± 52.41 g m^{-2}; assemblages within Procedural Controls contained 448.91 ± 245.54 g m^{-2} *C. edule* and Undisturbed Controls contained 484.09 ± 176.78 g m^{-2}. Therefore the Fishing treatment did result in a significantly-reduced biomass of *C. edule* within experimental plots. *C. edule* biomass was also found to increase under the factor Time (T_1 = 268.08 ± 124.13 g m^{-2}, T_2 = 413.35 ± 181.92 g m^{-2}), presumably due to the immigration and/or growth of the organisms over the summer period (Flach 1996).

Total macrofaunal biomass was greater at Thurstaston than at Warton Sands (mean total macrofaunal biomass for Thurstaston = 768.7 ± 429.0 g m^{-2}, for Warton Sands = 353.1 ± 283.6 g m^{-2}, Mann-Whitney U = 779, p <0.001). A total of 35 taxa were identified over both assemblages, with 33 at Thurstaston and 19 at Warton Sands.

Thurstaston showed greater macrofaunal diversity than Warton Sands (Shannon-Weiner mean $H'_{\text{Thurstaston}} = 1.30 \pm 0.37$; $H'_{\text{Warton Sands}} = 0.97 \pm 0.23$, Mann-Whitney $U = 742$, $p = 0.002$). Significant differences in faunal assemblage compositions were identified between the two shores (Table 3.1 and Figure 3.1). Faunal biomass within both assemblages was dominated by *C. edule*, contributing 45.3 ± 28.34% of total faunal biomass at Thurstaston and 52.9 ± 28.6% at Warton Sands. Other major contributors to faunal biomass at both assemblages were the bivalve *M. balthica* (contributing to 20.7 ± 13.1% at Thurstaston and 20.7 ± 15.2% at Warton Sands) and the gastropod *H. ulvae* (contributing 7.3 ± 9.2% at Thurstaston and 12.8 ± 21.8% at Warton Sands). Most of the difference between the two shores was derived from differences in the biomass of the bivalve species *C. edule*, *M. balthica* and *S. plana*, the polychaete *Hediste diversicolor* and the gastropod *H. ulvae* (Table 3.2). With the exception of *H. ulvae*, which showed a greater biomass at Warton Sands, these taxa were either absent at Warton Sands or present at much reduced biomass.

Table 3.1. PERMANOVA and pairwise *a posteriori* comparisons between faunal biomass compositions within two assemblages (TH = Thurstaston and WS = Warton Sands) under different experimental treatments (F = Fished, P = Procedural Control and C = Undisturbed Control) at two different times (1 = June 2007 and 2 = October 2007). Significant (p <0.05) differences in the PERMANOVA are shown in bold. For the pairwise *a posteriori* comparisons, only significant results (P <0.05) are shown. For example, TH, 1: F≠P means that there was a significant difference in assemblages at Thurstaston at Time 1 between the Fished and Procedural control plots.

Source of variation	df	MS	F	P(perm)	*A posteriori* comparisons
Shore	1	16940.02	18.60	**<0.01**	TH≠WS
Treatment(Shore)	4	2621.31	2.88	**<0.01**	TH: F≠C, P≠C; WS: F≠C
Time	1	549.68	0.60	0.644	
Shore × Time	1	618.02	0.68	0.586	
Treatment(Shore) × Time	4	2861.33	3.14	**<0.01**	TH, 1: F≠P, F≠C; TH, 2: F≠P, P≠C
Error	36	910.58			

Total macrofaunal biomass (including biomass of *C. edule*) present within assemblages was significantly reduced following the removal of *C. edule* (LMM, F = 16.27, p <0.001). There was also a significant increase of biomass with sampling time, with samples taken in October housing a greater biomass than those in June (LMM, F = 7.25, p = 0.010). However, when the biomass data for *C. edule* were excluded from the analysis, then, although total biomass at Thurstaston was still greater (LMM, F = 84.97, p <0.001), no significant changes under the experimental treatments were detected and hence, non-*C. edule* assemblage biomass was not significantly changed by the treatments. The removal of *C. edule* had a significant effect on biodiversity both with (Shannon-Wiener Index, LMM, F = 11.94, p = 0.002) and without (Shannon-Wiener Index, LMM, F = 4.56, $p < 0.05$) the *C. edule* data being included in the analyses. Fished plots showed increased biodiversity compared with both Procedural and Undisturbed Controls.

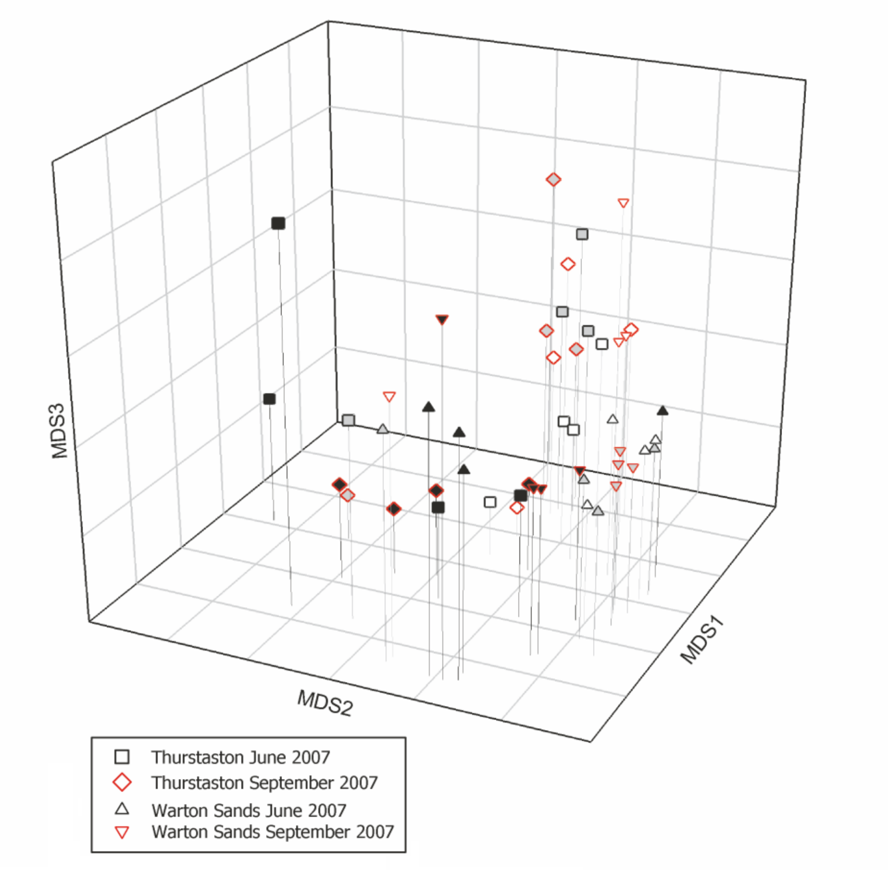


Figure 3.1. Three-dimensional non-metric multidimensional scaling (nmMDS) ordination of biomass-weighted community assemblages within experimental assemblages at two study shores. Sample treatment is indicated by symbol shading: solid black = Fished, grey = Procedural Control and white = Undisturbed Control. nmMDS Stress = 0.06.

PERMANOVA revealed no significant changes over Time and no Shore × Time interaction within faunal assemblage compositions within either assemblage (Table 3.1). However, significant differences were observed with regards to Treatment and within the Treatment × Time interaction (Table 3.1). *A posteriori* pairwise analyses were carried out on the significant outcomes of the PERMANOVA. At Thurstaston, differences in faunal biomass compositions were observed between the Undisturbed Control plots and both the experimentally-Fished and the Procedural Controls. SIMPER analyses (Table 3.2) revealed that much of the differences were due to a reduced biomass of the bivalves *C. edule*, *Macoma balthica*, and *Scrobicularia plana* in Fished plots and increased biomass of *Hydrobia ulvae* in Undisturbed Control plots. Procedural Control plots showed an increased biomass of *M. balthica* and reduced biomass of *C. edule*, *S. plana* and *H. ulvae* compared with Undisturbed Control plots.

Table 3.2. Output of the SIMPER analyses displaying the taxa responsible for 90% of the difference between experimental treatments (F = Fished, P = Procedural Control, C = Undisturbed Control) within two assemblages (TH = Thurstaston and WS = Warton Sands) both in terms of overall differences and over two sampling occasions. Only differences revealed as significant by *a posteriori* pairwise comparisons are displayed. Values represent the percentage contribution made to the differences by each taxon. Superscript *A* indicates that the former of the pairwise comparisons has the greater biomass, *B* indicates that the latter comparison has greatest biomass, e.g. Thurstaston has a greater biomass of *Hediste diversicolor* than Warton Sands.

		Thurstaston						Warton Sands
		Overall		Jun-07		Oct-07		Overall
Species	TH vs. WS	F vs. C	P vs. C	F vs. P	F vs. C	F vs. P	P vs. C	F vs. C
Hediste diversicolor	4.86^{A}						2.95^{B}	
Hydrobia ulvae	4.02^{B}	4.14^{A}	3.76^{B}	4.83^{B}	6.27^{A}		3.27^{B}	
Cerastoderma edule	54.40^{A}	64.74^{B}	65.77^{B}	68.15^{B}	57.98^{B}	73.95^{B}	61.68^{A}	83.60^{B}
Macoma balthica	15.03^{A}	11.21^{B}	10.81^{A}	17.26^{A}	18.59^{A}	7.60^{B}	7.47^{A}	8.79^{B}
Scrobicularia plana	14.36^{A}	10.17^{B}	10.78^{B}		9.70^{B}	10.21^{B}	15.64^{A}	

At Thurstaston, assemblages were also significantly-different between Treatments at the two sampling times. In June 2007, Fished plots were different from both Procedural and Undisturbed Control plots (Table 3.1). Fished plots had a reduced biomass of *C. edule* and increased biomass of *M. balthica* compared to both controls. Plots subjected to Fishing had a reduced biomass of *H. ulvae* compared with Procedural Controls, but a greater biomass than Undisturbed Control plots. In October 2007, Procedural Controls were significantly different from both Fished and Undisturbed Control plots, with most of the difference attributable to increased biomass of *C. edule*, *S. plana* and *M. balthica* within Procedural Controls compared to the other two treatments (Table 3.2). Procedural Control plots were found to have a decreased biomass of *H. ulvae* and *H. diversicolor* in relation to the Undisturbed Control plots.

At Warton Sands, the only significant difference in taxon biomass was between Fished and Undisturbed Control plots; most of the difference between the Treatments was due to decreased *C. edule* and *M. balthica* biomass within Fished plots (Table 3.2). This effect was irrespective of the factor Time.

3.3.2 Biological traits analysis

The first two axes of the FCA explained over 78% of variance, with 45% across fuzzy correspondence axis 1 (FCA1) and 34% of variance across axis 2 (FCA2) (Table 3.3). Much of the variance on FCA1 was represented within traits relating to faunal dispersal (propagule dispersal and adult mobility/dispersal potential), longevity and feeding-related traits (resource capture and food type) (Table 3.3). Ordination scores were compared for FCA1 and revealed a significant separation of samples under the factor Treatment (Kruskal-Wallis, H = 16.82, p <0.001), with Fished plots tending towards positive values and the Procedural and Undisturbed Control treatments tending towards negative values on FCA1 (Figure 3.2). No significant differences were detected on FCA1 under the factors Shore or Time. Fished samples tended towards a reduction in non-motile fauna, with an increased proportion of highly-motile fauna (adult mobility >100 m^2). Fished plots had an increased prevalence of relatively short-lived fauna (0.5-1 and 1-2 year modalities) and a reduced biomass of long-lived organisms (2-5 years). Scavengers were more prominent within Fished plots and these plots had reduced prevalence of active suspension feeders relative to the controls (Figure 3.3). The removal of *C. edule* therefore, explained much of the variability within the data (factor = Treatment within FCA1) and much of this variability was explained by traits relating to the dispersive abilities, longevities and feeding methods of fauna.

Much of the variance within FCA2 was explained by the living location and maximum size of taxa, the dispersive potential of adult organisms and the food resources exploited (Table 3.3). No separation of samples on FCA2 was found relating to the factors of Treatment or Time. Significant separation of samples was observed along FCA2 under the factor Shore (Kruskal-Wallis H = 32.62 p <0.001), with samples taken at Warton Sands tending towards positive values and Thurstaston tending towards negative values on FCA2 (Figure 3.2). Assemblages at Warton Sands tended towards a prevalence of smaller (0-50 mm), more motile (>100 m^2 $year^{-1}$) taxa, dwelling at the sediment-water interface and assemblages at Thurstaston tended towards larger (50-100 mm), deeper-dwelling (>20 cm) and less-motile (<10 m^2 year^{-1}) fauna (Figure 3.3).

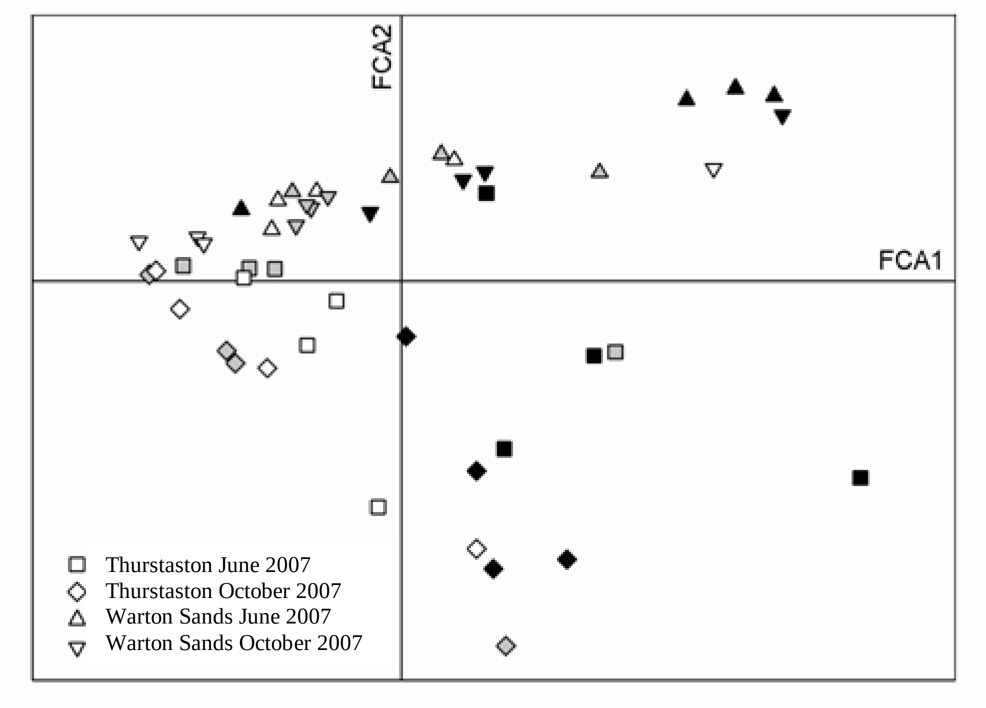
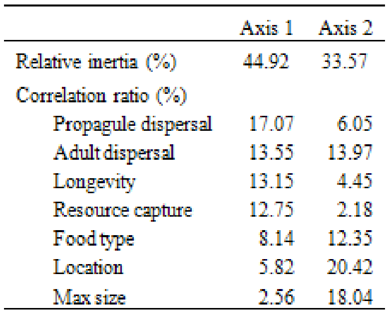


Figure 3.2. Ordination of the first two axes of the FCA for the two experimental assemblages. FCA1 explained approximately 45% of variance in the data and FCA2 explained approximately 34%. Sample treatment is indicated by symbol shading: solid black = Fished, grey = Procedural Control and clear = Undisturbed Control.

Table 3.3 Relative inertia and correlation ratios of biological traits on the first two axes of the fuzzy correspondence analysis. Correlation ratios represent the proportion of variance expressed by the different traits across each axis.



	Axis 1	Axis 2
Relative inertia (%)	44.92	33.57
Correlation ratio (%)		
Propagule dispersal	17.07	6.05
Adult dispersal	13.55	13.97
Longevity	13.15	4.45
Resource capture	12.75	2.18
Food type	8.14	12.35
Location	5.82	20.42
Max size	2.56	18.04

When *C. edule* were excluded from the BTA, no significant changes to the distribution of biological traits was observed on either FCA1 or FCA2 (Kruskal-Wallis H = 0.53 p = 0.766 and H = 1.21 p =0.547 respectively). This suggests that the differences in the prevalence of traits following the removal of large *C. edule* are directly caused by *C. edule*, rather than a shift in the underlying community structure. Differences were observed on FCA1 between the two Shores (H = 33.57 p <0.001) and there were significant differences between the two sampling Times on both Shores (Thurstaston: H = 10.08 p = 0.001; Warton Sands: H = 5.60 p = 0.018), with increased prevalence of deep-dwelling, relatively non-motile and larger trait characteristics between the June and

October sampling occasions. No significant differences were observed on FCA2 for any of the factors.

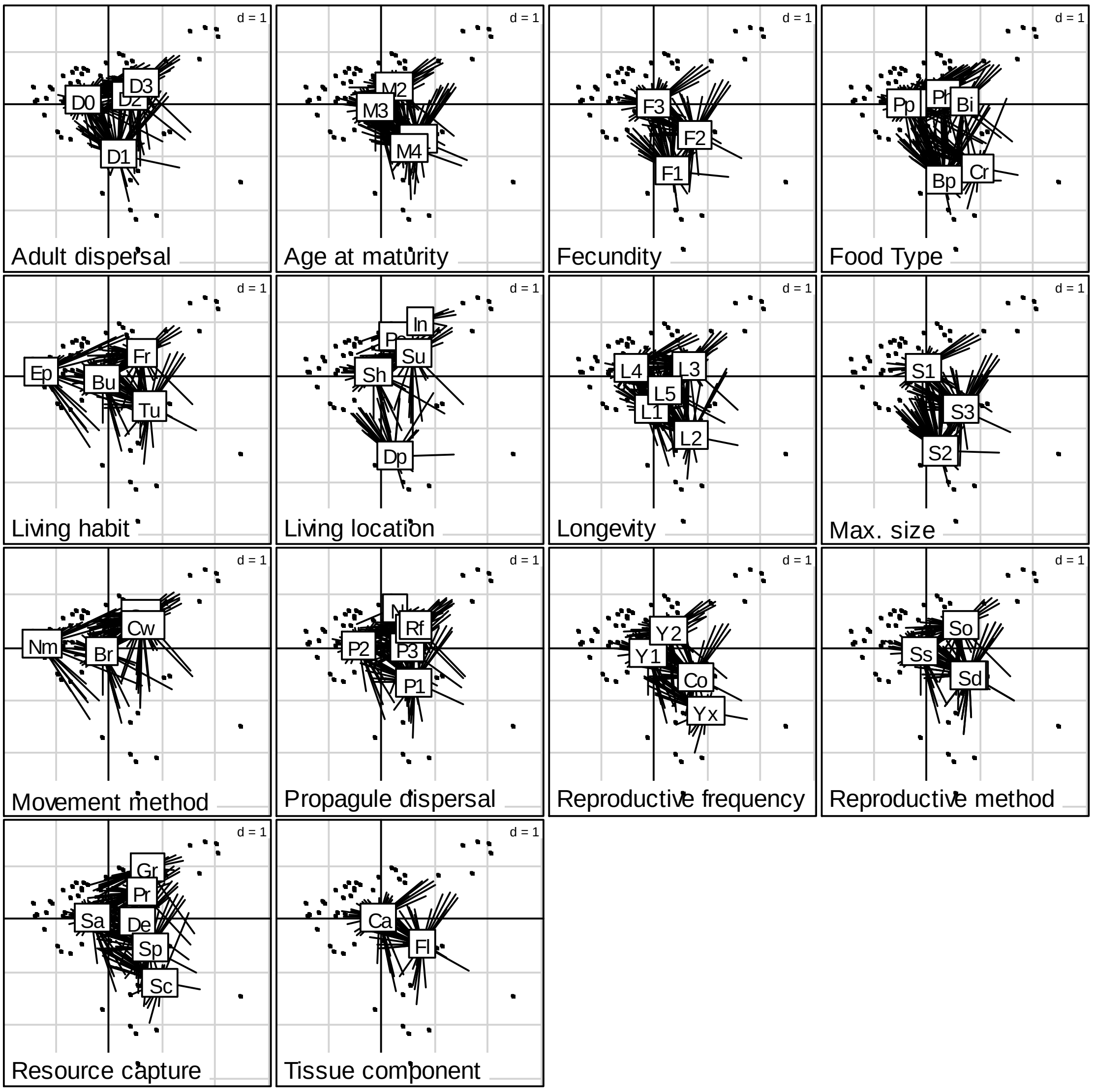


Figure 3.3. Ordination of biomass-weighted biological trait categories from the first two axes of the FCA, allowing interpretation of the traits responsible for the differences between assemblages. Points represent the FCA coordinates for each experimental plot (as in Figure 3.2) and label locations represent the centroid for each trait modality and lines link the plots to the modalities. Modality labels refer to Table 2.1.

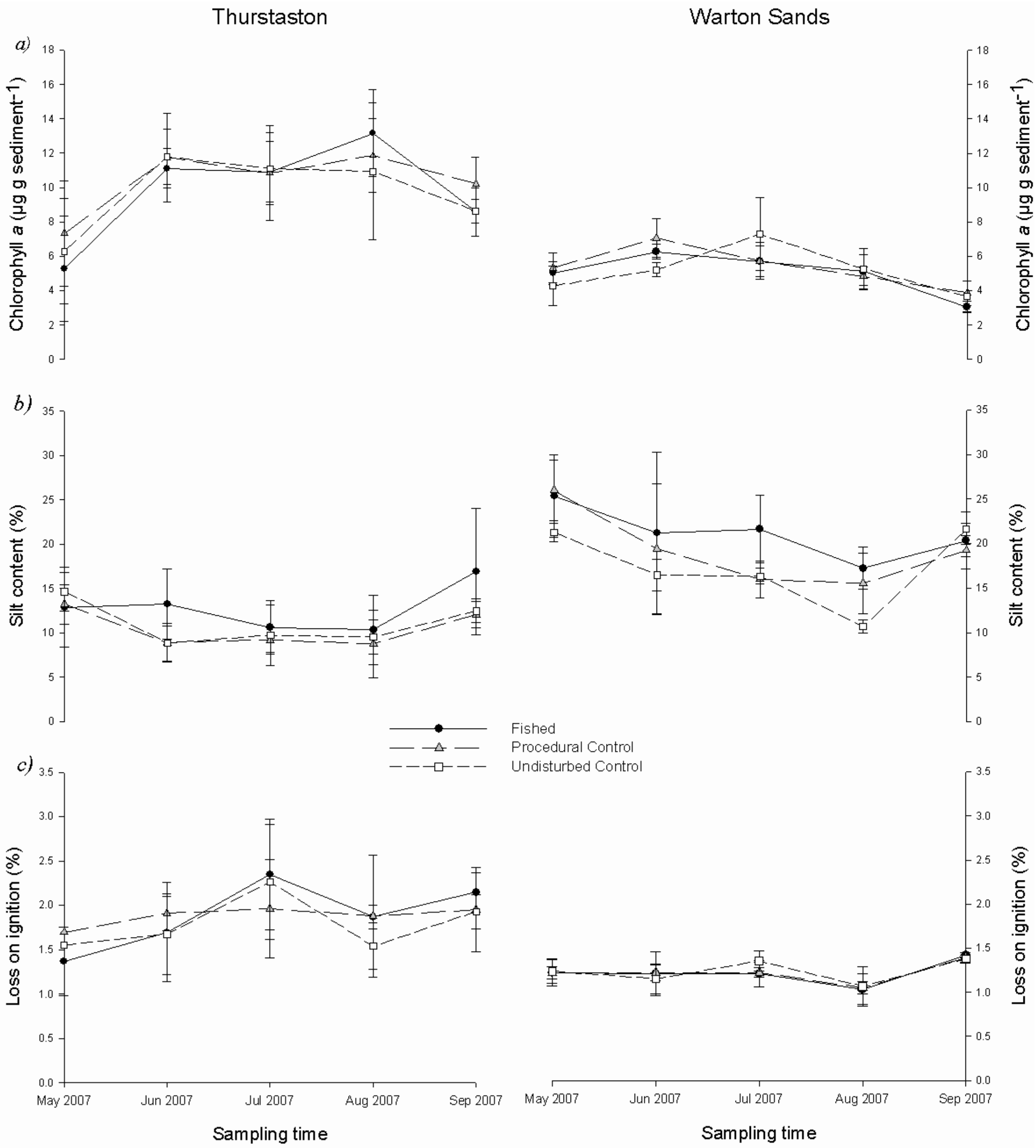


Figure 3.4. Mean values (± standard deviation) of the sediment parameters over four months following the experimental removal of *Cerastoderma edule* at two locations: *a)* chlorophyll *a* concentration (µg g dry sediment^{-1}), *b)* silt content (% <63µm) and *c)* organic matter (as % loss on ignition).

3.3.3 Sediment properties and chlorophyll a concentration

Sediment properties differed between Thurstaston and Warton Sands, with significantly higher concentrations of chlorophyll *a* (11.249 ± 1.867 µg chlorophyll *a* g sediment^{-1} and 6.005 ± 1.032 µg chlorophyll *a* g sediment^{-1} respectively) and organic matter content (1.928 ± 0.463% and 1.427 ± 0.179 respectively) at Thurstaston than at Warton Sands (Table 3.4, Figure 3.4). Sediment granulometry (indicated by silt content (63 µm)) at Warton Sands was less coarse than that at Thurstaston and changed over time at both shores (Table 3.4, Figure 3.4). No significant differences in chlorophyll *a* concentration or loss on ignition were observed with regards to experimental treatment. Sediment granulometry however did show significant change; sediments in Fished plots contained higher silt contents (3.99 ± 0.06%) than non-fished plots (3.70 ± 0.10%; Table 3.4).

Table 3.4. Summary of the linear mixed model analyses for the sediment parameters quantified to investigate ecological functions. Significant values ($\alpha = 0.05$) indicated in bold.

