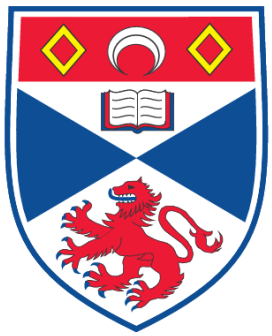
BIODIVERSITY AND ECOSYSTEM PROCESSES IN HETEROGENEOUS ENVIRONMENTS

Kirstie Elizabeth Dyson

A Thesis Submitted for the Degree of PhD
at the
University of St. Andrews



2008

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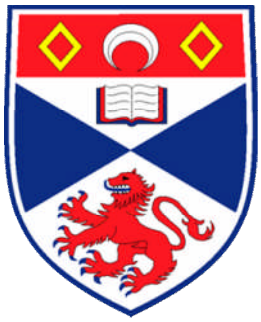

A thesis submitted in partial fulfilment of the requirements for the degree of Doctor of Philosophy, at the University of St Andrews

School of Biology
University of St Andrews
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Declarations

I, Kirstie Elizabeth Dyson hereby certify that this thesis, which is approximately 35000 words in length, has been written by me, that it is the record of work carried out by me and that it has not been submitted in any previous application for a higher degree.

Date Signature of candidate

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Contents

Abstract

The decline in biodiversity over the last decade has motivated researchers to investigate the relationship between species richness (biodiversity) and ecosystem function. Empirical approaches are becoming more realistic as more factors have been included. Spatial heterogeneity is an example. Heterogeneity is an inherent part of the environment and apparent in all habitat types creating a patchy, mosaic of natural landscape. Researchers have reported the extent of heterogeneity in the landscape, but surprisingly not yet included heterogeneity into biodiversity and ecosystem function (BEF) studies.

In recent years, empirical studies of marine systems have enhanced the BEF debate. Depauperate estuarine systems are ideal candidates for establishing model systems. In this study, estuarine microphytobenthos (MPB) were used as a response variable since the relationship between MPB and primary productivity is well-known. This relationship was exploited to employ MPB biomass as a proxy for primary productivity. Benthic chambers were used to assess the effect of macrofauna in single species and multi-species treatments on both ecosystem function and net macrofaunal movement. Heterogeneity was created through enriching sediment 'patches' with *Enteromorpha intestinalis*, providing areas of high and low nutrient. Heterogeneity, macrofaunal biomass, species richness, species diversity and flow were all varied in order to assess combined effects on the functioning of the system.

Heterogeneity was found to have a significant influence on ecosystem functioning and on macrofaunal movement, however, patch arrangement did not. MPB biomass was highest in patches containing organic enrichment suggesting that nutrients were obtained locally from the sediment/water interface rather than the water column. There was variation in MPB biomass with macrofaunal species, probably resulting from differences in behavioural traits. It was also evident that flow altered species behaviour, as there was a significant difference between static and flow treatments. This work shows the importance of heterogeneity for BEF relationships.

1. Introduction

1.1: Biodiversity and ecosystem function

The global cycles of elements and materials that keep life systems on Earth in balance would not be possible without the biogeochemical processing of the Earths' millions of biota. It is the biota, living organisms and associated activities, that make the Earth a truly unique place within the solar system (Loreau et al 2002). The Earths' biodiversity, that is all living species, play a role in keeping the Earth systems balanced. Many activities fundamental for human subsistence lead to biodiversity loss, conversely the existence of humans is also depended on species diversity for medicines, food, fibres, and other renewable resources. Less recognised is that biodiversity also influence other aspects of human well-being including access to clean water, crop growth and fresh air (Diaz et al 2006). Modern man is dramatically changing the distribution and abundance of biota and predicting the consequences of this is an important issue (Naeem et al 2002, Baumgartner 2007).

1.2: Species richness

The simplest measurement of biodiversity is the number of different species (species richness) that describes how many different species exist within an assemblage or community. Other more complex metrics include; balance of species across the community (species evenness) (Magurran 1998), the particular species present (species composition), the interactions among species, and the temporal and spatial variation in these properties (Symstad et al 2003). Each measurement has slightly different mathematical attributes; however the basic requirement is to provide a mathematical expression for the diversity of an assemblage that can be compared between samples, against time or correlated with functional attributes of the system.

1.3: Ecosystem function

Any metabolic process, or any transport or transfer of materials through an ecosystem can be described as an ecosystem function. Ecosystem functions, when beneficial to mankind, may be referred to as ecosystem services. Ecosystem services can be defined as the benefits human populations derive, directly or indirectly, from the biota (Chapin et al 1997). These ecosystem services include: disturbance regulation,

biological control, food production, raw materials, recreation and cultural resources. Biodiversity is expected to affect ecosystem functioning because the number and kinds of species present determine the specific traits represented in an ecosystem (Symstad et al 2003). Species traits such as feeding, burrowing, and movement may directly mediate energy and material fluxes or may alter abiotic conditions (e.g. disturbance, climate, and limiting resources) that regulate functional rates (Biles et al 2001, Heisse et al 2007).

1.4: Measuring ecosystem function

Ecosystem functioning is measured in a way that captures the specific metabolic process, transport or transfer of materials through an ecosystem. At first this would seem to allow for many types of measurements however after consideration it is often found to be quite limited. Metabolic processes are hard to measure directly, and therefore primary productivity is often measured. In addition, the standing stock of chlorophyll a (chl a) can be used, as a proxy for productive potential (Honeywill et al 2002) and this was the approach applied in this thesis.

1.5: Species Loss

There have been several key events in the last decade that have highlighted the fragile balance between technological progress, biodiversity and species loss (Diaz et al 2006). One such condition is the threat of global warming and the impact this might have on the world's biota; the influence of decreasing biodiversity and species loss cannot be overlooked as a potential major consequence. Species loss is happening locally, nationally and globally, and therefore genetic diversity is also decreasing as a result of this (Hooper et al 2005). As the human population rises and the demand on natural resources and space increases, fragmentation along with destruction of habitats, could cause species loss (Diaz et al 2006); thus possible impact on ecosystem processes and society is not yet know.

Declining biodiversity is a consequence of global change drivers (e.g. climate, Hardman-Mountford et al 2005, biological invasions, Arenas et al 2006, and land use Buckley and Roughgarden 2004). Species loss is not indiscriminate, on average animals with longer life spans, bigger bodies, poor dispersal capacities, more specialised resource use, lower reproductive rates and other characteristics that make

species more susceptible to human activity (Mckinney and Lockwood 1999, Raffaelli 2004, Wright et al 2006) are more likely to become extinct. It is generally considered that species loss will adversely affect ecosystem function, however the mechanisms underlying this principle are not clear (Loreau et al 2001, Naeem and Wright 2003, Balvanera et al 2006, Cardinale et al 2006, Hector and Bagchi 2007,). It has been shown that ecosystem function measurements may vary under a variety of extinction scenarios (Solan et al 2004), so the way in which species are lost (i.e. body size, rarity, behaviour traits) will be important in predicting ecosystem functioning.

The consequences of mass species loss to humans are potentially huge; these include changes in the functioning of ecosystems that provide crucial services such as nutrient cycling and photosynthesis. Such species loss would have a direct effect on material goods, causing a loss of crops, natural resources, and even medicines. There would also be a loss of non-market values such as the aesthetic beauty of biodiversity. The scientific challenge is to predict the importance of a reduction in biodiversity, to ultimately improve environmental policy in protecting habitats and species richness (Hooper et al 2005, Fischer and Young 2007).

1.6: Environmental variables

To enable predictions to be made about the importance of biodiversity on ecosystem functioning, the environmental variables that influence ecosystem function rates need to be recognised. An environmental variable (e.g. light, temperature, water flow, wind exposure, CO_2, nutrients, soil composition, etc) is a natural variable that can cause changes in the way a species behaves, and therefore alters ecosystem function (Ives and Carpenter 2007). For example, plants require light, however there is a balance between too much light that it becomes damaging and too little light that the plant can no longer effectively photosynthesise. There is no set amount of light that is perfect for every species as each one is different in its requirements and has evolved to suit different conditions. Therefore, by analogy, under different environmental drivers species will respond differently, ultimately affecting ecosystem functioning.

Hector et al (1999) investigated the impact of loss of plant diversity on ecosystem functioning at 8 European field sites. The results showed a difference in detail at each location, but overall a reduction of ecosystem function with loss of species.

Geographical location and species composition were associated with the effects of plant diversity, indicating that site location played an important role in generating diversity and hence ecosystem function. This indicates that some habitats due to the geographical location will be more susceptible to change than others.

1.7: The development of biodiversity and ecosystem function (BEF) theory

The relationship between biodiversity (species richness) and ecosystem function was considered first by Darwin (1859). He proposed that an area occupied by a large number of species is more ecologically stable than if occupied by a smaller number. MacArthur (1955) extended this theory and developed a model (Fig 1.1a), which he named the diversity-stability hypothesis. This theory extended Darwin's (1959) by suggesting that trophic groups, along with number of species, would increase stability of the system.

More recent theories about the relationship between biodiversity and ecosystem function have been developed since the early considerations of Darwin and MacArthur. Ehrlich and Ehrlich (1981) have been accredited with the Rivet hypothesis (Fig 1.1b) that considers all species to be important and play a role in the functioning of the ecosystem. This theory likened species to the rivets holding together the wing of an aeroplane, several rivets can be lost before the wing falls off however it is difficult to predicted which rivets and how many can be lost before the total collapse. This theory proposes that a few extinctions will not affect ecosystem function since the influence of different species overlap. The functional role played by the now extinct species will be compensated by other species, therefore masking ecosystem degradation.

Walker (1992) proposed the redundant species hypothesis (Fig 1.1c), which was an extension of the rivet hypothesis. This theory suggested that relatively few species are needed to sustain ecosystem processes and above this threshold any addition species have little effect on ecosystem function. As a result some species may be expendable in terms of ecosystem maintenance if other species take over or replace the extinct species functional role. This is an extension of the rivet hypothesis as it considers which rivets are most expendable in response to ecosystem changes. This theory also proposes species to be segregated into functional groups, and proposes

that extinction of a species within a functional group is less detrimental to the system than extinction of a species without a functional group substitute.

The idiosyncratic hypothesis (Fig 1.1d) proposed by Lawton (1994), suggested that the direction and extent of change in ecosystem function with changing diversity cannot be predicted. This is due to the complexity of the system and relationships between species and their role in ecosystem. Schlapfer and Schmid (1999) consolidated all these theories by graphically representing them (Fig 1.1). These hypotheses provided a clear picture of what needs to be tested or rejected, the null hypothesis of no relationship between biodiversity as an independent variable and ecosystem functioning as a depended variable.

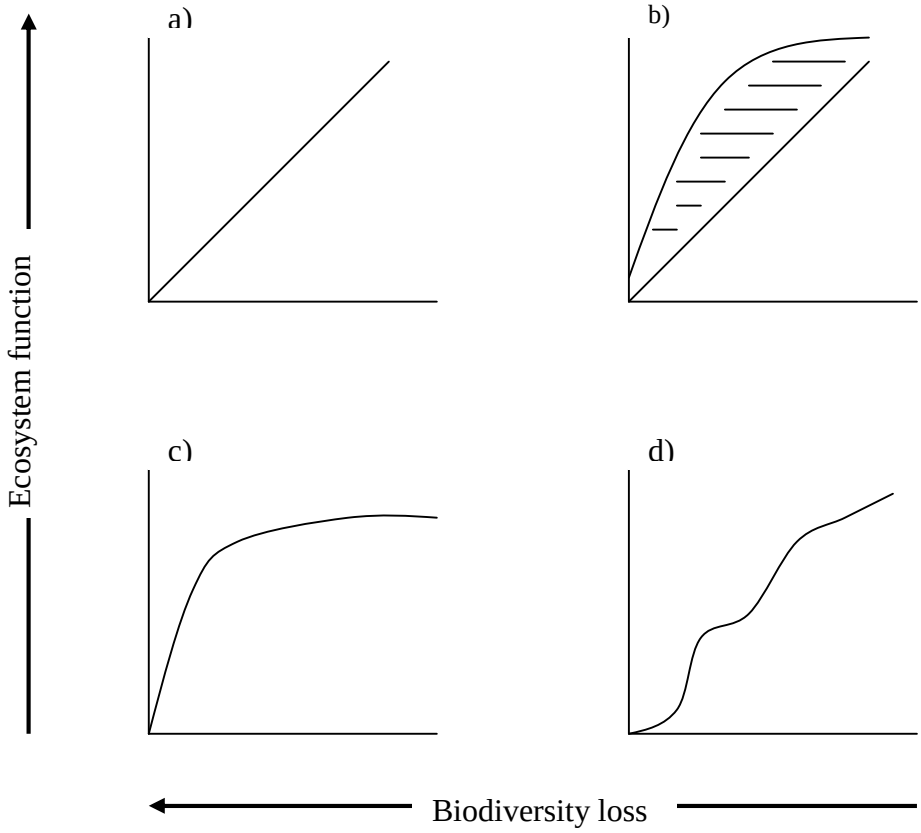


Fig 1.1 Graphical representation of the early theoretical relationships between biodiversity and ecosystem function (Schläpfer and Schmid 1999).

1.8: Early experimentation of BEF relationships

The graphical representation of the theories by Schläpfer and Schmid (1999) lagged slightly behind the experimental work that was developed to address the relationship between biodiversity and ecosystem functioning. Some of the early studies have been subjected to controversy (Naeem *et al* 1994, Tilman 1996, Chapin *et al* 1997 and Tilman *et al* 1997). This is largely due to the interpretation of the experimental response, whereby the experimental treatment has been affecting ecosystem function and not species richness *per se* (Naeem *et al* 2002).

In one of the early experimental tests, Naeem (*et al* 1994) constructed artificial ecosystem assemblages replicated in a sophisticated growth chamber called an Ecotron, to control environmental factors. This study found that loss of biodiversity might alter or impair the services that ecosystems provided. However a number of problems about the experimental design were highlighted, for example the ecosystem functioning effects observed could be influenced by the particular size and species of the plants chosen rather than diversity *per se.* This was shown in the experiment by including fast growing plant species that were only present in high-diversity systems and not present in the low-diversity systems; this has been named the 'selection probability effect' (Grime 1997; Wardle 1998). Therefore it could be explained that the high production shown in species rich assemblages could be due to these fast growing plants (Andre *et al* 1994). The experimental design also suffered from pseudoreplication, as sub-sets of species were sampled within bigger sets, this resulted in lower levels of diversity being nested in sets of higher diversity levels (Wardle 1998; Fukami et al 2001).

Another early experiment by Tilman *et al* (1996) found plant productivity and soil nitrogen utilisation increased with increasing plant species richness. This study used grassland plots with varying species richness, where species composition was randomly selected to avoid biases. The data showed that interspecific differences in the use of resources allowed more diverse communities to attain greater productivity, through the greater utilisation of limiting resources. However, this experimental design had a different problem, as species diversity was controlled by nutrient addition, it could not be determined if ecosystem function was being influenced by

species richness or directly through the nutrient treatment; this has been termed 'hidden treatment effect' (Huston 1997).

These early studies introduced more question than they addressed, the ensuing debate that surrounded the interpretations of the rapidly accumulating findings generated a sense that it was possible that biodiversity really did not matter (Naeem *et al* 2002). Lessons were learnt, however, the early designs and experiments taken forward into the new millennia included not only terrestrial studies, as traditional, but also freshwater, intertidal and marine habitats. The new challenges are for experimental designs to include a variety of environmental drivers that best represent the natural habitats and predicted scenarios of global change.

1.9: Studying BEF in aquatic environments

The seabed is the most extensive habitat on the planet, occupying at least 75% of the earth's surface. It therefore follows that fluxes of materials across the sediment-water interface and mechanisms that mediate and constrain those fluxes are likely to have global significance (Raffaelli *et al* 2003b). The ecological significance of estuaries as an interface between land and sea has long been appreciated (Rees 2003). More recently, the economic importance of estuaries and coastal embayments has been recognised for the wide range of processes that occur, from which the wider environment and human society benefits. Estuarine and coastal environment are some of the most productive and diverse communities on the earth and have a high value to human society (Costanza *et al* 1997, Meire *et al* 2005). The ecosystem services provided are of global importance to climate, nutrient budgets and primary producers (Falkowski *et al* 1998). Other services also include storage of sediment, flood defence and storm buffering, maintenance of water quality and support of coastal and marine food chains (Crooks and Turner 1999). In addition, humans benefit from activities including fishing, recreation, waste disposal and aquaculture. However, the contributions that coastal ecosystems make to these ecological processes are compromised by anthropogenically induced stresses including over-fishing, habitat destruction and pollution (Worm *et al* 2006).

The value of coastal and estuarine environments and their catchments has been demonstrated and therefore it is also important to investigating the relationships

between biodiversity and ecosystem functioning (Boogert *et al* 2006, Naeem 2006). The advantages of studying the coastal and estuarine systems are based round the knowledge and experiences already gained (Solan *et al* 2006). Common macrofauna species have been described and their patterns of abundance, biomass and life cycles are well-documented. Estuaries are species poor habitats in comparison to some marine and terrestrial environments. This enables a clearer relationship between species richness and function to be established; as the lower levels of diversity have the greatest change in ecosystem function (Lawton 1994, Tilman and Downing 1994). The tools and techniques that have been established enable the effective assessment of ecosystem functioning. Experimental systems are relatively easy to establish and response times are relatively rapid which permits a more complex design. Finally, the long history of research and results not originally used to demonstrate biodiversity and ecosystem function relationship can be re-interpreted in this way (Raffaelli 2003a). Using these numerous advantage to disentangle the relationship between biodiversity and ecosystem functioning in estuarine systems, much progress has already been achieved (for review see Covich *et al* 2004).

1.10: Measuring ecosystem function in estuarine environments

Many methods can be used to measure ecosystem function in the estuarine environment, however, the bioturbatory effects of the infauna influence them all. Therefore the affects of bioturbation, which include, mediating sediment shear strength, sediment resuspension, microbial diversity, nutrient release and primary productivity can all be used as functional measurements. As discussed in paragraph 1.4 (Measuring ecosystem function) this series of experiments uses a measure of standing stock (chl a) as a proxy for the productive potential of the systems to assess ecosystem function.

1.11: Bioturbation

Bioturbation is the mixing of sediment through biological processes; these include the actions of infauna, epifauna, fish and mammals that cause particulate movement (Cadée 2001). Bioturbation provides a number of important functions for the ecosystem, such as oxygenation of the sediment (Pelegri and Blackburn 1994) and enhances carbon and nutrient cycling (Aller 1982). Benthic infauna are major bioturbators of the sediment in marine and estuarine environments (Biles et al 2002,

Norling et al 2007). Infaunal species differ in their feeding behaviour and mode of movement, consequently creating different levels of disturbance to the sediment structure (Snelgrove 1999, Austin et al 2002).

1.12: Bioturbation and macrofaunal species

The macrofaunal species selected (*Hediste diversicolor, Hydrobia ulvae, Corophium volutator* and *Macoma balthica*), are known to be consumers of MPB, therefore there was an a priori reason to expect an effect on MPB biomass. This was detected through two different positive and negative effects, the positive effect is from macrofaunal bioturbation that releases nutrients from the sediment (Biles *et al.* 2001; Emmerson *et al.* 2001) needed for the growth of MPB. The negative effect is from macrofaunal grazing, where consumptions of large numbers of MPB can outstrip growth causing a reduction in biomass. The macrofauna used have different bioturbatory characteristics for example, *Hediste diversicolor* is a deep sediment bioturbator while *Hydrobia ulvae* bioturbates only the top few mm. These characteristics are variable and will depending on the prevailing environmental conditions such as water flow and availability of food. The behavioural traits associated with feeding and bioturbation for all the macrofauna selected for this series of experiments are described in the methods and materials (Chapter 2.4)

1.13: Bioturbation causes nutrient release

Bioturbation causes the release of nutrients from the sediment into the water column, fuelling primary production in the water column and at the sediment surface (Heip *et al* 1995). The nutrient within the system can come from external sources via the land or sea, and from within due to metabolic waste or decay. Nutrients are released as a result of several different processes in the ecosystem (Kristensen *et al* 1985) these include sediment disturbance from physical and biotic activities (Riedl *et al* 1972), metabolic activities of bacteria and mineral solubility and sorbtive mechanisms (McLusky 1981). Physical disturbances to the sediment through biotic, macrofaunal activity or abiotic processes such as water flow, has an effect on the release of nutrients. This can be directly through the sediment porewater (Paterson and Black 1999) or indirectly through facilitating microbial activity by increasing the surface area of the sediment, by promoting nutrient cycling (Loo *et al* 1996).

1.14: Bioturbation and microphytobenthos biomass

Microphytobenthos (MPB) is the term given to photosynthetic microscopic organisms adapted to surviving on estuarine and coastal sediments, and comprises of diatoms, euglenids and cyanobacteria (Consalvey et al 2004a). Bioturbation from macrofaunal disturbance of the sediment increases the release of nutrients, MPB utilises these limited resources during photosynthesis and the elevated levels increase MPB health and reproductive capability. Environmental variables such as nutrients, light, temperature, salinity and pH have all been demonstrated to affect the overall rate of photosynthesis (Colijn 1975, Admiraal 1977, Admiraal 1984). Bacteria in the sediment break down organic inputs, initially to NH_4-N then NO_2-N and finally NO_3-N, these compounds can then be utilised by MPB (Raven et al 1992). The nitrification of NH_4-N to NO_3-N is dependent on the depth of the oxic layer in the sediment. Oxygen concentrations in the sediment will have a large influence on nitrogen cycling, therefore bioturbation rates and depth of macrofauna will ultimately affect MPB biomass through resource availability.

1.15: Heterogeneity as an environmental variable

Most habitats are not uniform due to the distribution of organisms, and the heterogeneous nature of the habitat (Tilman 1994, Williams et al 2006). Habitat heterogeneity can be caused by biotic and abiotic factors. Environmental factors such as flow can cause erosion and deposition that will rearrange the sediment, sorting the sediment by particulate size, this action will cause heterogeneity in the landscape. Macrofauna species to some degree can engineer the habitat in which they live (Hastings et al 2007), either through behavioural traits (e.g. feeding, burrowing, tube building) or by just being present they can induce changes that cause heterogeneity. Habitat heterogeneity may also reflect fragmentation, the main cause of biodiversity loss (Wilcox and Murphy 1985), so understanding the relationship between heterogeneity and ecosystem functioning is of critical importance to biodiversity conservation.