		Chlorophyll *a*		LOI		Silt content	
Source	df	F	p	F	p	F	p
Shore	1	**127.42**	**<0.001**	**15.47**	**<0.001**	**52.21**	**<0.001**
Raked	1	0.18	0.673	0.04	0.841	0.16	0.690
Cockles Removed	1	0.30	0.589	1.75	0.198	**5.39**	**0.026**
Time	4	1.01	0.379	1.21	0.315	1.94	0.111
Shore × Raked	1	0.24	0.630	0.05	0.834	0.72	0.403
Shore × Cockles Removed	1	0.03	0.858	0.66	0.425	0.19	0.666
Shore × Time	4	0.42	0.797	1.78	0.145	**2.86**	**0.028**
Raked × Time	4	0.27	0.894	0.39	0.818	1.00	0.414
Cockles Removed × Time	4	0.16	0.960	2.11	0.091	0.86	0.491
Shore × Raked × Time	4	0.19	0.944	0.15	0.963	0.92	0.459
Shore × Cockles Removed × Time	4	0.05	0.994	1.15	0.342	2.43	0.054

To investigate the power of the experiment to detect significant changes, iterative analyses were carried out on the chlorophyll and organic matter data. Under the current design, a change in surface chlorophyll values of ~16% and a change in organic matter of ~2.5% would be necessary in order to give statistically significant changes.

3.4 Discussion

This study has shown that the removal of *Cerastoderma edule* led to a marked increase in faunal diversity within manipulated plots, irrespective of the pre-impact diversity at the site. This suggests that other taxa are prevented from establishing themselves by the presence of *C. edule* even within species-poor assemblages. Following the removal of large *C. edule,* other taxa established within the system, either exploiting the resources liberated by the loss of *C. edule* or in response to the physical disturbance of the habitat. Such changes have been reported in a range of systems (*e.g.* Tuck *et al.* 1998; Sparks-McConkey & Watling 2001; de Juan *et al.* 2007).

The observed changes to macrofaunal communities were reflected in changes to the distribution of biological traits within the assemblages, however this change was due to the removal of large *C. edule* themselves, rather than changes to the distribution of underlying traits following removal. Removal of *C. edule* resulted in an increased prevalence of motile, short-lived taxa, with increased occurrences of scavengers, coupled with a relative reduction in non-mobile, longer-lived suspension-feeding fauna. This adds support to the potential of *C. edule* acting as key contributors to ecological functioning, in that their removal significantly altered the distribution and prevalence of functionally-important biological traits. This occurred within both study assemblages and is consistent with other studies investigating the impacts of disturbance on benthic communities. At a smaller scale, for example Norkko & Bonsdorff (1996) found increased abundances of opportunistic taxa beneath experimentally-placed algal mats. Larger-scale analyses of North Sea benthos also found a trend towards increased numbers of motile predators and scavengers with high levels of fishing disturbance (Bremner *et al.* 2003b; Tillin *et al.* 2006).

Following the observed changes to the macrofaunal communities and distribution of biological traits within the two assemblages, it could be expected that changes to the delivery of the ecological functions would be evident. However, this was not the case with respect to the functions quantified here. *C. edule* is an active suspension feeder and a significant portion of its diet can be re-suspended microphytobenthos (Sauriau & Kang 2000; Page & Lastra 2003). Removal of *C. edule*, a principal contributor to macrofaunal biomass, would therefore reduce grazing pressure and result in an increased biomass of primary producers. This was not observed in the current study, with chlorophyll *a* concentration (as a proxy for primary producer biomass) showing no significant relationship to experimental treatment on either study shore.

Organic matter content within the sediment was also studied as a proxy for the overall community metabolism within the assemblages (van Duyl *et al.* 1992). A shift towards a more motile fauna for example, can influence the rate at which organic matter is incorporated into the sediment, influencing the resources available to the microbial community; this has subsequent impacts onto the provision of oxygen and the mineralisation of organic materials within the sediment (Gilbert *et al.* 1995; Fenchel 1996). Assemblages with shorter life histories and more generalist and opportunistic feeding methods would also influence the trophic relationships throughout the assemblages and hence also influence the overall metabolism within the system. Therefore, the removal of *C. edule* was expected to result in a change to the availability of organic matter within the benthos. Again, no significant relationship with experimental treatment was observed at either study site.

The third ecological indicator considered was the granulometry of sediments within the assemblages. This is important to the functioning of systems, impacting upon sediment porosity and hence the depth of the redox layer (Huettel & Rusch 2000) with implications for the settlement of larvae into the benthos (Pinedo *et al.* 2000; Duchêne 2004). *C. edule* have been shown to loosen and destabilise sediments (Flach 1996), leaving finer-grained particles more prone to resuspension, resulting in a coarsening of sediment granulometry (Ciutat *et al.* 2006; 2007). The findings of the current study concur with this: the removal of large *C. edule* resulted in a higher silt content within plots.

Within the current study, the removal of large *C. edule* had no significant impact upon the measured ecological functions relating to the biological productivity (as indexed by organic matter content and primary producer biomass) within the benthos. These responses, or lack of them, were common to both study shores and hence were independent of the initial biodiversity of the assemblages. Following the removal of large *C. edule*, both the species-rich assemblage at Thurstaston and the species-poor assemblage at Warton Sands showed a shift towards assemblages dominated by traits common to opportunist taxa. It appears therefore that ecological functions were conserved following the removal of large *C. edule*, regardless of the underlying macrofaunal diversity present at the sites. A number of possible explanations may account for this. Firstly, the statistical power of the current study may be insufficient to detect subtle changes to the measured functions. Iterative power analyses revealed that, with the natural variability observed in this study, chlorophyll and organic matter contents would have to alter by ~16% and 2.5% respectively to detect a significant change. Such changes are not unfeasible. Swanberg (1991) for example, observed that the presence of *C. edule* resulted in a change in microphytobenthic biomass the region of 90%. Therefore, it appears that surface chlorophyll and organic matter content did not change significantly under the experimental treatments. Two alternative explanations may also account for this observation: *i)* the removal of large *C. edule* and the subsequent changes to the macrofaunal communities (and therefore the distribution of biological traits) within the study systems has no effect on the investigated functions, or *ii)* other processes are buffering or masking the response of the systems to the removal of *C. edule* and the subsequent shift in assemblage and trait structure.

Previous, largely laboratory-based, experimental investigations have shown that *C. edule* can influence ecological functions, for example, enhancing primary productivity (Swanberg 1991); *C. edule* has also been shown to significantly alter near-bed hydrodynamics and sediment stability (Ciutat *et al.* 2007). Additionally, shifts in the distribution of biological traits within an assemblage have been shown to affect the delivery of ecological functions, for example changes to the prevalence of different methods of bioturbation affect the regeneration of nutrients and penetration of oxygen within sediments (Mermillod-Blondin *et al.* 2005; Waldbusser & Marinelli 2006; Gilbert *et al.* 2007). It is therefore likely that the removal of *C. edule* and the subsequent shift in biological traits would have some effect on the functions under investigation unless other factors were simultaneously changing and compensating for it.

The findings of the current study imply that the ecological functions measured are largely unaffected by significant reductions in the biomass of a potentially key contributor to ecological function. This is contrary to a number of other studies that have identified substantial changes to ecological functions following the removal of large taxa. Kanaya *et al.* (2005) for example, found that deposit-feeding bivalves had significant impacts on primary producer biomass and Volkenborn (2005) found that the exclusion of the lugworm *Arenicola marina* from intertidal plots had significant effects on a number of ecosystem functions. Conversely however, Bolam *et al.* (2002) found no effects of macrofaunal biomass or species richness on ecological functioning within a Scottish intertidal mudflat.

In the present study, BTA revealed significant changes to the prevalence of a number of traits within the macrofauna following the removal of large *C. edule*. However, there were no significant changes in the distribution of traits likely to impact upon the composition of the microbial community which primarily drives benthic primary production and microbial metabolism (Azam *et al.* 1993; Azam 1998; Paerl 1997).

Additionally, Franklin & Mills (2006) showed that microbial communities display a high degree of functional redundancy and even considerable changes to the microbial community composition are not reflected by changes to ecological functioning. Therefore, even if changes to the macrofaunal community did alter the microbial community, the substantial functional redundancy within these communities means that the measured functions were unlikely to be affected.

The conservation of functions observed in this investigation is likely, to some degree, to be related to the scale of the study. Subtle and/or small-scale changes in ecological processes are likely to be masked by the substantial natural variability over small scales and larger-scale processes occurring within the habitat (Kendrick *et al.* 1996). A larger-scale study would be more likely to reveal observable impacts (*e.g.* Watling *et al.* 2001; Falcão *et al.* 2003). However, as the aim of the current study was to assess the impacts of small-scale cockle hand-raking on ecological functioning, the spatial and temporal scales used reflected those used within the commercial fishery.

A limitation of the fuzzy-coding approach in BTA requires further consideration. This technique can provide only an indication, based on published and expert information, as to how a taxon may behave. It is unable, however, to provide any indicator of how an organism actually does act in a given situation. *Hediste diversicolor* for example, is able to utilise a number of feeding strategies, depending upon resource availability (Fauchald 1979) and *Pygospio elegans* can adopt a wide range of reproductive strategies (Gudmundsson 1985; Anger *et al.* 1986). Additionally, BTA does not account for ontogenetic differences within taxa. In this investigation, large *C. edule* were removed from assemblages and the remaining *C. edule* were scored identically to large *C. edule* for the purposes of BTA. However, it is likely that younger (*i.e.* smaller) individuals behave differently and display a different range of functional traits than adult individuals, for example displaying different feeding preferences (Sauriau & Kang 2000). It is impossible to ascertain which strategy or strategies an organism is utilising at any time and whether a change in conditions causes an organism to change its strategy as the fuzzy coding technique includes all the strategies that can potentially be used by a species. It is necessary to address whether conspecifics under different disturbance regimes adopt the same behavioural/life-history strategies as this would potentially affect how the assemblage is delivering ecological functions. Studies involving BTA should therefore aim to incorporate some inference of the behavioural responses of taxa within affected assemblages.

The conservation of the measured functions does not necessarily mean that fished systems will continue to deliver other ecological functions. The availability of food resources within the system for example, is a key aspect of ecological functioning (Frid *et al.* 2008). As well as being the target of a fishery, *C. edule* are an important food source to demersal fish and shorebird species and are a major contributor to macrofaunal biomass. Large-scale removal of *C. edule* will therefore affect the food availability within, and by definition, the functioning of, affected systems. Over longer timescales, this will potentially impact upon the regeneration and sustainability of *C. edule* stocks (Piersma *et al.* 2001; Kraan *et al.* 2007) and directly affect taxa relying on *C. edule* for food (Beukema & Dekker 2006). Therefore, the implications of small-scale, non-ubiquitous cockle harvesting depend upon the aspect of the system under concern. In terms of microbial and small-scale processes, there appears to be little impact; however the removal of large *C. edule* from the system does impact upon the total macrofaunal biomass within the system and the potential availability of food resources available within the system.

CHAPTER 4: BENTHIC DISTURBANCE AFFECTS INTERTIDAL FOOD WEB DYNAMICS: IMPLICATIONS FOR INVESTIGATIONS OF ECOSYSTEM FUNCTIONING

4.1 Introduction

A range of methods may be utilised in the investigation of the influence of taxa on the functioning of ecosystems. Traditionally, methods have been based largely on taxonomic approaches, investigating how the number of species (species richness), the evenness of species and other diversity indices influence the delivery of ecological functions (see Section 1.1 for further discussion). Increasingly, these methods are being replaced by methods that group organisms by the functional roles they play, taking account of how the ecology, behaviour and physiology of taxa mediates ecological functions rather than relying solely on nominal taxonomic approaches (*e.g.* Petchey *et al.* 2004; Wardle & Zackrisson 2005).

Functional group approaches map the occurrences of biological traits or phenotypes within an assemblage. Taxa are grouped by the strategies they have evolved in order to feed, reproduce, move *etc.* Different strategies affect ecological processes to different extents, for example different bioturbation methods have different impacts on the cycling and regeneration of nutrients within the benthos (Mermillod-Blondin *et al.* 2005). Therefore, if the identities of taxa within an assemblage differ, then the range of biological traits supplied by the assemblages may also differ. However, the functional group approach has some potential problems. Functional group approaches generally assume that taxa are rigid in the functional groups to which they belong, offering no potential for organisms to change the biological traits they are expressing. A fuzzy coding approach allows taxa to be assigned to more than one trait category (Chevenet *et al.* 1994), however, this approach still only provides a potential range of traits that an organism may be expressing. That is, it is impossible to define the actual, or realised, range of traits being expressed under any given environmental or experimental condition.

Within naturally-variable habitats, such as within the temperate estuarine intertidal, such uncertainty is of potential concern. In these habitats, taxa have to be able to respond to changeable environmental and ecological conditions, as factors such as salinity, temperature, food availability and the risk of predation often show substantial seasonal fluctuations (Hoch & Kirchman 1993; Froneman 2001). It is common therefore, for taxa within these habitats to show some degree of plasticity with regards to their expression of biological traits, for example conspecifics may adopt differing reproductive (Gudmundsson 1985; Wilson 1985; Anger *et al.* 1986), or feeding (Rönn *et al.* 1988; Esselink & Zwarts 1989; Vedel 1998) strategies depending on localised conditions.

With such a potential for variability in the expression of traits by taxa, it is difficult to discern the approach that a taxon is taking at any given time and this can influence the ecological roles that taxa are playing within an assemblage. Using approaches in which taxa are rigidly assigned to functional groups, it is impossible to identify the suite of traits an organism is expressing at any given time. Consider for example, some disturbance event that causes the taxa present within an assemblage to alter their expression of some trait (for example, feeding method), but not otherwise altering the distribution of species within the assemblage. This would mean that the ecological roles played by taxa have changed, for example, different feeding methods resulting in altered energy flow through the system. However, when examining the functional structure or functional diversity of the assemblage, this change in the realised distribution of traits would not be observed as the taxonomic composition, and hence the potential presence and prevalence of ecologically-important traits remains unaltered.

The aim of the current investigation was to assess the potential for taxa to alter the biological traits they are expressing following a disturbance event. This has implications for the applicability of functional group and biological trait analyses as a change in the trait expression of taxa can lead to functional group approaches giving a misleading depiction of the functional roles that taxa within an assemblage are playing.

One trait that is potentially highly susceptible to misinterpretation is the feeding mode adopted by organisms. Taxa living within the benthic intertidal often show a great deal of plasticity with regards to this trait and many will adopt different feeding preferences depending upon local conditions (see Fauchald & Jumars 1979 and references therein). As a substantial body of literature exists describing the feeding modes of taxa, this is an ideal model trait to test whether there is the potential for changes in the distributions of traits within an assemblage to be overlooked.

Additionally, the degree to which taxa express different feeding strategies has the potential to change under changing environmental conditions. The polychaete *Hediste diversicolor* for example, has been shown to exhibit preferences for suspension and deposit feeding, as well as acting as active predators depending upon the availability of food sources (Rönn *et al.* 1988; Esselink & Zwarts 1989; Vedel 1998). Furthermore, the feeding methods of taxa are of substantial ecological interest and trophic relationships within ecosystems play a fundamental role in the transfer of energy and organic carbon through the system and thus are a key contributor to ecosystem functioning (Frid *et al.* 2008). What is required therefore is a method that allows changes in the diets of taxa following disturbance to be investigated. This will permit testing of the hypothesis that disturbances to a faunal assemblage can result in a change to the feeding preferences of taxa within the assemblage to be tested.

4.1.1 Examination of feeding modes of fauna

Analysis of the gut contents of organisms is one method that has been utilised for the examination of taxa feeding behaviour, providing a direct indication as to what organisms have been feeding on immediately prior to sampling. In practical terms, this method generally requires study organisms to be fairly large. Additionally, the identification of stomach contents is a time-consuming and difficult task and is generally restricted to identifying food sources from undigestible body parts, such as amphipod cuticles and polychaete setae (*e.g.* Schubert & Reise 1986). In addition, this method provides no indication as to how taxa have been feeding over longer time periods, providing only an isolated observation making it impossible to extrapolate feeding behaviour beyond the time that samples were taken.

An increasingly commonly-used alternative is through the investigation of the ratios of stable isotopes within the tissue of organisms. The stable isotopes of carbon (^{13}C) and nitrogen (^{15}N) are the most commonly-examined isotopes in ecosystem ecology, though those of hydrogen (H), oxygen (O) and sulphur (S) are also used (Peterson & Fry 1987). This method is used to trace the passage of material and hence energy through an ecosystem. When organisms feed, atoms of the heavier nitrogen isotope tend to be retained and assimilated into the tissues of the consumer, while lighter isotopes are preferentially excreted (De Niro & Epstein 1978; Rau *et al.* 1983). The transfer of nitrogen is associated with ~3‰ enrichment (termed fractionation) of the heavier ^{15}N per trophic level and hence may be used as an estimate of the trophic level of taxa (*e.g.* Minagawa & Wada 1984; Vander Zanden & Rasmussen 1999; Post 2002). The ratio of ^{13}C to ^{12}C (termed δ^{13}C) within the tissue of an organism shows much less fractionation (~1‰ per trophic level, De Niro & Epstein 1978; Peterson & Fry 1987) and generally reflects the δ^{13}C value for the food material consumed by the organism (the 'You are what you eat' principle of Post 2002). Isotopic signatures can therefore be used to trace the flow of materials through a system, with δ^{13}C values reflecting those of food sources and taxa within a trophic level gathering around comparable δ^{15}N signatures (Vander Zanden & Rasmussen 1999; Post 2002).

The stable isotope approach allows analysis of feeding preferences over longer time periods than those provided by gut content analyses. Some tissues assimilate carbon and nitrogen more quickly than others. Metabolically-active tissues, such as gills and gonads, have a more rapid tissue turnover rate and hence incorporate the isotopic ratios of the food source more rapidly than muscle tissue (Paulet *et al.* 2006; Yokoyama & Ishihi 2006).

To analyse the potential for changes in the feeding behaviour of taxa to be overlooked within a functional trait-based approach, stable isotope signatures were analysed within taxa taken from experimental plots that had been subjected to experimental fishing activity. These manipulations were part of an investigation into the effect of a fishery for the cockle, *Cerastoderma edule* on ecological functioning (see Chapter 3).

4.2 Methods

4.2.1 Site description and faunal sampling

Experimental manipulations were carried out at two shores in north-west England, located within two macrotidal sedimentary estuaries at Thurstaston within the Dee estuary and Wharton Sands within Morecambe Bay (Figure 2.1). Macrofaunal assemblages at Thurstaston housed a greater species richness than those at Warton

Sands (Chapter 2). Field manipulations and faunal sampling were carried out between June and September 2007. At each shore, one of three experimental treatments was applied to four replicate 2 × 2 metre plots arranged in a randomised block design:

1. Fished – surface sediments of plots hand-raked (to a depth ca. 10 cm) using methods analogous to those used by commercial *Cerastoderma edule* fishers. Raked sediments were passed through a 20 mm wide square mesh and retained cockles removed. All other fauna returned to the plots

2. Procedural Control – as F, but all organisms (including *C. edule*) returned to the sediment

3. Control – undisturbed plots

Four months following the initial manipulations, faunal assemblages within the experimental plots were sampled and macrofauna fixed with 4% formaldehyde solution and stored in 70% ethanol solution. Taxa that were present within at least three of the four replicate plots of each treatment were selected for stable isotope analysis. At Thurstaston, these fauna consisted of the bivalves *Cerastoderma edule*, *Macoma balthica* and *Mya arenaria* and the annelids *Tubificoides benedii*, *Eteone longa* and *Hediste diversicolor*. The bivalves *Cerastoderma edule* and *Macoma balthica* and the polychaetes *Pygospio elegans* and *Nephtys hombergii* were selected for Warton Sands. These taxa display a range of feeding modes and have a wide range of potential diets (Table 4.1) and are likely to be important contributors to ecosystem functioning, representing 79.3% of macrofaunal biomass at Thurstaston and 93.8% of macrofaunal biomass at Warton Sands (Chapter 3).

4.2.2 Sample preparation and stable isotope analysis

The individuals of each species that were selected for stable isotope analyses were of similar size. This is because adult organisms are likely to show different proportions of isotopes, for example due to increased apportionment of gonad tissue growth, compared with juveniles (Lorrain *et al.* 2002). Preparation of faunal samples differed depending upon the taxonomic group of fauna. The annelids *Tubificoides* *benedii, Eteone longa* and *Pygospio elegans* were used whole, while those of *Hediste diversicolor, Nephtys hombergii* had their chitinous jaws removed. The bivalves, *Cerastoderma edule*, *Macoma balthica* and *Mya arenaria* were removed from their shells and muscular foot and gut tissue removed under a stereo microscope, retaining metabolically-active gills and viscera for analysis. Chitinous polychaete jaws and bivalve muscle tissue typically have longer turnover rates and hence take a longer period of time to assimilate changes to dietary isotope content than the more metabolically-active viscera (Tieszen *et al.* 1983; Lorrain *et al.* 2002). Given the relatively short time period of the experimental manipulation (four months), such tissues were considered inappropriate and so were excluded from this study. Samples were rinsed in deionised water and dried at 60°C for 48 h. Dried samples were then thoroughly homogenised to powder using a mortar and pestle.

Dried powdered aliquots (ca. 1.25 mg) were packed into 8 × 5 mm tin capsules. Samples were analysed for ratios of stable isotopes (^{13}C and δ^{15}N) using the elemental analyser of a continuous flow isotope ratio mass spectrometer (PDZ Europa 20/20, UC Davis Stable Isotope Facility, Davis, California). All isotope data are expressed in standard δ notation, comparing the ratio of the heavy:light isotope to a standard, using the equation:

$$\delta^{a}X = [(R_{sample}/R_{standard} - 1)] \times 10^{3}$$

where ^{a}X is ^{13}C or ^{15}N and R is the ratio ^{13}C/^{12}C or ^{15}N/^{14}N. δ values (measured as ‰) reflect the ratio of heavy to light isotopes within a sample, compared to the standard reference materials Pee Dee Belamite (PDB) for carbon and atmospheric N_2 (AIR) for nitrogen.

It is important to note that because faunal samples were fixed and preserved in formalin and ethanol respectively, this may potentially affect the interpretation of the data. These compounds have been shown to have some, though generally limited, effect on δ^{13}C and δ^{15}N values (Sarakinos *et al.* 2002). Therefore, caution is urged when comparing the values of δ^{13}C and δ^{15}N observed in this study. The principle aim of this investigation however is not to investigate food-web dynamics *per se* in the study systems, but to investigate whether disturbances have the potential to result in changes to the feeding behaviour within a faunal assemblage. Therefore, because all faunal tissue samples spent an equal amount of time in preservative fluids and the tissues of conspecifics were treated in the same manner, comparisons between treatments are valid.

Table 4.1. Taxa selected for stable isotope analysis to determine the impacts of benthic disturbance on the realised feeding niche in benthic assemblages. The taxa chosen show a number of feeding modes with the potential to exploit a range of food resources.

Taxon	Feeding mode(s)	Primary food resource(s)
Cerastoderma edule	Suspension/Re-suspended benthos	Plankton, particulate matter, microphytobenthos[1, 2, 3]
Macoma balthica	Deposit/Suspension	Plankton, microphytobenthos[4]
Mya arenaria	Suspension	Plankton[5]
Tubificoides benedii	Deposit	Particulate matter[6, 7]
Eteone longa	Predator	Invertebrates[8, 9]
Hediste diversicolor	Opportunistic	Plankton, particulate matter, invertebrates[10, 11, 12]
Pygospio elegans	Deposit/Suspension	Plankton, particulate matter[8]
Nephtys hombergii	Predator	Invertebrates[8]

Superscript numbers indicate information sources: 1 = Kamermans (1993), 2 = Kamermans (1994), 3 = Sauriau & Kang (2000), 4 = de Goeij & Luttikhuizen (1998), 5 = Nichols (1985), 6 = Powell *et al.* (1983), 7 = Giere *et al.* (1984), 8 = Fauchald & Jumars (1979), 9 = Michaelis & Vennemann (2005), 10 = Rönn *et al.* (1988), 11 = Esselink & Zwarts (1989), 12 = Vedel (1998).

4.2.3 Statistical analyses

Isotope ratio values were compared using general linear model ANOVA. The factors employed in the models were the Shore from which the organisms were removed and the Species of the organism. In order to distinguish between the effects of cockle removal and those of experimental procedure (*i.e.* plots with cockles removed had also undergone raking), two factors were used for experimental treatment. The factor Raked was used to distinguish between plots that had or had not undergone any form of raking; thus, this allows Fished and Procedural Control treatments to be distinguished from Controls. The Cockles Removed factor allowed Fished treatments to be distinguished from Procedural Controls and Controls. Shore and Species were treated as fixed factors and the two treatments treated as random factors. Where significant results were obtained, *post-hoc* pairwise comparisons were made between factors using the Bonferroni pair-wise procedure. Analyses were carried out with the software package SPSS version 16.

4.3 Results

Within the undisturbed Control treatment, significant differences were observed in the ratios of carbon and nitrogen isotopes between investigated species (GLM ANOVA, df = 7, $F_{Carbon} = 3.577$, p = 0.030; $F_{Nitrogen} = 8.907$, p = 0.001). This shows that the species present were utilising a range of food sources (differing in their $\delta^{13}C$ values, Table 4.2) and occupying multiple trophic levels (differing in their $\delta^{15}N$ values, Table 4.3), with taxa generally regarded as being predators, *i.e. Eteone longa* and *Nephtys hombergii*, showing higher $\delta^{15}N$ values than those associated with primary consumption, in particular *Pygospio elegans*, *Macoma balthica* and *Mya arenaria* (Figures 4.1 & 4.2). The $\delta^{13}C$ and $\delta^{15}N$ values obtained in the current investigation compared reasonably with values obtained by others (Table 4.4).

Some taxa showed significant differences in their $\delta^{13}C$ values between control and experimentally raked plots at Warton Sands (Shore × Species × Raked, df = 2, F = 4.190, p = 0.020). These effects were not apparent at Thurstaston (Figure 4.1). Bonferroni-adjusted pairwise comparisons of species between treatments showed that both the cockle *Cerastoderma edule* and the polychaete *Nephtys hombergii* showed significant reductions at Warton Sands in their $\delta^{13}C$ values within raked plots compared to controls (mean differences of 1.327‰ and 1.863‰ respectively, both p-values <0.001, Figure 4.2). No significant differences were observed in $\delta^{13}C$ values between controls and plots with cockles removed (Shore × Species × Cockled, df = 2, F = 0.494, p = 0.613).

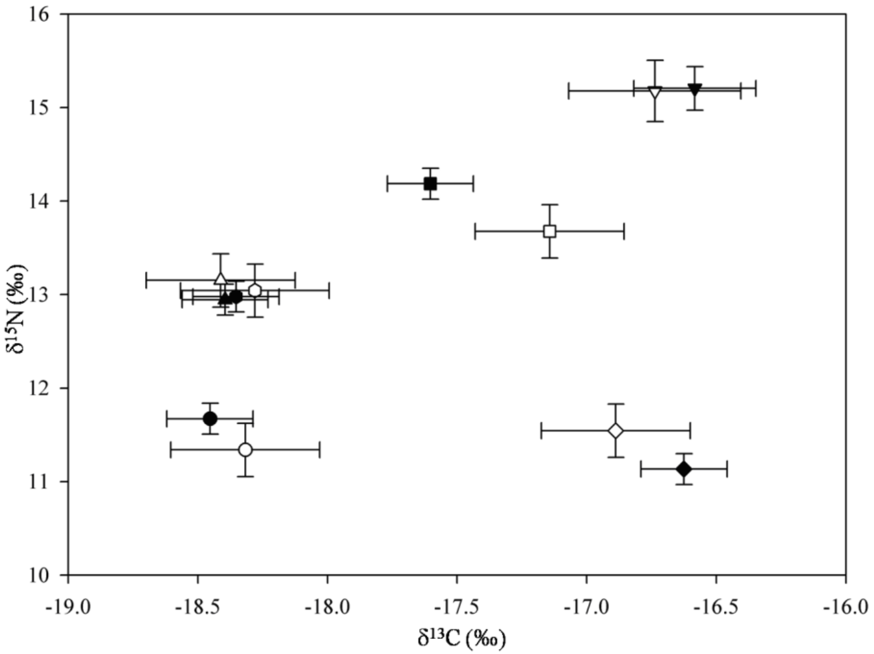


Figure 4.1. Mean δ^{13}C and δ^{15}N values (± S.E.) for macrofaunal taxa investigated within assemblages at Thurstaston (▲ = *Cerastoderma edule*, ▼ = *Eteone longa*, ■ = *Hediste diversicolor*, ● = *Mya arenaria* and ♦ = *Macoma balthica*) subjected to experimental raking (black symbols) or undisturbed controls (white symbols).

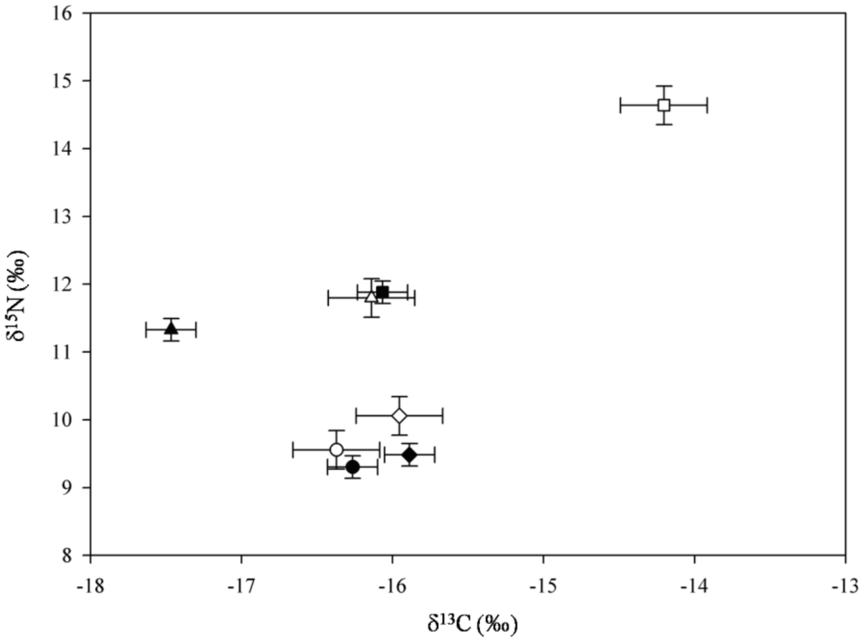


Figure 4.2. Mean δ^{13}C and δ^{15}N values (± S.E.) for macrofaunal taxa investigated within assemblages at Warton Sands (▲ = *Cerastoderma edule*, ■ = *Nephtys hombergii*, ● = *Pygospio elegans* and ♦ = *Macoma balthica*) subjected to experimental raking (black symbols) or undisturbed controls (white symbols).

Table 4.2. P-values from the output of Bonferonni-adjusted pairwise comparisons of δ^{13}C values for individuals belonging to eight taxa taken from undisturbed Control plots at two intertidal shores, a) Thurstaston and b) Warton Sands. Values in bold indicate significant differences at α = 0.05.

a)

	C. edule	*E. longa*	*H. diversicolor*	*M. balthica*	*M. arenaria*
C. edule					
E. longa	**<0.001**				
H. diversicolor	**0.001**	0.053			
M. balthica	**<0.001**	1.000	0.164		
M. arenaria	1.000	**<0.001**	**0.001**	**<0.001**	
T. benedii	1.000	**<0.001**	**0.003**	**<0.001**	**1.000**

b)

	C. edule	*M. balthica*	*N. hombergii*
C. edule			
M. balthica	**0.002**		
N. hombergii	**<0.001**	**0.008**	
P. elegans	0.259	0.577	**<0.001**

Table 4.3. P-values from the output of Bonferonni-adjusted pairwise comparisons of δ^{15}N values for individuals belonging to eight taxa taken from undisturbed Control plots at two intertidal shores, a) Thurstaston and b) Warton Sands. Values in bold indicate significant differences at α = 0.05.

a)

	C. edule	*E. longa*	*H. diversicolor*	*M. balthica*	*M. arenaria*
C. edule					
E. longa	**<0.001**				
H. diversicolor	**0.005**	**<0.001**			
M. balthica	**<0.001**	**<0.001**	**<0.001**		
M. arenaria	**<0.001**	**<0.001**	**<0.001**	1.000	
T. benedii	1.000	**<0.001**	**0.003**	**<0.001**	**<0.001**

b)

	C. edule	*M. balthica*	*N. hombergii*
C. edule			
M. balthica	**<0.001**		
N. hombergii	**<0.001**	**<0.001**	
P. elegans	**<0.001**	0.885	**<0.001**

At both shores there were significantly-different δ^{15}N values between raked and control plots (Species × Raked, df = 7, F = 34.544, p = 0.004). Bonferroni-adjusted pairwise comparisons of species between treatments showed that δ^{15}N values within the tissues of *Nephtys hombergii* and the bivalve *Macoma balthica* were significantly lower within raked plots compared to controls (mean differences for *N. hombergii* 2.760‰, p <0.001; *M. balthica* 0.492‰, p = 0.039, Figure 4.1). No significant differences in δ^{15}N values between controls and plots with cockles removed were observed (Shore × Species × Cockled, df = 2, F = 0.710, p = 0.496).

Table 4.4. Stable carbon and nitrogen isotope signatures (‰) identified within previous investigations for the tissues of taxa examined within the present study.

Taxon	δ^{13}C (‰)	δ^{15}N (‰)	Location	Source†
Cerastoderma edule	-17.9 ± 0.9	8.2 ± 1.1	Western France	1
	-21 to -24	16 to 26	Westerschelde estuary	2
	-19.7*	15.4*	Westerschelde estuary	3
	-20.2*	14.2*		3
Nephtys sp	-19*	18.4*		3
	-17.2*	18.7*		3
N. hombergii	-18.1 ± 0.9	11.5 ± 0.6	NW France	4
	-19.8 ± 0.1	10.8 ± 0.1	NW France	4
Eteone sp	-15.8*	17.6*	Westerschelde estuary	3
	-17.4*	20.6*		3
Macoma balthica	-17.7*	13.5*		3
	-18.4*	14.6*		3
Pygospio elegans	-17.6*	14.6*		3
	-16.9*	15.8*		3
Mya arenaria	-19.6*	13.7*		3

* Estimated values

† Sources: (1) Kang *et al.* (1999); (2) Rossi *et al.* (2004); (3) Herman *et al.* (2000); (4) Bodin *et al.* (2008).

Of the eight species investigated, three showed evidence of significant differences in feeding behaviour following experimental raking. Differences were observed in the food sources of taxa (reduced δ^{13}C values of *Cerastoderma edule*), the trophic level at which taxa are feeding (reduced δ^{15}N values of *Macoma balthica*) or both (reduced δ^{13}C and δ^{15}N values of *Nephtys hombergii*). However, there was no evidence of any changes to feeding behaviour in the other taxa when a biomass dominant taxon (*Cerastoderma edule*) was removed from the studied assemblages.

4.4 Discussion

This study has shown that analyses based on functional groups or biological traits (hereafter termed functional-trait analyses) have the potential to give a misleading representation as to the ecological roles that taxa within an assemblage are carrying out. This has significant implications for the application of these measures in the assessment of ecosystem functioning. Experimental plots on two study shores were subjected to experimental raking of surface sediments and to simulated fishing of the cockle *Cerastoderma edule*. After four months, the feeding habits of three of the eight species investigated, as indexed by the ratios of the stable isotopes of carbon and nitrogen within body tissues had altered compared to controls. This suggests differences in the dietary compositions of conspecifics within plots subjected to the raking treatment and hence a difference in feeding behaviour compared with control plots.

Significantly-reduced δ^{15}N values within the tissues of the suspension- and surface deposit-feeding bivalve *Macoma balthica* and significant reductions in both δ^{15}N and δ^{13}C values for the predatory polychaete *Nephtys hombergii* were evident following surface sediment raking compared to controls. As the heavier ^{15}N isotope accumulates up the food web, tissues of consumers tend to be enriched by 2-4‰ relative to the food resource consumed (Vander Zanden & Rasmussen 2001; Post 2002; McCutchan *et al.* 2003). The observed differences in δ^{15}N values for *M. balthica* were much below this level (~0.5‰). The Zeroth Rule (O'Leary *et al.* 1992) encourages substantial caution regarding δ values below 1‰, due to the variability inherent in the handling and preparation of tissue samples, as well as variations intrinsic to the samples themselves. This observation therefore will not be considered further here.

The observed differences in δ^{15}N values for *N. hombergii* were within the range of values consistent with a shift in trophic level. The data suggest that *N. hombergii* within raked plots were feeding at a lower trophic level than those in control plots. However, trophic interactions within natural assemblages are complex; the high degree of connectivity between taxa within food webs means that it is futile to attempt to assign taxa to rigid and discreet trophic levels (Polis & Strong 1996). Instead, δ^{15}N values within the tissues of taxa often lie along a continuum from taxa with diets dominated by primary producers at one end to those feeding as top predators at the other, with only limited clustering of values into discrete trophic levels (*e.g.* Garcia *et al.* 2006; Garcia *et al.* 2007). The data, therefore suggest that

N. hombergii within experimentally-raked plots were feeding on average at a lower trophic level than those in controls. Within raked plots therefore, the diet of *N. hombergii* contains more primary consumers (for example, algivorous molluscs and polychaetes) than those within control plots, which were feeding on a greater proportion of secondary consumers (such as other polychaetes and crustacea). *Nephtys* have been observed to feed on a wide range of taxa (Fauchald & Jumars 1979) and hence such a difference in feeding preferences is not unfounded. The observed differences in δ^{13}C values between raked and control treatments were also consistent with a shift in the feeding behaviour of *N. hombergii*, supporting the argument that *N. hombergii* were feeding closer to the base of the food chain.

The suspension- and surface deposit-feeding cockle, *Cerastoderma edule* showed no significant differences in δ^{15}N values with treatment, implying that cockles were feeding at the same trophic level across all treatments. However, δ^{13}C values in raked plots were significantly lower to those in controls. This implies that, although feeding at the same trophic level, the food sources being consumed and assimilated differed between the treatments. Cockles feed primarily on primary producers, taking algae from both the phytoplankton and the microphytobenthos (Sauriau & Kang 2000). A review of >870 algal δ^{13}C values by France (1995) showed that microphytobenthos (MBP) have higher mean δ^{13}C values than phytoplankton (δ^{13}C values of -17 ± 4‰ and -22 ± 3‰ respectively), with the difference attributed to a greater diffusion resistance for dissolved inorganic carbon within the benthos. The significantly lower δ^{13}C values in raked plots suggest that *C. edule* within these plots had diets containing a higher proportion of phytoplankton than those in control plots. This is likely to be related to the physical disturbance of surface sediments in raked plots resulting in a reduction in the MPB community. Benthic algal communities tend to re-establish over a timescale of days to weeks (Tolhurst *et al.* 2008) and hence would necessitate a short-term shift in *C. edule* feeding towards a diet dominated by phytoplankton. Human impacts within intertidal estuarine assemblages can therefore propagate into altered food web dynamics. Other studies have also found changes to feeding behaviour with human impacts, with evidence reported within microbial assemblages (*e.g.* Garstecki & Wickham 2001), subtidal systems (*e.g.* Ramsay *et al.* 1996) and modelled systems (*e.g.* Coll *et al.* 2008). To the author's knowledge however, this is the first study to show that anthropogenic disturbance leads to altered food web dynamics of biomass-dominant taxa through investigation of the foods assimilated by taxa.

The significant differences in isotope ratios between conspecifics under different treatments were observed only at Warton Sands, with the exception of the *Macoma balthica* data which showed significantly lower $\delta^{13}C$ values in raked plots independent of shore. *Nephtys hombergii* was only present at Warton Sands and hence could only be investigated at this shore. *Cerastoderma edule* however, was present within both study shores but only showed significant effects of treatment at Warton Sands, with no significant differences observed at Thurstaston. This implies that the degree of plasticity shown by taxa following a disturbance is to some degree likely to be dependent on the ecology of, and communities present within systems at a local scale. Therefore any changes to feeding behaviour following a disturbance that are displayed by taxa in one study area cannot necessarily be assumed to occur within different assemblages or even within the same assemblage within different seasons and/or within conspecifics at different stages of their life-cycles. This is because taxa can show plasticity over temporal scales, both seasonal (*e.g.* Esselink & Zwarts, 1989) and ontogenic (*e.g.* Sauriau & Kang 2000) as well as over spatial scales (*e.g.* Rönn *et al.* 1988; Esselink & Zwarts 1989; Vedel 1998).

In the present study, three of the eight macrofaunal taxa investigated showed significant changes in stable isotope ratios following experimental raking. That is, over one third of the studied taxa showed changes to their feeding behaviour following benthic disturbance. These three taxa represent a substantial proportion of the macrofaunal biomass of the study shores. *C. edule* represents ~63% of total faunal biomass at Thurstaston and ~82% at Warton Sands; *M. balthica* represents ~11% of the total faunal biomass at both Thurstaston and Warton Sands and *N. hombergii* represents ~1% of the total faunal biomass at Warton Sands (Chapters 2 & 3). These taxa therefore represent a substantial proportion of the organic carbon within the study systems and are likely to contribute substantially to ecosystem processes. Therefore, this change may have potentially substantial impacts on food web dynamics and hence the delivery of ecosystem functions and also affect the validity of the inferences that can be made using functional-trait approaches in the assessment of ecosystem functioning. For example, *C. edule* is the dominant contributor to macrofaunal biomass within the study shores and following physical disturbance to the benthos went from feeding largely from the benthos to a diet consisting of more material from the pelagos. This means that following disturbance, there has been a proportional increase to benthic-pelagic coupling within the system (albeit over the short term) and this has implications for the transfer and processing of energy and nutrients between the pelagos and the benthos (Loo & Rosenberg 1996). Despite this, a functional-trait analysis of the assemblage would reveal no change and hence no implications for benthic-pelagic coupling and ecological processes.

Analyses of the functional roles that taxa play are based on databases of species characteristics (*i.e.* grouping taxa by the functional role they play, or the building of species-by-trait matrices) and such studies treat these properties as fixed elements. While the use of fuzzy coding of trait preferences recognises the diversity of the potential roles of taxa within assemblages (*e.g.* Bremner *et al.* 2003a)**,** it is unable to specify how, over temporal and spatial scales, these roles are expressed. Thus, the findings of the current study confirm the cautions of others (*e.g.* Statzner *et al.* 1997; Haybach *et al.* 2004; Bremner 2008) in that the interpretation of any functional-trait analysis must be made carefully. Within the existing literature, there are often conflicting views with regards to how a specific taxon should be assigned to functional groups. A diversity of food preferences (*e.g.* Esselink & Zwarts 1989; Vedel 1998) or a variety of reproductive methods (*e.g.* Hannerz 1956; Gudmundsson 1985; Anger 1986) have been reported for individuals of many species and it is difficult to ascertain the strategy being employed by a taxon at any given time. It is important when assigning functional traits to taxa to consult as large a range of information sources as possible. Although online resources such as BIOTIC (available from the webpage http://www.marlin.ac.uk/biotic/) and AnAge (available at http://genomics.senescence.info/species/) are very useful as starting points to obtain trait information, it is always beneficial to look beyond these resources as the range and depth of species-trait information that these sources provide is often limited.

Functional-trait approaches do not provide a comprehensive view of ecosystem functioning; as yet no index is able to incorporate the necessary information. Such an approach would have to bring together the biological, physical and chemical aspects of an ecosystem at the micro-, meio-, macro- and mega- scales (Bremner 2008). Ecosystem functions themselves are, by definition, by-products of the activities and behaviour of organisms within a system (Naeem *et al.* 2002) and analysis of these activities through the investigation of the functional traits of taxa can provide an indication as to the potential functioning of the system.

It is evident therefore, that there is no single measure or index that can alone provide all the information required to assess and quantify ecosystem functioning. Functional trait analyses however are able to provide more information as to the functional processes within a system than taxonomic approaches. Nevertheless, due to the inherent plasticity within natural systems and the knowledge gaps in our current understanding of many ecological processes, it is essential that any approach utilised is supported by direct measurements and considered experimental design when investigating ecosystem functioning.

CHAPTER 5: DENSITY-DEPENDENT EFFECTS OF COCKLES ON NUTRIENT AVAILABILITY

5.1 Introduction

Due to the high densities at which they may be found, as well as the relatively large size of the organism, the cockle *Cerastoderma edule* (L.) is a dominant contributor to macrofaunal community biomass within many temperate tidal flat systems (*e.g.* Beukema 1991; Reise *et al.* 1994; Chapter 2) and is the target of a lucrative fishery. As dominant members of the macrofauna, cockles have the potential to exert a substantial influence over a number of ecosystem processes and functions. Reduced cockle abundance within an assemblage causes significant changes to the prevalence of filter feeding organisms within intertidal assemblages (Chapter 3). Filter feeders are important with regards to benthic-pelagic coupling (*e.g.* Riisgård 1991) and the linkages between the benthos and the pelagos play a vital role in the productivity of coastal systems (Cloern 1982). Bivalves have the potential to influence a number of ecological processes; studies on freshwater unionid mussels have shown that the rates of these processes scale linearly with the biomass of mussels present (Vaughn *et al.* 2004). Other studies have also shown that the density of taxa within a habitat can have a substantial influence on ecological processes within a system, either directly (*e.g.* through effects on excretion-derived nutrient availability, Vaughn *et al.* 2008), or through influences on the associated communities (Creed *et al.* 2009). It is apparent therefore, that the density of a taxon within a system, in particular a biomass-dominant taxon, has the potential to influence the ecological functions operating within that system. The impacts of these influences however, are difficult to predict *a priori*, due to the diversity of possible responses by other taxa within the system.

Many studies are based on laboratory and/or mesocosm manipulations of organism densities (*e.g.* Doering *et al.* 1987; Widdicombe *et al.* 2004; Karle *et al.* 2007) and this makes the application of the findings of these studies to ‘real-world’ scenarios difficult. This is because the processes and functions investigated are likely to be affected differently within natural systems and to different extents by all the organisms present from micro- to megafauna as well as by abiotic processes within a habitat. Therefore, limiting studies to very few taxa (*e.g.* Mermillod-Blondin *et al.* 2004) cannot provide a reliable picture as to how an individual or a small number of taxa may affect functioning within a ‘natural’ assemblage. Natural assemblages house multiple taxa under the influence of several abiotic processes (for example tidal activity and climatic factors) that are absent from, or only approximated within mesocosm investigations (*e.g.* Orvain *et al.* 2004; Gilbert *et al.* 2007; Bulling *et al* 2008.). Field manipulations are also associated with some inherent disadvantages. From practical and financial perspectives, the establishment of *in situ* experiments is labour-intensive and often costly potentially limiting the design of *in situ* experiments. From an ecological perspective, experiments carried out *in situ,* although providing a better impression as to the response of a 'natural' system to manipulations than mesocosm studies, inherently come with numerous potential 'hidden treatments', for example, interactions between organisms not manipulated as part of the experimental treatments (*sensu* Huston 1997). This makes it difficult to ascertain the causality of any observed effects. However, a main benefit of *in situ* investigations is that they provide an indicator as to the effects on a natural system and hence these 'hidden treatments' can actually be seen as favourable, in that they show how complex and dynamic systems may respond to manipulations, unlike those using stringently-controlled mesocosm investigations. It is preferable to strive to carry out *in situ* investigations in order to gain an accurate account of the effects of organism density on ecological processes.

The aim of the current study was to manipulate the density of the cockle *Cerastoderma edule in situ* within a temperate intertidal flat and to measure the impacts upon an ecological function within the system. The model function selected in the current study was the concentration of ecologically-important nutrients (ammonium, silicate, phosphate and combined nitrate and nitrite) within interstitial porewaters. Nutrient availability was selected as a proxy for ecosystem functioning as nutrient availability is often a limiting to primary productivity within coastal habitats (Oviatt *et al.* 1995; Downing *et al.* 1999).

Experiments investigating effects on nutrients often measure the flux rates of nutrients between the sediment and the water column (*e.g.* Nickell *et al.* 2003; Macreadie *et al.* 2006). Within the subtidal, benthic chambers are used, confining a volume of water from which subsamples may be removed over time, nutrient concentrations measured and flux rates inferred. Within such investigations, it is often necessary to sample over a period of many hours to days (*e.g.* Webb & Eyre 2004). Within the intertidal however, retaining a volume of water within a benthic chamber for sufficient time to measure significant changes is impractical due to the ebb and flow of tides. There is the potential for advances such as the development of microelectrodes and micro-benthic chambers to overcome this issue (*e.g.* Chapman & van den Berg 2005; Fang *et al.* 2007), meanwhile however, alternative measures must be used to quantify nutrients.

Nutrient availability, as indexed by porewater nutrient concentrations taken from a fixed depth, was selected as an appropriate measure. From an experimental perspective, changes to nutrient concentrations within *in situ* experimental systems are likely to occur over short timescales (*e.g.* Kobayashi *et al.* in press), making them suited to short-term investigations of ecological functioning. However, some precautions must be taken with this approach. Porewater samples can only provide a 'snapshot' measurement of nutrient concentrations, relevant only to the time that the sample was taken. Flux measurements on the other hand, provide an indication of the rate at which investigated nutrients are entering or leaving the system over time. Additionally, within sandy sediments, porewaters can only practically be removed from sediments at depths below the first few centimetres, as closer to the sediment surface, porewaters are rarely retained. This means that some caution must be taken before relating porewater concentrations at depth to processes occurring at the sediment surface, as the two may not necessarily relate. Despite such limitations however, the availability of nutrients within interstitial waters can be seen as an important driver of ecological functioning and thus the measurement of this property is justified, with influence for example, on benthic primary production (Underwood 2002).

It was expected that increasing densities of *Cerastoderma edule* would affect the concentration of nutrients within porewaters as bivalve densities have been shown to affect concentrations elsewhere. Marinelli & Williams (2003) for example, found increased loss of silicate from sediments with increasing density. Sandwell *et al.* (2009) showed that increasing densities of the bivalve *Austrovenus stutchburyi in situ* increased the loss of ammonium within a New Zealand tidal flat system. Bartoli *et al.* (2001) found that increasing densities of the clam *Tapes philippinarum* strongly enhanced the availability of silicate, phosphate and ammonium within the Po River delta, northern Italy. Although these studies measured nutrient fluxes and the current study is concerned with nutrient concentrations, it is likely that changes to nutrient fluxes would be reflected by changes to nutrient concentrations.

Increasing cockle density (and hence biomass) serves to increase the amount of metabolising tissue per unit area and thus increase the amount of excreted material entering the system. Therefore, it was predicted that the nutrient concentrations, in particular NH_4, a substantial component of bivalve excretions (*e.g.* Prins & Smaal, 1989; Gibbs *et al.* 2005), would show a positive relationship with cockle density.

5.2 Methods

5.2.1 Site description

In situ manipulations of cockle density and porewater sampling was carried out between 23 June and 2 July 2008 at Thurstaston within the Dee estuary in north-west England (Figure 2.1). Cockles are a dominant contributor to macrofaunal community biomass in this area, representing ~50% of faunal biomass (Chapter 2). Thurstaston has historically supported a cockle fishery; however, prior to the current study, fishing for *C. edule* had not taken place for 5 years (DEFRA 2008).

Preliminary sampling (Appendix A) showed that cockles at Thurstaston were present at a mean biomass of 402 ± 151.4 g m^{-2} with beds dominated by year one individuals. Smaller numbers of larger year class two cockles were also present (see Appendix A).

5.2.2 Experimental design and set up

Adult *Cerastoderma* were collected at low tide from the mid-shore at Thurstaston. Surface sediments were dug to a depth of ca. 15 cm and washed through a 10 mm mesh in the field. The cockles retained were used to seed the experimental plots during the same low tide.