Ecosystem functioning may be substantially affected by heterogeneity (Hovel and Lipcius 2001). Research shows that ecosystem functions that are affected by heterogeneity include maintenance of species diversity (habitat) as well as material and energy cycles (Franklin 2005). The importance of environmental heterogeneity in

determining species interactions in marine and freshwater environments has been demonstrated, at landscape (km) (Ellingsen and Gray 2002, Sanvicente-Anorve et al 2002, Bengtson et al 2006) regional (m) (Noren and Lindegarth 2005, Hovel and Lipcius 2001) and local (cm) (Hewitt et al 2002) scales. Some studies now include links to related functional attributes, for example, Jesus et al. (2005) provided a detailed analysis of microphytobenthos (MPB) distribution on the surface of an estuarine mudflat linked to the photosynthetic functionality at a cm scale.

Spatial heterogeneity at a variety of scales is a well-recognised feature of benthic habitats and the tractability of these systems under experimental conditions makes them a good model for investigating the ecosystem-level effects of heterogeneity. Therefore, it is surprising that the natural heterogeneity of ecosystems has rarely entered into the experimental analysis of ecosystem function (Cheng *et al.* 2007; Holzschuh *et al.* 2007). Enhancing our understanding of ecosystem function, particularly at larger scales, must therefore include investigation of heterogeneity effects and consider how to integrate these effects into the overall habitat performance (Hawkins 2004; Raffaelli 2006).

1.16: Macrofaunal response to heterogeneity

Heterogeneity within a system can be generated artificially; one way this can be done is by the addition of organic material (e.g. detrital material). Decaying macro-algae within an estuarine system is a good example and increases the organic content and, under the right conditions, supply nutrients locally within the sediment (Raffaelli 2000). MPB can also utilise these resources to enhance productivity and biomass (Admiraal, 1984). Field observations of localised enrichment have been shown to influence macrofaunal distribution within the estuary, *Hediste diversicolor* and *Hydrobia ulvae* are extensively found in areas with high localised enrichment whereas *Corophium volutator* are not (Raffaelli 1991; Lawrie *et al.* 2000; Raffaelli 2000).

1.17: Flow as an environmental variable

The surrounding fluid shapes the benthic ecosystem, and the influence of flow is an important component to be included in studies simulating estuarine and coastal areas.

In estuaries, flow can be the product of processes not apparent in fresh water systems. These include tidal effects and the mixing of water of varying salt concentration and density (McLusky 1981). Fluid dynamics in marine systems effect the type and size of substrata in benthic environments, the spatial configuration of habitat patches, the distribution of resources and the structure of biotic communities, including species richness (Austen et al 2002).

The difficulties of generating and characterising flow in laboratory experiments have limited the number of studies investigating biodiversity, ecosystem functioning and flow relationships. However one such study by Biles et al (2003) investigated the effects of flow on ecosystem functioning in an estuarine system using in situ benthic chambers. Flow was found to have a highly significant effect on ecosystem functioning in chambers containing macrofauna; however in macrofauna-free chambers, (controls) there was no flow effect. This indicated that flow generated an effect through promoting behavioural changes in macrofaunal bioturbatory activities causing greater disruption to the sediment and ultimately ecosystem function. Due to the change in behavioural activity flow has on macrofauna and the consequences it may have on ecosystem function, it is therefore important to consider this environmental driver in future biodiversity and ecosystem function studies.

1.18: Summary

Biodiversity and ecosystem functioning studies have evolved from mainly being carried out in terrestrial systems, and limited to species richness (biodiversity) as a driver for ecosystem functioning. The importance of including factor such as flow and heterogeneity into the system mean that studies now use a wide selection of drivers and include many ranges of habitats to create a more realistic paradigm for explaining the relationship between biodiversity and ecosystem functioning. Estuaries are depauperate systems, containing relatively few species and therefore limiting the choice of macrofauna that can be used in experimental systems, the species used in these experiments must therefore represent a large proportion of the macrofaunal composition. In addition, due to the relatively small numbers of species found in estuaries there is sound knowledge of the life-cycle, history and traits of all species. Therefore estuarine areas provide ideal habitats with proven methodologies

for investigate biodiversity and ecosystem function studies (Biles *et al* 2003, Ieno *et al* 2006).

1.19: Aims and objectives

This thesis will be the first to examine the influence of flow and heterogeneity on the relationship between biodiversity and ecosystem functioning for marine depositional systems. The objectives of this study were to develop a series of experiments that would build from each other in increasing complexity. In theory an unlimited number of drivers influence the biodiversity-ecosystem function relationship and the increasing complexity of experiments will to improve the models and the potential of the predictions made. However this may be a little ambitious in three years, so for this thesis the focus will be on including heterogeneity to address the following objectives:

1. Conduct experiments using macrofauna to investigate the effect of individual species on ecosystem functioning in a heterogeneous (2 patches) environment.
2. Conduct similar experiments using the same macrofauna to investigate the effects of species richness (biodiversity) on ecosystem functioning in a heterogeneous (2 patches) environment
3. Conduct experiments using macrofauna to investigate the effects of individual species on ecosystem functioning in both flow and static conditions in a heterogeneous (2 patches) environment.
4. Conduct experiments using macrofauna to investigate the effects of individual species on ecosystem functioning in a heterogeneous (multi patch) environment.

2. Materials and Methods

2.1: Study Site

The Ythan Estuary Newburgh Aberdeenshire (N 57° 20.085', W 02° 0.206') is a small estuary on the North East coast of Scotland. The Ythan was classified an SSSI in 1971 and included within the Forvie National Nature Reserve (NNR) in 1979. It is a Special Protection Area (SPA) due to its internationally important populations of wintering waterfowl where it regularly supports a population of 20,000 visiting birds. The estuary is about 8 km in length, with an average width of 300 m and has a tidal range of approximately 2.5 m. The sediment is muddy sand (mean particle size = 49.79 µm, silt content 61.5%), with an organic carbon content of 3.84%.

The site was chosen for the diversity and ample supply of macrofauna, and the close proximity to Oceanlab, Aberdeen University.

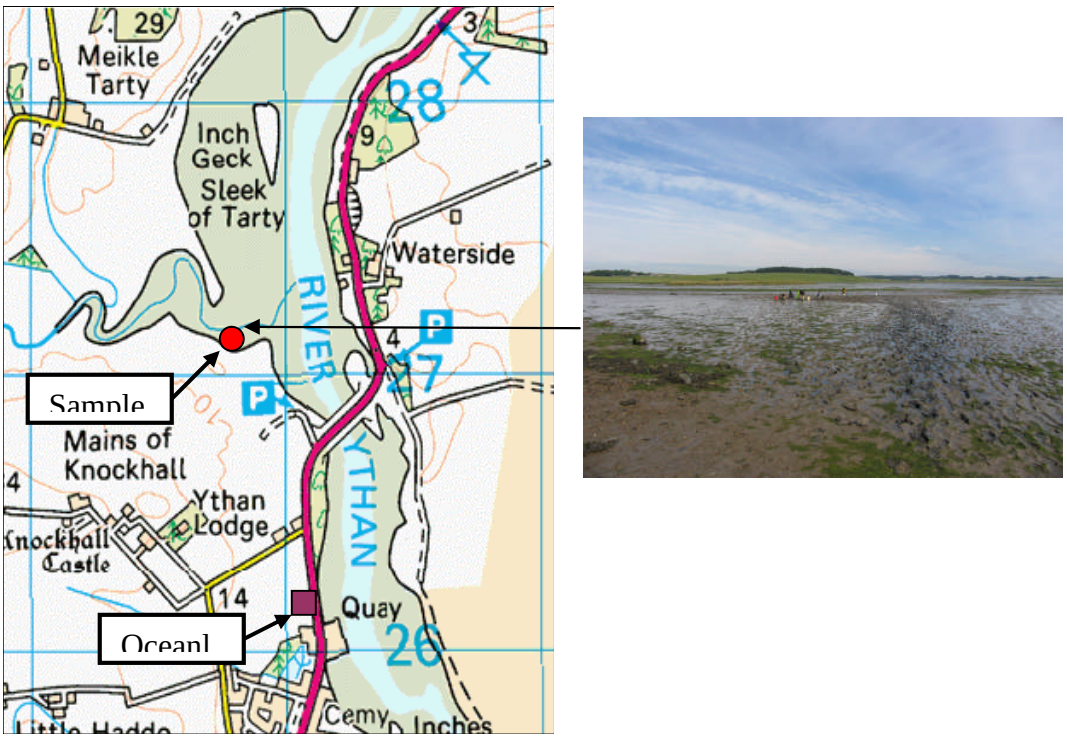


Fig 2.1: O.S. map of Ythan estuary and the sample site in the Sleek of Tarty, Ythan estuary, Aberdeenshire (Image courtesy of multimap at http://multimap.co.uk).

2.2: Collection of sediment

The top 10 mm of sediment was collect from the Sleek of Tarty mudflat, on the Ythan estuary. Dark brown patches where surface pigmentation was obvious were targeted for collection as it was assumed that these areas would be higher in microphytobenthos (MPB) biomass than unpigmented sediments (Consalvey *et al* 2004a). The sediment was brought back to the laboratory and sieved for the removal of unwanted macrofauna and macro-algae.

Past experiments have achieved the removal of macrofauna from the sediment using two alternative method, i) sieving the sediment through a 500 μm mesh (Ieno *et al* 2006), and ii) freezing the sediment for 24 h to kill all living macrofauna (Emmerson *et al* 2001). However, freezing the sediment kills but does not remove the macrofauna from the sediment and the decaying matter would be a source of nutrients to the system. Additionally, preliminary experiments demonstrated that freezing sediment decreased microphytobenthos (MPB) biomass, which would therefore affect a response variable from the planned experiments. Due to these concerns, in this series of experiments, the sediment was sieved (Fig 2.2) to preserve MPB integrity and to remove unwanted macrofauna from the system.

Fig 2.2 Sieving sediment into water bath

The sediment was sieved into a seawater bath through 500 μm mesh to remove macrofauna (Fig 2.2); the sediment was then left to settle for 24 h in order to retain

the finer particulate fraction (<63 μm). Excess water was removed and the settled sediment was homogenised to slurry that facilitated distribution between mesocosms.

2.3: Collection of macrofauna

The macrofaunal species selected for this work were known to have different functional characteristics and to be consumers of diatoms. Therefore there was an *a priori* reason to expect an affect on MPB biomass, both through the direct effect of grazing (Kamermans 1994, Smith *et al* 1996, Hagerthey *et al* 2002) and through the indirect influence of nutrient release through sediment bioturbation (Emmerson *et al* 2002).

Corophium volutator and *Macoma balthica* were collected from the banks of the Sleek of Tarty (Fig 2.3). Sediment containing these species was sieved on site using a 500 μm mesh, and the sieved material, containing the selected species plus organic material, shells, and stones, was taken back to the laboratory for further cleaning.

Fig 2.3: Collecting *Corophium volutator* and *Macoma balthica* from the Sleek of Tarty

The collection of *Hediste diversicolor* was achieved by digging into the sediment to a depth of 200 mm and turning it over to reveal the polychaete worm burrows. The sediment was than peeled apart along the burrow until the animal was found, this was then picked out and placed into a bucket full of seawater (Fig 2.4) and taken to the laboratory for further cleaning.

Fig 2.4: Digging for *Hediste diversicolor* on the Ythan estuary.

Hydrobia ulvae is a surface dwelling macrofaunal species that was separated as a side product during sediment collection (paragraph 2.2; Fig 2.5). During the sediment sieving process *Hydrobia ulvae* were collected into a container filled with seawater and retained in the laboratory.

Fig 2.5: Sediment collection containing *Hydrobia ulvae*.

All macrofaunal assemblages brought back to the laboratory were treated to remove all unwanted organic matter and the unwanted macrofauna species. The required macrofauna were held in aerated tanks for no longer than 48 h before being

introduced into the experiment. Macrofauna were added to the mesocosms on day 0, where they were confined to their patch of sediment by a Perspex divider. Macrofauna were observed for 30 min after addition to the mesocosm to ensure that all animals were behaving normally and apparently healthy, and any animals that were not were replaced.

2.4: Macrofaunal description

The polychaete worm *Hediste diversicolor* constructs a burrow in the surface layers of sediment. An examination of the gut contents of small *Hediste diversicolor* by Perkins (1958) showed this species to be highly adaptive with many methods of feeding. These include surface feeding whereby resources are scavenged from the sediment surface, or sub-surface feeding that involves feeding on fragmented particulate organic material within the substratum. Finally *Hediste diversicolor* can suspension feed (Riisgård 1991) which involves the secretion of a mucus net near the entrance to the burrow; the burrow is then irrigated by undulations of the body so that a current of water passes through the net. Small particles carried by the current are trapped on the net, and when sufficient quantity has accumulated the net and the particles are eaten together (Harley 1950).

The gastropod *Hydrobia ulvae* is a surficial grazer consuming organic matter including MPB (Defew *et al* 2002a, Hagerthey *et al* 2002). *Hydrobia ulvae* grazes on the sediment surface during periods of tidal exposure and will burrow a few mm under the sediment surface during periods of high tide (Barnes 1986). *Hyrobia ulvae* also has the ability to migrate large distances by floating on the surface tension of the advancing and receding tide. This allows *Hydrobia ulvae* an alternative method of planktonic feeding and dispersion from high-density areas (Green 1968).

Macoma balthica is a bivalve that can feed by one of two methods (Olafsson 1986). Either by a grazing at the sediment surface by extending the siphon and feeding on the sediment surface (de Goeij 1998), in doing so leaves the characteristic furrows on the sediment surface about 5 mm wide (Swennen & Ching 1974). *Macoma balthica* can also suspension feed while still buried in the sediment since a siphon can be extended through the sediment and used to feed on particulate organic matter, suspended in the water column at the surface (Kamermans 1994, de Goeij 1998).

The amphipod *Corophium volutator* constructs a U-shaped burrow in the top 200-400 mm of the sediment. The burrow is irrigated by *Corophium volutator* and aerated by the consequent transfer of water. Irrigation of the burrow is turbulent and results in fine sediment being suspended in the water column (Green 1968). *Corophium volutator* is a detritivore consuming organic matter and the associated micro-organisms (http://www.marlin.ac.uk).

Table 2.1: Feeding mode and functional type of macrofauna (Green 1968, http://www.marlin.ac.uk)

Species names	Detritvore	Grazer	Scavenger	Preditor	Filter feeder	Sufical modifier	Biodiffuser	Regenerator	Suspension feeder
H. diversicolor	*	*	*	*		*	*		*
H. ulvae	*	*				*			
M. balthica	*	*			*	*			
C. volutator	*	*				*	*	*	*

2.5: Algal collection for nutrient enrichment

The macro algae, *Enteromorpha intestinalis**, was collected from the Ythan estuary and used as a source of organic nutrient enrichment. The algae was washed to remove fauna and particulate matter, and the clean algae were then left to air dry for up to 24 h. The air-dried algae was placed in an oven at 60°C for 2 h to remove all moisture but preserve the organic content. The algae was then milled to a fine powder (Fig 2.6) and weighted into 1 g portions for use to enrich a patch in a mesocosm.

* NB: recent genetic testing changed this species to *Ulva intestinalis*; the reader should carry this change forward throughout this thesis.

Fig 2.6: Powdered *Enteromorpha intestinalis* used for sediment enrichment

2.6: Mesocosms

Mesocosm are semi-enclosed systems of varying sizes and composition. They are containers used in all areas of science to create a controlled micro-habitat. Inputs to the system can be controlled and therefore any change in the output is accountable to variation in input.

Table 2.2: Experimental designs for the thesis outline.

Chapter	Type	Mesocosm	*H. diversicolor*	*H. ulvae*	*M. balthica*	*C. volutator*
3	Single species	2 patch, static	*	*	*	*
4	Single species	2 patch, flow	*	*		*
5	Multi species	2 patch, static	*	*	*	*
6	Single species	Multi patch, static	*	*		*

2.6.1: Static mesocosms

Static mesocosms have been used successfully (Emmerson *et al* 2001, Raffaeli *et al* 2003a, Ieno *et al* 2006) to determine the effects of macrofaunal behaviour on ecosystem functioning. In the present study, the mesocosm design for static experimental conditions were non-transparent plastic aquaria (21 × 15 × 14 cm; Fig 2.7). Mesocosm were covered with a transparent film to allow light to penetrate but prevent water loss.

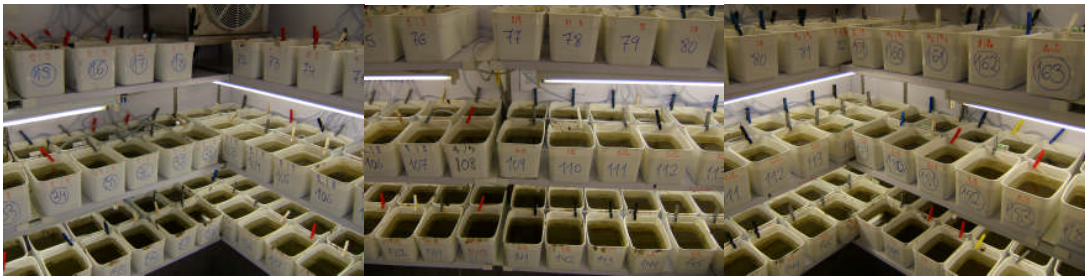


Fig 2.7: Static mesocosms

2.6.2: Flow mesocosms

Water flow is required to simulate a realistic estuarine or coastal environment. Difficulties in generating and characterising flow in experiments have, however, limited the number of studies that have used flow. Biles *et al* (2003) successfully used flow chambers to show the modifying effect of flow on ecosystem function, the flow chambers were set to reproduce a flow velocity profile present in natural aquatic systems. In this study, the flow regime used has been set up using the example from Biles (2003), therefore all flow chambers were set to 34 RPM approximate to 6 cms^{-1}.

The chambers consisted of a Perspex cylinder (200 mm dia., 300 mm height), with a removable lid. The lid housed the motor and paddle device (revolving skirt) that produced the flow. The motor was a RS 440.082 capable of generating speeds from 21 to 84 RPM, creating velocities of 0.14 to 12 cm s¯1. A central mains controller powered the motors with the capability of varying the speed of individual mesocosms. The revolving skirt (150 mm dia.) near the top of the chamber was responsible for generating flow (Fig 2.8). The revolving skirt created an annular flow with greatest

bottom shear stress towards the outer edge of the chamber. Shear stress varied across the bed due to wall effects (friction at the wall) and the action of the skirt (Biles 2002).

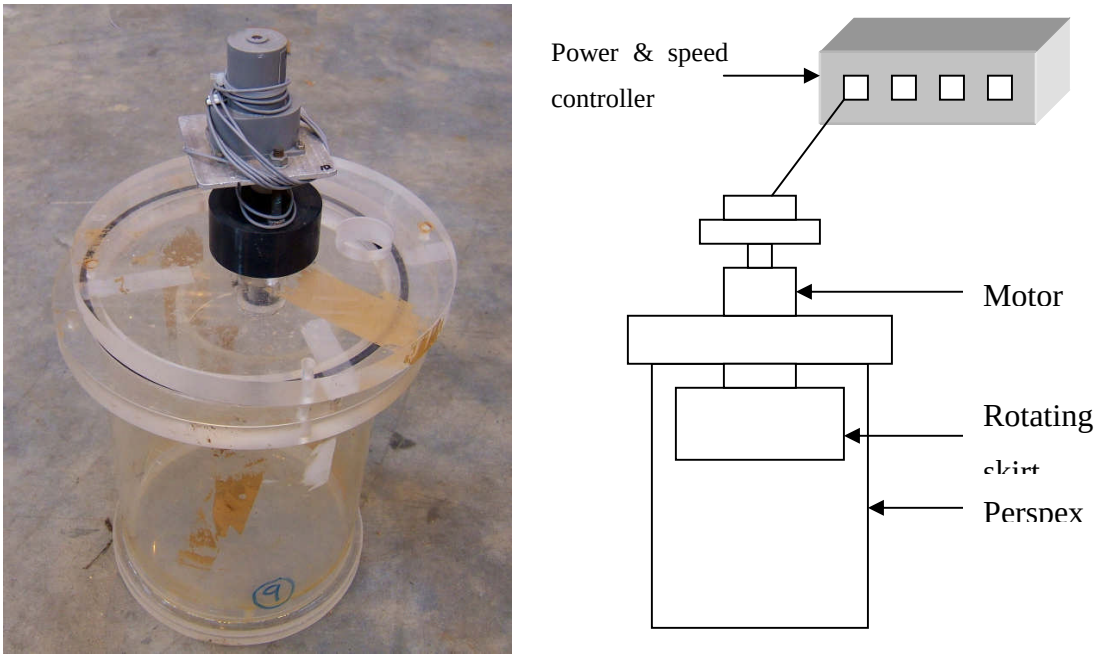


Fig 2.8: Flow mesocosm with a diagrammatic representation of the set up.

2.6.3: Multi patch (hexagonal) mesocosms

The multi patch mesocosm was made up of inter-joining hexagons, the hexagonal shape was chosen for the tessellating qualities and closest shape to a circle in order to minimise edge effect. The hexagons are approximately 900 mm across, which is the naturally occurring patch size for the experimental species in the Ythan estuary (Lawrie *et al* 2000).