A total of 56 plots measuring 32 × 32 cm (corresponding to an area of approximately 0.1 m^2) were arranged in a randomised block design. In order to minimise disturbance to experimental plots and to reduce the potential for *C. edule* to migrate between treatments, the border of each plot was separated from other plots by at least 2 metres. Initially, the surface sediments (to a depth of ca. 15 cm) were dug from the area encompassing each plot and the surrounding 10 cm. This sediment was carefully broken apart by hand in order to reduce damage to the sediment structure and *Cerastoderma* removed and sediment returned to the plots.

Each plot was assigned to one of fourteen cockle density treatments, containing 0, 1, 2, 3, 4, 5, 7, 10, 15, 20, 25, 30, 40 and 50 cockles per 0.1 m^2 plot and four replicates established for each treatment. Mean ambient adult (*i.e.* those retained on a 10 mm mesh sieve) cockle densities at Thurstaston at the time of sampling were 3.50 ± 1.18 individuals per 0.1 m^2. Cockles were randomly-distributed on the surface of experimental plots and gently pushed half-way into the sediment. Examination of plots on the following day suggested that the majority of cockles had burrowed into the sediment.

5.2.3 Nutrient collection and analysis

Experimental plots were sampled nine days after cockle density manipulation. This duration was chosen as it allowed as great a time as possible after the establishment of treatments whilst still being practical in terms of the time required for sampling during daylight hours. Porewater extractions commenced one hour prior to low tide. A disposable plastic pipette was placed into the sediment of each experimental plot to a depth of 5 cm and ~20 ml of porewater extracted by suction using sterilised syringes and stored on ice and in the dark until return to the laboratory, within 4 hours of collection. A depth of 5 cm represented the closest depth to the sediment surface from which a suitable volume of porewater could be retrieved. There was the potential for experimental treatments to lead to fluctuations in the depth of the redox potential discontinuity (RPD) and therefore to affect nutrient concentrations. However, as bioturbators of sediments (Flach 1996), cockles are likely to increase the depth to which oxygen-rich waters can penetrate (*sensu* Reise & Volkenborn 2004). Upon examination of the sediments at the end of the experiment, there was no indication that the RPD had travelled close to the 5 cm sampling depth and thus RPD depth was not considered likely to approach the sediment surface sufficiently to interfere with the investigation. Water samples were passed through pre-washed 0.2 µm filters in order to remove particulate material and then frozen (at -80 °C). Prior to analysis, samples were allowed to defrost in darkness at 4 °C for 24 hours and concentrations of ammonium, silicate, phosphate and nitrate and nitrite (summed and expressed as NO_x) were determined using a SEAL Analytical AutoAnalyzer 3 continuous flow auto-analyser.

After porewater samples had been extracted, the surface sediments of the experimental plots were dug to a depth of ca. 15 cm and the number of cockles within each plot enumerated. The variable degree to which cockles were retained within the plots produced a range of cockle densities within the experimental plots (2-47 cockles per plot); because of this, a fixed factor analysis of variance was deemed inappropriate for the analysis of porewater nutrient concentrations. As the density of cockles retained within the plots was distributed along a range of densities, regression analysis was deemed the most appropriate measure. One problem inherent in this approach however, is that it is impossible to ascertain the time period over which cockles had been present at the density observed on Day 9. However, Schuitema (as cited in Flach 1996) observed only limited movement of adult cockles, with an average of only 7% of cockles moving a distance of ~4 cm in one week. These findings are also supported by Coffen-Smout & Rees (1999) who also found only limited movement of cockles following disturbance. Similar behaviour has also been observed for other Veneroida (*e.g.* Sandwell *et al.* 2009). Therefore, after initial experimental manipulations, published research and personal observations suggest that cockle densities stabilised within a short period of time and then remained stable. Regression analysis was carried out with cockle density as the predictor variable and nutrient concentration as the response. Simple linear regression was used, unless a polynomial regression described the data significantly better (at $\alpha = 0.1$).

5.3 Results

5.3.1 Cockle retention

Nine days after manipulating adult cockle densities, experimental plots showed a variable degree of cockle retention (Figure 5.1). Even within a single treatment, some plots gained cockles and others lost cockles; for example the 30 cockles 0.1 m^{-2} treatment emerged with a range of cockles between 7 and 47 adults per 0.1 m^2 plot (mean = 27.3 ± 16.7 cockles m^{-2}; Figure 5.1) nine days after experimental manipulation. This within-treatment variation is likely to be due to a combination of the inefficient removal of cockles during experimental set-up, migration of cockles into and out of treatments and cockles failing to re-burrow into the sediment or feeding at the sediment surface and hence left prone to being washed away by tidal action or removed by predation. It is however, not the initial density manipulation treatments, but the cockle densities present nine days following experimental manipulation that were used to investigate impacts of density on nutrient availability. However, the final cockle densities did not differ significantly from the initial treatments (χ^2_{364} = 359.333, p = 0.559). Analyses can still be carried out, as cockle densities are likely to have stabilised within a short period of time (see Section 5.2.3), though some caution is required when interpreting these data.

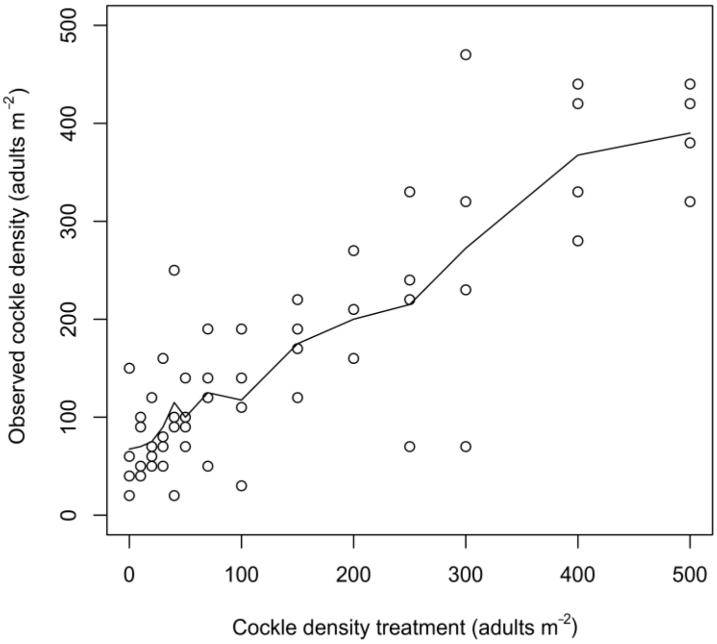


Figure 5.1. Retension of adult cockles within density-manipulation treatments. Cockle densities were manipulated within experimental plots on a tidal flat at Thurstaston, north-west England. Nine days after initial manipulation, cockle densities retained within plots were enumerated. Solid line represents the mean number of adult cockles retained in each treatment.

5.3.2 Cockle density-nutrient concentration relationship

All nutrient concentrations showed a high degree of variability in their response to cockle density (Figures 5.2-5.5). This variability made fitting appropriate models to the data difficult and this was reflected by the low observed r^2 values for all nutrients measured (ranging between -0.010 and 0.450). No significant influence of cockle densities was found with respect to the concentration of summed nitrate and nitrite (as NO_x) with differences in NO_x concentrations with increasing cockle density not differing significantly from zero (linear regression, r^2 = -0.010, $F_{1, 54}$ = 0.497, p = 0.484, Figure 5.2). A small but not significant positive relationship between cockle density and porewater silicate concentration was also found (linear regression, r^2 = 0.043, $F_{1, 54}$ = 3.483, p = 0.067, Figure 5.3).

Cockle density had a significant though small effect on the concentration of phosphate in sediment porewaters with concentrations increasing linearly with cockle density (linear regression, r^2 = 0.056, $F_{1, 54}$ = 4.251, p = 0.044, Figure 5.4). Though statistically significant, this relationship appears to be driven by a single point reflecting a high phosphate concentration and high cockle densities (1.134 mg l^{-1} phosphate with 440 cockles m^{-2}). Deletion of this point from the regression analysis results in no significant effect of cockle density on phosphate concentration (linear regression, r^2 = 0.003, $F_{1, 53}$ = 1.182, p = 0.282).

The greatest influence of cockle density related to the concentration of ammonium within sedimentary porewaters, with ammonium concentrations revealing a highly-significant cubic relationship with increasing cockle density (cubic regression, r^2 = 0.450, $F_{3, 52}$ = 15.990, p = <0.001, Figure 5.5). Ammonium concentrations remained relatively low and stable at cockle densities below ca. 300 adults m^{-2} plot, but increased substantially when cockle densities rose above this level.

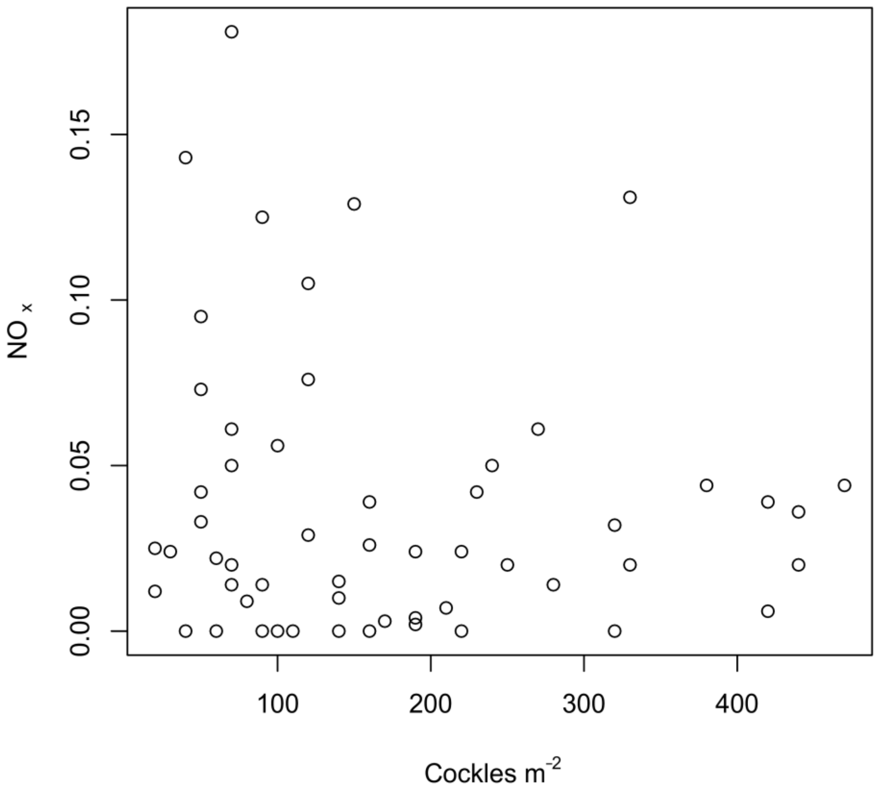


Figure 5.2. Relationship between summed porewater nitrate and nitrite concentration (mg l^{-1}) and cockle density within plots subjected to experimental cockle density manipulation. No significant effect of cockle density was observed, r^2 = -0.010, p = 0.497.

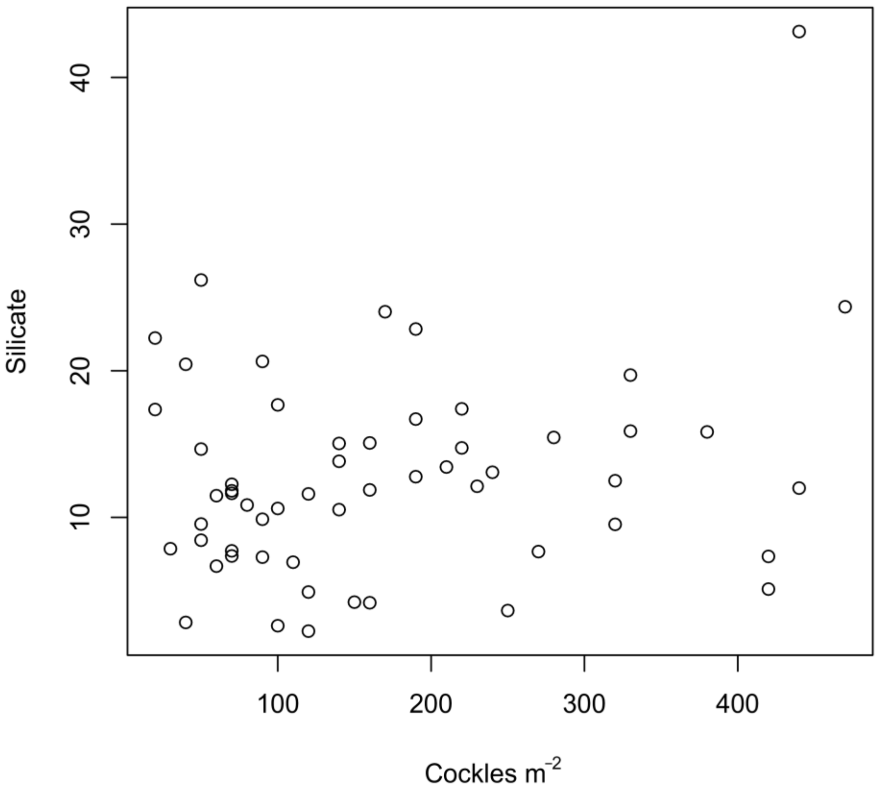


Figure 5.3. Relationship between porewater silicate concentration (mg l^{-1}) and cockle density within plots subjected to experimental cockle density manipulation. No significant effect of cockle density was observed, r^2 = 0.043, p = 0.067.

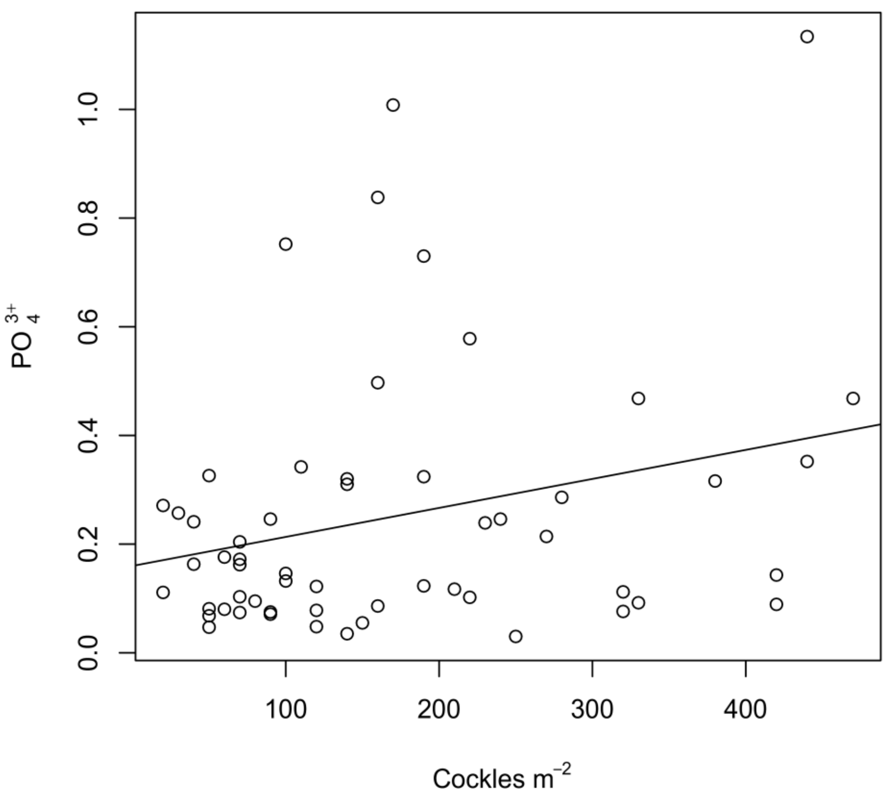


Figure 5.4. Relationship between porewater phosphate concentration (mg l^{-1}) and cockle density within plots subjected to experimental cockle density manipulation. The trend line represents linear regression prediction, with $r^2 = 0.056$, $p = 0.044$. The equation of the model is $y = 0.1598 + 0.0054x$, where x is cockle density (0.1 m^{-2}) and y is phosphate concentration (mg l^{-1}).

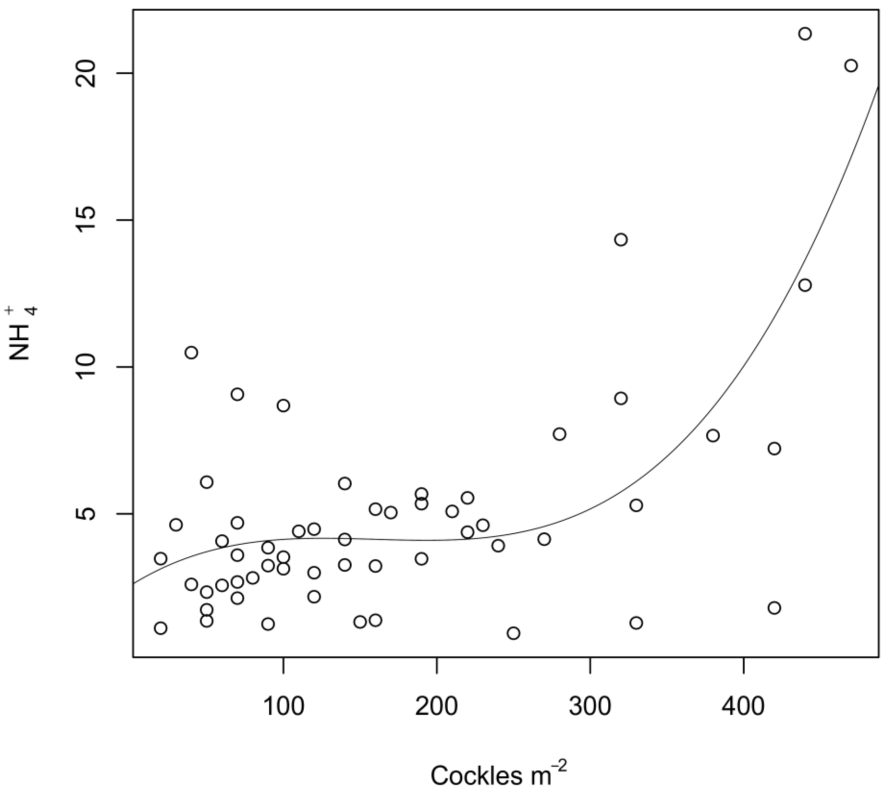


Figure 5.5. Relationship between porewater ammonium concentration (mg l^{-1}) and cockle density within plots subjected to experimental cockle density manipulation. The trend line represents cubic regression prediction, with $r^2 = 0.450$, $p < 0.001$. The equation of the model is $y = 5.0778 + 17.4734x + 10.8056x^2 + 5.5218x^3$, where x is cockle density (0.1 m^{-2}) and y is ammonium concentration (mg l^{-1}).

5.4 Discussion

Experimental manipulation of the density of the cockle *Cerastoderma edule* has revealed a significant impact of cockle density on the availability of nutrients within sedimentary porewaters, with potentially significant implications for benthic ecological functioning. The main role of cockles on the nutrient regeneration aspect of ecosystem functioning relates to the excretion of nitrogenous wastes.

Ammonium concentrations increased significantly with increasing cockle density, presumably at least in part due to excretion by the cockles. Cockles are therefore potentially important mediators of benthic primary production as ammonium is the preferred nitrogen source for marine primary producers. Nitrate and nitrite (hereafter, NO_x) and dissolved silicate however, showed no significant response to increasing cockle densities and although phosphate concentrations did show a significant increase, this was driven by a single and anomalous data point. Such an anomalous point is likely to be the result of sampling equipment (pipettes) removing porewaters from macrofaunal burrows, which have been shown to cause peaks in nutrient concentrations at depth (Mortimer *et al.* 1999a; 1999b). With this point removed, no significant effect of cockle density was observed on phosphate concentrations.

Two explanations might explain the observed relationship between cockle densities and nutrient concentrations in sedimentary porewaters. Firstly, there is the possibility that, of the measured nutrients, cockles only have an effect on ammonium concentrations, with no influence on silicate, phosphate or NO_x. This however is unlikely. Many studies have shown that suspension-feeding bivalves can have large impacts on the availability of a number of nutrients, including NO_x, silicate and phosphate, as well as on ammonium (*e.g.* Dame & Libes 1993; Fréchette & Bacher 1998; Dowd 2005). These impacts may be derived from the direct excretion of nutrients or the ejection of nutrient-rich biodeposits into the sediment, the physical activity of the bivalves, or the indirect mediation of other communities, from microbes to other macrofauna (Kautsky & Evans 1987; Huettel & Gust 1992; Herman *et al.* 1999; Marinelli *et al.* 2002; Hewitt *et al.* 2006). As biomass-dominant members of the macrofauna, it is likely therefore that cockles do directly contribute to, or in some way mediate, the supply of nutrients to interstitial porewaters. An alternative explanation for the observed response is that *Cerastoderma edule* do have an influence on the concentrations of other nutrients in the benthic environment, but that this effect is masked by other processes within, or components of, the system. These potential masking effects may be a result of abiotic and/or biotic factors.

An abiotic explanation behind the observed lack of a response to non-ammonium nutrients is the physical activity of cockles. The 'shaking' activity of cockles may loosen and destabilise surface sediments (Flach 1996; Coffen-Smout & Rees 1999) and this may potentially alter the movement of particles and hence porewaters within the sediment. In addition, the physical activity of cockles will result in disturbance to the sediment surface, making it topographically uneven. Huettel *et al.* (1996) found that a small degree of unevenness in sediment topography (small mounds, z = 2.5 cm) enhanced both the influx of surface water into sediments and the efflux of porewater from sediments to a depth of 5.5 cm. Such disturbances could therefore enhance the exchange of dissolved nutrients between the sediment and the water column and hence mask the impacts of the increased nutrient load entering the sediments with increasing cockle densities. Similar effects have been observed elsewhere; Thrush *et al.* (2006) for example, found that at decreased densities of the bivalve *Austrovenus*, surface sediments were less disturbed with a corresponding reduction in the degree of porewater exchange.

In addition to potentially destabilising the sediment structure, with repercussions for the flux of porewaters across the sediment-water interface, there is also the potential for physical activities to enhance the depth to which oxygen-rich waters are able to penetrate into the sediment. This can influence the availability of dissolved phosphates within the sediment. In the presence of oxygen, phosphate ions readily adsorb onto the surfaces of clay particles and ferric minerals, hence limiting their availability to the microphytobenthos (Hayes 2002; Sommer *et al.* 2003). The River Dee catchment is iron-rich (Ixer & Vaughan 1982) and thus the oxygenation of sediments in this iron-rich environment may account for the lack of a response to phosphate concentrations with increasing cockle densities. Enhanced oxygenation of surface sediments therefore may explain the observed relationship with phosphate. It does not however, account for the similar (*i.e.* non-significant) relationship with silicate. Additionally, the high silicate concentrations commonly found within benthic habitats can result in the dissolution of adsorbed phosphates and their release into interstitial waters (Tallberg & Koski-Vähälä 2001); therefore, an increase in phosphate concentration with increasing cockle density would still be expected. This suggests that another explanation is required to explain the lack of a response to phosphate and silicate concentrations, but a significant response to ammonium concentrations with increasing cockle density. One such explanation is through a biotic pathway. Prior to this, some consideration must be given to the nature of the response of ammonium to increasing cockle densities.

A positive cubic relationship with ammonium was observed with increasing cockle density (and logically, biomass), with ammonium concentrations increasing significantly as adult cockle densities exceeded ca. 300 individuals m^{-2}. At substantially-increased cockle abundances/biomasses, the input of faunal excretion and egestion material increases and this is likely to be the source of the increased ammonium concentrations observed at higher cockle densities. When adult cockle densities were in the range of ca. 100-300 adults m^{-2} however, ammonium concentrations showed little change with increasing cockle abundances, remaining largely constant, suggesting some degree of buffering with regards to ammonium concentrations within porewaters.

Swanberg (1991) investigated the influence of adult cockles on the fluxes of nutrients within experimental laboratory mesocosms and found that the presence of cockles increased ammonium concentrations at a rate of approximately 0.08 mg l^{-1} $cockle^{-1}$ d^{-1}. This rate is likely to vary between individuals and cannot be assumed to be the same across cockle populations in different geographic locations, or even in different laboratories and mesocosms; it does however provide a guide to the magnitude of the ammonium concentrations that may be expected to be present within the system with increasing cockle density. Comparison between estimated ‘expected’ ammonium concentrations with increasing cockle density, based on the findings of Swanberg (1991) (using a linear response model as observed by Sandwell *et al.* 2009) and the ammonium-cockle density model based on the findings of the current study, shows that the observed ammonium concentrations at moderate densities (ca. 100-300 adult cockles m^{-2}) were substantially less than those that might be expected (Figure 5.6).

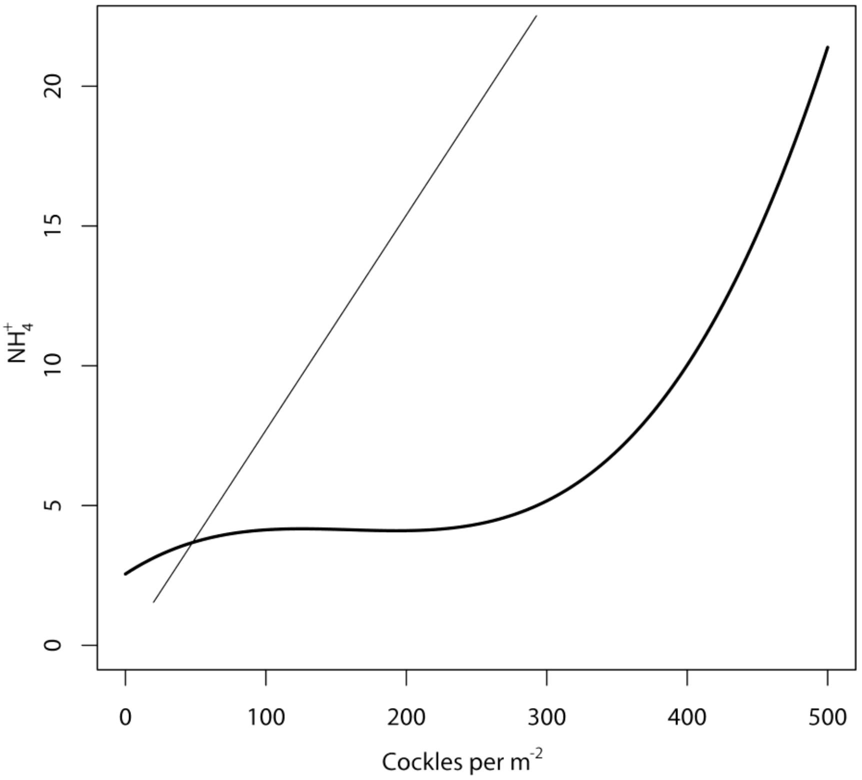


Figure 5.6. Relationship between cockle density (m^{-2}) and the concentration of ammonium (mg l^{-1}) within interstitial porewaters. Lines represent the cubic model obtained within the current study (bold) and the linear approximation obtained from Swanberg (1991).