The mesocosm consisted of an outside tank (length 1000 mm, width 750 mm, height 150 mm). In the tank, an inner boundary marked the shape of 22 tessellating hexagons (6 × 4), consisting of 4 rows containing either 5 or 6 hexagon (Fig 2.9a). The multi patch mesocosm was capable of housing a 22-hexagon divider, this was placed into the tank within the Perspex boundary markers to isolate each patch (Fig 2.9b).

a) b)

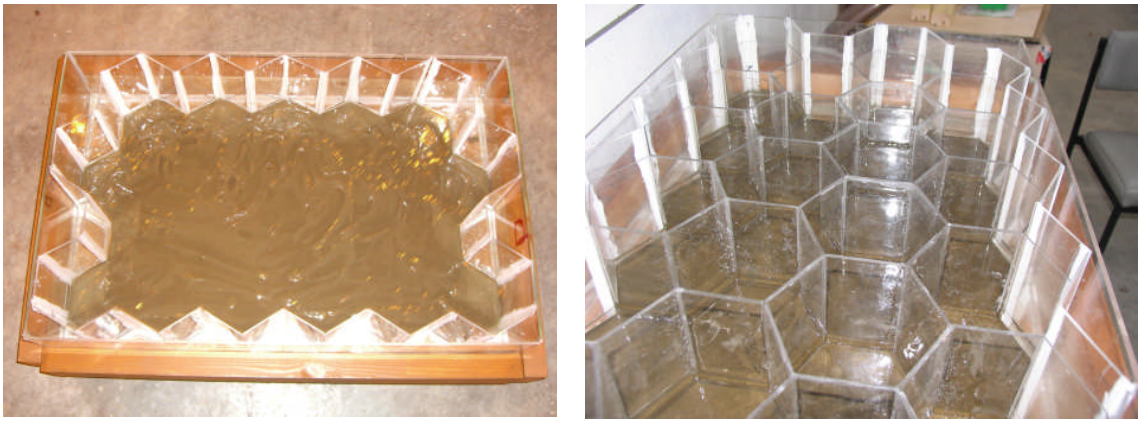


Fig 2.9a Multi-patch mesocosm without divider, **Fig 2.9b** Hexagon divider in mesocosm.

2.7: Mesocosm set up and experimental testing

All mesocosms had a sediment depth of 30 mm and were filled with seawater (UV-sterilised, 10 μm pre-filtered, salinity ≈ 33). Seawater was siphoned and refilled after 24 h to remove the initial flux of nutrients released from the sediment during the disturbance of sieving (Emmerson *et al* 2001, Ieno *et al* 2006). Care was taken during filling to retain the structure of the sediment surface to reduce nutrient flux and preserve the MPB biofilm. The mesocosms were held in a constant-temperature room (11 ± 2.0 ºC) with a 12:12 h light-dark regime (1 × 26 mm Ø white fluorescent tube per 8 mesocosms, model GE F36W/35; 36W, 3500ºK, 80-100 μE m^2 s^{-1}).

2.8: Incorporating heterogeneity

Powdered *E. intestinalis* was used to create heterogeneity in the experimental environment. The simple mesocosms (chapters 3-5) contained only two patches, created by dividing the mesocosm in half. Under experimental conditions powdered algae could be added to neither, either or both patches to create variation in the system (1 g of powdered algae in a patch was equivalent to 126 g m^{-2}, that is found natural in the Ythan estuary, Raffaelli, 2000). For this arrangement, the mesocosm was considered as a whole where both patches were measured to give a total (average) reading for the two patches (Fig 2.10a). Enrichment was as follows, E|E, E|NE or NE|NE, where "|" is the interface between the two patches, E is an enriched patch and

NE is a non-enriched patch. The patches were also measured individually to give a reading for each patch, the reading was taken from the left side of each experimental mesocosm which has been termed the 'focus' patch and the 'neighbouring' patch was coded with the enrichment type e.g. E or NE, thus four interface types were formed, E|E, E|NE, NE|NE and NE|E (Fig 2.10b).

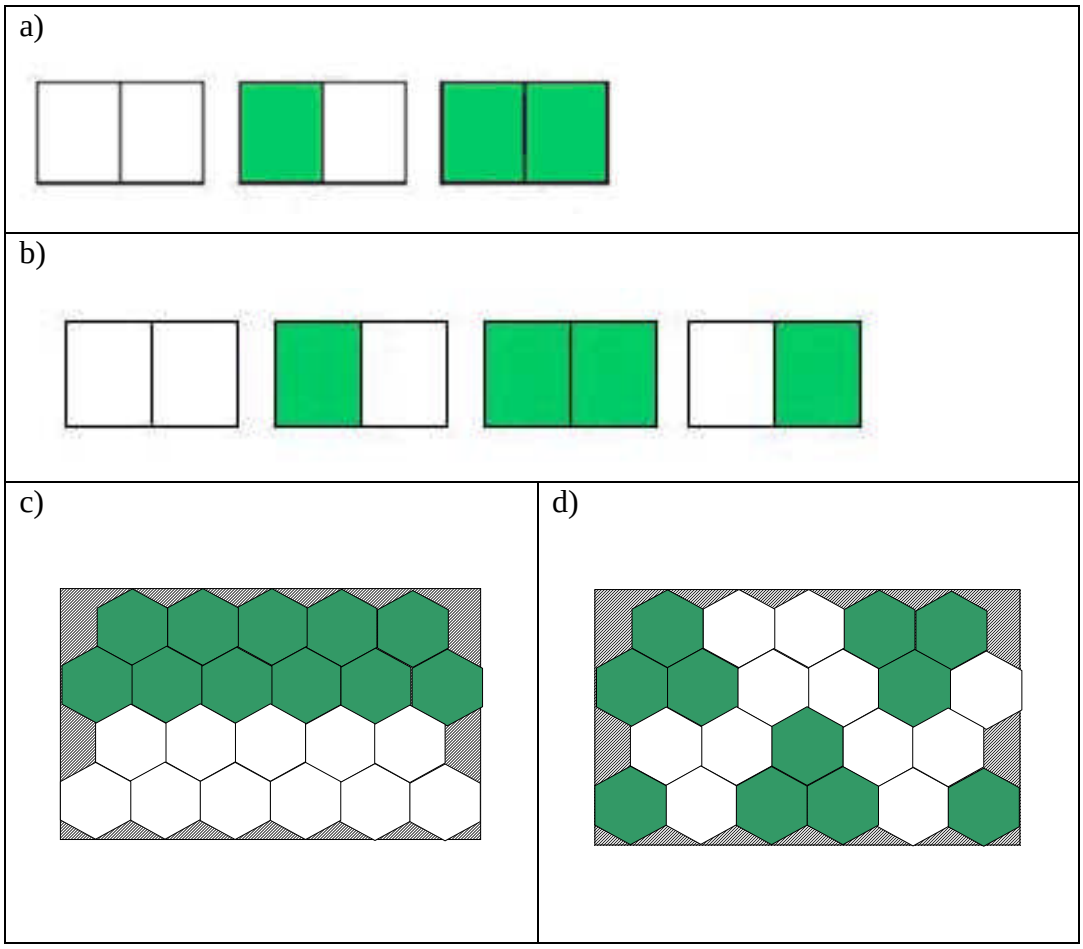


Fig 2.10: Mesocosm enrichment for two-patch and multi-patch experiments, a) is the simple enrichment design for mesocosm experiment, b) is the simple enrichment design for two-patch experiments, c) is the basic design for multi-patch experiments and d) is the fragmented design for the multi-patch experiment. Where all green patches represent enrichment with powdered *Enteromorpha intestinalis* and white patches represent non-enrichment.

There were two designs for the multi patch mesocosms (Fig 2.10c & d). The first arrangement was similar to the simple two patch experiment previously described

where the mesocosm was divided in half, however the multi-patch design was on a larger scale and each half consisted of a total of 11 patches (Fig 2.10c). The second design was fragmented and had 5 enriched areas (total of 11 patches) surrounded by non-enriched patches (Fig 2.10d).

2.9: Macrofaunal biomass

Macrofaunal biomass was either determined by counting individuals (*Hediste diversicolor* and *Macoma balthica*) or by wet weight (*Hydrobia ulvae* and *Corophium volutator*). Macrofaunal biomass was standardised for each species, the maximum biomass used was the natural carrying capacity for each individual species in the Ythan estuary (Table 2.3).

Table 2.3: Natural carrying capacity of macrofaunal species of the Ythan estuary.

Natural density (%)	0%	25%	50%	75%	100%
Corophium volutator	0g	0.25g	0.50g	0.75g	1.00g
Hydrobia ulvae	0g	0.50g	1.00g	1.50g	2.00g
Macoma balthica	0	1 indi*	2 indi*	3 indi*	4 indi*
Hediste diversicolor	0	1 indi*	2 indi*	3 indi*	4 indi*

* indi = individual/s

2.10: Macrofaunal movement

The standardised biomass of macrofauna were added to the mesocosm on day 0 and confined to their patch for 24 h. This period was need for the macrofauna to make burrows and become established in the patch. Once the dividers were removed on day 1 the macrofauna were free to move within the whole mesocosm. The experiment was run for 10 d, the dividers were then placed back into the mesocosm, and the sediment was removed from each patch, sieved and the macrofauna biomass recorded. *Hediste diversicolor* and *Macoma balthica* were counted directly at the sieving stage, however, obtaining a wet weight for *Corophium volutator* and *Hydrobia ulvae* was not possible at this stage due to time constraints and to avoid problems with variability in wet weights. Therefore, the individual numbers of *Hydrobia ulvae* and *Corophium volutator* were counted at a later date.

Counts for each patch were divided by the total count for the whole mesocosm to give a patch percentage. The percentage data was then used with the initial wet weight to produce a measurement in grams for each patch. Net movement could then be assessed as a migration of macrofauna away from the focus patch or immigration into the focus patch.

Calculations

Start (Day 0)

Wet weight:	Xw	Right patch
	Yw	Left patch
	$Xw + Yw$	Total mesocosm weight

Termination (Day 10)

Counts:	Xc	Right patch count
	Yc	Left patch count
	$Xc + Yc$	Total mesocosm count
	$\frac{Xc}{Xc + Yc} \times 100 = Xc\%$	Percentage in focus patch
Weights:	$\frac{X + Y}{100} \times Xc\%$	Focus patch weight

2.11: Ecosystem function measurement

The ecosystem function selected was a measurement of primary productive potential of the system as assessed through the standing stock of MPB (Chl a). This was achieved by measuring microphytobenthos (MPB) biomass using a pulse-amplitude modulated (PAM) fluorescence meter. The value (Fo^{15}) obtained by this mechanism is a proxy for Chlorophyll a (Honeywill *et al* 2002, Consalvey *et al* 2004b) and hence the potential for primary production. Measurements of MPB biomass were taken on day 6, this interval was appropriate because the combination was optimum for the MPB biomass and species activity (Defew *et al* 2002b). Mesocosms were dark-

adapted for 15 min, to optimise MPB biomass estimates from the Fo^{15} output (Honeywill *et al* 2002).

The Hansatech™ FMS2 with a blue measuring light was used during this study to measure MPB fluorescence. The FMS2 was set Gain = 99; Modulation Frequency Level = 3; Minimum Fluorescence Measurement Duration 2.8 μs. The saturation beam was set to 60 bits; which was calibrated to 10 000 μ mol $m^{-1}s^{-1}$ and lasted for 1 s. The setting and calibration are after Honeywill (2001). The probe was always 4 mm from the sediment/biofilm surface.

2.12: Data analysis

A GLS (Generalised Least Squares) (Pinheiro & Bates, 2000) statistical mixed modelling approach was used to assess the experimental hypotheses in Chapters 3 and 5. GLS allows for heterogeneity of variance within variables in a linear regression framework by incorporating variance-covariate terms linked with these variables. As a first step, a linear regression model was fitted. Model validation was applied to verify that underlying statistical assumptions were not violated; normality of residuals was assessed by plotting theoretical quantiles versus standardised residuals (Q-Q plots), homogeneity of variance was evaluated by plotting residuals versus fitted values, and influential data points were identified using Cook's distance method (Quinn and Keough 2002). The validation procedure showed that there was no evidence of non-linearity but there was evidence of unequal variance among the explanatory variables. A GLS framework was preferential over linear regression using transformed data because it retains the structure of the data whilst accounting for unequal variance in the variance-covariate terms. Analyses were performed using the 'R' statistical and programming environment (R Development Core Team 2005) and the 'nlme'package (Linear and nonlinear mixed effects models; Pinheiro *et al* 2006). The GLS model was refined by manual backwards stepwise selection using maximum likelihood (ML) to remove insignificant terms, and the final model was presented using restricted maximum likelihood (REML) (West *et al* 2007). The highest potential level of interaction that was assessed under these analyses was the three-way interaction terms. The statistical outputs of these models are based on the comparisons of the first level within each term with all other levels; no other within level comparison is

possible. To assess the importance of individual independent variables, a likelihood ratio test was used to compare the full optimal model with models in which the independent variable, and all the interaction terms it was involved in were omitted.

A LMM (Linear mixed model) (West *et al* 2007) statistical mixed modelling approach was used to assess the experimental hypotheses in Chapters 4 and 6. LMM allows, in addition to heterogeneity of variance, for random effects and auto-correlation to be incorporated into the model. The basic model validation procedure is the same as GLS, however prior to manual backwards stepwise regression the base model was tested for random and auto-correlation parameters, the AIC value was used as an indication of improvement. LMM was then refined in the same way as the GLS model.

3. Ecosystem function for single species in static mesocosms

3.1: Introduction

Biodiversity and ecosystem functioning studies in the shallow water marine systems have been limited in number and scope compared to terrestrial studies. However, marine systems are amenable to experimentation and manipulation and to-date several studies in marine systems have supported the hypothesis that biodiversity can have a significant effect on ecosystem function (Emmerson *et al* 2001, Raffaelli *et al* 2003b, Ieno *et al* 2006). A strong empirical approach is being developed using marine systems to test ecosystem theory concerning biodiversity-ecosystem function relationships. System designs are becoming more complex allowing for more variables to be included and tested.

Heterogeneity is a natural feature of the landscape and it is rare to find an entirely uniform habitat (Lovett *et al* 2005). Organisms exhibit a patchy distribution often reflecting the varied nature of the system. Heterogeneity has functionally important consequences for the productivity and services provided by an ecosystem and considering its recognised importance it is perhaps surprising that heterogeneity has not been included into ecosystem function experimental designs before now. The relative importance of habitat variation (e.g. "patchiness") on macrofaunal movement is not well-known, although evidence from laboratory experiments have shown the macrobenthic species can be selective with clear habitat preferences (Meadows 1964; Benedetti-Cecchi et al 2003; Fraschetti et al 2005). For example, field observations have shown that *Corophium volutator* are not found in areas that have high levels macro algae (Raffaelli 1999 and 2000). Therefore variation in habitat is likely to trigger a different movement response depending on species identity.

In this study, mesocosm experiments were developed to ascertain the effects of heterogeneity, species identity and species density on MPB biomass and net macrofaunal movement. MPB biomass was measured as a proxy for ecosystem function (production) using a pulse modulating amplified (PAM) fluorescence meter. A bipartite heterogeneous environment was artificially created in experimental microcosms by the addition of a nutrient source (powdered *Enteromorpha intestinalis*) to create two patches (enriched or non-enriched) in each test system.

3.2: Materials and Methods

3.2.1: Sediment and macrofauna collection

The collection and treatment of sediment and macrofauna are described in Chapter 2 (Materials and Methods).

3.2.2: Experimental design

The experimental designed included 396 mesocosms, divided randomly and equally between two runs, to determine the effects of macrofaunal species identity, macrofaunal species biomass and algal enrichment on MPB biomass (Fig. 3.1). Two patches were established in each mesocosm. The deposit-feeder *Hediste diversicolor* (Polychaeta), the surficial grazer *Hydrobia ulvae* (Gastropoda), the regenerator *Corophium volutator* (Crustacea) and the suspension/deposit-feeding (bivalve) *Macoma balthica* were added to the mesocosms on day 0. Macrofauna were confined to the initial patches where they were added for a period of 24 h by using Perspex dividers. Combinations of macrofaunal biomass (0, 25, 50 and 100% of natural macrofauna density in the Ythan estuary in either the left and right patches. i.e. 16 possible combinations) were established for all possible interface combinations of patch arrangements (E|E, E|NE, NE|E and NE|NE where '|' represents the interface and E = enriched and NE = non-enriched, the measured patch is on the left of '|', neighbouring patch is coded for on the right) for each of the 4 macrofaunal species (Fig 3.1). For *Macoma balthica* and *Hediste diversicolor*, whole individuals were counted and 4 individuals patch^{-1} was taken as analogous to the natural density on the Ythan estuary. For *Hydrobia ulvae* and *Corophium volutator*, the natural wet weight biomass was determined and appropriate proportional wet weights added to the mesocosms.

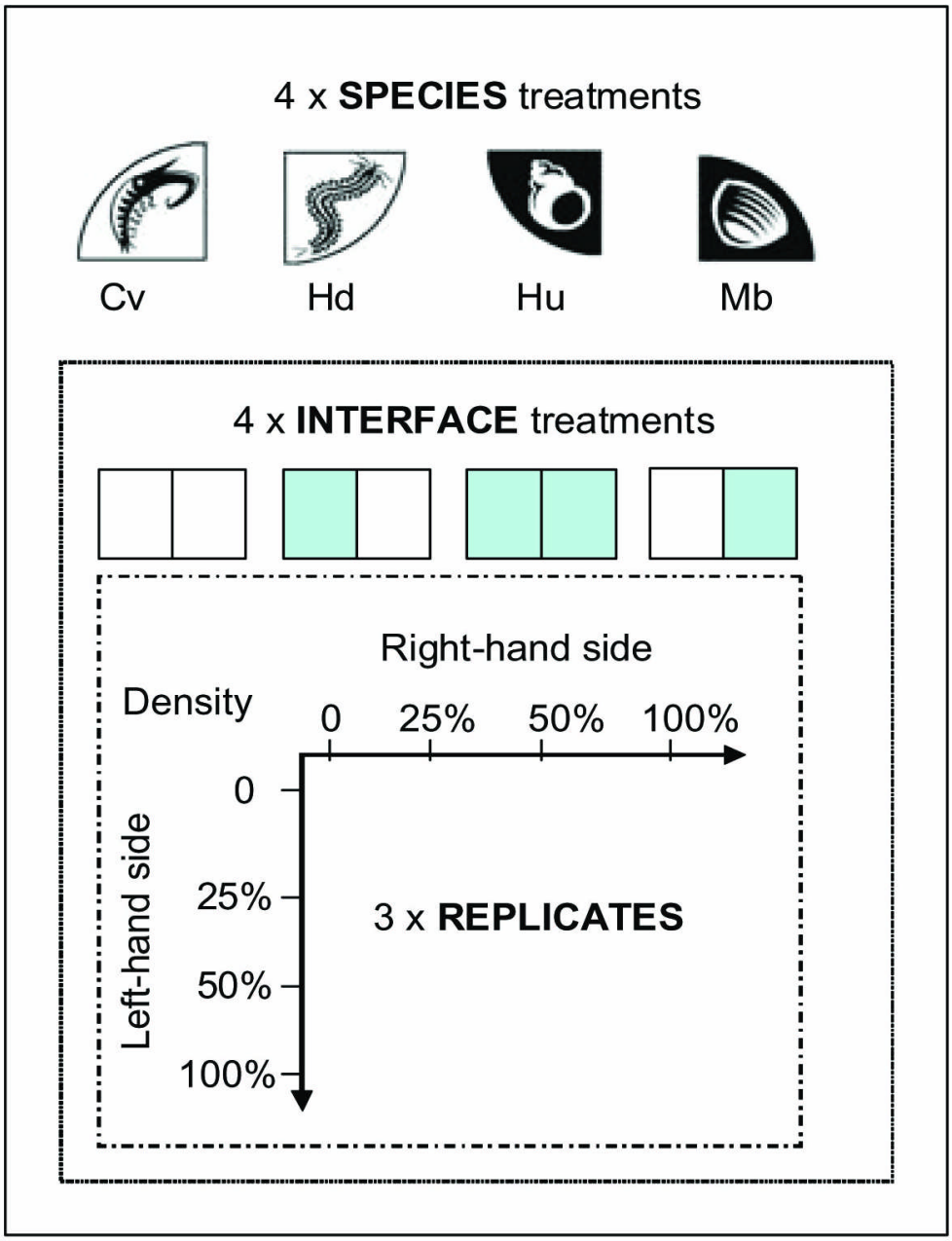


Fig 3.1: Overview of experimental design. The species-density gradients across the patch interface were established at the start of the experiment, using the relative levels of 0%, 25%, 50% and 100% of natural density at the study site. These combinations were used for each of the four interface treatments (enriched is green and non-enriched is white), and every species density-interface combination was used for each of the four species (*Corophium volutator* (Cv), *Hydrobia ulvae* (Hu), *Macoma balthica* (Mb) and *Hediste diversicolor* (Hd)).

Each configuration was replicated 3 times. An emergent property of the experimental design allowed the influence of the differential between the initial and final biomass of the macrofauna set in adjacent patches to be analysed. For this, the initial differential was expressed numerically as the relative biomass difference between the measured patch and the adjacent patch. A score of "4" was given when the maximum biomass of macrofauna were in the measurement patch with no macrofauna in the adjacent patch; a score of 0 was given when the biomass distribution was equal between adjacent patches, and "-4" given when the maximum biomass of macrofauna were in the non-measurement patch, with no macrofauna in the measurement patch.

3.2.3: Fluorescence measurements

Fluorescence readings were taken on day 6 of the experiment. This time period was chosen as an appropriate length of time that best captures the changes caused by species behaviour without the fluorescence levels dropping below a reliable limit as a consequence of the laboratory conditions, as discussed by Defew *et al* (2002b). The measurable output of the PAM fluorescence meter is Fo^{15} (no units) this value is representative of the chlorophyll *a* biomass present on the sediment surface (Serôdio *et al* 2001, Honeywill *et al* 2002) and can therefore be used as a proxy for primary production (Consalvey *et al* 2004b).

Two fluorescence readings were taken $patch^{-1}$, enabling the MPB biomass to be assessed as a whole mesocosm (average of 4 readings) and also at a patch level, where the measured 'focus patch' (average of 2 readings) was taken noting the enrichment code for the 'neighbouring patch'; the code is either enriched or non-enriched. This arrangement allows ecosystem function to be modelled at two spatial scales. Measured as a whole mesocosm where the effects of algae are shown by 3 types of treatment (E|E, E|NE & NE|NE), and measured at the patch level, where the effects of interface are shown by 4 treatment types (E|E, E|NE, NE|E & NE|NE)

3.2.4: Macrofaunal net movement

Macrofaunal net movement measurements were taken on day 10. The content of each patch was isolated using a Perspex divider, collected and sieved and the macrofauna counted as described in Chapter 2. Net macrofaunal movement was measured by unit change from the focus patch (a positive value was movement from the focus patch to

the neighbouring patch and a negative value was movement from the neighbouring patch to the focus patch).

3.2.5: Data analysis

The data were analysed using a linear regression with a generalized least squares (GLS) estimation, as described in Chapter 2. To explain the data, three models were used.

- Model 1; Fo^{15} (whole mesocosm) ~ f (algae, species identity, species density, starting density differential)
- Model 2; Fo^{15} ($patch^{-1}$) ~ f (interface, species identity, species density, starting density differential)
- Model 3; Movement ~ f (species identity, interface, species density, starting density differential)

Where:

Species identity: macrofauna species (*Hediste diversicolor*, *Hydrobia ulvae*, *Macoma balthica* and *Corophium volutator*)

Species density: standardised ordinal macrofaunal biomass level within a mesocosm (1, 2, 3, 4, 5, 6 and 8)

Algae: enrichment treatment within a mesocosm (E|E, E|NE and NE|NE, where E = enriched, NE = non-enriched and | is the boundary between the two patches)

Interface: enrichment treatment of a patch with consideration to neighbouring patch (E|E, E|NE, NE|NE and NE|E, where results from the left patch are used in conjunctions with a code for right patch)

Starting density differential: macrofaunal density differences between left and right patches (left – right) at the start of the experiment (-4, -3, -2, -1, 0, 1, 2, 3, 4).

3.3: Results

3.3.1: Algal effects (whole mesocosm model)

The optimum model for algae treatment was a linear regression with a generalized least squares (GLS) extension (allowing for unequal variance within species identity, heterogeneity and species density). It incorporated three single factors and three two-way interaction terms (Table 3.1). The three significant two-way interaction terms within the model were; species identity × algae (L-ratio = 22.54, d.f. = 25, p = <0.001; Fig 3.2), species identity × species density (L-ratio = 22.56, d.f. = 28, p = <0.0001; Fig 3.3) and algae × species density (L-ratio = 16.85, d.f. = 29, p = <0.001; Fig 3.4).

Table 3.1 Significant interaction and variance-covariate terms for algal model.

Term type	Significant factors
Single factors	Species identity
	Algae
	Species density
Two-way interactions	Species identity × algae
	Species identity × species density
	Algae × species density
Variance-covariate terms	Species identity × algae × species density

The single factor that had the greatest influence on the model was species identity (L-ratio = 291.62, AIC = 4609.59, d.f. = 19, p<0.0001), followed by heterogeneity (L-ratio = 121.31, AIC = 4443.28, d.f. = 21, p<0.0001) and species density (L-ratio = 107.06, AIC = 4437.03, d.f. = 25, p<0.0001).

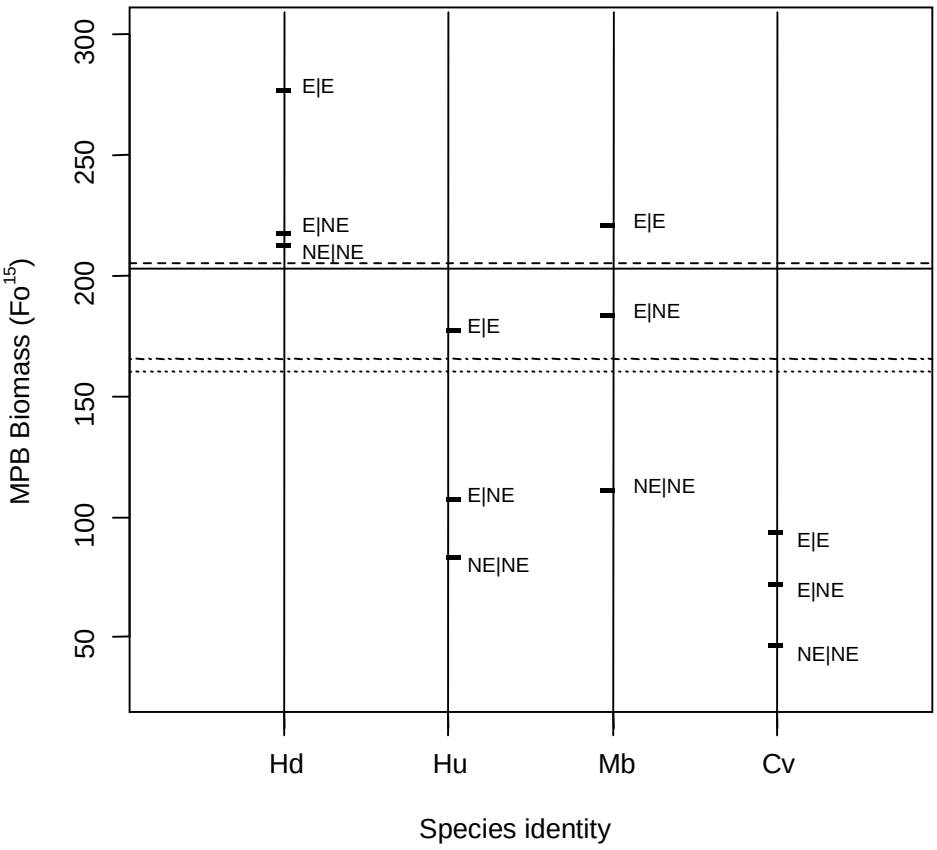


Fig 3.2: Graphical representation of the two-way interaction term species identity × algae. Vertical lines represent species identity *Hediste diversicolor* (Hd), *Hydrobia ulvae* (Hu), *Macoma balthica* (Mb), and *Corophium volutator* (Cv). Horizontal bars represent predicted values from the optimal regression model for each algae treatment. The four horizontal lines are averaged for control mesocosms (containing no macrofauna) at interface treatment E|E (——), E|NE (- - -), NE|E (—·—) and NE|NE (····). As the GLS framework allows for different spread in the data, individual data points are omitted for clarity.

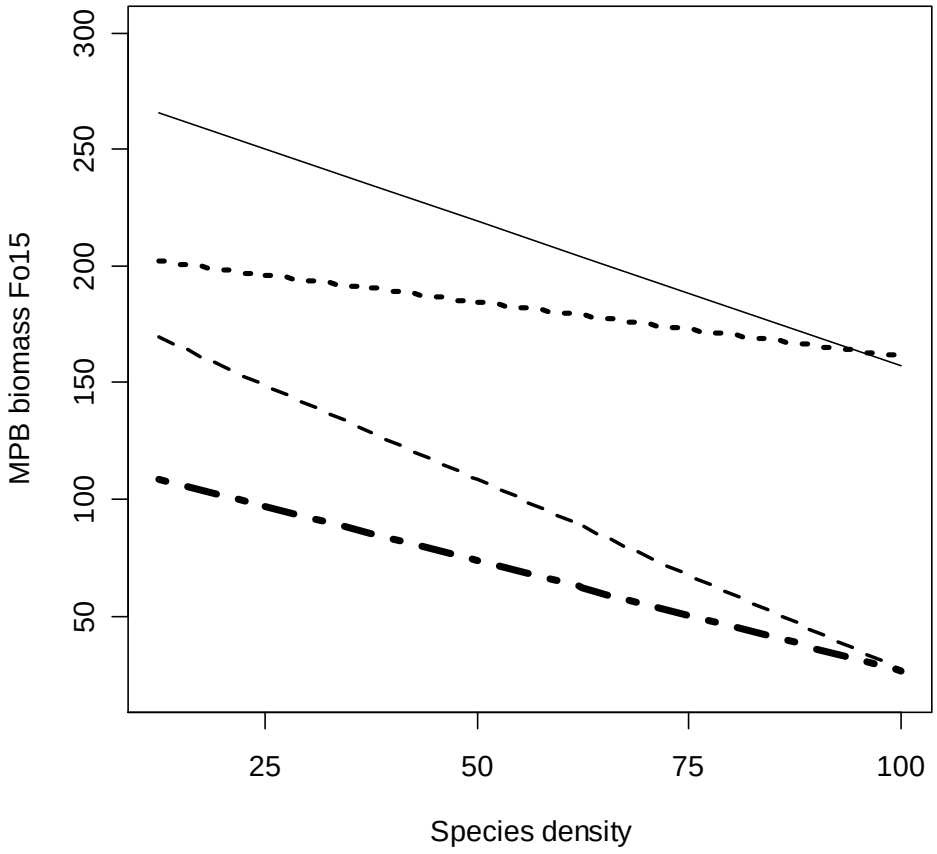


Fig 3.3: Graphical representation of the effect of the two-way interaction term species identity × species density. Lines represent species identity *Hediste diversicolor* (——), *Hydrobia ulvae* (– – –), *Macoma balthica* (· - - - ·), and *Corophium volutator* (—— –). Species density is a percentage of the natural densities found in the Ythan estuary. As the GLS framework allows for different spread in the data, individual data points are omitted for clarity.

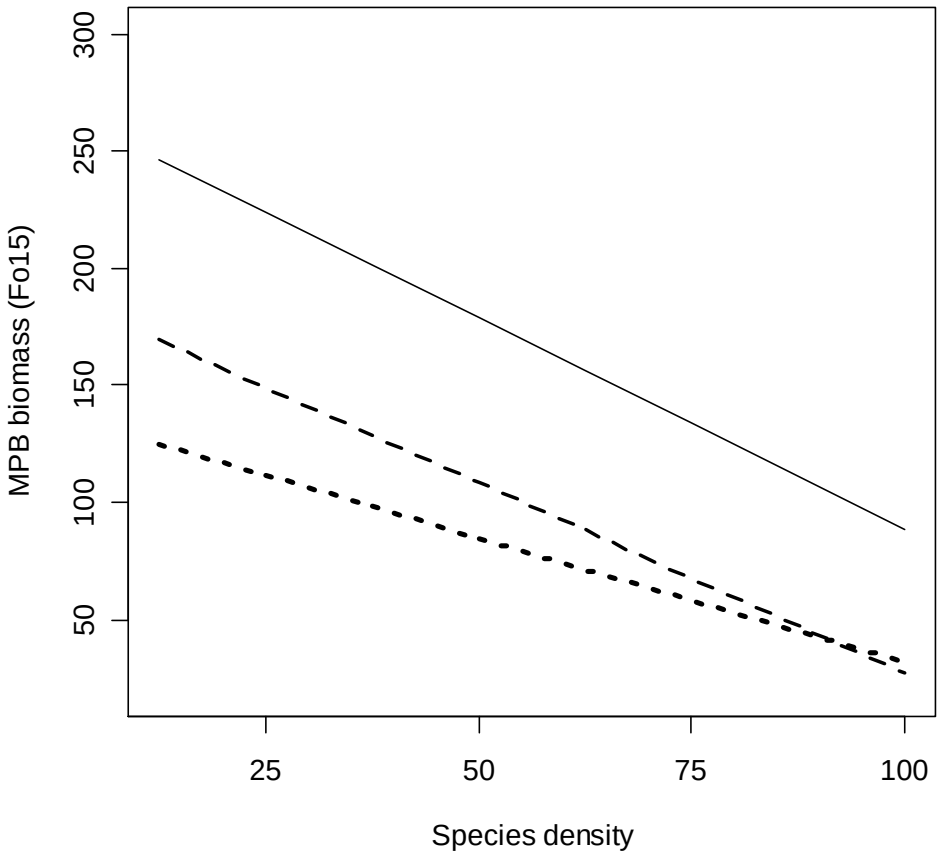


Fig 3.4: Graphical representation of the effect of the two-way interaction term heterogeneity × species density. Lines represent heterogeneity, E|E (——), E|NE (– – –), and NE|NE (·- - - -), where E is an enriched patch, NE is a non-enriched patch and "|" is the interface between each patch. Species density is a percentage of the natural densities found in the Ythan estuary. As the GLS framework allows for different spread in the data, individual data points are omitted for clarity.

There was a consistent pattern such that enriched mesocosms (E|E) maintained higher levels of MPB biomass than both half-enriched (E|NE) and non-enriched (NE|NE), for all macrofaunal species treatments, NE|NE had the lowest MPB biomass and E|NE MPB biomass fell between the other two treatments but the distribution was species specific (Fig 3.2). *Hediste diversicolor* at all algae treatments and *Macoma balthic* when fully enriched had the least effect on MPB biomass (highest levels), the other species had a greater effect with *Corophium volutator* having the greatest impact on MPB biomass (lowest levels).

The interaction between individual species identity and species density caused an overall reduction in MPB biomass as the density of each species increased (Fig 3.3). MPB levels varied, with *Hediste diversicolor* having least effect on MPB biomass (highest MPB biomass), *Macoma balthica* and *Hydrobia ulvae* having similar effects and *Corophium volutator* having the most (lowest MPB biomass). As species density increased, the rate of decline in MPB was very similar for all species with the exception of *M. balthica* where the slope of the regression was shallower than for the other species (Fig 3.3).

The interaction between species density and algae showed an overall reduction in MPB biomass as the density of each species increased (Fig 3.4). Starting levels varied with algal treatment with E|E having least effect on MPB biomass, followed by E|NE then NE|NE having the strongest effect. As species density increased, the rate of decline in MPB was very similar between E|E and E|NE treatments; however, the slope of the regression for NE|NE was different from E|E.

3.3.2: Influence of neighbouring patches (Interface model)

The optimum patch model was a linear regression with a GLS extension (allowing for unequal variance within species identity, interface type and species density) incorporating four single factors and four two-way interaction terms (Table 3.2). The significant two-way interaction terms within the model were; species identity × interface (L-ratio = 39.18, d.f. = 33, $p<0.0001$; Fig 3.5), species identity × species density (L-ratio = 38.13, d.f. = 39 $p<0.0001$; Fig 3.6), interface × species density (L-ratio = 24.15, d.f. = 39 $p<0.0001$; Fig 3.7) and species density × initial density differential (L-ratio = 4.42, d.f. = 41, $p = <0.05$; Fig 3.8).

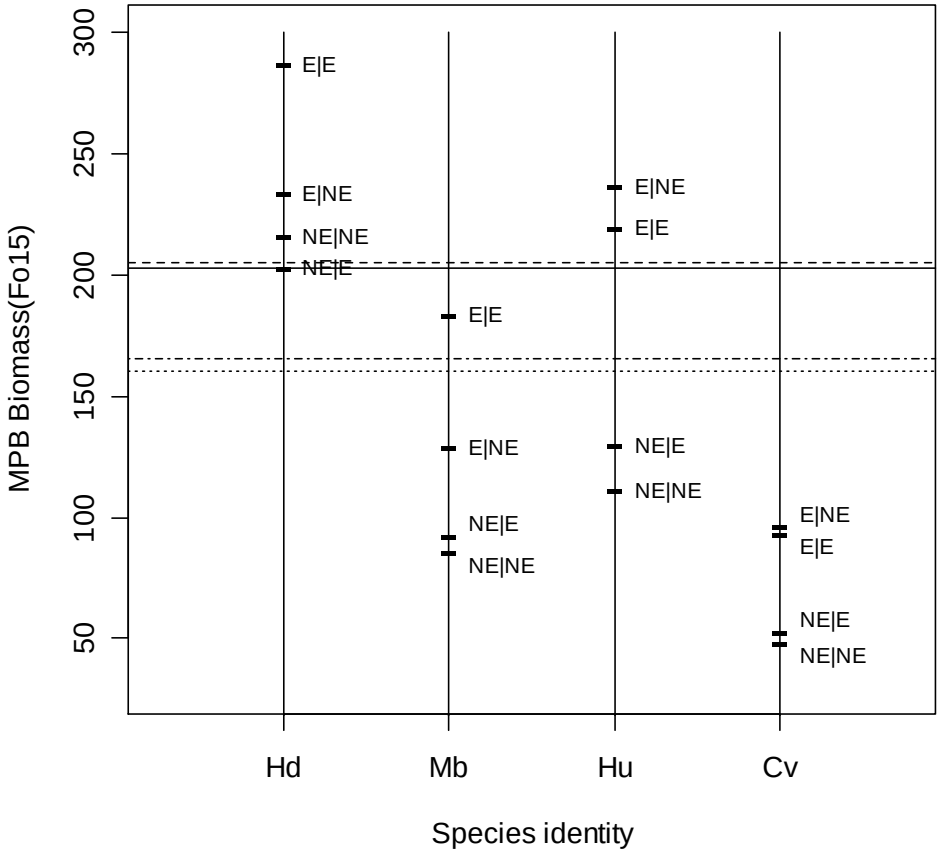


Fig 3.5 Graphical representation of the effect of the two-way interaction term species identity × interface. Vertical lines represent species identity *Hediste diversicolor* (Hd), *Macoma balthica* (Mb), *Hydrobia ulvae* (Hu), and *Corophium volutator* (Cv). Horizontal bars represent predicted values from the optimal regression model for each heterogeneity treatment, 'patches' are represented by the expression on the left of '|' while neighbouring patches are on the right. The two horizontal lines are the averaged for control mesocosms (containing no macrofauna) at interface treatment E|E (——), E|NE (- - -), NE|E (—— -) and NE|NE (·····). As the GLS framework allows for differential spread in the data, individual data points are omitted for clarity.

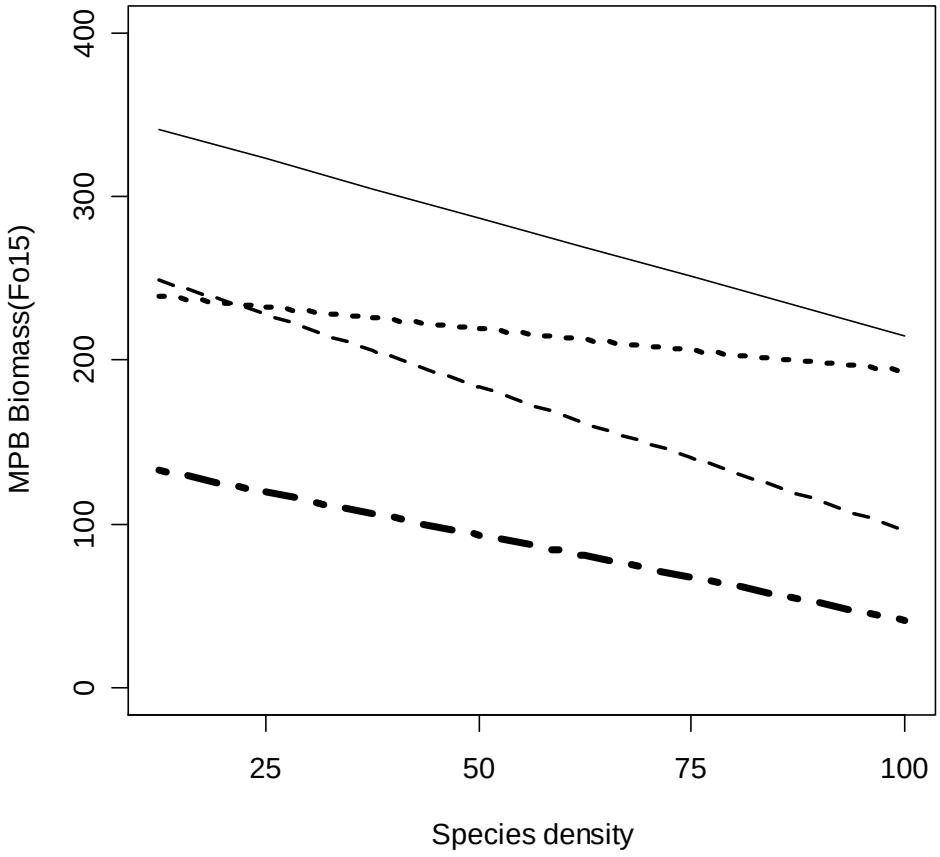


Fig 3.6: Graphical representation of the effect of the two-way interaction term species identity × species density. Lines represent species identity *Hediste diversicolor* (——), *Hydrobia ulvae* (– – –), *Macoma balthica* (· - - - ·), and *Corophium volutator* (— —). Species density is a percentage of the natural densities found in the Ythan estuary. As the GLS framework allows for different spread in the data, individual data points are omitted for clarity.

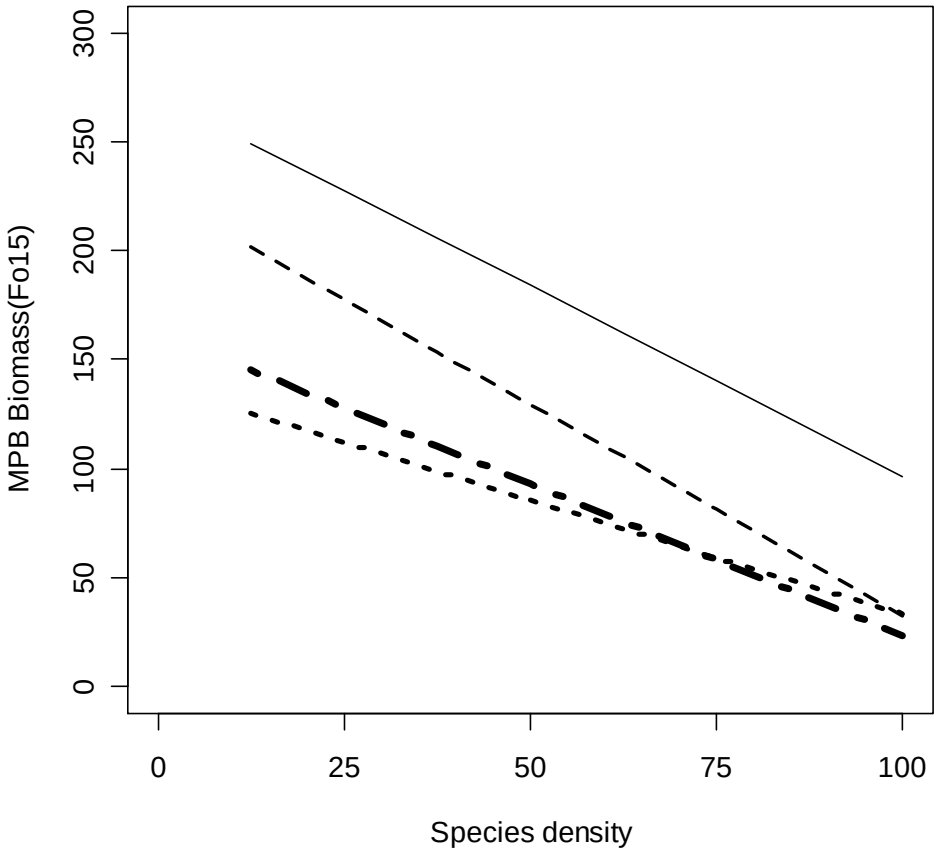


Fig 3.7 Graphical representation of the effect of the two-way interaction term interface × species density. Lines represent interface, E|E (——), E|NE (– – –), NE|NE (·– – –·), and NE|E (—— –), where E is an enriched patch, NE is a non-enriched patch and "|" is the interface between each patch. Analysis is based on the left patch and coded for neighbouring patch on the right. Species density is a percentage of the natural densities found in the Ythan estuary. As the GLS framework allows for different spread in the data, individual data points are omitted for clarity.