A possible sink for this ‘missing’ ammonium is the microphytobenthos (MPB). Benthic algae rapidly remove substantial quantities of dissolved nutrients, doing so more readily than planktonic primary producers (Granéli & Sundbäck 1985). There is the potential therefore for the observed buffering of ammonium concentrations with increasing cockle density to be a result of the uptake of ammonium by the MPB community. How then, does this explain the observed rapid increase in ammonium concentrations when densities exceed ca. 300 adult cockles m^{-2}? This may be explained by the limitation of other nutrients within the system.

The discussion of nutrient limitation in this study requires some clarification. In particular is the issue of whether nutrient concentrations measured within porewaters at 5 cm depth can realistically be discussed in relation to MPB communities present within the top few centimetres of sediment. This is more likely to be problematic within compacted and fine-grained sediments, where the majority of chlorophyll is concentrated to the 2 mm or so closest to the surface (de Brouwer & Stal 2001; Kelly *et al.* 2001). Within more sandy sediments however, such as those examined in the current study, chlorophyll is evenly-distributed with sediment depth (Perkins *et al.* 2003; Kromkamp & Forster 2006); therefore, there is the potential for nutrients present at 5 cm to influence MPB communities.

MPB communities tend to be diatom-dominated (Egge & Aksnes 1992). This dominance however, is dependent on the availability of silicate required for the construction of protective frustules. In the pelagos, silicate often becomes a major limiting factor to diatom community growth (Raguenueau *et al.* 2002), though within the benthos, dissolved silicate is generally abundant and thus is not considered to be limiting (Hackney *et al.* 2000; Leynaert *et al.* 2009). Phosphate however, is often considered limiting to primary production within the benthos, due to the relative scarcity of the nutrient compared with other ions, as well as the ready adsorption of the ion on to iron and clay in the presence of oxygen (Lever & Valiela 2005). It is phosphate limitation that may explain the observed buffering of ammonium concentrations with increasing cockle density.

The mean atomic ratios of nitrogen, silicon and phosphorus observed in the current study were in the region of 30:81:1. The ratios of N:Si:P were much in excess of the Redfield-Brzezinski ratios of 16:15:1 (Redfield 1958; Brzezinski 1985) as well as the MPB-adjusted N:P ratio of 17:1 (Hillebrand & Sommer 1999). This suggests that phosphate is limiting to primary production within the benthos. According to Liebig’s law of the minimum (de Baar 1994), when one resource (*i.e.* one nutrient) becomes scarce relative to the demand for that resource, even if other resources are abundant, then primary producer growth becomes limited. In this case, it is likely that phosphates, as the most scarce nutrients, are limiting to MPB growth and hence the ability of the MBP to continue to uptake ammonium is compromised. This therefore may explain the ‘buffering’ of ammonium with increasing cockle densities until densities exceed ca. 300 adult cockles m^{-2}. The observed values of silicate lend some support to this. Suspension feeders are typically associated with an increase in silicate concentrations with increasing density (*e.g.* Magni & Montani 2006). In the current study however, such an increase was not observed, with silicate concentrations showing no significant response to increasing cockle densities. If MPB are rapidly removing the additional silicate derived from cockles, then this may explain the lack of a response.

The Redfield-Brzezinski ratio (atomic ratios of C:N:Si:P = 106:16:15:1) suggests that the nutrient concentrations measured should support approximately 26.6 mg l^{-1} of carbon within algal tissues. Organic carbon makes up approximately one quarter of the total biomass of benthic diatoms (McIntire & Wulff 1969) and hence this corresponds to a MPB biomass of approximately $26.6 \times 4 \approx 100$ mg l^{-1} being supported by the nutrient concentrations available within the porewater. This can be compared to observations of the MPB biomass supported within the study area. Mean surface chlorophyll *a* measurements in undisturbed plots in June 2008 (Chapter 3) gave values of 11.249 ± 1.867 µg chlorophyll *a* g sediment^{-1}. The studies of Banse (1977) found C:Chl*a* ratios in healthy microalgae to range between 10 and 100. de Jonge (1980) found a C:Chl*a* ratio of 40 g $C_{organic}$ g Chl*a*$^{-1}$ within estuarine MPB. This suggests in the region of $11.249 \times 4 \approx 450$ µg $C_{organic}$ g sediment^{-1} within the MPB community at the site. As approximately 0.25 of diatom biomass is consists of organic carbon, this corresponds to a total MPB biomass of approximately $4 \text{ x } 450 \approx 2$ g kg^{-1} sediment (following McIntire & Wulff 1969).

The inferred biomass of MPB present within the benthos (ca. 2 g kg sediment^{-1}) is greater than that seemingly able to be supported by the nutrient concentrations available within the porewater (ca. 0.1 g kg^{-3}) by an order of magnitude. A number of explanations may account for this discrepancy. Firstly, the values used for the calculation of the algal biomass values are necessarily vague. The value of the C:Chl*a* ratio alone may range between 10-100 (Banse 1977). Since the value used presently was 40, reflecting the findings of de Jonge (1980) for microphytobenthos within the Ems estuary on the Dutch-German border, this value is potentially erroneous by a factor of two to four. The minimum C:Chl*a* ratio found in the microphytobenthos by de Jonge (1980) was 10.2. Using this value would correspond to a total MPB biomass much closer to that estimated to be supported by the nutrients available, a MPB biomass of 0.5 g kg^{-1} sediment. Another source of error is that mean values were used when estimating the proportion of MPB biomass made up by organic carbon. These values were used only as a guide and are likely to be subject to great variability across temporal or spatial scales and with differing MPB species compositions (*e.g.* McIntire & Phinney 1965; McIntire 1968). Finally, these calculations are based on the assumption that sedimentary porewaters are the only nutrient source that MPB utilise within the benthic environment. However, MPB are known to remove a substantial quantity of nutrients from the water column itself (Tobias *et al.* 2003; Andersson & Brunberg 2006) and so do not depend exclusively on the availability of benthic nutrients.

It may be argued therefore that the discrepancies between the inferred MPB biomass present and that able to be supported by the available nutrients are not so large as to be explained by anything other than inaccurate parameter estimations. MPB uptake therefore, does represent a viable sink for the increased influx of phosphates and silicates derived from increased cockle densities. Other studies also support this. The addition of cockles can be viewed as a manipulative experiment, effectively adding nutrients to the sediment. Increased nutrient concentrations have been shown to stimulate the growth of (and hence the nutrient uptake by) benthic microalgae (Posey *et al.* 1995; Morris & Keough 2003). However, such responses have not been observed universally; O'Brien *et al.* (2009) for example found minimal only impacts of increased nutrients on primary producer biomass, attributing this to localised sedimentary, light or hydrological parameters.

MPB therefore, are a potentially viable sink for an increased nutrient load arising from enhanced cockle densities. Future work should incorporate studies of the response of the MPB community to increased cockle density. However, although chlorophyll *a* observations provide a reliable indicator as to the algal biomass present, the relationship between algal biomass and chlorophyll *a* content may be rather variable (de Jonge & Colijn 1994). Additionally, knowledge of MPB biomass alone does not necessarily indicate the quality of the algal community, in terms of, for example the lipid content of algal cells and these measures may differ significantly, with potential consequences for the functional roles played by the MPB assemblage (Rossi & Lardicci 2002).

There is the potential therefore for cockles to exert a control on algal growth within the benthos, with this influence potentially extending to pelagic algae if cockles are present at sufficient densities (*c.f.* Prins & Smaal 1994). Increased cockle densities have significant effects on the concentration of ammonium within interstitial waters, with concentrations increasing substantially at high cockle densities. Ammonium is an important resource for the functioning of ecosystems, being the preferred nitrogen source for uptake by many microphytes, including the majority of diatoms that represent a major component of the benthic primary producer community (see Dortch 1990 and references therein). However, no relationship between the other nutrients investigated with increasing cockle density was observed.

It is difficult to fully ascertain the cause, or causes behind the lack of a response in the non-ammonium nutrients to increasing cockle density. Past work, largely based within laboratory mesocosms suggests that phosphate and silicate concentrations would also have been affected by the presence of cockles or other suspension feeders (Doering *et al* 1987; Swanberg 1991; Peterson & Heck 1999). This is one of the challenges of *in situ* field investigations: there are always likely to be a number of inherent 'hidden treatments' (*sensu* Huston 1997) present that may support or contradict the effects of the known experimental treatments. This means that the interpretation of such investigations must be taken with caution. Contrary to mesocosm-based studies however, *in situ* studies provide the best insights into the impacts of experimental treatments on a 'natural' system. Therefore, although controlled laboratory-based investigations suggest that a greater biomass of cockles should result in greater concentrations of ammonium, silicate and phosphate entering interstitial porewaters, the current field-study shows that these presumptions do not necessarily translate within a natural ecosystem. Within such a system, physical factors, such as sediment porosity, granulometry and hydrodynamics, as well as ecological factors related to the microphytobenthos and the co-habiting community can exert an influence to mask these responses.

CHAPTER 6: THIRTY YEARS OF COCKLE STOCK VARIATION: THE ECOLOGICAL CONTROLS WITHIN MORECAMBE BAY

6.1 Introduction

Populations of marine benthic taxa are known to fluctuate over time (*e.g.* Beukema *et al.* 1993; Paugy *et al.* 1999) including the abundance the cockle, *Cerastoderma edule* (Williams *et al.* 2004). A range of factors are likely to influence the strength of cockle populations. Some of these factors are likely to have a greater influence on adult cockles whereas others are likely to be more influential on juvenile cockle spat populations. The drivers that are responsible for fluctuations in cockle populations are of particular interest to both ecologists and fisheries managers and these drivers may consist of abiotic and/or biotic factors.

6.1.1 Abiotic factors

Cockle populations have shown statistically significant links with climatic conditions (Coosen *et al.* 1994). Cockles are found across a wide geographic range, with populations found in habitats ranging from subtropical Africa in the south to Scandinavia in the north (Jonsson & Andre 1992; Gam *et al.* 2008). Cockles therefore are able to survive and reproduce over a broad range of temperature regimes. However, cockles have been shown to respond adversely to uncharacteristically severe temperatures. For example, the fecundity of cockle populations in the Wadden Sea was found to be temperature-dependent, with higher numbers of smaller eggs produced at higher temperatures; these smaller eggs contained a lower proportion of lipids, potentially reducing the survivability of larvae (Honkoop & van der Meer 1998). Severe temperatures have also been associated with significant cockle mortality events. Such events may be associated with both uncharacteristically hot (Hancock 1971) and cold (Beukema *et al.* 2002) temperatures. This suggests therefore that cockles are likely to be susceptible to uncharacteristic extremes of temperature.

Cerastoderma edule, lives within estuarine intertidal habitats. As such, individuals are subjected to fluctuating degrees of salinity. This is a further source of stress that may influence the strength of cockle populations. However, cockles are able to tolerate a wide range of salinities. Cockles within the Baltic Sea tolerate salinities in the region of 10 (Brock 1980) though Ysebaert *et al.* (2002) predicted optimum salinity for cockles in the Schelde estuary in the region of 25. Therefore, the fluctuations of salinity within estuarine regions are generally not substantial enough to produce mass mortality of cockles alone (*e.g.* Kater *et al.* 2006). However, when other factors are involved, such as temperature extremes, cockles may display a reduced tolerance to salinity extremes (Ibing & Theede 1975).

Within the estuarine intertidal, sediment granulometries are also susceptible to change (Frostick & McCave 1979; Anderson 1983) and different sediment granulometry compositions house a different range of taxa (Jones & Frid 2009). Cockles have a limited range of preferred sediment granulometries, with preferred grain size diameter in the region of 0.1 mm, with extremes of granulometry resulting in substantially-altered burrowing times (Alexander *et al.* 1993). However, Alexander *et al.* (1993) found that, despite the limited 'preferred' range, cockles were able to burrow into a wide range of granulometries, and Huxham & Richards (2003) suggested that hydrodynamics rather than granulometry *per se* is likely to be more influential to cockle distribution

Cockles feed at the sediment surface during tidal inundation. As a result, there is also the potential for cockles to be displaced by tidal action. However, Armonies (1994) showed that within the Wadden Sea, tidal activity was only of minor importance for distributing cockles across a small bay (ca. 1 km^2) with high tidal velocity.

6.1.2 Biotic factors

A number of biotic factors are also likely to influence the population strength of taxa. These pressures can be broadly divided into two categories: intra- and interspecific factors.

Intraspecific competition occurs when individuals within a species compete for some resource. In the intertidal, such resources are living space and food resources, with the competition for these resources increasing with increasing density of individuals. Density-dependence within cockle populations has received a good deal of attention. A primary reason behind density-dependent mortality in cockles appears to be related to recruitment of juveniles. The recruitment of cockle spat into cockle beds has been shown to be negatively-correlated to adult cockle densities (*e.g.* Andre & Rosenburg 1991). This negative correlation is related to both the digestion of planktonic cockle larvae by adult cockles (Kristensen 1957; Andre *et al.* 1993), and the direct competition between settled cockles for food and space (Hancock 1973). As a result, large recruitments of cockles have been observed after severe winters, with recruitment increased following mass mortalities of adults (Beukema 1985).

Predation is also a major factor affecting *C. edule*. Wetland birds such as the oystercatcher *Haematopus ostralegus* gather on UK shores in substantial numbers over winter and spring months (Dare & Mercer 1973). A substantial portion of the diet of oystercatchers consists of *C. edule,* particularly over winter when oystercatchers have been found to feed exclusively on cockles (Dare & Mercer 1973). Birds therefore have the potential to impact upon the strength of cockle populations within the intertidal (Norris *et al.* 1998) where they feed primarily on larger cockles (Zwarts *et al.* 1996) though smaller cockles may be consumed if availability necessitates (Ens *et al.* 1996). Birds however are not the only predatory taxa that may potentially decimate cockle populations. Epibenthic predators, for example demersal fish such as plaice *Pleuronectes platessa* and the shrimp *Crangon crangon* are also potentially-important consumers of cockles, able to remove in excess of 90% of annual cockle production, feeding preferentially on the smaller juvenile cockles (Oh *et al.* 2001; Pihl 1985; Beukema & Dekker 2005). Cockles are also subject to intensive predation by humans, with UK cockle fisheries landing over 11000 tonnes of cockles in 2007, with landings valued in excess of £7 million (Shellfish News 2008).

6.1.3 Morecambe Bay cockle fishery and current study

In the UK, Sea Fisheries Committees oversee the inshore fisheries within six miles of the coast. This therefore includes all of the UK's cockle fisheries. The North West and North Wales Sea Fisheries Committee (NWNWSFC) has overseen the cockle fisheries within Morecambe Bay since 1890. This agency publishes up to four reports per year, detailing the status of active fisheries throughout the region, including estimates of the status of commercially-fished stocks, fishery landings and other related information, for example extreme weather events. These reports contain both numerical and descriptive information. The quality of this information varies between fishery target species and by year, tending to reflect the then current fishing trends and concerns in the region. Only seldom do these reports contain any numerical estimate of the total abundance or biomass of taxa within the region.

However, the form of the descriptions for each fishery is given in sufficiently-standard and consistent form to allow estimations to be made as to the relative strength of the stocks of a number of taxa and for these to be compared between years. These reports contain information for cockle and cockle spat populations, demersal fish and shrimp fisheries.

Morecambe Bay in the north-west of England (Figure 2.1) is also home to oystercatchers occurring in numbers deemed to be of 'international importance', with in excess of 65 000 individuals, representing approximately 6.5% of the European population of the species (Ramsar 2008). Oystercatchers therefore have the potential to directly impact on the strength of cockle stocks within Morecambe Bay.

As well as indications of biotic components that may affect cockle populations, long data sets of meteorological factors are also recorded at a number of stations throughout the UK under the control of the Met Office (formerly Meteorological Office). The measured factors include records of the monthly temperature minima and maxima and hence can provide an accurate measure of the temperature extremes that taxa in the region are subjected to over time.

The aim of the current study was to investigate the fluctuations of cockle and cockle spat populations within Morecambe Bay over a thirty year period in relation to possible environmental and biological drivers. The source of much of the data examined is semi-quantitative and thus the findings must be viewed as indicators of future areas of research, rather than a definitive indication of the drivers of cockle and cockle spat population fluctuations. Despite the semi-quantitative nature of the data, such approaches to time series analyses have been used successfully in other investigations, for example examining the fluctuations in populations of the shrimp *Crangon crangon* (Duran 1997) and in terrestrial investigations of lemmings (Blomqvist *et al.* 2002) and these approaches have provided useful insights into the long-term fluctuations of the study-taxa.

6.2 Methods

6.2.1 Species data 1: Fishery stocks

Quarterly reports of the North West & North Wales Sea Fisheries Committee (NWNWSFC) between 1974 and 2004 inclusive were systematically searched for information regarding the strength of the stocks of a number of taxa. Information was sufficient for, and hence estimates could be made for, the cockle *Cerastoderma edule* and its spat; demersal fish, consisting largely of plaice *Pleuronectes platessa* and flounder *Platichthys flesus*; and the brown shrimp *Crangon crangon* within Morecambe Bay. Data were taken from the March NWNWSFC reports, which provide information relating to the winter fisheries, when effort and landings are at their peak.

Stock estimates were made on a five-point scale ranging from 1 = scarce to 5 = highly-abundant, using information from the following measures:

1. Account was made of the fishery landings of each species or species group. Often, the NWNWSFC reports provide numerical data of landings, for example the September 1976 report stated: “There have been steady but low landings of cockles from scattered patches of cockles in Morecambe Bay, mainly off Flookburgh […] the figure of around 200 cwt. [ca. 10 tonnes] from this area was about the same as for the third quarter of last year”.

2. Knowledge of fishery landings alone is not sufficient to gain an accurate measure of stock vitality. This is because landings are intrinsically-dependent on the fishing effort being made. Fishing effort is the product of a range of conditions, including biological, environmental, economic and sociological factors. Some indication of the fishing effort made could be gleaned from the NWNWSFC reports, in terms of the numbers of full and part time fishermen and their distribution of effort in terms of the numbers of fishermen working on boats or on foot/on tractors, for example the March 1979 report states that “Between six to ten fishermen were engaged in this fishery […] taking six to eight bags each per tide”. Such information did not provide enough information to allow catch per unit effort to be accurately

calculated, but was sufficient to provide an indication as to the fishing effort involved for each stock within Morecambe Bay.

3. For the cockle, *Cerastoderma edule*, information was often provided as to the brood stock strength within the area. For example, the September 1982 report states that cockle beds were "densely populated with small cockles […] present in greater numbers than have been observed for thirty years". This information was taken into account when estimating cockle spat strength.

4. NWNWSFC reports tended to give some general reports of the stock states, for example the October 1988 report stated that shrimp fishermen regarded the quarter as being "one of the best for several years". This information was useful in conjunction with the other sources in order to gain an indication of the relative abundances over a number of years.

The descriptions of the states of each fishery were reported in a suitably standard and consistent form to allow their use in assigning a score to each as a measure of the relative health of the fishery within Morecambe Bay. Information within the NWNWSFC reports between 1974 and 2004 were assigned scores based on the report descriptions (Table 6.1) as an estimate of the populations of adult (*i.e.* fishery-target, >2.5 cm maximum shell width) cockles, cockle spat, shrimp and demersal fish, with each population scored independently.

Table 6.1. Criteria used to estimate the health of stocks based on the NWNWSFC fishery assessment reports. Reports were systematically searched for information on a number of taxa and the scoring criteria used to provide an index of the population strength of each taxon.

Index	Scoring criteria
1	No commercial fishery, mention of 'scarcity' of the taxon
2	Light, but low landings, 'thinly populated' grounds, low fisher returns resulting in reduced effort
3	Commercially-viable fishery with mention of 'reasonable landings'
4	Good returns of 'high quality' catches from 'well-populated' grounds, attracting an increased effort
5	As 4, but even better landings of 'high quality' organisms

6.2.2 Species data 2: Birds

An indicator of the intensity of bird predation on the cockle beds was inferred using information on the populations of the oystercatcher *Haematopus ostralegus* in

England between 1974 and 2004. Information is gathered by the British Trust for Ornithology (BTO) as part of their Wetlands Bird Survey (WeBS) which gathers regional count data for a number of wetland bird species and published online (see www.bto.org/webs). These values are provided as an index derived from a general additive model (Marchant *et al.* 1990). For each year between 1974 and 2004, these data were assigned a score from 1 to 4 depending upon whether index values fell into the first, second, third or fourth quartile (from lowest to highest abundance). At the time of writing, oystercatcher abundance data were only available for all of England, however additional information was often provided in the NWNWSFC reports on oystercatcher abundances within Morecambe Bay itself. The September 1978 report for example reports “large numbers of oystercatchers feeding on [cockle] spat in September”. When this information differed from the score obtained using the BTO index values for the whole of England, the NWNWSFC reports were given priority. Comparison of the oystercatcher indices for the whole of England with those combined with information from the NWNWSFC reports showed very little difference (< 0.7%) between the two in terms of the ability of the data to explain variations in the cockle abundance data.

6.2.3 Environmental data

To investigate the vulnerability of Morecambe Bay cockles to extremes of weather, long term data was retrieved from the Met Office (available at www.metoffice.gov.uk). Data was gathered from the nearest weather station to Morecambe Bay at Newton Rigg in Cumbria. Data for the minimum and maximum temperatures (°C) of the first quarter (January to March) of each year from 1974 to 2004 provided an indication as to the thermal stress that cockle populations had been subjected to.

6.2.3 Statistical analyses

Populations of marine fauna often show some degree of cyclicity over time (*e.g.* Gröger & Rumohr 2006; Frid *et al.* 2009a). To examine the degree of cyclicity in the population estimates of adult cockles and cockle spats, autocorrelation and partial autocorrelation functions (ACF’s and PACF’s respectively) were calculated as outlined by Royama (1992) and Crawley (2007). Autocorrelation functions are measures of the correlation between population sizes at time t and a later time $t + j$

where j = lag. Autocorrelation analyses are influenced by population values j units in the past, as well as j-1, j-2… j-n; that is, ACF's are influenced by all the intermediate lag times and not just the lag time under investigation. PACF's control for the influence of populations at intervening lag times and measures the covariance of the population sizes separated by the lag and are useful in the interpretation of broad patterns in time-series data (Turchin 1990).

As the population estimates consist of categorical data, the suitability of the use of autocorrelation analyses was inferred from Spearman's nonparametric correlation coefficients which were calculated for each autocorrelation analysis. No difference between the statistical significance for the autocorrelation calculations and the Spearman's rank correlation coefficients were detected in any of these analyses and hence ACF investigations were carried out and correlograms interpreted with reference to Royama (1992), with spikes outside the Bartlett bounds considered statistically significant ($\alpha = 0.05$).

The extent of any changes to cockle population estimates were examined in relation to extreme values of the potential explanatory variables. This is because populations tend to show some degree of resilience or buffering to changes, either physiological (*e.g.* Theede *et al.* 1969) or ecological (*e.g.* Reusch 1998) in nature. This implies that the responses of a system to increasing stress are likely to be minor, until the strength or intensity of the stressor goes beyond some 'tipping' point which exceeds the buffering ability of the population, after which the population displays a substantial change (*sensu* functional redundancy, Walker 1992). To investigate this, extreme values of population indices and temperature values were explored alongside changes to cockle and spat index values. An extreme change was defined as those years in which index values changed by a value of ± 2 or more.

To explore more subtle drivers and the potential roles of multiple drivers of cockle and spat population indices, a number of models (or hypotheses) were examined using Akaike's Information Criteria (AIC) as described by Burnham & Anderson (2001). A total of eight linear regression models were examined to determine the potential drivers of adult cockle population strength. Eleven linear models were assessed for cockle spat data. Cockle and cockle spat indices were tested against minimum and maximum temperatures and indices of demersal fish and oystercatcher abundance, as well as the interactions between each of the biotic and abiotic factors. In addition, the cockle spat indices were also investigated as a function of shrimp abundances and interactions between shrimp population indices and each of the abiotic factors were also investigated. This is because unlike adults, juvenile cockles are susceptible to predation by shrimp (Beukema & Dekker 2005). The models tested were selected *a priori* and were based on the consideration of rational ecological theory and hence serves to avoid the problems associated with ‘data dredging’ (Anderson *et al.* 2001). The difference between each model and the best-fitting model (Δ_i) was calculated and analysed following the guidance of Burnham & Anderson (2001), using:

$$\Delta_i = \mathrm{AIC}_i - \mathrm{minAIC}$$

with models having $\Delta_i \leq 2$ having substantial support (or evidence), models with $4 \leq \Delta_i \leq 7$ have much less support and $\Delta_i > 10$ being effectively unsupported by the data. The ‘best’ fitting models were determined through calculation and comparisons of the relative Akaike weights (w_i) of the models (Burnham & Anderson 2001) using:

$$w_i = \exp(-\Delta_i/2)/\Sigma\exp(-\Delta_i/2)$$

which provides the weight of evidence of one particular model compared to the other models, with all w_i values summing to one.