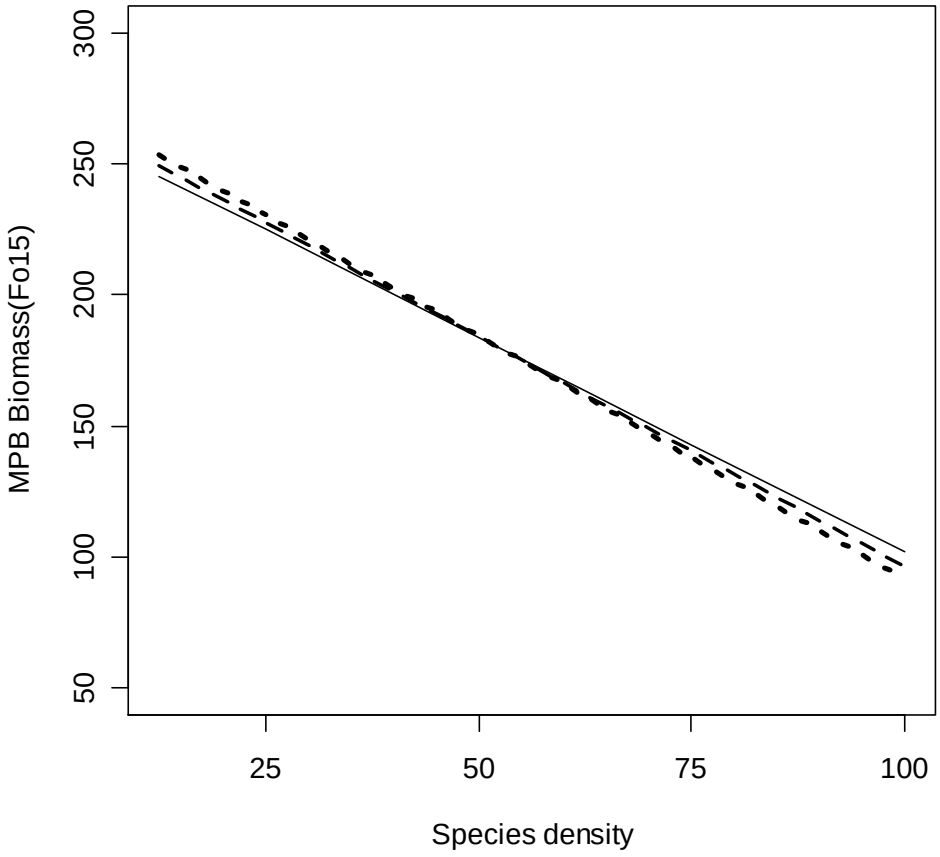


Fig 3.8 Graphical representation of the effect of the two-way interaction term species density × initial density differential. Lines represent the initial density differential, -4 (——), 0 (– – –), 4 (·– · – ·), where initial density differential ranges from a maximum density in the right-hand patch and no macrofauna in the left-hand patch (-4) to a maximum density in the left hand patch and no macrofauna in the right-hand patch (4). Species density is a percentage of the natural densities found in the Ythan estuary. As the GLS framework allows for different spread in the data, individual data points are omitted for clarity.

Table 3.2 Significant interaction and variance-covariate terms for interface model

Term type	Significant factors
Single factors	Species identity
	Interface
	Species density
	Initial density differential
Two-way interactions	Species identity × interface
	Species identity × species density
	Interface × species density
	Species density × initial density differential
Variance-covariate terms	Species identity × interface × species density

Species identity was the single factor that has the greatest influence on the model (L-ratio = 494.39, AIC = 9306.87, d.f. = 27, $p<0.0001$), followed by interface type (L-ratio = 214.37, AIC = 9026.85, d.f. = 27, $p<0.0001$), species density (L-ratio = 173.43, AIC = 8999.91, d.f. = 34, $p<0.0001$) and starting density difference (L-ratio = 9.78, AIC = 8848.26, d.f. = 2, $p<0.01$). The MPB biomass in non-macrofaunal control mesocosms was also compared. These analyses showed that the focus patches had a significant effect on MPB biomass, whilst neighbouring patches had no significant effect (Two-way ANOVA: focus patch $F = 5.93$, d.f. = 1, $p = <0.05$; neighbouring patch $F = 0.26$, d.f. = 1, $p = 0.627$), indicating that bottom up processes were fuelling MPB biomass.

However, while the fully enriched condition (E|E) maintained the highest biomass of MPB for *Hediste diversicolor* and *Hydrobia ulvae* this was not the case for *Macoma balthica* or *Corophium volutator* where the heterogeneous condition (E|NE) maintained the highest level of biomass. Within the interaction species × heterogeneity, *Macoma balthica* × E|NE ($p = 0.018$) and *Corophium volutator* × E|NE ($p = 0.016$) and *Corophium volutator* × NE|E ($p = 0.029$) were significant compared with *Hediste diversicolor* × E|E. The nature of the interaction was to increase the influence of the E|NE condition, (Fig. 3.5) so that for these two species, the interface condition positively influenced MPB biomass.

For each species, there was an overall reduction in MPB biomass with increasing density (Figure 3.6). At low density levels, *Hediste diversicolor* had least affect, *Macoma balthica* and *Hydrobia ulvae* had similar effects, and *Corophium volutator* had the greatest affect on MPB biomass. As species density increased, the rate of decline in MPB biomass was similar for all species with the exception of *Macoma balthica*, where the MPB decline was less pronounced ($p = 0.0036$).

The interaction species density × interface showed an overall reduction in MPB biomass as the density of each species increased in all treatments (Fig. 3.7). The rate of change in MPB biomass was similar between E|E, E|NE and NE|E. At low densities, MPB biomass varied with interface treatment, with the highest biomass associated with the enriched patches E|NE and E|E.

The interaction species density × initial density differential was also significant, but weak ($p = 0.0355$). Model visualisation (Fig. 3.8) indicates that the level of MPB biomass declined as species density increased. The rate of decline was greatest in mesocosms with the maximum biomass in the focus patch and zero biomass in the neighbouring patch (score 4), followed by treatments where initial densities were evenly distributed between patches (0), and for mesocosms with the maximum biomass in the neighbouring patch and zero biomass in the focus patch (-4).

3.3.3: Movement model

The optimum net movement model was a linear regression with a GLS extension (allowing for unequal variance within species identification and interface treatment) incorporating four single factors, six two-way interaction terms and two three-way interaction terms (Table 3.3). The significant three-way interaction terms within the model were; species identity × interface × species density (L-ratio = 63.88, d.f. = 57, $p<0.0001$; Fig 3.9) and species identity × interface × initial density differential (L-ratio = 23.75, d.f. = 57, $p<0.01$; Fig 3.10).

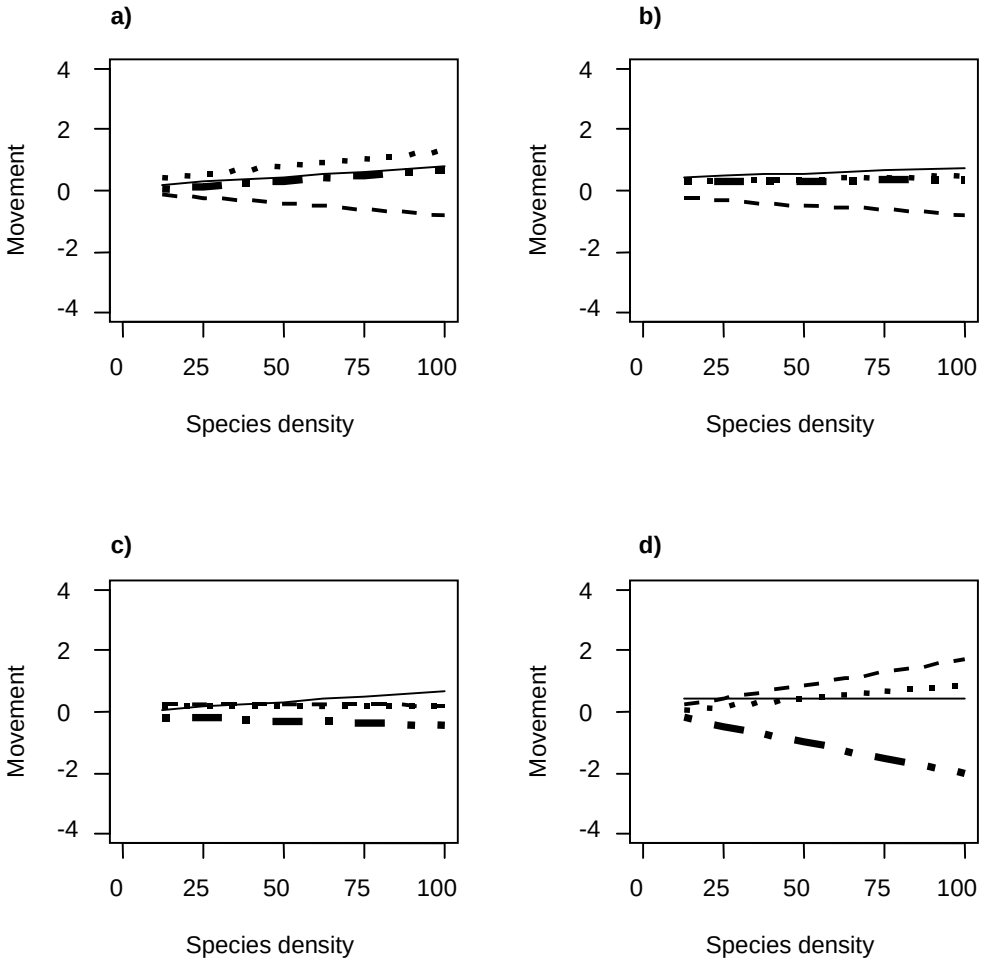


Fig 3.9: Graphical representation of the effect of the three-way interaction term species identity × interface type × species density on net macrofaunal movement for (a) *Hediste diversicolor*, (b) *Hydrobia ulvae*, (c) *Macoma balthica*, and (d) *Corophium volutator*. Lines represent predicted values from the optimal regression model for heterogeneity treatments where: (——) both patches were enriched, E|E; (– – –) only a single patch on the left was enriched, E|NE; (· · · · ·) no patches were enriched, NE|NE; and (— – —) only a single patch on the right was enriched, NE|E. Net movement is represented by the relative change in macrofaunal biomass within a given patch, corresponding to directional migration out from (-4) or into (4) the right-hand patch. Species density ranges from no macrofauna to 100% of natural density. As the GLS framework allows for different spread in the data, individual data points are omitted for clarity.

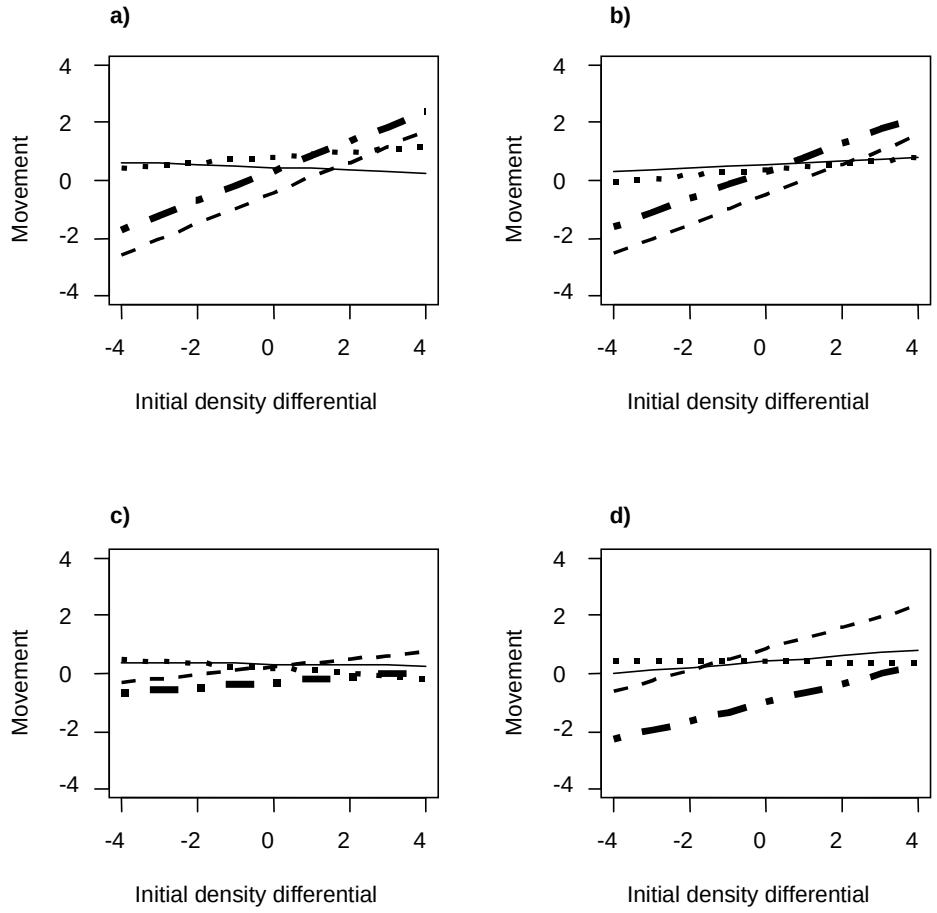


Fig 3.10 Graphical representation of the effect of the three-way interaction term species identity × interface type × starting density differential on net macrofaunal movement for (a) *Hediste diversicolor*, (b) *Hydrobia ulvae*, (c) *Macoma balthica*, and (d) *Corophium volutator*. Lines represent predicted values from the optimal regression model for heterogeneity treatments where: (——) both patches were enriched, E|E; (– – –) only a single patch on the left was enriched, E|NE; (· · · · ·) no patches were enriched, NE|NE; and (—· —) only a single patch on the right was enriched, NE|E. Net movement is represented by the relative change in macrofaunal biomass within a given patch, corresponding to directional migration out from (-4) or into (4) the right-hand patch. Starting density differential ranges from a maximum density in the right-hand patch and no macrofauna in the left-hand patch (-4) to a maximum density in the left hand patch and no macrofauna in the right-hand patch (4). As the GLS framework allows for different spread in the data, individual data points are omitted for clarity.

Table 3.3 Significant interaction and variance-covariate terms for net movement model.

Term type	Significant factors
Single factors	Species identity
	Interface
	Species density
	Initial density differential
Two-way interactions	Species identity × interface
	Species identity × species density
	Species identity × initial density differential
	Interface × species density
	Interface × initial density differential
	Species density × initial density differential
Three-way interactions	Species identity × interface × species density
	Species identity × interface × initial density differential
Variance-covariate terms	Species identity × interface

The single factor that had the greatest influence on the model was initial density differential (L-ratio = 429.29, d.f. = 49, $p<0.0001$), followed by species identity (L-ratio = 276.45, d.f. = 30, $p<0.0001$), interface (L-ratio = 201.13, d.f. = 30, $p<0.0001$) and species density (L-ratio = 79.20, d.f. = 49, $p<0.0001$).

The 3 way interaction between species identity × interface × species density showed an overall increase in net movement with species density, *Corophium volutator* demonstrated greatest net movement followed by *Hediste diversicolor* and *Hydrobia ulvae* while *Macoma balthica* did not move significantly (Fig 3.9c). Graphical representation of the two heterogeneous interface treatments reveals that *Corophium volutator* show a strong net movement away from enriched sediment (Fig 3.11d), and *Hediste diversicolor* moved towards enriched patches (Fig 3.11a). *Hydrobia ulvae* and *Macoma balthic* showed no net movement (Fig 3.11b & c).

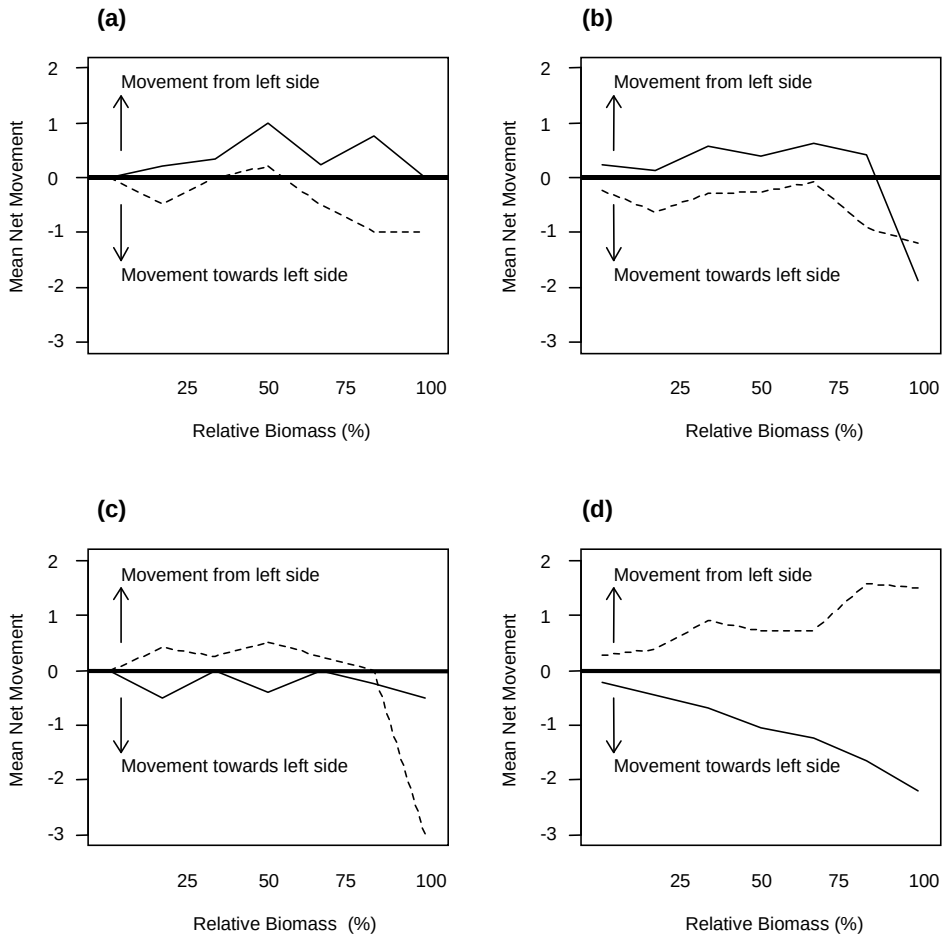


Fig 3.11: Graphical representation of the effect of the three-way interaction term species identity × interface type × species density on net macrofaunal movement for (a) *Hediste diversicolor*, (b) *Hydrobia ulvae*, (c) *Macoma balthica*, and (d) *Corophium volutator*. Lines represent predicted values from the optimal regression model for two heterogeneity treatments where: (——) only a single patch on the right was enriched, NE|E, and (– – –) only a single patch on the left was enriched. Net movement is represented by the relative change in macrofaunal biomass within a given patch, corresponding to directional migration out from (-3) or into (2) the right-hand patch. Species density ranges from no macrofauna to 100% of natural density. As the GLS framework allows for different spread in the data, individual data points are omitted for clarity.

H. diversicolor, *H. ulvae* and *C. volutator* all showed strong movement in relation to the interaction between starting density difference and interaction treatments (Fig. 3.10). For *Hediste diversicolor* and *Hydrobia ulvae*, the responses to initial density difference dominated responses to interface treatments. Both species showed similar patterns of response, moving away from higher starting density areas. *Macoma balthica* showed no response to initial density differential.

3.4: Discussion

3.4.1: Algal enrichment

The mesocosms with the highest MPB biomass were algal enriched and those with the lowest had not been enriched. This indicates that the addition of powdered *Enteromorpha intestinalis* was having the desired effect and creating a heterogeneous environment. However, nutrient release is also controlled by the amount of oxygen in the sediment so, within this artificial system, oxygen distribution in the sediment is largely dependent on bioturbation. It might be considered that macrofaunal movement will increase in patchy environments (Levinton and Kelaher 2004) and, therefore increasing the levels of bioturbation and consequent nutrients release which would have a positive effect of MPB biomass. In this scenario, the heterogeneous mesocosms would have higher MPB biomass than the homogeneous ones. However this was not the case and the fully enriched mesocosms that contain double the amount of algae had the highest MPB biomass.

3.4.2: Macrofaunal biomass

In general, the MPB biomass decreased with increasing macrofaunal biomass. MPB biomass was lowest in mesocosms that were not enriched and with the highest numbers of macrofauna. All macrofaunal species in this experiment are known to be consumers of diatoms (Hagerthey *et al* 2002, Kamermans 1994, Smith *et al* 1996), therefore increasing the number of species or biomass would increase MPB consumption causing a decrease in the level of MPB biomass. In contrast to this, increasing the numbers of macrofauna within the mesocosm also increases bioturbation, releasing more nutrients and ultimately increasing the resources for MPB growth. The balance between grazing and growth is species specific as all the

macrofauna have different bioturbatory characteristics, for example *Hediste diversicolor* is a deep sediment bioturbator that allows oxygen to penetrate deeper into the sediment than *Hydrobia ulvae* which only bioturbates the top few mm. Therefore increased species density decreased MPB biomass but the rate of decline will depend on the behavioural characteristics of the individual species and the balance between MPB production and consumption.

3.4.3: Species identification

At all density levels and enrichment treatment *Macoma balthica* had little influence on MPB biomass. *Macoma balthica* is predominantly a deposit feeder that has the ability to switch to siphon feeding from the water column (Kamermans 1994, de Goeij & Luttikhuizen, 1998), a feeding behaviour not impacting on MPB biomass. During this type of feeding the main body of *Macoma balthic* does not move from the burrow reducing bioturbation rates, nutrient release and MPB biomass.

Mesocosms containing *Hediste diversicolor* produced the highest MPB biomass, possibly due to its relatively large size and bioirrigatory capacity (Magni and Montani 2006) increasing nutrient turnover, as well as stimulating microbial activity (Hansen and Kristenen 1997). *Hediste diversicolor* has an alternative method of feeding that could impact in MPB by reducing biomass, however these methods were not observed in this study and MPB levels remained highest compared to the other species.

Hydrobia ulvae is an active surficial grazer, so although bioturbation of the surface sediment occurs, releasing nutrients, their feeding behaviour greatly reduces MPB biomass. *Hydrobia ulvae* impact MPB biomass, where the positive effects of shallow bioturbation and the negative effects of grazing are finely balanced.

Corophium volutator had a negative effect on MPB biomass. In past studies this species has proven to be a highly active bioturbator producing high levels of NH_4-N (Biles *et al* 2002, Raffaelli *et al* 2003a, Emmerson *et al* 2001). *Corophium volutator* is highly active and re-suspends particulate material (Smith *et al* 1996, Hagerthey *et al* 2002), due to these behavioural trails and past findings, the low MPB biomass is probably attributable to a turbidity effect. The highly visible re-suspended particulate

material reduces the amount of light available for photosynthesis at the sediment surface effecting MPB production.

3.4.4: Interface

Nutrients from the enriched sediment can reach the MPB either directly from the sediment/water interface or be released into the water column and mixed across the chamber. Nutrients released in the water column would become available for the whole mesocosm and any MPB biomass response would be seen over the entire mesocosm. However if the MPB were obtaining nutrients locally, from the sediment/water interface, there would only be an MPB response in the treated sediment. Overall the highest MPB biomasses were in patches that had been enriched and lowest in those that had not been enriched, no matter what the neighbouring patch type. This would indicate that the major source of nutrients for MPB is derived locally from the sediment/water interface rather than the water column itself.

3.4.5: Movement and species identity

There was no net movement of *Macoma balthica* during any of the species density or heterogeneity treatments. This is reflected in the distribution of MPB biomass for this species, no movement or bioturbation would result in little or no movement-stimulated nutrient release from the sediment and therefore no enhanced growth of primary producers (MPB).

Corophium volutator had a strong response where net movement was away from enriched patches towards non-enriched patches, this pattern has been observed in the field (Raffaelli *et al* 1999, Lawrie *et al* 2000). This movement could be expected to release higher levels of nutrients (influencing MPB biomass) in the heterogeneous treatments, where more movement would be expected, however, this was not the case and the highest levels of MPB biomass were in enriched patches.

It was assumed in this work that the amount of movement made by macrofauna is reflected in the amounts of MPB biomass due to the release of nutrients and the positive effects this has on MPB biomass. However, due to the combined effects of algal enrichment, which has been shown to contain higher MPB biomass than non-enriched sediment the only way of assessing the amount of movement would be to

track the macrofauna over time. This would be easier for surface dwelling macrofauna like *Hydrobia ulvae*, as they are visible. However it would be more difficult for sediment dwelling macrofauna like *Hediste diversicolor* and *Corophium volutator* that spend most of their time in the sediment, unseen. Possible tracking methods might include the ingestion of a radioactive isotope or the use of a thermal imaging camera.

Hediste diversicolor and *Hydrobia ulvae* showed a positive net movement to enriched patches, again this has been observed in the field (Raffaelli 2000, Hull 1987). Movement was probably induced by the increase availability of food (MPB) in the enriched patches, and therefore a greater amount of time would be spent foraging in these patches.

3.4.6: Movement and interface

For all motile species, an increase in density resulted in greater net movement, the degree of movement was an interaction with species identity and interface. It would be predicted that macrofauna that moved at low densities would also move in the same direction at higher densities and that the latter movement would be greater as there would be more animals to move. The greatest net movement was from *Corophium volutator*, then *Hediste diversicolor* and the least from *Hydrobia ulvae*. All three species consume MPB and at increasing densities the amount of MPB consumed would also increase. If the assumption made in this study holds true and movement, especially for *Hydrobia ulvae* and *Hediste diversicolor*, is induced by food resources then the amount of food available in the mesocosm may vary over time. On day 10 food resources may have dwindled and the difference between enriched and non-enriched patches may be insignificant, therefore the amount of net movement observed might not reflect net movement at a time when patch differential is at maximum. Net movement on day 10 would be a watered down version of events, further investigation is needed to find the optimal day that best represents maximal net movement.

3.5: Conclusion

Heterogeneity, species identity, and species density all influenced the MPB biomass distribution. The experimental set up was based on a tried and tested methodology (Ieno *et al* 2006, Bulling *et al* 2007) and all statistical models followed appropriate procedures (Pinheiro & Bates 2006, West *et al* 2007). Heterogeneity was successfully achieved in the mesocosms by algal enrichment (*Enteromorpha intestinalis*), which caused an increase in MPB biomass.

The use of PAM fluorescence enabled variation between patches to be distinguished in a non-destructive manner and this revealed the pathway of nutrient to primary production to be through the sediment water interface rather than directly from the water column. It was assumed that species identity, through behavioural characteristics including bioturbation rates and feeding methods, altered the balance between the positive effects of bioturbation and the negative impact of MPB consumption. Net movement patterns provided further evidences to support field observation of the directional movement of macrofauna away from or toward nutrient enriched areas, however there is no evidence to suggest that heterogeneity increased movement.

Future developments should include multi species interactions to find the effects of species richness on ecosystem function in a heterogeneous habitat. Time laps measurement for macrofaunal movement should be taken to find if there are movement differences between heterogeneous and homogeneous mesocosms.

4. Ecosystem function for single species in flow mesocosms

4.1: Introduction

Water flow is an important structuring component of estuarine and coastal systems and is an important consideration when attempting to recreate these habitats in experimental systems. The effect of flow, both on the sediment fauna and the physical sediment matrix, has been documented (Denny 1993, Paterson and Black 1999). There is growing recognition of the importance of flow on all life history stages of macrofauna. The orientation, size and shape of the organism, as well as the environment, all determine the relative importance of hydrodynamic forces on both deposit and suspension feeding macrofauna (Wildish, 1977). The classification of species feeding behaviour can include several alternative descriptions allowing for the plastic behavioural traits of macrofauna during varying environmental conditions (Pearson 2001). Switching feeding behaviour is thought to result from a change in particle concentration in the water column, affecting food supply rates (Fauchald and Jumars 1979, Miller *et al* 1992, Loo *et al* 1996). Concentration of particulates in the water column is largely a result of flow (Patterson and Black 1999), therefore behavioural changes in feeding regime may be modified by flow. The ability of macrofauna to switch feeding mode presents a considerable advantage to species living in dynamically variable benthic environments, such as estuaries (Vogel 1994).

Previous work by Biles *et al* (2003) found that flow generated an affect through promoting changes in bioturbatory activity of the infauna causing greater disruption to the sediment. Habitat heterogeneity has also proven to be an important component when considering biodiversity and ecosystem function (Dyson *et al*, 2007, Bulling *et al*, Submitted). This study tests the additional influence of flow combined with those variables tested in chapter 3, species identity, species density and heterogeneity on MPB biomass (proxy for ecosystem function) and macrofaunal movement in an experimentally-replicated marine benthic system.

4.2: Material and Methods

4.2.1: Sediment and macrofauna collection

The collection and treatment of sediment and macrofauna are described in Chapter 2 (Materials and Methods)

4.2.2: Experimental design

The experimental design included 126 separate mesocosms incorporating 3 algal treatments, 3 biomass levels, 3 species, 2 flow conditions, 3 replicates and 9 controls, which were divided randomly between eleven runs. The effect of macrofaunal species identity, macrofaunal species biomass, algal enrichment and flow were determined using MPB biomass as a proxy for ecosystem function (Fig. 4.1). Two patches (heterogeneity) were established in each mesocosm and macrofauna were only introduced into the left (focus) patch. The deposit-feeder *Hediste diversicolor* (Polychaeta), the surficial grazer *Hydrobia ulvae* (Gastropoda), and the regenerator *Corophium volutator* (Crustacea) were added on day 0. Macrofauna were confined to their initial patches for 24 h using Perspex dividers. Combinations of macrofaunal biomass (0, 25, and 100% of natural density of the Ythan estuary were added to the left 'focus' patch) were established for all possible algal combinations of patch arrangements, as described in Chapter 3 (E|E, NE|E, and NE|NE where '|' represents the interface and E = enriched and NE = non-enriched) for each of the 4 macrofaunal species (Fig 4.1). For *Hediste diversicolor*, whole individuals were counted and 4 individuals patch^{-1} was taken as analogous to the natural density on the Ythan estuary. For *Hydrobia ulvae* and *Corophium volutator*, the natural wet weight biomass was determined and appropriate proportional wet weights added to the mesocosms. Flow and static mesocosms were set up, where flow was established the velocity was set to 6 cm s^{-1} (Biles *et al* 2003). Variation in timing between multiple runs was an unavoidable effect of this design that might be influenced by seasonal trends in the MPB biomass response and therefore "run" (equivalent to season trends) was included in the model as a random factor.

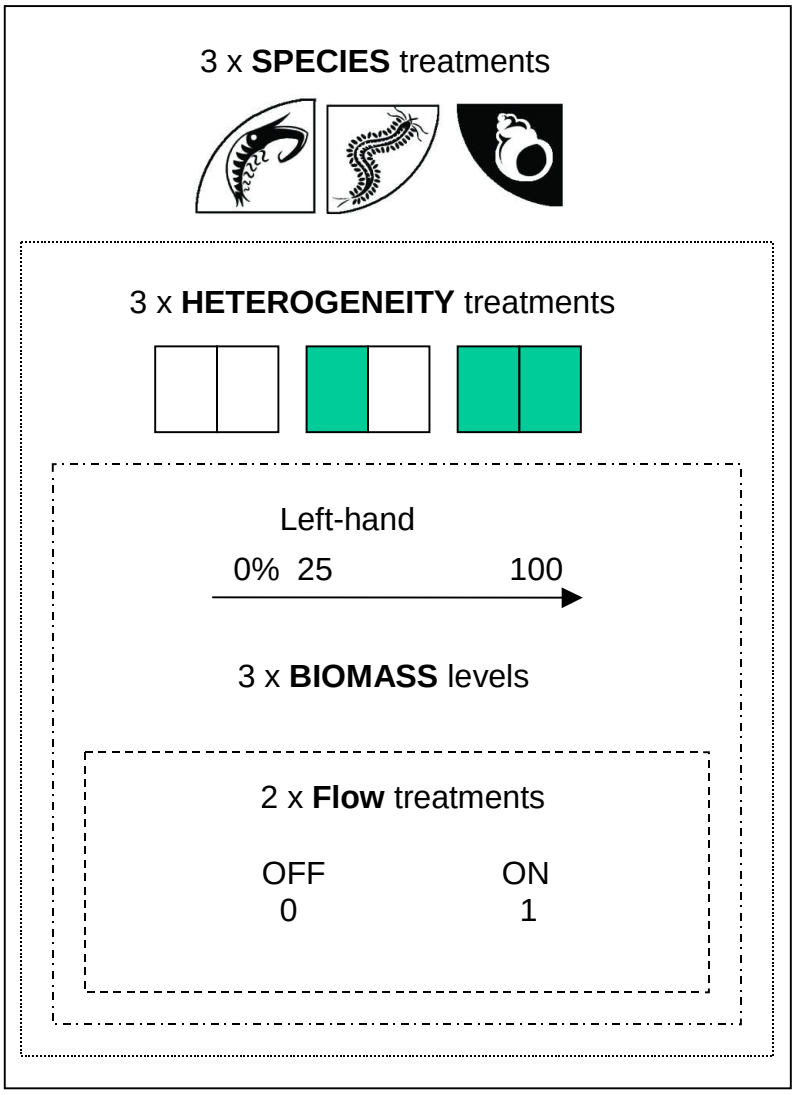


Fig 4.1: Overview of experimental design. The species biomass gradients across the patch interface were established at the start of the experiment, using the relative levels of 0%, 25%, and 100% natural density at the study site. All species were placed at the start of the experiment in the left hand patch (Focus patch). These combinations were used for each of the three interface treatments (enriched is green and non-enriched is white), every species density-interface combination was used for each of the four species (*Corophium volutator* (), *Hydrobia ulvae* () and *Hediste diversicolor* ()) and all combinations were treated in either flow or static conditions.

4.2.3: Fluorescence measurements

Fluorescence readings were taken on day 6 of each run, this day was chosen as an appropriate length of time that best captured the changes caused by species behaviour without the fluorescence levels dropping below a reliable limit as a consequence of the laboratory conditions, as discussed by Defew et al (2002b). The measurable output of the PAM fluorescence meter is Fo^{15} (ratio measurement, no units) and this value is representative of the chlorophyll a biomass present on the sediment surface (Serôdio et al 2001, Honeywill et al 2002) and can therefore be used as a proxy for primary production (Consalvey et al 2004b). Two fluorescence readings were taken $patch^{-1}$, enabling the MPB biomass to be assessed as a whole mesocosm (average 4 readings).

4.2.4: Movement measurements

The relocation of macrofauna between patches during the experimental period was assessed on day 10. Each patch was isolated using a divider, the mud collected and sieved and the macrofauna counted as described in Chapter 2. Net macrofaunal movement was measured by unit change from the focus patch (a positive value is net movement from the focus patch to the neighbouring patch and a negative value is net movement from the neighbouring patch to the focus patch).

4.2.5: Data analysis

All data was analysed using a linear regression, with a generalized least squares (GLS) estimation to allow for heteroscedasticity for the data within the model selected. Two models were applied, one for the MPB response variable and the other for the net movement response variable, both models required a mixed effects extension to the linear regression to take into account the random effect 'run' which is an artefact of the experiment being run in batches. To explain the two models;

- Model 1; $Fo^{15} \sim f$ (algae, species identity, species density, flow, run)
- Model 2; Movement $\sim f$ (interface, species identity, species density, flow, run)

Where;

Species identity: macrofauna species (*Hediste diversicolor*, *Hydrobia ulvae* and *Corophium volutator*)

Species density: standardised macrofauna biomass with a mesocosm (0, 25 and 100% of the carrying capacity of the Ythan estuary)

Algae: enrichment treatment within a mesocosm (E|E, NE|E and NE|NE, where E = enriched, NE = non-enriched and | is the boundary between the two patches)

Flow: movement of water within the mesocosm (0 = no flow, 1 = flow)

Run; the number of the experimental batches taken over time (n = 10).

4.3: Results

4.3.1: MPB biomass model

This model was a linear mixed model (LMM) based on a linear regression with a GLS extension (allowing for unequal variance within the run factor and random effects also within the run factor) it incorporated four single factors and three two-way interaction terms (Table 4.1). The significant two-way interaction terms within the model were species identity × species density (L-ratio = 27.72, d.f. = 22, $p<0.0001$; Fig 4.2), species identity × flow (L-ratio = 8.74, d.f. = 22, $p<0.05$; Fig 4.3) and algae × species density (L-ratio = 13.33, d.f. = 22, $p<0.01$; Fig 4.4).

Table 4.1 Significant interaction and variance-covariate terms for the algal model.

Term type	Significant factors
Single factors	Species identity
	Algae
	Species density
	Flow
Two-way interactions	Species identity × species density
	Species identity × flow
	Species density × algae
Random effect	Run
Variance-covariate terms	Run

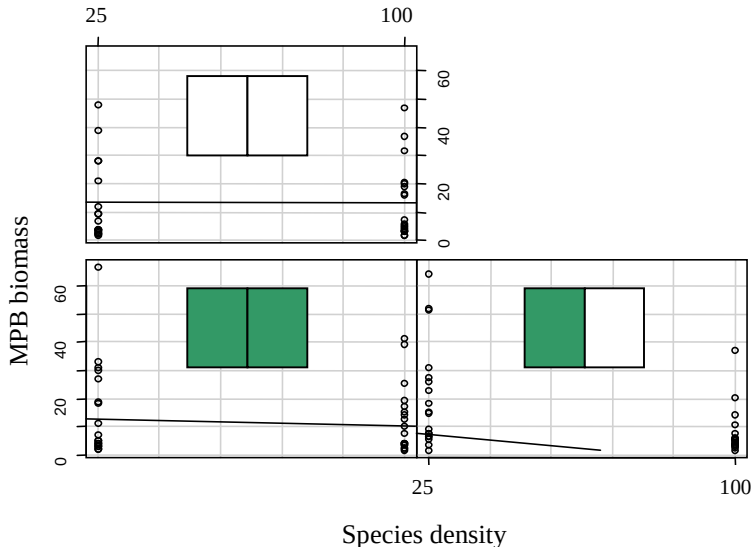


Fig 4.2: Graphical representation of the two-way interaction term species density × algal enrichment. Algal enrichment treatment is represented by a diagrammatic plan of the mesocosm divided into two patches where E is an enriched patch (green) and NE is non-enriched patch (white). Species density is a percentage of the natural densities found in the Ythan estuary.

The single factor that had the greatest influence on ecosystem function was species identity (L-ratio = 52.18, d.f. = 18, $p<0.0001$), followed by species density (L-ratio = 46.84, d.f. = 19, $p<0.0001$), flow (L-ratio = 20.32, d.f. = 21, $p<0.001$) and algae (L-ratio = 20.14, AIC = 1110.20, $p<0.001$). To assess the influence of flow on MPB biomass comparison to control mesocosms (no macrofauna) revealed that there was no significant difference between flow and no flow (static) treatments (One-way ANOVA: F = 1.63, d.f. = 1, p = 0.220).

The interaction between individual species density and algae showed the greatest reduction in MPB biomass at algal treatment NE|E followed by E|E, and there was no reduction in algal treatment for NE|NE (Fig 4.2). At the lowest species density, MPB biomass was the same for all species.

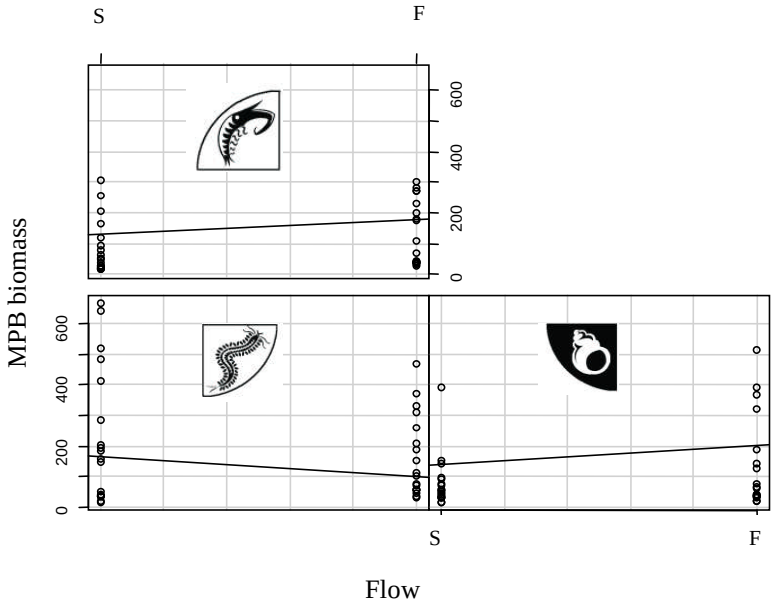


Fig 4.3: Graphical representation of the two-way interaction term species identity x flow. Species identity is represented by diagrammatic pictures in each box, *Hediste diversicolor* (), *Hydrobia ulvae* () and *Corophium volutator* (). Flow treatment is represented by S (Static) and F (flow).

The interaction between flow and species identity showed that flow treatments were species specific. *Hydrobia ulvae* and *Corophium volutator* had higher MPB biomass levels during flow condition while *Hediste diversicolor* had higher MPB biomass levels during no flow conditions. MPB biomass varied in static conditions, with *Hediste diversicolor* having least effect (highest MPB biomass), followed by *Hydrobia ulvae* and *Corophium volutator* having the greatest (least MPB biomass) (Fig 4.3). However in flow conditions *Hydrobia ulvae* had highest MPB biomass followed by *Corophium volutator*, and *Hediste diversicolor* had the lowest levels.

The interaction between species identity and species density demonstrated that increased density of *Hediste diversicolor* and *Corophium volutator* reduced MPB biomass (Fig 4.4). However, increased density of *Hydrobia ulvae* had no effect on MPB biomass. MPB biomass varied, with *Hediste diversicolor* having least effect,

followed by *Hydrobia ulvae* and *Corophium volutator* having the greatest. As species density increased, the rate of decline in MPB was varied for all species, *Hediste diversicolor* produced the greatest decline, followed by *Corophium volutator*. *Hydrobia ulvae* produced no decline in MPB biomass.