6.3 Results

6.3.1 Cyclicity of cockle populations

Population estimates of adult cockles and cockle spat showed much variability over time (Figure 6.1). Autocorrelation (ACF) and partial autocorrelation function (PACF) calculations revealed little significant cyclicity of cockle or cockle spat populations. Adult cockle population estimates showed the suggestion of a damped oscillatory pattern of ACF values, displaying some degree of symmetry with an oscillation approximating to 25 years (Figure 6.2); however, the short length of the time series (30 years) in comparison to the length of the supposed cycle (25 years) means that the assertion of such a cycle cannot be made robustly. Significant partial autocorrelations were only evident within the adult cockle data with a lag of 1 year

(Figure 6.2) and hence adult cockle abundance estimates were only significantly correlated with those from the previous year.

Correlations of juvenile cockle populations between different years did not differ significantly from the assumption of white noise. As with adult cockles, there was some evidence however of a degree of cyclicity in ACF values over time, with the suggestion of an oscillatory cycle apparent over a timescale of approximately 12 years in cockle spat population dynamics (Figure 6.3).

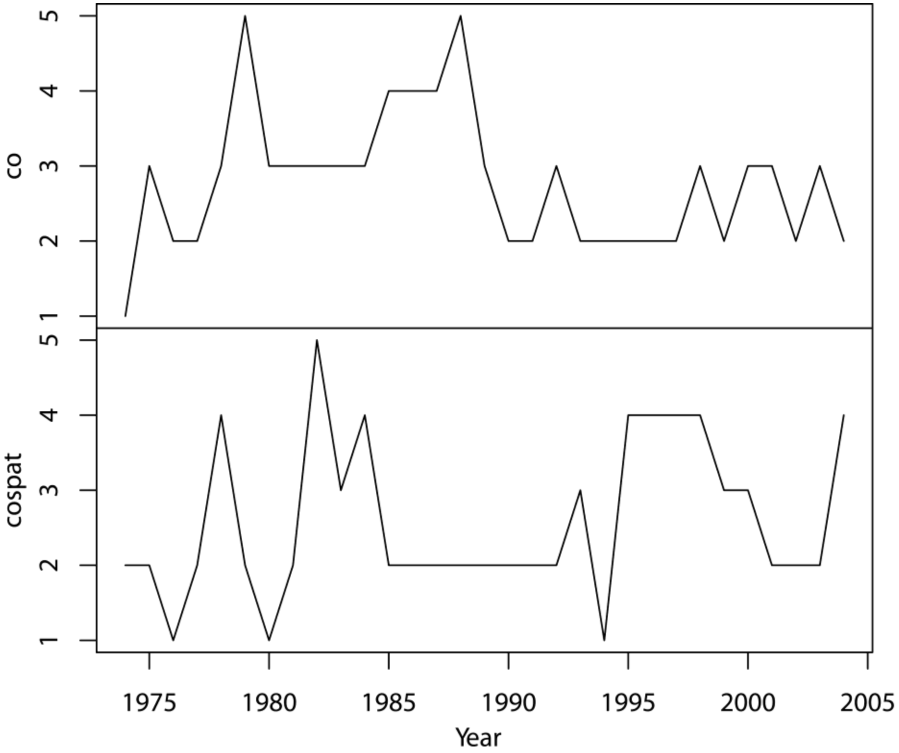


Figure 6.1. Indices of adult cockle (co) and cockle spat (cospat) population abundances over a thirty year time period within Morecambe Bay. Indices were ascertained from information within the reports of the NWNWSFC.

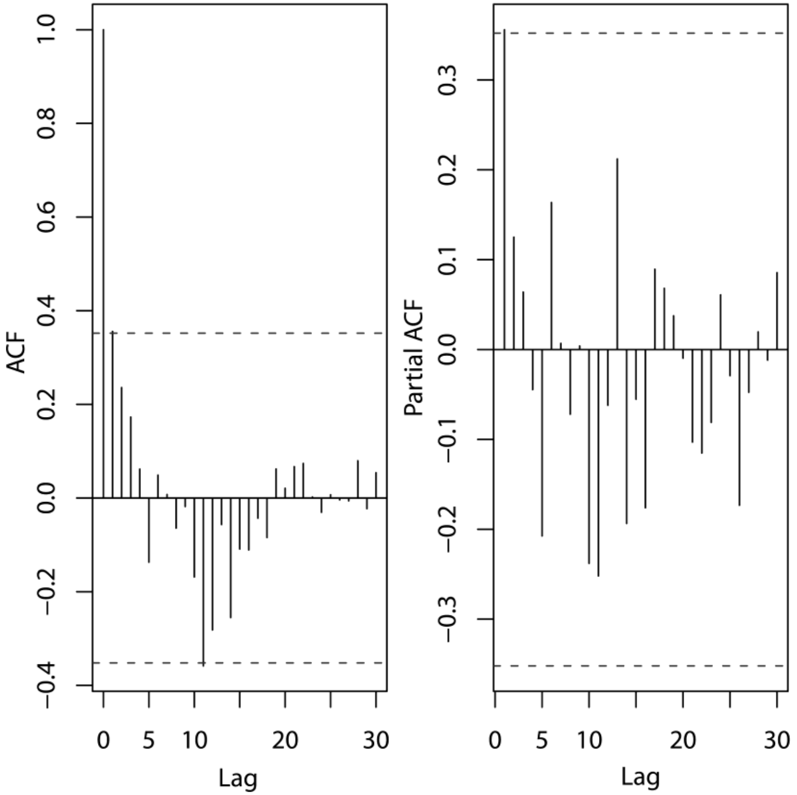


Figure 6.2. Autocorrelation and partial autocorrelation function plots of cockle abundance indices over a range of lag times. Spikes crossing the dotted lines (Bartlett bounds) indicate a significant autocorrelation (95% confidence level) at that lag.

There was some indication, though not significant, of cross-correlation between adult and juvenile cockle abundance estimates, with the suggestion of a positive correlation with cockle spats controlling for adult stocks with a lag of three years (Figure 6.4a). There was however, no evidence of a corresponding correlation of adult cockle abundance on cockle spat population strength (Figure 6.4b). Therefore, cockle spat population estimates were not correlated with estimates of adult cockles, but adult cockles did show evidence of a correlation with spat abundances with a lag of three years.

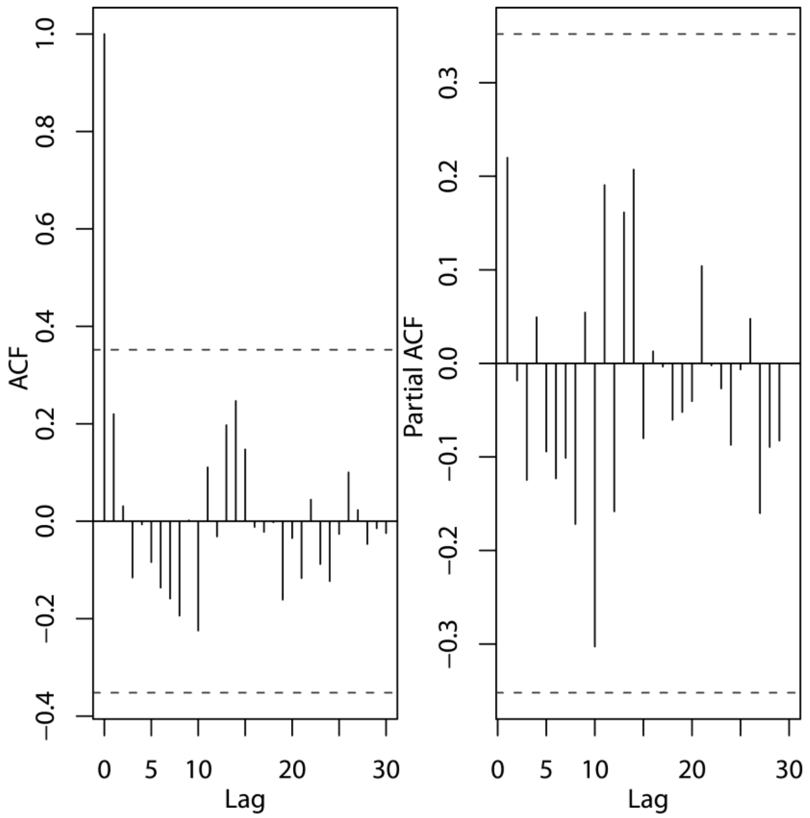


Figure 6.3. Autocorrelation and partial autocorrelation function plots of cockle spat abundance indices over a range of lag times. Spikes crossing the dotted lines (Bartlett bounds) indicate a significant autocorrelation (95% confidence level) at that lag.

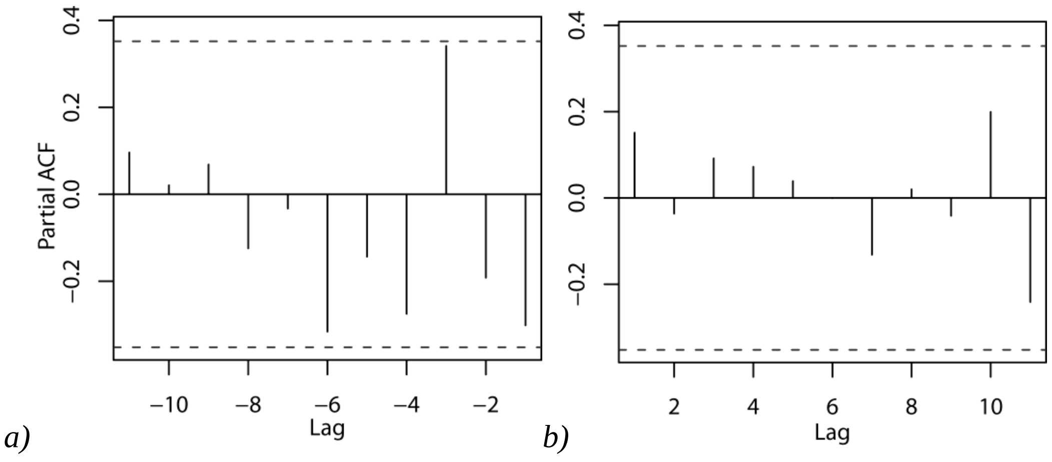


Figure 6.4. Cross-correlations indicating the partial autocorrelation functions of cockle population estimates based on NWNWSFC reports. Bars represent partial autocorrelation values of *a)* cockle spat, controlling for cockle spat and adult cockles and *b)* adult cockles, controlling for cockles and cockle spat. Spikes crossing the dotted lines (Bartlett bounds) indicate significant autocorrelation (95% confidence level) at that lag.

6.3.2 Factors contributing to extreme population changes

Adult cockles showed two large recruitment events, in 1975 and 1979 and two marked population reductions, in 1980 and 1989 (Figure 6.5). These losses indicate a period in which the mortality of cockles was substantially greater than the recruitment into the fishery. Both loss events occurred following particularly cold winters. No relationship was observed with extremes of temperature maxima or oystercatcher abundances (Figure 6.5).

Cockle spat indices showed large recruitment peaks in 1982 and 1995 and lesser peaks in 1978 and 2004. Loss events occurred in 1979, 1983, 1985 and 1994 (Figure 6.6). No relationships between the spat recruitment or loss events and the investigated parameters were evident. However, the substantial peak in 1982 did occur two years following the extremely low winter temperatures and the subsequent reduction in the adult cockle population strength.

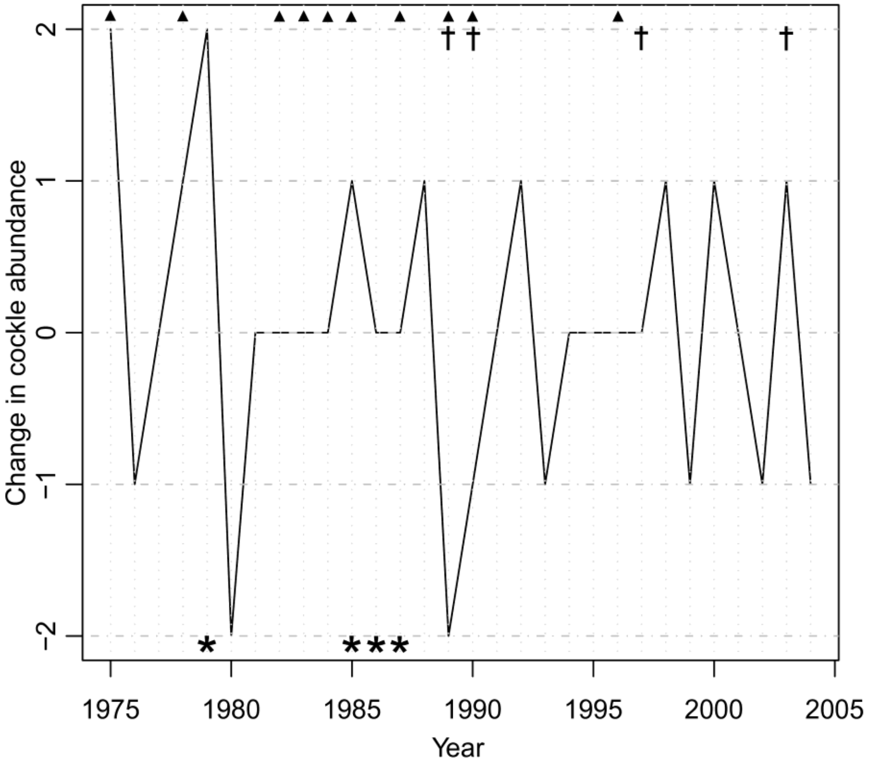


Figure 6.5. Relative changes to cockle index values (t – t-1) over the study period. Symbols indicate years displaying lowest 10% winter temperatures (*), highest 10% temperatures (†) and highest oystercatcher abundance indices (index value = 4, ▲).

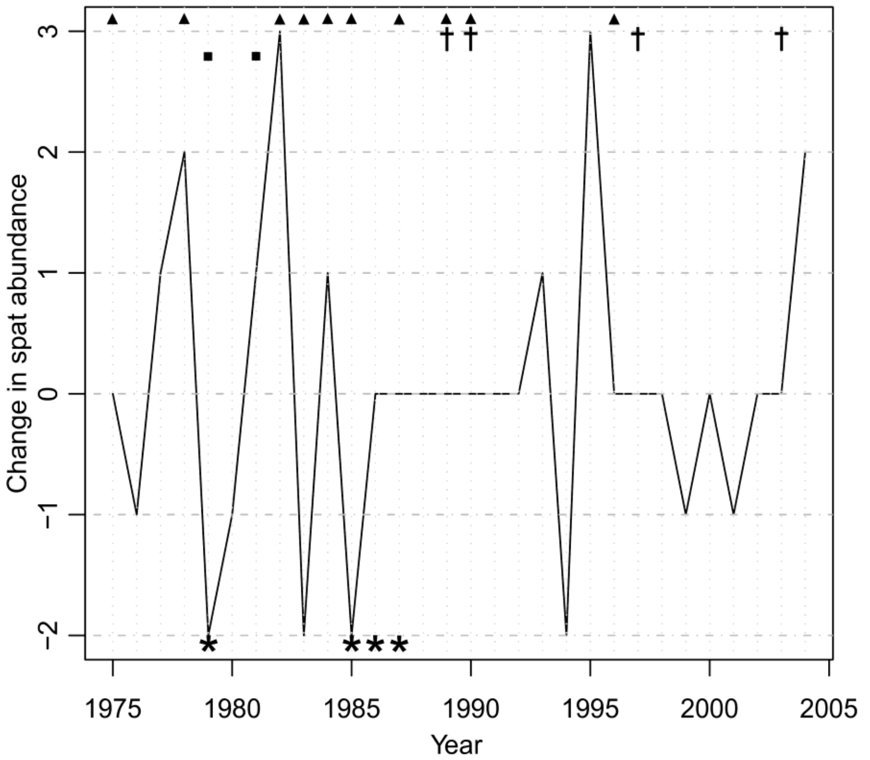


Figure 6.6. Relative changes to cockle spat index values over the study period. Symbols indicate lowest and highest winter temperatures and oystercatcher abundances (refer to Figure X) and demersal fish abundance maxima (index value = 5, ■).

6.3.3 Model validity: adult cockles

The ability for the variation in the populations of oystercatchers, demersal fish and shrimp (Figure 6.7) and the temperature measurements obtained from the Met Office (Figure 6.8) to explain the variability in the cockle and cockle spat data was investigated using Akaike's Information Criterion (AIC). Demersal fish abundances interacting with winter minimum temperatures provided the best-fitting model to explain the variability in adult cockle populations (Table 6.2). This model explained the variability in the adult cockle data three times more powerfully than the second-most powerful, which modelled cockle abundance as a function of maximum temperatures and demersal fish abundances (Table 6.2). The least powerful model was that measuring an index of oystercatcher abundance against the index of adult cockle abundance, with the model being effectively unsupported by the data (*i.e.* $\Delta_i >$ 10). The model containing only demersal fish estimates was also only weakly-supported by the data.

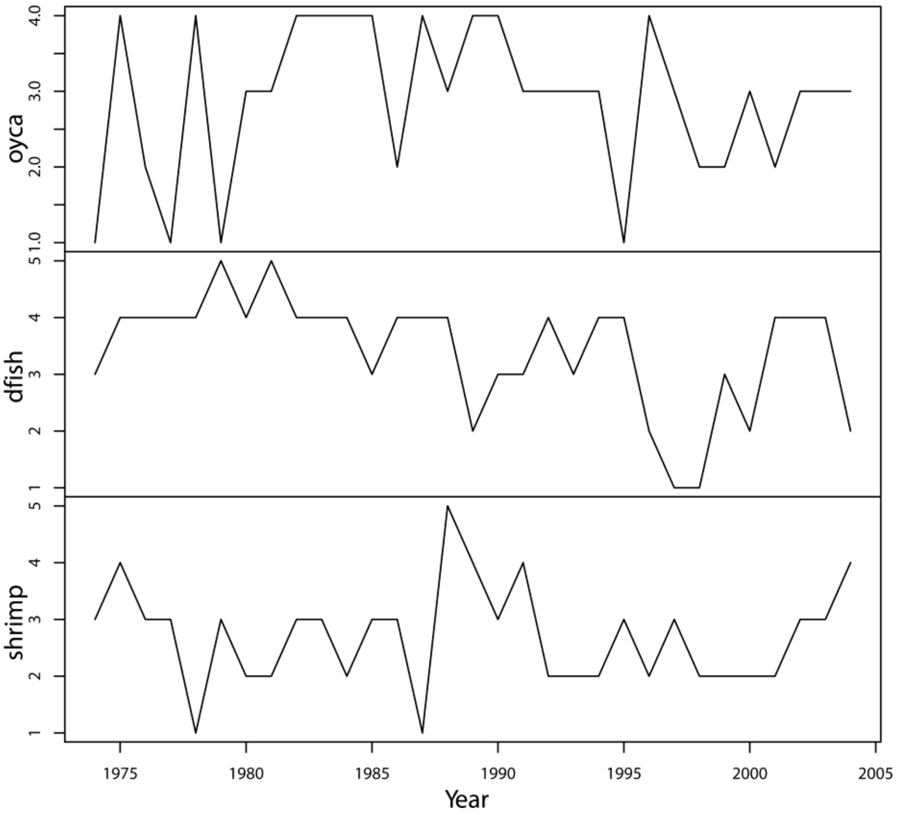


Figure 6.7. Indices reflecting oystercatcher (oyca), demersal fish (dfish) and shrimp abundances within Morecambe Bay over a thirty year period.

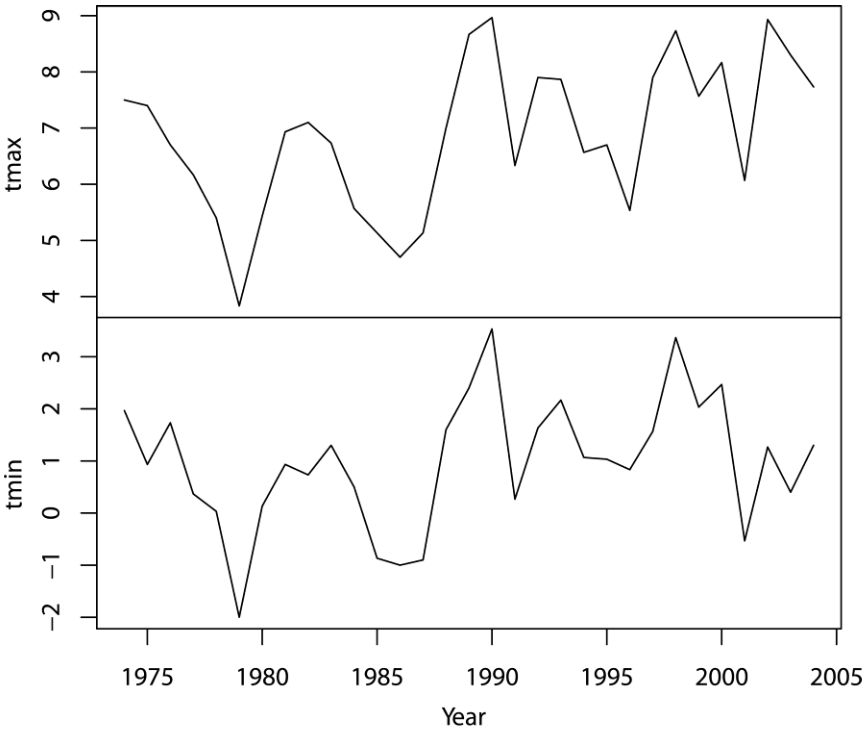
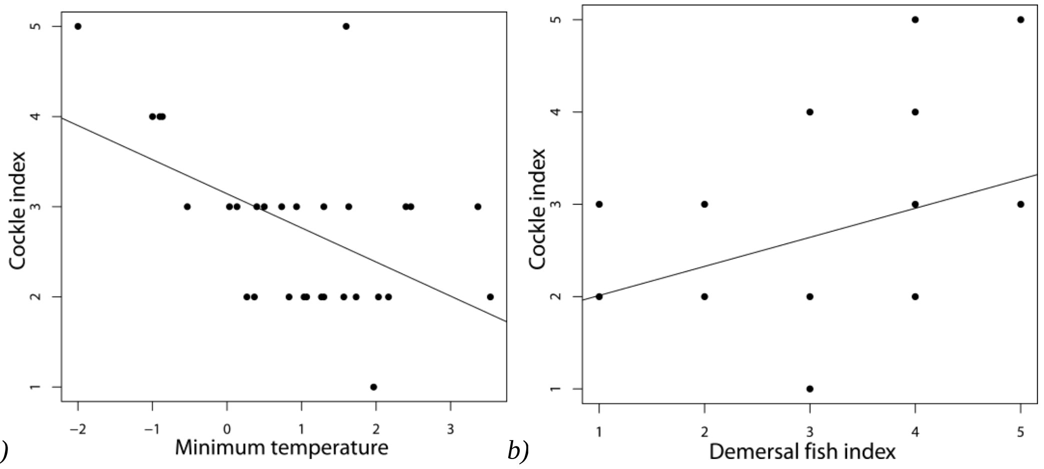


Figure 6.8. Met Office measurements of maximum and minimum winter temperatures (tmax, tmin, °C) at Newton Rigg, Cumbria measured over a thirty year period.

Table 6.2. AIC of linear regression models of cockle population indices against biotic and abiotic factors. Δ_i indicates the AIC of a model compared to the AIC of the 'best' model. Akaike weights (w_i) indicate the relative strength of each model.

Model	AIC	Δ_i	w_i
Single factor: Biotic			
Demersal fish	83.749	8.81	0.006
Oystercatchers	87.07	12.131	0.001
Single factor: Abiotic			
Temperature min.	78.26	3.321	0.096
Temperature max.	80.094	5.155	0.039
Combined factors			
Temperature min. × Demersal fish	74.939	0	0.507
Temperature max. × Demersal fish	77.179	2.24	0.165
Temperature min. × Oystercatchers	78.571	3.632	0.082
Temperature max. × Oystercatchers	78.121	3.182	0.103



a) *b)*

Figure 6.9. Cockle abundance index correlations with *a)* minimum temperature (°C) (y = 3.161-0.347x, r^2 = 0.224) and *b)* demersal fish abundance index (y = 1.830 + 0.292x, r^2 = 0.090). Trend lines represent linear regression model fit.

Winter adult cockle populations showed a negative relationship with minimum winter temperatures, with strongest cockle population strengths observed when minimum temperatures were at their lowest (Figure 6.9a). A positive relationship was apparent between adult cockle population indices and those for demersal fish, with greater fish abundances apparent when adult cockle abundances were also at their greatest (Figure 6.9b).

6.3.4 Model validity: cockle spat

Models that investigated the drivers of cockle spat population strengths showed a different response to those for adult cockles. The most powerful model was that modelling cockle spat abundance as a function of demersal fish abundance. This was more than six times as powerful as the second-placed model (Table 6.3). The remaining ten models all had reasonably-similar power to explain the variability in the data ($3.6 > \Delta_i < 8.7$).

Cockle spat abundances were negatively-correlated with those for demersal fish (Figure 6.10). Years in which the estimated juvenile stock strengths were greater generally had lower estimated populations of demersal fish within Morecambe Bay. Years with stronger demersal fish population estimates had lower cockle spat index values.

Table 6.3. AIC of linear regression models of cockle spat population indices against biotic and abiotic factors. Δ_i indicates the AIC of a model compared to the AIC of the 'best' model. Akaike weights (w_i) indicate the relative strength of each model.