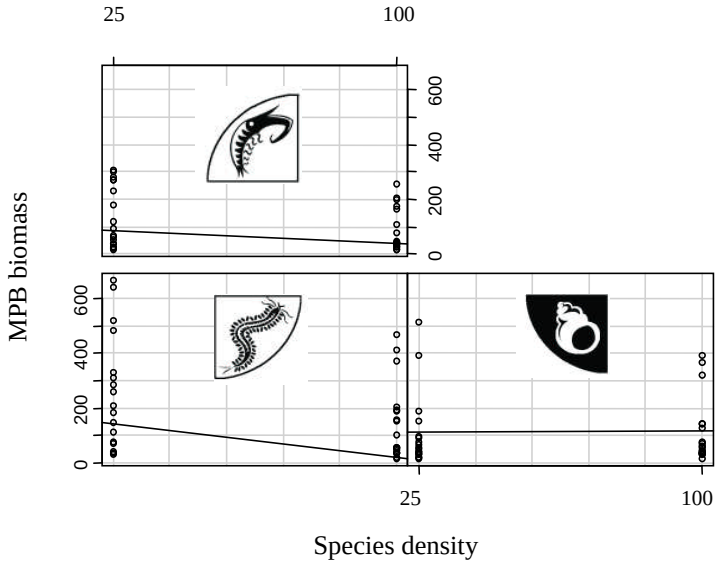


Fig 4.4: Graphical representation of the effect of the two-way interaction term species identity × species density. Species identity is represented by diagrammatic pictures, *Hediste diversicolor* (), *Hydrobia ulvae* () and *Corophium volutator* (). Species density is a percentage of the natural densities found in the Ythan estuary.

4.3.2: Movement model

The model for net movement was a linear mixed model based on a linear regression with a GLS extension (allowing for unequal variance within the species density and random effects within the run factor) that incorporated three single factors, three two-way interaction terms and one three-way interaction term (Table 4.2). The significant three-way interaction term within the model was species identity × species density × algae (L-ratio = 11.04, d.f. = 17 $p<0.05$; Fig 4.5).

Table 4.2: Significant interaction and variance-covariate terms for the net movement model.

Term type	Significant factors
Single factors	Species identity
	Species density
	Algae
Two-way interactions	Species identity × algae
	Species identity × species density
	Species density × algae
Three-way interaction	Species identity × species density × algae
Random effect	Run
Variance-covariate terms	Species density

The single factor that had the greatest influence on the model was species density (L-ratio = 109.59, d.f. = 12, $p<0.0001$), followed by species identity (L-ratio = 38.92, d.f. = 9, $p<0.001$) and algae (L-ratio = 17.02, d.f. = 9, $p = 0.149$). Flow was included into the initial model and was found to be insignificant for all interactions terms and at the single factor level and was therefore excluded from the optimal model.

The interaction between species identity × species density × algae revealed that for all species there was greater net movement out of the focus patch at the highest species density. However, in relative terms, *Corophium volutator* showed little net movement compared to the other two macrofaunal species. The greatest net movement for both *Hediste diversicolor* and *Hydrobia ulvae* was for the algal treatment NE|E, were net movement was away from the focus patch into the neighbouring patch. There was not as much net movement in the other two algal treatments (E|E & NE|NE). This was not the case for *Corophium volutator* where the greatest net movement was found in the NE|NE mesocosm and there was less net movement in the other two algae treatments (E|E & NE|E).

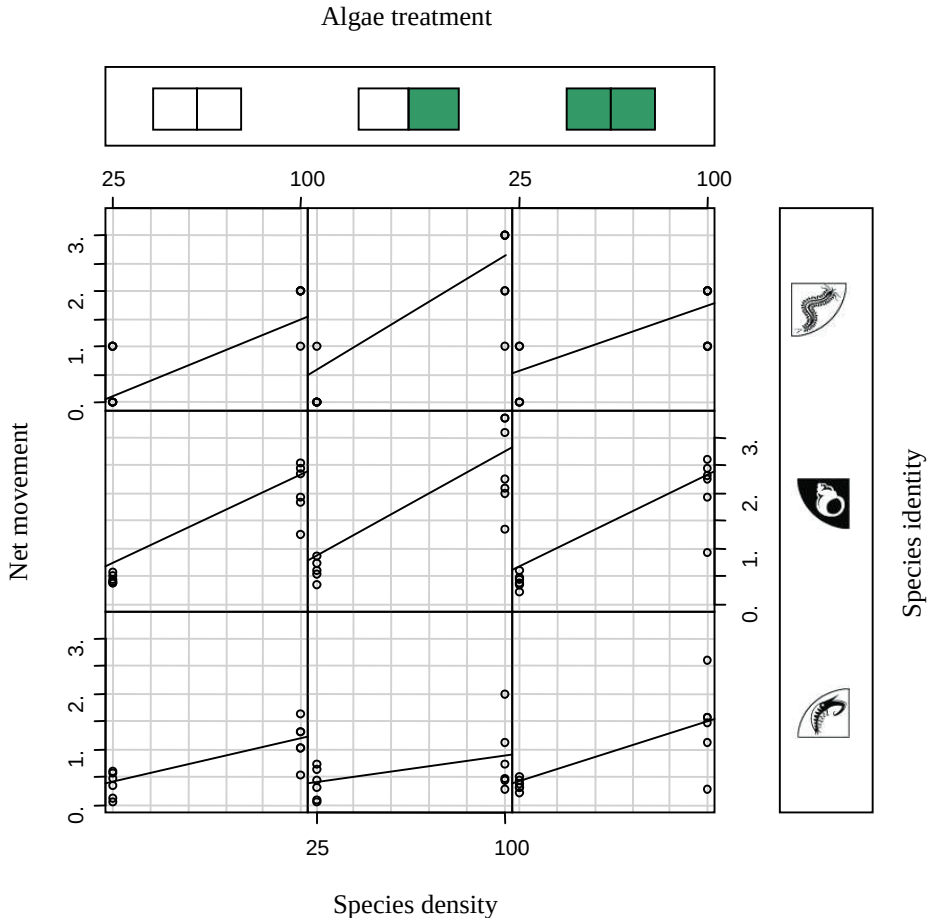


Fig 4.5: Graphical representation of three-way interaction species identity × species density × algae treatment. Species identity is represented by diagrammatic pictures, *Hediste diversicolor* (), *Hydrobia ulvae* () and *Corophium volutator* (). Species density is a percentage of the natural densities found in the Ythan estuary. Algae treatment is represented by a mesocosm plan where algal enrichment is E (grey) and non-enriched is NE (white). Species density is a % of the natural densities found in the Ythan esturay. Net movement is a measure of standard densities found in the neighbouring patch (left) after 6 days having started in the focus patch (right).

4.4: Discussion

4.4.1: Microphytobenthos

Flow, species identity, species density, and algal enrichment were significant factors influencing MPB biomass in this experimental system. The direct effect of flow on MPB in experimental conditions is unknown, however under natural conditions tidal flow is thought to be one of the possible triggers for migration of diatoms into the sediment (Round & Palmer 1966). The downward migration of diatoms is thought to provide protection from scouring as the tidal water floods and ebbs. During these submerged periods, in the natural environment, the light may also be limited due to the turbidity of the flooding tide that would prevent photosynthesis (Consalvey *et al*, 2004a). However, Perkins (1958) observed the presence of diatoms during periods of submersion, this was possibly due to the local clarity of the water, allowing photosynthesis at flow energy below the critical entrainment point. In this experiment, flow rates were consistent with ambient velocities of the Ythan estuary (Biles *et al* 2003), this reduced the risk of surface scouring that would eradicate the MPB from the sediment. Observations during this study showed dark brown patches of MPB were present during periods of illumination in both the static and flow mesocosms.

4.4.2: Flow

Flow had a significant effect on the MPB biomass when interacting with species identity. It was interesting to note that the effect of flow on MPB biomass levels was species specific. This was expected since an *a priory* understanding of the chosen macrofauna would suggest different effects on ecosystem function, this is thought to be due to the difference between bioturbation and feeding characteristics of each species (Biles *et al* 2003, Raffealli *et al* 2003a). Under flow conditions *Hediste diversicolor* reduced MPB biomass levels whereas *Hydrobia ulvae* and *Corophium volutator* increased MPB biomass levels over the 6 day testing period. Flow could affect MPB biomass in two ways; firstly, it could cause a change in behaviour in the macrofauna (Fauchald and Jumars 1979, Miller *et al* 1992, Loo *et al* 1996). Secondly it could cause changes to the physical environment by altering the temperature stratification in the water column, oxygen concentrations or disturbing the sediment/water interface.

Changes in the feeding behaviour of the macrofauna could be influenced by flow, since animals can adjust feeding behaviour to utilize different food sources (Taghon *et al* 1980). *Corophium volutator* creates burrows that it builds and keeps clear by ejecting sediment into suspension. It is possible that the flow was enabling *Corophium volutator* to be less active in creating their own flow, this would have the advantage of reducing particulate matter in the water column, therefore allowing greater amounts of light penetration, and increasing photosynthesis to achieve higher levels of MPB biomass. In contrast the deposit feeder (*Hediste diversicolor*) can take advantage of environmental conditions and switch from suspension feeding to surface deposit feeding depending on food resources. Deposit feeding may have a greater impact under the present conditions given that this produces more sediment disturbance and will have a greater influence on the surface MPB (Smith *et al* 1996, Riisgård and Kamermans 2001), ultimately reducing MPB biomass.

Besides the direct influence flow has on macrofauna, it would also influence the physical environment within the mesocosm (Nowell & Jumars 1984). Flow disrupts the sediment/water interface, and could enhance flux and distribute the nutrients being released through bioturbation into the nutrient depleted zones within the mesocosm, causing an overall increase in MPB biomass. Nutrients can be limiting for photosynthesis (Admiraal 1984) in natural ecosystems, and this is likely to be more extreme in confined systems.

4.4.3: Species density

Species density had a significant effect on MPB biomass, interacting with both species identity and algal treatments in this mesocosm experiment. The effect of increasing species density on MPB biomass was species specific, increased density of both *Hediste diversicolor* and *Corophium volutator* had a negative effect on MPB biomass (low MPB biomass). In contrast increased densities of *Hydrobia ulvae* had no effect. Species density can affect MPB biomass either through consumption (Hagerthey *et al* 2002), or through physical disturbance of the sediment surface (Defew *et al* 2002a). MPB is one of the major components of the diet of the macrofaunal species used in this experiment (Hagerthey *et al* 2002). An increase in density will intensify the consumption of MPB. If consumption were above the rate of production there would be a reduction in MPB biomass. An increase in

macrofaunal biomass would also increase the disturbance cause at the sediment surface due the increased number of burrows, and the maintenance of burrows. There was a species density affect on *Hydrobia ulvae* in Chapter 3, so it was surprising not to see a response in this experiment. It is possible that the highest densities were not enough to cause a response however, due to the density being the same as in Chapter 3 this is unlikely.

4.4.4: Species identity

Species identity had a significant effect on MPB, interacting with both flow and species density. Visual observation of *Hediste diversicolor* revealed this species to be deposit feeding; this is consistent with observations with the static mesocosms in chapter 3 (Bulling *et al* Submitted, Dyson *et al* 2007). It was assumed that elevated levels of MPB was due to *Hediste diversicolor* feeding behaviour, deposit feeding at depth caused bioturbation and the release of nutrients but also left the surface sediment undisturbed. Biles *et al* (2003) found that mesocosms containing macrofauna had higher concentrations of NH_4-N in the water column in the flow treatment compared to static treatment. It would therefore be expected in this study that mesocosms under flow conditions (Biles *et al* 2003) would have a higher MPB biomass than in static treatments. This was correct for *Hydrobia ulvae* and *Corophium volutator*, however this was not true for *Hediste diversicolor* where MPB biomass was lower compared to static conditions. The changes in MPB biomass under flow conditions, although not supported by visual evidence, could be due *Hediste diversicolor* switching from deposit feeding at depth in static conditions to surface deposit feeding during flow conditions. This change in feeding behaviour would reduce bioturbation and associated nutrient release that would impact directly through the consumption of diatoms.

Hydrobia ulvae are active surficial grazers that consume MPB (Defew *et al* 2002a, Hagerthey *et al* 2002). There was elevated MPB biomass in flow mesocosms compared to static conditions, the underlying mechanism is likely to be associated with changes in behaviour, promoting macrofauna to bioturbate the sediment more actively under flow conditions (Biles *et al* 2003).

Corophium volutator interacted with both flow and density, where flow had a positive effect and increased density had a negative effect on MPB biomass. The lower levels of MPB biomass at increased species density were also shown in Chapter 3. It was assumed that the low levels of MPB biomass were due to this species being highly active and re-suspending particulate material, increasing turbidity and reducing the levels of light able to penetrate to the sediment surface (Dyson *et al* 2007). Under flow conditions MPB biomass increases compared to static conditions. Reduced turbidity maybe due to *Corophium volutator* being less active under flow conditions, the current will help to provide particulate matter for food and assist oxygen exchange. Therefore the need to create a current through movement is reduced, resulting in fewer disturbances to the sediment surface and a greater chance for diatom growth. This would be relatively easy to investigate in the future by measuring water column turbidity and comparing control mesocosms (no macrofauna) with mesocosms containing *Corophium volutator*.

The impact on MPB biomass under flow conditions can be positive or negative depending on species identity, and the behavioural change that takes places under flow conditions. Biles *et al* (2003) found that ecosystem function was increased under flow condition no matter what the species. In this study, where a different measurement of ecosystem function was used, it has been shown that species identity can have either a positive or negative impact. It is therefore important to consider the limitation of the experimental design and the possible misinterpretations when relating to the natural environment.

4.4.5: Enrichment

Algal enrichment was a significant factor interacting with species density. Increased density reduced the MPB biomass levels in the heterogeneous mesocosm, however there was no effect in the homogeneous treatments. In chapter 3 a significant interaction between algal enrichment and species density was also found. The general trend for all algal enriched treatments was a reduction in MPB biomass, the increased grazing and associated physical impact on the sediment surface was thought to be the cause. Here, flow could be reducing the effect of increased species density on MPB biomass, however this would not explain the MPB biomass reduction in the heterogeneous mesocosm.

4.4.6: Movement

The net movement of macrofaunal species was measured by counting the number of individuals that were in the neighbouring (right) patch after the 6-day test period. In this experiment macrofauna were positioned at the start of the test in the left patch, so net movement is a measurement of species leaving the focus (left) patch. Species identity, species density and algae were all significant factors in this system, however flow was insignificant for all interaction terms and as a single factor, and was therefore not included in the final model.

The net movement pattern of *Corophium volutator* was different to that of *Hediste diversicolor* and *Hydrobia ulvae*. Both *Hediste diversicolor* and *Hydrobia ulvae* showed the greatest net movement in NE|E treatments, this is most likely due to food availability (Bulling *et al* Submitted). In this treatment, all species started the experiment in the non-enriched patch that was lower in MPB biomass and only *Hydrobia ulvae* and *Hediste diversicolor* moved to the enriched patch that was higher in MPB biomass. Although *Corophium volutator* is a consumer of MPB, previous observations in the field (Raffaelli *et al* 1991, Rossi 2006) and mesocosm experiment (Bulling *et al* 2007) has shown lower dispersion within enriched patches and movement away from enriched patches respectively. Therefore there was no expectation in this experiment for *Corophium volutator* to move into the enriched patch of the heterogeneous mesocosm, even when moving would mean a more readily available food source and less competition from neighbours. In the non-enriched mesocosm the effect of competition caused greater net movement and produced an even spread of *Corophium volutator* over the mesocosm.

4.5: Conclusion

Flow and spatial heterogeneity within the experimental mesocosm system played an important role in determining MPB biomass, interacting with both macrofaunal species identity and density. Control mesocosm were successfully established, there was no significant difference between flow and static systems as expressed by MPB biomass therefore this demonstrated that all changes could only be attributed to macrofaunal behaviour. Algal treatment was also shown to be an important factor

where patches of enrichment were created resulting in an elevated MPB biomass. The experimental set-up was based on a tried and tested methodology (Ieno *et al* 2006, Bulling *et al* 2007, Dyson *et al* 2007) and all statistical models followed appropriate procedures (Pinheiro & Bates 2006, West *et al* 2007).

Flow was an important interacting factor influencing MPB biomass, but had no influence on net macrofaunal movement. Biles *et al* (2003) showed that flow increased ecosystem function and suggested that changes in macrofauna behaviour was the underling mechanism for this increase. My data showed that the influence of flow was not always an increase in ecosystem function but was clearly species specific.

The difference in the findings between these two studies is most likely due to the different methods of measuring ecosystem function. Biles *et al* (2003) measured nutrients in the water column, which are the first steps to sustaining life as they are required for primary produces. My study used a proxy for primary production (MPB biomass) to assess ecosystem function, a step further up the food chain. This shows that caution is need when generalising ecosystem function results since depending on the method of measuring the output or result may vary (Hector and Bagchi 2007). Mesocosm designs are useful tools for manipulating the environment by limiting and controlling the number of factors that can affect the ecosystem response. However, these systems can only represent a small proportion of possible natural habitat interactions, and once scaled to the size of a natural system might not be a true representation.

5. Ecosystem function for multiple species in static mesocosms

5.1: Introduction

The effects of biodiversity on ecosystem processes have received considerable attention due to the concern that the loss of biodiversity may impair ecosystem functioning and, ultimately, the ecosystem services on which humans rely (Tilman, 1999; Duffy, 2002). In the past, it was generally accepted that diversity was controlled by disturbance and productivity, and that ecosystem functioning was controlled by the traits of the dominant resident species (Tilman *et al*, 1996; Naeem and Li, 1997). However, resent research implies that diversity is as important as composition in determining ecosystem functioning, and a more generalised hypothesis is, that changes in species richness has a measurable effect on ecosystem processes (Naeem *et al*, 1994; Raffaelli *et al*, 2003b).

Studies investigating species richness effects have compared functional rates (ecosystem processes) from monoculture ecosystem to rates from multi-culture experiments (Emmerson *et al*, 2001; Raffaelli *et al*, 2003a; Bruno *et al*, 2006). The relative affects of species combinations can be compared against the functional effects of individual species. For example, increasing the biomass of *Corophium volutator* increases the nutrient flux across the sediment interface in a linear manner against biomass. This can be used to predict the effect of a certain biomass of *Corophium volutator* on nutrient flux. If this is repeated for each species then predications can be made on the expected effect of species combinations of known biomass (Emmerson *et al* 2001)

The predicted effects of species combinations (species richness) are estimated by comparing the ecosystem function measurement of all monocultures that contribute to the mixture. The monoculture with the highest ecosystem function measurement is then compared to the ecosystem function measurement of the multi species culture. If the multi species culture is the highest value, then the system has exceeded the predicted value and is said to be ‘overyielding’. If the multi species culture is the lowest value, than the system has not reached the predicted value and is said to be ‘underyeilding’ (Emmerson *et al* 2001). However, there is general confusion over

what constitutes a diversity effect and how to untangle effects on ecosystem properties based solely on species diversity from the usually much stronger effects of species identity and composition (Drake 2003, Richmond *et al* 2005).

The tight controls needed to obtain unambiguous interpretation of cause-effect relationships in experiments have a related "costs" in terms of replicating natural systems. To increase the realism of mesocosm systems, the numbers of variable factors included into the experimental design are increased. This adds to the realism of the system but increases the complexity of the experimental model and the sophistication of the statistics required to interpret it.

In this experiment, three variables were used, species richness, heterogeneity and macrofaunal biomass. Dyson *et al* (2007) revealed significant interactions between species density and ecosystem heterogeneity in single species experiments. Therefore it might be expected that these terms would also be significant with the additional complexity of species richness. Many terrestrial (Naeem *et al* 1994, Tilman 1996, Hector *et al* 1999) and marine studies (Emmerson *et al* 2001, Raffaelli *et al* 2003a) have shown the effects of species richness being either complimentary, by increasing expected additive values of combined single species ecosystem function (over yielding), or having a negative effect, by reducing expected ecosystem function (under yielding). The null hypothesis is that species richness has no effect on ecosystem function in these experimental systems.

5.2: Methods and Materials

5.2.1: Sediment and macrofauna collection

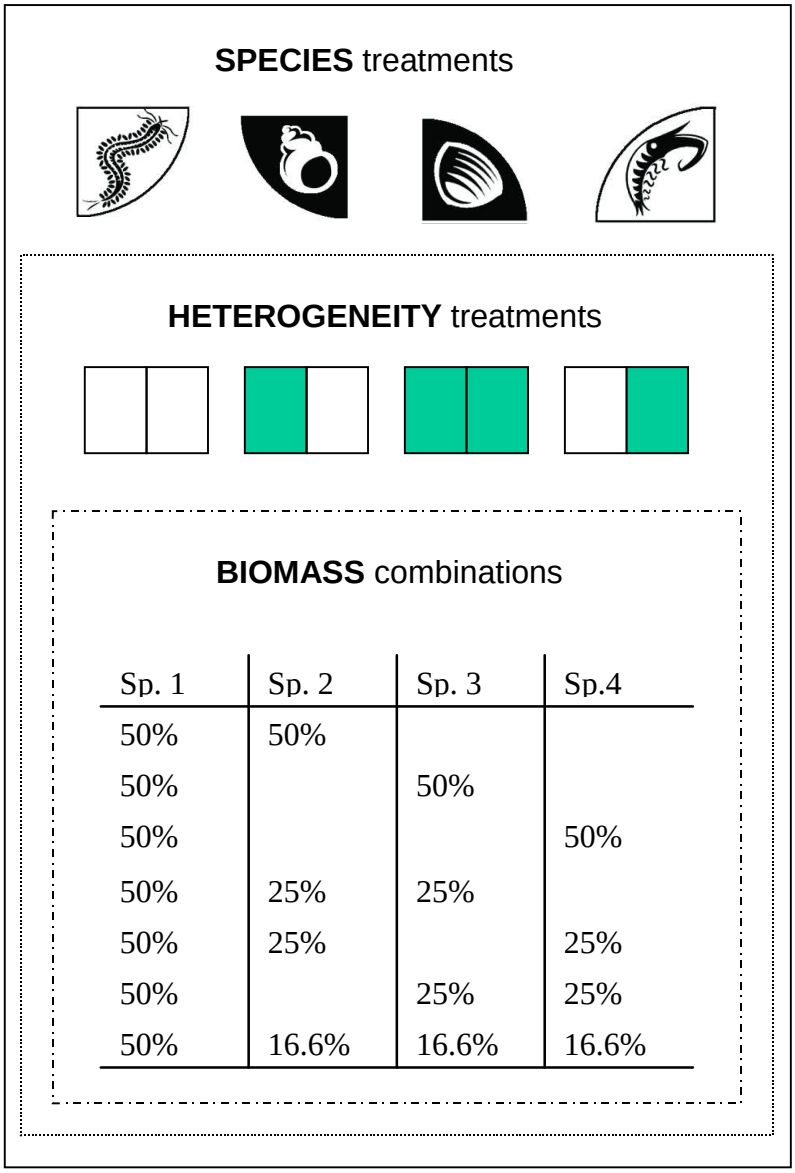
The collection and treatment of sediment and macrofauna are described in Chapter 2 (Materials and Methods).