Model	AIC	Δ_i	w_i
Single factor: Biotic			
Demersal fish	90.519	0	0.587
Oystercatchers	95.415	4.896	0.051
Shrimp	95.879	5.360	0.040
Single factor: Abiotic			
Temperature min.	95.469	4.950	0.049
Temperature max.	96.058	5.539	0.037
Combined factors			
Temperature min. × Demersal fish	94.33	3.811	0.087
Temperature max. × Demersal fish	94.147	3.628	0.096
Temperature min. × Oystercatchers	97.906	7.387	0.015
Temperature max. × Oystercatchers	97.365	6.847	0.019
Temperature min. × Shrimp	98.319	7.800	0.012
Temperature max. × Shrimp	99.217	8.698	0.008

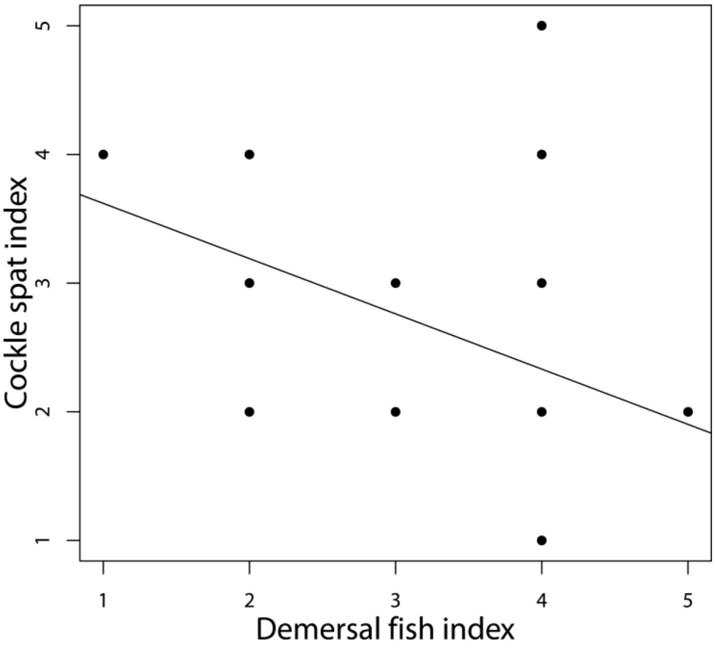


Figure 6.10. Cockle spat abundance correlation with estimates of demersal fish population strength ($y = 4.111 - 0.440x$, $r^2 = 0.154$). Trend line represents linear regression model fit.

6.4 Discussion

The availability of ecological data over the timescales necessary to ascertain multi-generation long-term trends are generally lacking. This is particularly so for investigations of the marine benthos, where studies of ‘long-term’ dynamics rarely cover in excess of ten-to-fifteen years (*e.g.* Currie & Small 2005; Borja *et al.* 2006; Seitz *et al.* in press). Long-lived taxa within benthic systems however, may live for many years (*e.g.* McIntyre 1970; Gilbert 1973) and, despite the ecological relevance of such data, few long-term (>15 years) time series are extant (but see for example Link 2004; Bêche *et al.* 2006; Frid *et al.* 2009b). As a result of the paucity of long-term ecological data, other approaches must be considered in order to investigate temporal changes to the populations of organisms. This investigation has used a novel, semi-quantitative approach based on NWNWSFC fishery status reports to investigate the fluctuations in cockle and cockle spat population strengths within Morecambe Bay, north-west England over a thirty year time period. This information has indicated variations in aspects of a number of ecosystem functions over a thirty year period within an estuarine system and has suggested a number of potential avenues of future investigation.

Prior to discussion of the drivers behind cockle stocks fluctuations in Morecambe Bay however, a caution regarding the limitations of this study must first be considered. Although the data within the NWNWSFC reports are given in a consistent and standard form, these reports are inherently based on communication from a number of fisheries and fisheries supervisory workers. As such, the accuracy of any reports is potentially subject to some inter-annual variability. In addition, these data cannot provide a definitive identification of the drivers responsible for fluctuations in the cockle and cockle spat populations. The nature of the data limits its interpretation to the identification of correlations. Also, the available data can only be correlated with the cockle populations when they were measured in the field. This inherently misses any variability in spatfall success and as such, this investigation can only examine factors relating to post-settlement mortality. Despite these limitations, the hypotheses (*i.e.* correlations) tested were selected *a priori* and based on sound ecological theory, rather than ‘data dredging’ for all possible models (Anderson *et al.* 2001). This means, therefore that the correlations provided within the current investigation can provide a valuable indication as to the drivers behind cockle fluctuations in Morecambe Bay and aid in the development of future investigations. Unlike most experimental observations, which are predominantly restricted to manipulations at localised scales, this approach allows the investigation of population dynamics at the scale of an entire coastal fishery.

Cockle and cockle spat populations within Morecambe Bay showed some evidence of cyclicity over the thirty year time series, with a suggestion that populations cycled over a period of ca. 25 and 12 years respectively. The reasonably-symmetrical nature of these oscillations is indicative of an oscillatory endogenous component in the population dynamics of cockles within Morecambe Bay (Nisbet & Gurney 2004). Other studies have generally revealed much shorter cycles of cockle population strengths. Within the Wash region, cockle population strengths have been shown to cycle, with wavelengths found in the region of 2-3 (Dare *et al.* 2004) and 7-8 years (Duran 1997). The long timescale oscillations observed within Morecambe Bay will not however be considered further here as generally, for statistically significant cycles or oscillations to be identified within a time series, the length of the time series should be much in excess of the length of these cycles (see Turchin & Berryman 2000; following Hunter & Price 1998).

ACF and PACF showed that populations of adult cockles were influenced by those of spat following a lag of three years. The length of this lag is longer than might be expected. This is because it is likely that the time between a peak in cockle spat abundances and the lagged peak in adult cockle abundances would reflect the maturation of cockles. That is, it would reflect the time scale between cockle spatfall and cockle maturity. However, the three year lag suggested within the NWNWSFC data, is longer than the typically-observed time taken for *Cerastoderma edule* to mature to adult status and reach sexual maturity. Iglesias & Navarro (1991) for example, found cockle populations to reach sexual maturity in their second year. This might imply that over the thirty year period, cockles within Morecambe Bay had a tendency for a relatively prolonged development, possibly due to stressful environmental conditions (Iglesias & Navarro 1991). The adult cockles as inferred from the NWNWSFC reports however, do not simply refer to all sexually-mature cockles. Instead, the reports are concerned with the fishable stock of cockles. Within the Morecambe Bay fishery, cockles are defined as fishable when they are retained on a square mesh with sides measuring 20 mm. This corresponds to cockle lengths in the region of ≥25 mm (Bill Cook, NWNWSFC *pers. comm.*). Hancock & Franklin (1972) showed that the majority of cockles of shell length in excess of 25 mm within the Burry Inlet, South Wales, were three years of age or older. This suggests that the years in which adult cockle population strengths at Morecambe Bay were higher and three years following peaks in cockle spat populations, then the adult peak was dominated by cockles aged three years or older. However, demographic data for Thurstaston on the Dee estuary suggests that two-year old cockles often measured in the region of 25 mm (Figure A.2, Appendix).

There was no evidence in the partial- and autocorrelation function investigations that the cockle spat population strengths were influenced by the strength of the adult cockle populations. There was however, some suggestion of one substantial cockle spat recruitment event following a large reduction in adult cockle numbers following a particularly cold winter. Such occurrences have been observed elsewhere (Beukema *et al.* 2002), however, within the current study, this observation was not consistent. That is, large spatfalls did not occur despite other substantial adult cockle population reductions occurring following a severe winter.

It is axiomatic that the potential for successful recruitment is greater when the abundance of sexually-mature adult cockles is greater. The observation that cockle spatfall is largely unaffected by adult cockle population strength must therefore be due to factors other than simply the amount of gametes being released. The factors affecting the recruitment strength of cockles are many, ranging from environmental and hydrodynamic factors, with cockles spending up to five weeks within the plankton (Lebour 1938; Creek 1960) to biological effects. The observed relationship therefore is likely to be more influenced by the survival of released gametes and settled spat than it is by the abundances of gametes released and this would benefit from further research. The amount of time that *Cerastoderma edule* larvae can potentially spend in the water column suggests that factors beyond those at a local scale may be important to larval survival, with the potential for large dispersal distances over the 5 week larval development period. However, the hydrology of Morecambe Bay means that larvae tend to be retained within the Bay, as well as accumulated from other areas (Fox *et al.* 2006), suggesting that the primary drivers behind larval mortality are likely to be localised in scale.

Variability in the cockle spat population data was best explained by changes in demersal fish assemblages. When demersal fish populations were at their strongest, cockle spat abundances were typically reduced. This observation was as might be expected. Although demersal fish may feed upon larger cockles, there appears to be some preference for younger juvenile cockles (*e.g.* Berghahn 1987). This therefore offers an explanation as to the lack of a negative relationship between adult (or fishery-viable) cockles and fish, but a relationship observed with cockle spat.

The NWNWSFC data indicated that variability in adult cockle populations were negatively-correlated with winter minimum temperature and positively correlated with demersal fish abundances within Morecambe Bay over the thirty year time period, with stocks at their strongest following colder winters and when demersal fish populations were at their strongest. This finding may appear counter-intuitive. Firstly, cold periods have been shown to result in large-scale cockle mortalities (Crisp 1964; Hancock 1971; Beukema 1985), suggesting that cockle populations would display a positive correlation with minimum winter temperatures, *i.e.* with reduced cockle mortalities with higher minimum temperatures (and hence a milder winter). Such a finding was observed in the current study, with reductions observed in adult cockle populations following the relatively severe winters of 1979 and 1985-1987. These extreme events aside however, adult population strengths showed a negative relationship to increases of temperature.

Periods of mild winter temperatures have been shown to reduce cockle stocks within the Wadden Sea (Beukema 1992) and this trend appears to be replicated within Morecambe Bay. The possible explanations behind this are numerous. Concerning abiotic factors, the increased temperatures themselves may be directly responsible for cockle mortality, with increased air temperatures associated with increased heat and desiccation stress to individuals (Ansell *et al.* 1981). However, in the current study, the ability of maximum temperatures to explain variations in cockle population indices was weak, suggesting that increased temperatures themselves were not a primary driving factor behind reduced cockle population strengths in Morecambe Bay. Biotic factors may explain this relationship.

Warmer weather may bestow some benefit upon other organisms within the system that may have some deleterious impacts on adult cockle health. Cockles are host to a range of parasitic organisms, being particularly susceptible to infestation by trematodes which utilise cockles as intermediate hosts (de Montaudouin *et al.* 1998; Lassalle *et al.* 2007). These parasites are potentially deleterious to the health of cockles. For example, some common cockle parasites are associated with the gill or foot tissue of cockles (de Montaudouin *et al.* 1998; Jensen *et al.* 1999). It is possible therefore, that parasitic infestation may leave cockles with potentially reduced respiratory efficiency and/or a reduced burrowing capability (Lauckner 1984; Carballal *et al.* 2001; Thieltges 2006). The impacts of trematode infestation has been shown to impact upon the survival of *Cerastoderma edule* and other members of benthic sedimentary assemblages and this is exacerbated under changed climatic conditions (Jensen *et al.* 1996; Gam *et al.* in press).

The current findings therefore suggest a climate-driven effect on cockle populations. Some further evidence for this is seen by comparing the timings of peaks and troughs in the landings of other cockle fisheries. Cockle landings across the UK as a whole showed similar peak timings to those observed in the current study, with peak landings in the mid-1980s following comparatively low landings in preceding years (Dare *et al.* 2004). Cockle landings in the Wadden Sea also showed peaks during the mid 1980s (Dijkema 1992). Despite landing data alone being insufficient to infer the strength of stocks, being related to fishing effort as well as cockle abundance, there is sufficient indication to support the case of there being one or more common driver such as climate behind the observed fluctuations across such a large geographic range.

This therefore suggests that with predicted changes to climate, there will be impacts to cockle populations and beyond. Firstly, the reproductive potential, development and transmission of parasites is potentially highly sensitive to temperature (Poulin & Mouritsen 2006), with increased temperatures corresponding to an increased reproductive potential of parasites, particularly trematodes (Mouritsen 2002). Therefore if winter conditions become milder, then the climate-control of parasites is weakened, leaving cockle populations more prone to infestation (*e.g.* Jonsson & Andre 1992; Gam *et al.* in press). Parasite-related mortality therefore, may be a possible driver behind the observed lower adult cockle population strengths in milder winters.

The model that best explained the variability in adult cockle population indices also included demersal fish abundance as an interaction term; that is, as well as being negatively correlated with minimum winter temperatures, adult cockle populations were positively correlated with demersal fish abundances. Again, this is an apparently counter-intuitive finding. As predators on benthic organisms, it was predicted that years in which demersal fish were highly-abundant would have correspondingly-reduced adult cockle populations due to increased predation pressure and thus result in reduced adult cockle population strength. However, despite demersal fish, as well as minimum winter temperatures, providing the best explanation of adult cockle data, this relationship does not necessarily infer any degree of causality. That is, these observations are merely correlations, in that years with higher cockle abundances also demonstrated increased demersal fish abundances. A number of explanations may account for such an observation. Firstly, the observed data may not be indicating that high demersal fish abundances lead to high cockle abundances, but rather the opposite. That is, it is more likely that when cockle abundances are greater, demersal fish migrate to tidal flat areas in order to exploit the increased food availability. Such observations are common, with predator populations moving to exploit food resources as they become available (*e.g.* Bradshaw *et al.* 2002; Alves *et al.* 2003). A number of other potential explanations are possible that are unable to be explored further with the data available, for example increased influx of river-borne food or nutrients, storm events releasing pulses of sediment-based nutrients into the system with subsequent stimulation of production, with benefits for both cockle and demersal fish populations.

The findings of the current study provide information not relating simply to the drivers of cockle populations, but also provide an indication of changes to the functioning of the ecosystem over a large temporal (30 year) and spatial (Morecambe Bay) scale. As biomass-dominant members of the faunal community (Chapter 2), cockles have a potentially substantial impact on the functioning of estuarine systems, from acting as a major food resource for epibenthic predators and birds (Drinnan 1957; Pihl 1985) to playing a role in the cycling of nutrients (Chapter 5). Therefore, it is apparent that changes to the abundance of cockles over time can have an impact on the functioning of a system. Given that long-term ecological data sets are generally uncommon and the fact that often the taxa that records are maintained for tend to be substantial contributors to ecosystem biomass and/or important contributors to system functioning (cockles in the current study, see also for example Baker & Clapham 2004; Furness 2007; Cardinale *et al.* 2009), then similar approaches to those described here provide a suitable method of generating long-term datasets across a range of ecosystems, hence potentially allowing longer-term insights into ecosystem function than those data sets currently available.

The current study therefore, offers an indication as to the major drivers of *C. edule* population strengths within Morecambe Bay. Adult mortality is seemingly most highly-correlated with temperature, with increased mortality in milder winters; past work suggests that this may be related to physiological stress and/or parasite-derived mortalities. Cockle spat on the other hand, are seemingly driven primarily by predatory mechanisms, with strong demersal fish populations corresponding to reduced cockle spat population strengths. Cockle spat also showed a suggestion of an influence on adult cockle populations, with no corresponding influence of adult populations on spat. This suggests therefore, that it is the survival of cockle spat, rather than the survival of adults that are the major drivers of post-settlement cockle population strength. Similar relationships have been observed in other bivalve taxa, for example the mussel *Mytilus edulis* (Troost *et al.* 2009) and the soft clam *Mya arenaria* (Bowen & Hunt 2009) showed little influence of spawning stock density on recruitment. Dare *et al.* (2004) also discuss the high variability of cockle-recruitment data that show no systematic relationship to adult spawning stock strengths within a number of estuaries. Future investigations should concentrate on these findings, investigating the roles of epibenthic predation, in particular demersal fish on cockle spat. In addition, the role of parasitic infection on adult cockles should be explored further, taking into account the roles of temperature on cockle mortality.

The methods carried out here provide useful indications of multi-decadal population trends within assemblages. This provides a measure of the relative fluctuations of species abundances and allows inferences to be made of the ecological functioning within these assemblages. The semi-quantitative techniques used here can be applied to other sources of data within other habitats, with these methods particularly promising in areas where ecological surveys are non-existent or insufficient, but where long-term information is available for certain taxa, for example through fishery landing data and game hunting. Such analyses allow analyses of the functioning of systems and drivers behind fluctuations in populations to be carried out and provide indications of the possible major drivers behind population health.

CHAPTER 7: GENERAL DISCUSSION

Organisms within ecosystems influence a range of biological, biochemical and physical activities, processes and properties within these systems and are vital to the delivery of key ecological goods and services, commonly referred to as ecosystem or ecological functions (Naeem *et al.* 2002; Millennium Ecosystem Assessment 2005; Bremner *et al.* 2006b; Frid *et al.* 2008). As biomass-dominant members of benthic infaunal assemblages in temperate regions, the cockle *Cerastoderma edule* has the potential to contribute to or mediate a number of these functions, either directly, for example acting as a store of organic carbon, or indirectly, for example, disturbance to sediments by cockle movement affecting the cycling of nutrients within the benthic environment (Figure 7.1).

The findings of this thesis show that cockles are potentially key contributors to ecological functioning within temperate tidal flat systems. The work also indicates that the removal of cockles on a large-scale has the potential to change the delivery of a number of functional processes or properties. These changes are related both to the removal of cockles themselves as well as through the associated disturbance to the benthic environment (Figure 7.2, Table 7.1).

Cockles can affect the availability of nutrients in porewaters (Chapter 5; Figure 7.2) and directly contribute to a substantial portion of the macrofaunal biomass within these systems (Chapters 2 & 3; Figure 7.2). These studies also revealed effects beyond simply the abundance of *Cerastoderma edule*. Disturbance to benthic habitats by cockle hand raking resulted in two of the eight members of the biomass-dominant fauna investigated to display altered feeding preferences and this has potential repercussions for benthic-pelagic coupling and ecological functioning within benthic systems (Chapter 4; Figure 7.2). In addition, removal of cockles caused a shift in the distribution of functionally-important biological traits within the benthos (Chapter 3; Figure 7.2). These findings therefore, support the notion that *C. edule* is a key contributor to or mediator of ecological functioning within temperate tidal flats and thus might be considered a ‘key’ species with regards to the functioning of these habitats (*sensu* ‘keystone species’ of Paine 1969, Figure 7.2). However, the studies described here highlight some implications for ecological investigations and for the management of ecosystems.

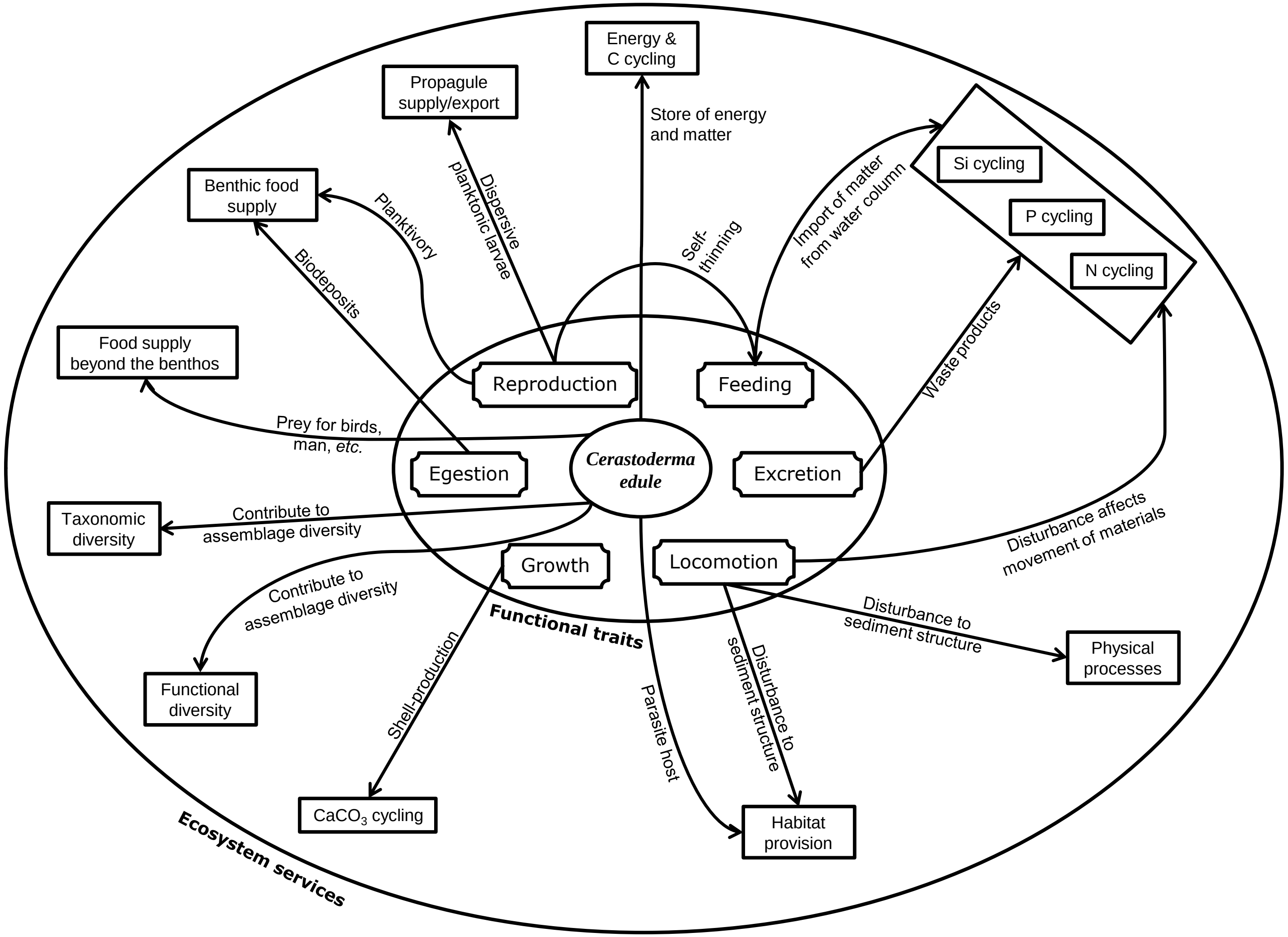


Figure 7.1. Conceptual model of the possible influence of *Cerastoderma edule* and the functional traits it provides on the provision of ecosystem goods and services within the marine benthic environment (as outlined by Millennium Ecosystem Assessment 2005; Bremner *et al.* 2006b; Frid *et al.* 2008).

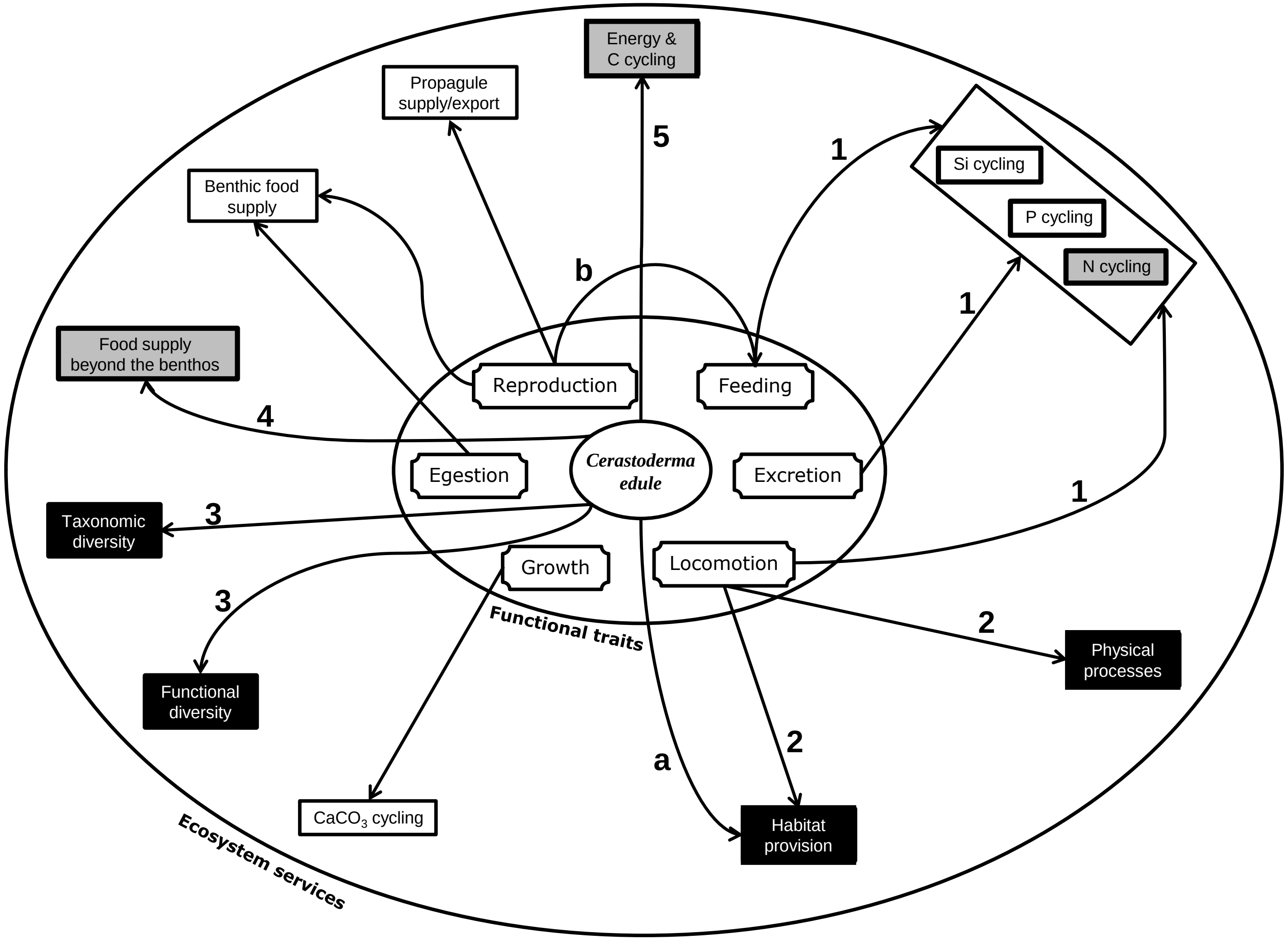


Figure 7.2. Conceptual model of the effects of large-scale removal of *Cerastoderma edule* on the provision of ecosystem goods and services within the marine benthic environment based on the findings of this thesis. The investigated functions (thick borders) were enhanced or increased (black), reduced or impaired (grey) or unaffected (white) by removal of cockles. Alpha-numeric arrow labels refer to Table 7.1.

Table 7.1. Predicted responses to ecological functions following the theoretical large-scale removal of the cockle *Cerastoderma edule*. Responses are based on the findings within this thesis, with the relevant chapter numbers indicated. Codes correspond to Figure 7.2, referring to either direct evidence within the associated chapter (1-5) or where an association is suggested or hypothesised by the data (a-b).

Code	Functional response	Chapter
1	No effect on Si, P or NO_x, but reduced sedimentary NH_4 with reduced cockle density	5
2	Reduced median sediment particle size following reduced cockle density	3
3	Increased taxonomic and functional diversity within assemblages	3
4	Reduced cockle density	3
5	Change to feeding behaviour of taxa and transfer of energy through system	4
a	Hypothesised effect of parasite-induced mortality	6
b	Hypothesised density-dependent self-thinning - likely to be reduced	6

It was shown (Chapter 3; Cesar & Frid 2009; Figure 7.2) that, despite the removal of substantial biomasses of *C. edule,* a number of ecological processes within the benthos were not significantly affected. In addition, despite the experimental manipulation of cockle densities significantly influencing ammonium concentrations (Chapter 5; Figure 7.2), impacts on other nutrients investigated were not apparent. These findings might understate the potential importance of the roles of cockles for the functioning of tidal flat systems and this may be seen as an argument for the authorisation of the unrestricted commercial removal of *C. edule* from sedimentary habitats. However, the large-scale removal of cockles from the Wadden Sea system had significant implications to primary productivity (Beukema *et al.* 1998). Therefore, while the investigations described here suggest that the removal of cockles at the magnitude of a small-scale hand-gathering fishery might have little effect on a number of ecological processes, care must be taken before extrapolating these findings to the scale of whole systems as small-scale hand-gathering for example occurs at a patchy scale, with impacted areas surrounded by relatively large areas of undisturbed habitat. Larger scale disturbances, such as mechanical dredging inherently impact on a larger area of the habitat and thus have larger-scale impacts on the system (*e.g.* Ferns *et al.* 2000).

Intertidal sedimentary ecosystems are by their nature highly dynamic, with species compositions changing substantially over both temporal and spatial scales (Chapter 2; Bachelet *et al.* 2000; Rossi *et al.* 2001). As well as spatial differences, these assemblages also differed over the seasonal scale, with assemblages containing considerably greater macrofaunal biomass in autumn, due to recruitment and growth of individuals, in particular bivalves, over the summer months. This inherent dynamism at the scale of entire estuaries and/or habitats might mask the impacts of relatively small-scale variations and impacts to macrofaunal abundance, even if, as in the current thesis, the taxa affected are dominant contributors to the macrofaunal biomass. This is a likely explanation as to why the laboratory observations of others (*e.g.* Swanberg 1991) did not translate to the field investigations of the present studies. This means that hypotheses formulated based on the findings from laboratory or micro- or mesocosm (hereafter mesocosm) investigations do not necessarily translate to field-based investigations (see also Kimball & Levin 1985; Goñi-Urriza *et al.* 1999; Shi *et al.* 1999).

The inability for the results of laboratory investigations to consistently translate into field studies is generally seen as a 'problem' for field experiments. That is, it may be argued that the multitude of 'hidden treatments' in field studies (Huston 1997) add too much variability and too many unknowns to field experiments, making their interpretation difficult. It is for this reason that many investigations utilise laboratory mesocosms as models of study systems (Odum 1969). However, the perceived difficulty of interpreting field studies need not necessarily be seen as a detracting factor for arguments against the use of *in situ* investigations. This is particularly so in the field of ecology and especially so when investigators are attempting to gain an insight into the functioning of entire systems. A primary aim of investigations of ecological functioning is to ascertain the effects of some change, whether changes to species and/or functional group composition, or faunal biomass (*e.g.* Chapter 3, Mermillod-Blondin *et al.* 2003; Olsgard *et al.* 2008), on the functional processes within the ecosystem. It is obvious therefore, that attempting to recreate the vast number of uncertainties that are present within natural systems within laboratory conditions is practically impossible. In fact, hidden treatments aside, few laboratory investigations even attempt to recreate one of the most obvious factors present within 'natural' intertidal systems, *i.e.* tidal ebb and flow (*e.g.* Ieno *et al.* 2006; Bulling *et al.* 2008, but see for example Richardson *et al.* 1993).

Laboratory mesocosm investigations are also restricted by scale, primarily spatial scale, which means that observations from such investigations cannot readily be transferred to the ecosystem-scale (Schindler 1998). As well as spatial scale, Petersen *et al* (2003) also classified ecological complexity as a further scaling variable, defined as species, functional and habitat diversity. Within mesocosm assemblages, the distribution of taxa and functional groups are generally incomparable to those of the natural system and trophic complexity, ecological network linkages and food-chain length is generally substantially reduced compared to those within natural systems (*e.g.* Marinelli & Williams 2003; Griffin *et al.* 2009; Worsfold *et al.* 2009). In addition, mesocosms themselves, in terms of the size and shape of experimental containers can also influence the response of study taxa (*e.g.* Petersen *et al.* 2003) which not only makes extrapolation to the system-scale difficult, but also comparisons with other mesocosm experiments.

Although the *in situ* investigations described within this thesis only manipulated *C. edule* densities at a relatively small scale (Chapters 3 & 4), the scale from which the impacted system could respond was not restricted. Within confined laboratory mesocosms however, the only aspects of the system (*i.e.* the experimental mesocosm) that are able to respond to an experimental manipulation or stressor are those aspects of the system that were initially introduced to the mesocosm by the investigator. This therefore removes the potential for new taxa to enter the exploited system and for the horizontal or vertical transport of materials to contribute in some way to its functioning following a disturbance. Therefore, many factors argue for the use of *in situ* investigations over mesocosm studies. However, this does not imply that mesocosm studies should be abandoned. On the contrary, mesocosm studies have contributed a number of key findings, without which the field of ecosystem functioning would be much less-developed (*e.g.* Naeem *et al.* 1994; Hulot *et al.* 2000; Cardinale *et al.* 2002). This suggests therefore that mesocosm investigations are ideal systems in which to formulate ecological hypotheses, however to test these hypotheses, where possible (in terms of practical, ethical and financial aspects) investigations should be carried out within the study systems themselves. This is the only way to ascertain the response of a natural system to some change or impact. Additionally, the influence of ‘hidden treatments’ can indicate novel components of systems that may be involved in driving ecosystem functioning and hence allow hypotheses to be further-refined and developed. This is particularly pertinent, as taxa often respond in a manner differing to what may be predicted *a priori*, as was shown with the response of *C. edule* populations to ecological drivers (Chapter 6).

The findings of this thesis also suggest a number of future research directions. Within marine systems, microbial communities potentially play a major role in the regeneration of nutrients and the cycling of compounds. Within the benthos, microbial benthic algae contribute a substantial proportion of the primary productivity (MacIntyre *et al.* 1996) and this community has been shown to be influenced by macrofaunal assemblages and *vice versa* (*e.g.* Dyson *et al.* 2007; Kang *et al.* 2006). Experimental manipulation however, showed no impact of the removal of *C. edule* on the concentration of chlorophyll *a* on a tidal flat (Chapter 3). Despite this, increased cockle densities did show an influence on the availability of ammonium within sedimentary porewaters and modelling based on previous studies suggested that uptake of this additional nutrient supply by microphytobenthos (MPB) represented the likely sink (Chapter 5). Despite chlorophyll *a* concentrations themselves remaining seemingly unaffected by cockle density, there is the potential for the quality and/or composition of the MPB communities to be affected. Such changes may have impacts upon the ecological functioning within the benthos (*e.g.* Philippart *et al.* 2007; Méléder *et al.* 2005).

This study also highlights the fact that there are a number of knowledge gaps with regards to the phenotypes expressed by taxa within benthic assemblages. With feeding behaviour of taxa as a model trait, it was shown in Chapter 4 that taxa can alter their behaviour and hence the biological trait(s) they are expressing due to some external stressor. It was also observed that these differences were not consistently expressed by conspecifics within different assemblages. This has implications with regards to the use of biological traits in investigations of ecological functioning, in that investigators have little knowledge as to how taxa are expressing those traits that have the potential to change. The use of fuzzy scoring (Chevenet *et al.* 1994) allows taxa to be assigned to numerous traits. However, this approach effectively only provides investigators with a wide range of potential functional roles carried out within assemblages, providing no indication as to the actual behaviour expressed by taxa at any time. A future direction for ecological functioning investigations could be for investigators to move away from scoring based on species averages, *i.e.* scoring all individuals according to the mean value for that species, towards using an approach based on individual organisms (see Reiss *et al.* 2009 and references therein). In practical terms however, such an approach is likely to be restricted to the size fractionation of organisms, due to the relative ease of separating organisms by their size (*c.f.* Edgar 1990). That is, an individual approach to functional measures is likely to be limited to those functions that correlate predictably to the age and/or size of an organism, for example fecundity (Sprung 1983), living location (Arsenault & Himmelman 1996; Ryer *et al.* 2007) or feeding activity (Wood & Buxton 1996; Woods & Schiel 1997). The applicability of this approach however, depends largely upon the investigators knowledge of the size-related activity of taxa within the study system.

Regardless of the approach or approaches adopted, it is clear that before the distribution of taxonomic assemblage compositions can be used to infer ecological functioning, substantial improvement needs to be made as to our understanding of the natural history of species within ecosystems. Despite intertidal assemblages having been studied intensively over a long time-scale (*e.g.* Stopford 1951; Sanders *et al.* 1962; Paine 1966), a number of functionally-important key aspects of even common taxa are unknown. For example, the spionid polychaete *Pygospio elegans* is an abundance-dominant member of temperate infaunal communities (Chapters 2-4; Beukema *et al.* 1999; Bolam & Fernandes 2003) and have been shown to adopt a number of reproductive strategies (Hannerz 1956; Anger *et al.* 1986). Despite this, peer-reviewed publications on the fecundity of this species (in terms of the number of oocytes released in each spawning event) is lacking and thus investigators are restricted to inferring a value for fecundity from studies at the family level (*e.g.* Yokoyama 1990; MacCord & Amaral 2007). Such gaps in ecological knowledge can potentially reduce the quality of the information gained within these investigations. Despite the field of descriptive ecology being rather unfashionable, greater effort must be made into investigations of the ecologically-relevant behaviour of taxa. Without such knowledge, the interpretation of even the most rigorous investigations is inherently limited.

In addition to refining how ecologists investigate ecosystem functioning as a measure of the functional diversity of study assemblages, it is also important to define what is meant by ecosystem functioning and to consider whether investigations of ecological functioning are measuring a sufficient number of functions. Each organism within an assemblage contributes to a number of ecosystem functions (Gamfeldt *et al.* 2008) and with increasing numbers of functions measured, the importance of individual species to sustaining ecosystem functioning also increases, that is the functional redundancy of taxa decreases as the number of ecological processes assessed increases (Hector & Bagchi 2007; Gamfeldt *et al.* 2008). Clearly, the more ecosystem functions that are investigated, then the more informative a view is gained to the functioning of and interrelationships within the system and the responses of these aspects to changes to biodiversity. This is similar to including increased numbers of biological traits in analyses giving a better indication of the functional complexity of an assemblage (Bremner *et al.* 2006a). A substantial number of investigations however, only measure single response variables and use this single proxy to infer the health of an entire system. Knowledge of the inherent complexity of ecological systems suggests that this approach means that much ecologically-valuable knowledge is potentially lost. Future work therefore, should strive to measure multiple ecological functions and the effects of changes to assemblage compositions on the delivery of these functions (Reiss *et al.* 2009).

The focus of this thesis has been to investigate the ecological roles that the biomass-dominant cockle *Cerastoderma edule* plays within temperate sedimentary tidal flats and the potential for anthropogenic actions to affect this role. The findings suggest that cockles have the potential to be key contributors to ecosystem functioning (Figure 7.2). However, this response seems to have a substantial degree of redundancy associated with it. As some perturbation moves a system close to a sudden change (*i.e.* a regime shift or tipping point), then it becomes increasingly difficult to predict the future trajectory, including the recovery dynamic, of the impacted assemblage and the functional properties of the system. In part, the development of predictions is hampered by a lack of knowledge as to the behaviour and the traits of the organisms and how these traits are expressed. Therefore, the future advancement of the field of marine ecology and developing predictive tools for managers and ecologists requires increased efforts in classical marine biology through investigations of ‘classical’ biology.

APPENDIX

DOES THE NUMBER OF MOUTHS MATTER? INVESTIGATION OF THE SUITABILITY OF TAXON BIOMASS IN ECOLOGICAL INVESTIGATIONS

A.1 Introduction

Investigations of species impacts on ecosystem functions tend to use biomass to enumerate the amount of an organism or taxonomic group present within an assemblage (*e.g.* Olsgard *et al.* 2008; Bremner *et al.* 2003b). The primary reason for this is that biomass data provide a more accurate indication as to the apportionment of ecosystem resources, such as organic carbon, that organisms represent within a system, and therefore a better insight into the contribution to ecosystem processes and functions, than abundance data (Chiarucci *et al.*, 1999).

Enumerating taxa using biomass data alone may however mean that some important aspects of ecosystem functioning are overlooked. This is because some processes or functions may be more influenced by the abundance of individuals, rather than solely the biomass. For example, juveniles and small adults often show increased metabolic activity per unit body mass than fully-grown adults (Normant *et al.* 2004). Therefore, if an assemblage contains juveniles of a given taxon representing a high proportion of the faunal biomass, then there is the potential for the sum of their feeding, respiration, egestion and excretion rates to differ to that of an assemblage with its biomass dominated by fewer larger individuals, despite the total faunal biomasses not differing. This has the potential to impact on the delivery of ecosystem functions as it is these metabolic processes that impose direct impacts on and hence mediate the functioning of ecosystems (Naeem *et al.* 2002).

The metabolic activities of macrofauna can have a substantial influence on the cycling of nutrients within intertidal systems (*e.g.* Mortimer *et al.* 1999a). Egestion and excretion by benthic fauna means that sediment porewaters act as a source of nutrients within the benthos. These nutrients are readily utilised by primary producers and the presence and behaviour of macrofauna have been shown to influence the growth of algae within the benthos through the increased turnover of nutrients (*e.g.* Bianchi & Rice 1988). The impact of macrofauna on primary production also extends beyond the benthos, influencing the availability of nutrients to, and hence controlling the growth of, coastal phytoplankton assemblages (Prins & Smaal, 1994; Denis & Grenz 2003).

Cockles have the potential to affect the availability of nutrients within sediment porewaters, through excretion, feeding and oxygenation within the upper sediment layers, with subsequent impacts on the microphytobenthic (MPB) and macrofaunal communities (Swanberg 1991; Rossi *et al.* 2008). The aim of this investigation was to carry out manipulations of cockle abundances *in situ* whilst maintaining cockle biomass in order to assess whether the number of cockles present at a consistent biomass within an assemblage affects ecosystem function. This would allow future investigators to ascertain whether studies of biomass alone are suitable for investigations of ecological functioning or whether some indication as to taxon abundance and/or population structure is required.

The availability of nutrients within sediment porewaters was chosen as the model ecosystem function. This is because cockles have been shown to directly influence the composition of nutrient porewaters (Swanberg 1991). Nutrient availability in coastal areas is often a major limiting factor on the growth of primary producers (Oviatt *et al.* 1995; Downing *et al.* 1999) and therefore is an important ecosystem property to investigate. Additionally, nutrient concentrations in the experimental systems are likely to respond more rapidly to experimental manipulation than other functions, such as primary producer biomass or organic matter within the sediment. Porewater sampling is also relatively straightforward and repeated measurements of porewater concentrations may be taken with less physical disturbance to sediments than the removal of the volumes of sediment required for analysis of primary producer biomass or organic matter content.

A.2 Methods

A.2.1 Ambient cockle densities and size-class distributions

Studies were carried out within the mid-shore zone of the tidal sedimentary flat at Thurstaston within the Dee estuary, north-west England (Figure 2.1). Prior to experimental manipulations of cockle *Cerastoderma edule* densities, a preliminary investigation of the size-distribution and density of cockles was taken. Sediments were dug to a depth of ca. 15 cm and sediments passed over a 10 mm mesh *in situ* and returned to the laboratory. Cockles retained were measured using vernier callipers (shell length at maximum width ± 0.05 mm), wet weight ascertained (± 0.0001 g) and age class ascertained by examination of shell growth rings. These cockles were used to ascertain ambient cockle densities for the calculation of experimental densities.

A.2.2 Abundance-biomass manipulations

Cockles were gathered (at Day 0) from the mid-tide shore level at Thurstaston and sorted in the field using callipers, separating them into two categories, with 'large' cockles measuring ≥21 mm and 'small' cockles measuring 14-20 mm at their maximum shell width. These cockles were used to seed experimental plots measuring 320 × 320 mm (corresponding to a surface area of approximately 0.1 m^2). Prior to the addition of cockles, all plots were carefully dug to a depth of ca. 150 mm and sediments carefully broken apart by hand in order to locate and remove cockles. Plots were marked out in the sediment using a length of bamboo cane at each corner and assigned to six replicates of the following treatments:

1. Maximum abundance 1 – containing an abundance of only 'small' cockles at ambient biomass
2. Minimum abundance 1 – containing an abundance of only 'large' cockles at ambient biomass
3. Intermediate abundance 1 – containing equal biomasses of 'small' and 'large' cockles at ambient cockle biomass
4. Control – sediments dug and cockles present removed by hand

Each 0.1 m^2 plot, except for controls, therefore contained cockles at approximately local ambient biomass. The aim of this investigation was not to investigate ecosystem functioning *per se*, but rather to 'force' a response in an ecosystem property and to assess whether experimental conditions (*i.e.* the initial abundance or biomass of cockles) affected the intensity of the response. The results of Chapter 3 show that, despite cockles having the capacity to affect macrofaunal communities, their impact on ecosystem processes, such as primary production and organic content of sediments was potentially more limited. To increase the likelihood of a response in porewater nutrient concentrations therefore, a duplicate set of treatments were also established, in which cockle biomass was present at double the ambient biomass:

5. Maximum abundance 2 – containing an abundance of only 'small' cockles at double ambient biomass

6. Minimum abundance 2 – containing an abundance of only ‘large’ cockles at double ambient biomass
7. Intermediate abundance 2 – containing equal biomasses of ‘small’ and ‘large’ cockles at double ambient cockle biomass

This approach therefore necessitated a total of 42 0.1 m^2 experimental plots to be established. Each plot was sampled on a daily basis (Days 1-9), extracting ~20 ml porewater using a sterilised plastic syringe from a depth of 50 mm. Experimentally-manipulated plots however, were likely to be susceptible to predation, primarily from birds and demersal fish, as well as potentially susceptible to the migration of cockles away from plots. Therefore, four duplicate plots of each treatment were also established and treated in exactly the same manner as the experimental plots. That is, porewater samples were also removed from these plots, though the extracted fluid was discarded. These plots were used as a guide in order to measure how successfully plots are able to maintain cockle abundances: on Day 3 and Day 6, two of the ‘guide’ plots from each treatment were destroyed and the number of cockles present enumerated to investigate how the manipulated cockle densities had been retained. On the final sampling day, Day 9, the experimental plots themselves were destroyed and cockle densities enumerated. This meant that a total of 60 plots were required for the study.

A.3 Results

A.3.1 Ambient cockle densities and biomasses

Preliminary sampling showed that cockles at Thurstaston were present at a mean biomass of 402 ± 151.4 g m^{-2}. Cockle beds were dominated by smaller individuals consisting of year one individuals with a mean maximum shell width of 16.70 ± 2.45 mm (Figure A.1). Larger individuals consisted of year two cockles and had a mean maximum shell width of 25.62 ± 2.84 mm. The size of cockle shells (as maximum shell width) provided a good indicator of the biomass of the organism. Year class one cockles had a mean wet weight of 1.08 ± 0.41 g, whilst the cohort dominated by year class two cockles had a mean wet weight of 4.89 ± 2.13 g (Figure A.2).

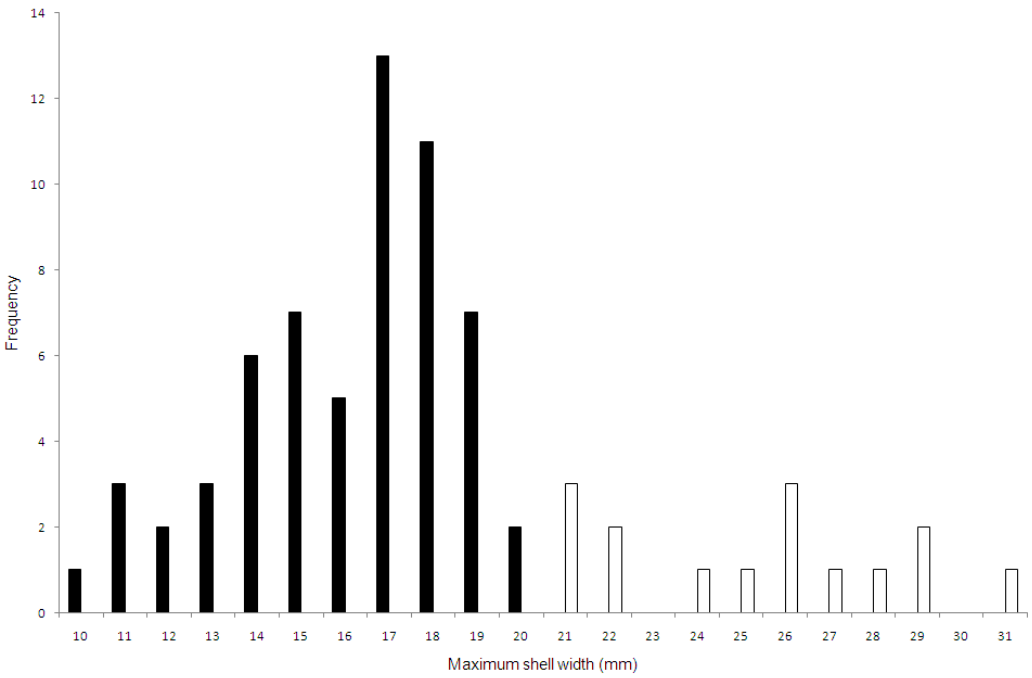


Figure A.1. Size-frequency distribution of cockles sampled at Thurstaston, within the Dee estuary. Cockles were dominated by two year classes: year one (black) and year two (white).

A.3.2 Abundance-biomass manipulations & logistical constraints

From the ambient size-density distributions, the cockles required for the experimental seeding manipulations were:

1. Maximum abundance 1 – containing 40 small cockles per plot
2. Minimum abundance 1 – containing 8 large cockles per plot
3. Intermediate abundance 1 – containing 20 small and 4 large cockles per plot
5. Maximum abundance 2 – containing 80 small cockles per plot
6. Minimum abundance 2 – containing 16 large cockles per plot
7. Intermediate abundance 2 – containing 40 small and 8 large cockles per plot

Therefore, a total of 1080 ‘small’ and 216 ‘large’ cockles would be required for the experimental manipulations alone. Including the guide plots, this means that a total of 1800 ‘small’ and 360 ‘large’ cockles would be required for this investigation. With ambient biomass of 402 g *C. edule* m^{-2}, in excess of 300 plots would have to be extracted in order to gain sufficient cockle abundances. Over a single tidal cycle, therefore, such a number was deemed practically impossible to obtain. It was considered inappropriate to collect cockles over two or more low tides, due to logistical concerns and shifting tide times. It was deemed that keeping cockles alive in the laboratory would be difficult given the lack of available facilities at the University of Liverpool. In addition, movement away from the habitat and the necessary storage of *C. edule* would introduce an additional stress to the cockles and would hence act as a potentially confounding factor.

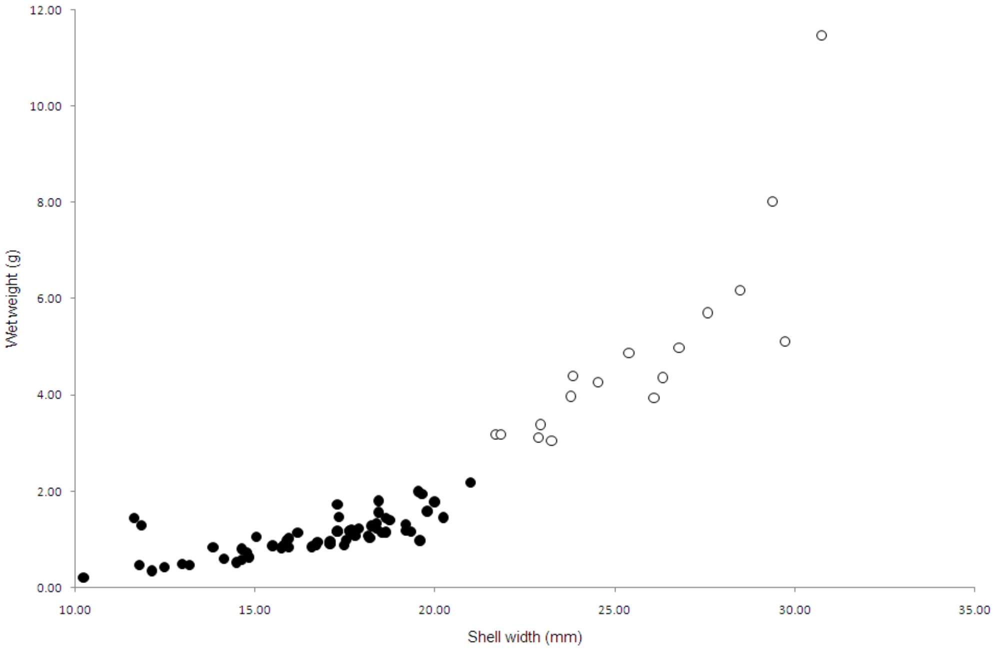


Figure A.2. Cockle size-weight relationship, showing wet weight as a function of maximum shell width. Cockles were dominated by two year classes: year one (black) and year two (white).

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