5.2.2: Experimental design

The experiment was designed to include four algal treatments, 4 biomass levels of the selected macrofauna, and 4 species of macrofauna. All treatments had 22 species × biomass combinations for each of the four algal treatments. All treatments were repeated in triplicate, equalling 66 mesocosms for each algal treatment, making a total of 264 mesocosms (Fig 5.1). To reduce the number of permutations, the macrofauna were only introduced into the left patch of the mesocosms. The experiments were run in two randomly selected batches due to the space constraints within the control temperature room.

5.2.3: Fluorescence measurements (Fo^{15})

Fluorescence readings (FMS2) were taken on day 7. This time period was chosen as an appropriate length of time that best captures the changes caused by species behaviour without the fluorescence levels dropping below a reliable limit as a consequence of the laboratory conditions, as discussed by Defew *et al* (2002b). The measurable output of the PAM fluorescence meter is Fo^{15} (ratio, no units) which is a proxy for the chlorophyll *a* (Chl a) content on the sediment surface (Serôdio *et al* 2001, Honeywill *et al* 2002) and can therefore be used as a proxy for potential primary production (Consalvey *et al*, 2004b).



Sp. 1	Sp. 2	Sp. 3	Sp.4
50%	50%		
50%		50%	
50%			50%
50%	25%	25%	
50%	25%		25%
50%		25%	25%
50%	16.6%	16.6%	16.6%

Fig 5.1: Overview of experimental design. The species density gradients across the patch interface were established at the start of the experiment, using the relative levels of 0%, 16.6%, 25% or 50% natural density of species at the study site. Therefore, multiple species combinations were made to total 100% natural densities. The biomass combinations were repeated for each of the four species (*Corophium volutator* (), *Hydrobia ulvae* (), *Macoma balthica* () and *Hediste diversicolor* ()). All species were placed in the left hand patch (Focus patch) at the start of the experiment. These combinations were used for each of the four interface treatments (enriched = green and non-enriched = white), and experiments were repeated in triplicate.

Two fluorescence readings were taken per patch and averaged to reduce the effects of the inherent natural heterogeneity, giving total replication of n = 3. MPB biomass could also be considered over the whole mesocosm (n = 4). The patch where measurements were taken was known as the 'focus patch', the adjacent patch, known as the 'neighbouring patch' was code with the algal treatment (enriched or non-enriched). This arrangement allows ecosystem function to be modelled at two spatial scales. Measured as a whole mesocosm where the effects of algae are shown by 3 types of treatment (E|E, E|NE & NE|NE), and measured at the patch level, where the effects of interface are shown by 4 treatment types (E|E, E|NE, NE|E & NE|NE).

5.2.4: Movement measurements

Macrofaunal net movement measurements were taken on day 10. Each patch was isolated using a Perspex divider, the sediment was then collected and sieved and the macrofauna counted (as described in Chapter 2). As all macrofauna were introduced into the left 'focus' patch, net macrofaunal movement was a measure of emigration from or migration into this patch. Therefore movement out of the focus patch would give a negative value and movement into the focus patch would give a positive value.

5.2.5: Data analysis

The data was analysed using a linear regression with a generalized least squares (GLS) estimation, as described in Chapter 2. The models used were:

- Model 1; $Fo^{15} \sim f$ (algae, BS *Hediste diversicolor*, BS *Hydrobia ulvae*, BS *Macoma balthica*, BS *Corophium volutator*)
- Model 2; $Fo^{15} \sim f$ (interface, BS *Hediste diversicolor*, BS *Hydrobia ulvae*, BS *Macoma balthica*, BS *Corophium volutator*)
- Model 3; *Hediste diversicolor* Movement ~ f (interface, BS *Hydrobia ulvae*, BS *Macoma balthica*, BS *Corophium volutator*)
- Model 4; *Hydrobia ulvae* Movement ~ f (interface, BS *Hediste diversicolor*, BS *Macoma balthica*, BS *Corophium volutator*)
- Model 5; *Macoma balthica* Movement ~ f (interface, BS *Hediste diversicolor*, BS *Hydrobia ulvae*, BS *Corophium volutator*)

- Model 6; *Corophium volutator* Movement ~ *f* (interface, BS *Hediste diversicolor*, BS *Hydrobia ulvae*, BS *Macoma balthica*)

Where:

Algae: enrichment treatment within a mesocosm (E|E, E|NE and NE|NE, where E = enriched, NE = non-enriched and | is the boundary between the two patches)

Interface: enrichment treatment of a patch with consideration to neighbouring patch (E|E, E|NE, NE|NE and NE|E, where results from the left patch are used in conjunctions with a code for right patch)

BS: standardised biomass (all species combinations added to 100% of the natural macrofaunal density found in the Ythan estuary).

The data were analysed further to compare 'actual' values measured during species richness experiments with predicted additive values from the single species experiments (Chapter 3). The optimal fluorescence models from the single species experiments were bootstrapped (× 1000) to predict an 'expected' (mean) additive value for species in combination, plus a lower and upper confidence interval (95%). Bootstrapping is a statistical method for estimating the sampling distribution of an estimator by repeated sampling with replacement from the original sample. The predicted data was then used in conjunction with the 'actual' values to assess functional capacity under different species richness combinations. It was not possible to bootstrap all the data in this experiment due to the computational limitations. Therefore data was selected for one species (*Hediste diversicolor*) to examine the predicted and actual measurement with all interface treatments.

5.3: Results

The study revealed significant effects of heterogeneity, *Hediste diversicolor* biomass, *Hydrobia ulvae* biomass, *Macoma balthica* biomass and *Corophium volutator* biomass on MPB biomass. *Hydrobia ulvae* was the only species that interacted with heterogeneity and had a significant effect on net movement. A net movement model for *Macoma balthica* was unnecessary, as this species did not move.

5.3.1: Mesocosm model

The optimal model for algal treatment was a linear regression with a GLS extension (allowing for unequal variance within *Corophium volutator* biomass and *Hydrobia ulvae* biomass) that incorporated five single factors, ten two-way interaction terms and three, three-way interaction terms (AIC = 2090.08, d.f. = 28; Table 5.1).

Table 5.1 Significant interaction and variance-covariate terms for algae model.

Term type	Significant factors
Single factors	Algae
	H. diversicolor biomass
	H. ulvae biomass
	M. balthica biomass
	C. volutator biomass
Two-way interactions	Algae × *H. diversicolor* biomass
	Algae × *H. ulvae* biomass
	Algae × *M. balthica* biomass
	Algae × *C. volutator* biomass
	H. diversicolor biomass × *H. ulvae* biomass
	H. diversicolor biomass × *M. balthica* biomass
	H. diversicolor biomass × *C. volutator* biomass
	H. ulvae biomass × *M. balthica* biomass
	H. ulvae biomass × *C. volutator* biomass
	M. balthica biomass × *C. volutator* biomass
Three-way interactions	Algae × *M. balthica* biomass × *C. volutator* biomass
	H. diversicolor biomass × *H. ulvae* biomass × *M. balthica* biomass
	H. diversicolor biomass × *C. volutator* biomass × *M. balthica* biomass
Variance-covariate term	*C. volutator* biomass × *H. ulvae* biomass

The single factor that had the greatest influence on the model was *Hediste diversicolor* biomass (L-ratio = 37.38, AIC = 2111.45, d.f. = 20, p = <0.0001), followed by *Corophium volutator* biomass (L-ratio = 37.73, AIC = 2109.81, d.f. = 19, p = <0.0001), *Macoma balthica* biomass (L-ratio = 36.58, AIC = 2106.66, d.f. = 18, p = <0.001) and the influence of *Hydrobia ulvae* was not significant (L-ratio = 9.10, AIC = 2085.18, d.f. = 21, p = 0.2454).

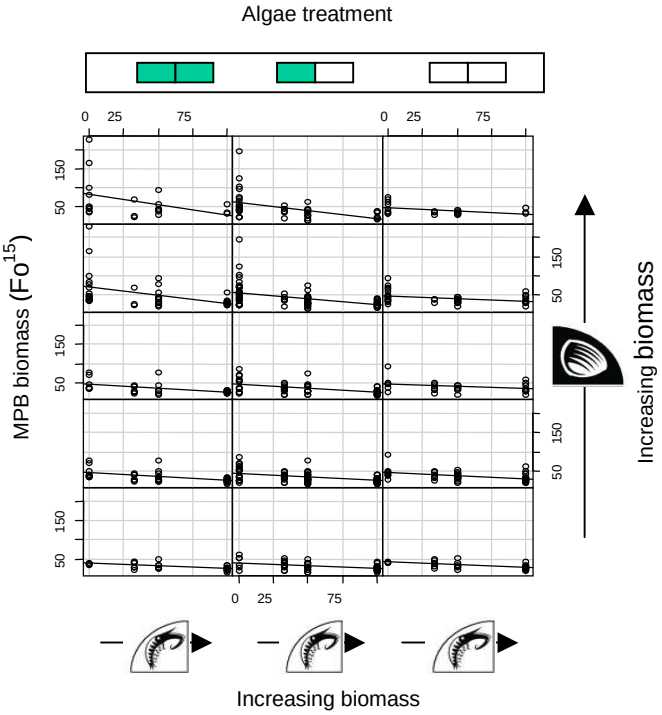


Fig 5.2: Graphical representation of the three-way interaction term, algae × *C. volutator* biomass × *M. balthica* biomass. Algal enrichment treatment is represented by a diagrammatic plan of the mesocosm divided into two patches where E is an enriched patch (green) and NE is non-enriched patch (white). Species identity is represented by diagrammatic pictures, *Macoma balthica* ( ) and *Corophium volutator* ( ). Species density is a percentage of the natural densities found in the Ythan estuary. Lines represent the linear relationship between variables with model adjustments; data points are present but have not been model adjusted, these points must be used with caution.

The three significant three-way interaction terms within the model were: Algal treatment × *Macoma balthica* biomass × *Corophium volutator* biomass (L-ratio = 8.28, AIC = 2094.36, d.f. = 26, p = <0.05; Fig 5.2), *Hediste diversicolor* biomass ×

Hydrobia ulvae biomass × *Macoma balthica* biomass (L-ratio = 8.11, AIC = 2096.19, d.f. = 27, p = <0.01; Fig 5.3) and *Hediste diversicolor* biomass × *Corophium volutator* biomass × *Macoma balthica* biomass (L-ratios = 15.04, AIC = 2103.115, d.f. = 27, p = <0.001; Fig 5.4).

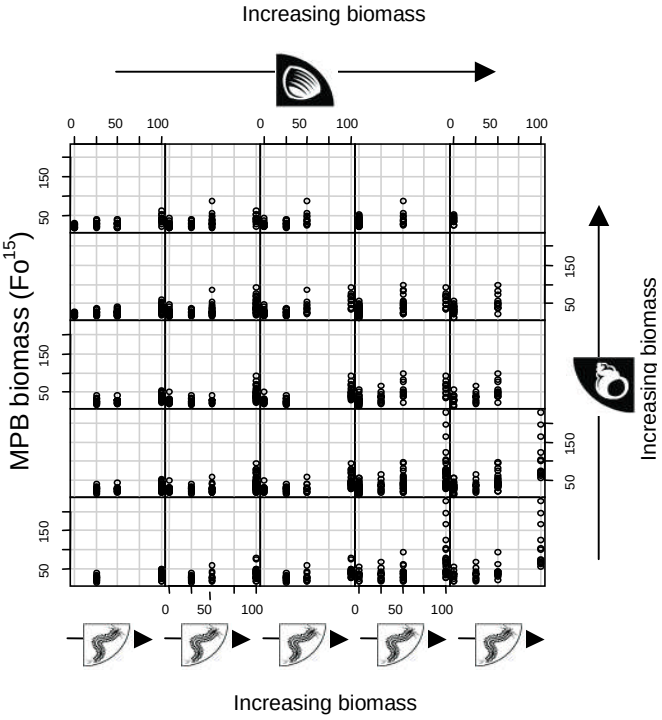


Fig 5.3: Graphical representation of the three-way interaction term, *H. ulvae* biomass × *M. balthica* biomass × *H. diversicolor* biomass. Species identity is represented by diagrammatic pictures, *Macoma balthica* (), *Hydrobia ulvae* () and *Corophium volutator* (). Species density is a percentage of the natural densities found in the Ythan estuary.

Algal treatments influenced MPB biomass with fully enriched treatments (E|E) having the highest levels and non-enriched treatments (NE|NE) having the lowest levels of MPB (Fig 5.2). Interactions of the other macrofaunal species with either *Corophium volutator* (Fig. 5.3) or *Hydrobia ulvae* (Fig 5.4) resulted in lower MPB biomass as macrofaunal densities increased. *Macoma balthica* and *Hediste diversicolor* had the opposite effect on MPB biomass levels where increased macrofaunal densities also increased MPB biomass. Therefore within this interaction term the highest levels of

MPB biomass was achieved in mesocosms with maximum enrichment and maximum *Macoma balthica* biomass levels and/or *Hediste diversicolor* and no/minimum levels of *Corophium volutator* or *Hydrobia ulvae* present (Fig 5.3 & 5.4).

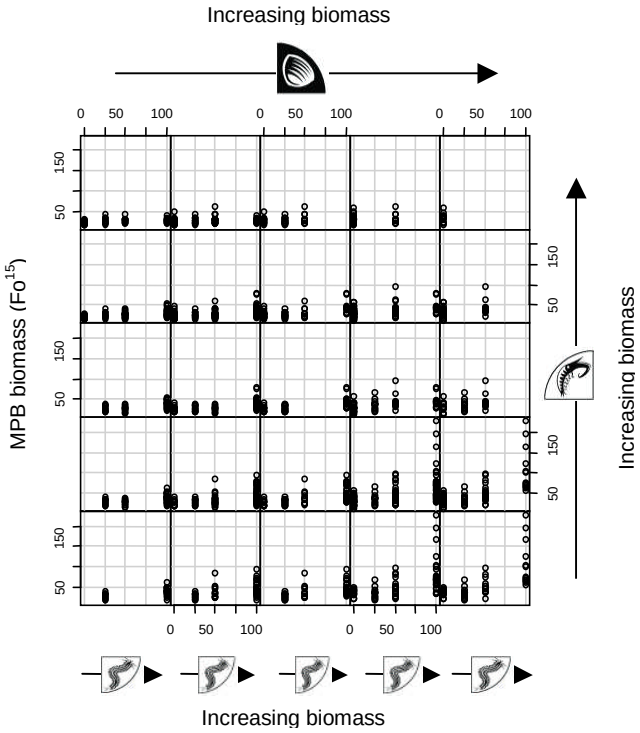


Fig 5.4: Graphical representation of the three-way interaction term, *Corophium volutator* biomass × *Macoma balthica* biomass × *Hydrobia diversicolor* biomass. Species identity is represented by diagrammatic pictures, *Macoma balthica* (),*Corophium volutator* () and *Hediste diversicolor* (). Species density is a percentage of the natural densities found in the Ythan estuary.

Actual measurements of MPB biomass were far lower than predicted (Fig 5.5), however, despite the actual values being lower, the difference between interface treatments shown (Fig 5.2) was supported. The predicted values for two species combinations were highest for *Hediste diversicolor* and *Macoma balthica*, although lower than predicted this combination did have actual measurements that were higher than the other two species combinations in all interface treatments. The predicted

values were higher for three species combinations and higher still for the four species combination, however for *Hediste diversicolor* the actual three and four species combinations were not higher than the two species combinations.

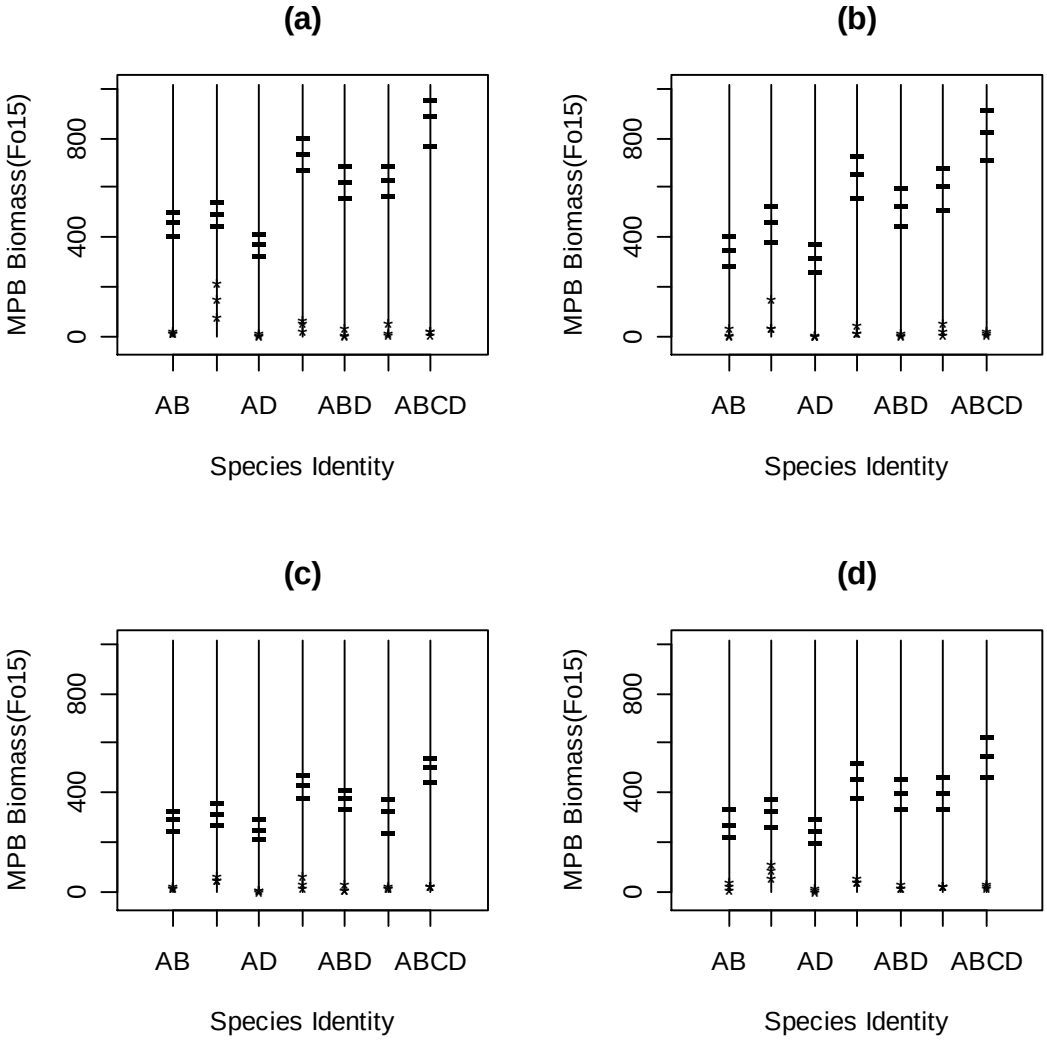


Fig 5.5: Graphical representation of the low, medium and high predicted values (-) taken from the bootstrapped data and actual values (∗) taken from the multi species experiments (n = 3), where species in combinations are (A) *Hediste diversicolor,* (B) *Hydrobia ulvae*, (C) *Macoma balthica* and (D) *Corophium volutator*. Each interface treatment is plotted, fully enriched E|E (a), half enriched E|NE (b), non-enriched NE|NE (c), and half enriched NE|E (d).

5.3.2: Interface model

This model was a linear regression with a GLS extension (allowing for unequal variance within *Corophium volutator* biomass and *Hydrobia ulvae* biomass factors) incorporating five single factors, ten two-way interaction terms and five three-way interaction terms (AIC = 2109.81, d.f. = 40; Table 5.2).

Table 5.2 Significant interaction and variance-covariate terms for interface model.

Term type	Significant factors
Single factors	Interface
	H. diversicolor biomass
	H. ulvae biomass
	M. balthica biomass
	C. volutator biomass
Two-way interactions	Interface × *H. diversicolor* biomass
	Interface × *H. ulvae* biomass
	Interface × *M. balthica* biomass
	Interface × *C. volutator* biomass
	H. diversicolor biomass × *H. ulvae* biomass
	H. diversicolor biomass × *M. balthica* biomass
	H. diversicolor biomass × *C. volutator* biomass
	H. ulvae biomass × *M. balthica* biomass
	H. ulvae biomass × *C. volutator* biomass
	M. balthica biomass × *C. volutator* biomass
Three-way interactions	Interface × *H. diversicolor* biomass × *C. volutator* biomass
	Interface × *H. diversicolor* biomass × *M. balthica* biomass
	Interface × *M. balthica* biomass × *C. volutator* biomass
	H. diversicolor biomass × *H. ulvae* biomass × *M. balthica* biomass
	H. diversicolor biomass × *C. volutator* biomass × *M. balthica* biomass
Variance-covariate terms	*C. volutator* biomass × *H. ulvae* biomass

Interface treatment was the single factor that had the greatest influence on the model (L-ratio = 104.60, AIC = 2166.41, d.f. = 16, p<0.0001), followed by *Corophium volutator* biomass (L-ratio = 64.90, AIC = 2140.72, d.f. = 23, p<0.0001), *Hediste diversicolor* biomass (L-ratio = 63.12, AIC = 2148.92, d.f. = 28, p<0.0001), *Macoma balthica* biomass (L-ratio = 42.04, AIC = 2127.85, d.f. = 28, p<0.0001) and *Hydrobia ulvae* biomass (L-ratio = 27.45, AIC = 2117.26, d.f. = 30, p = 0.01)

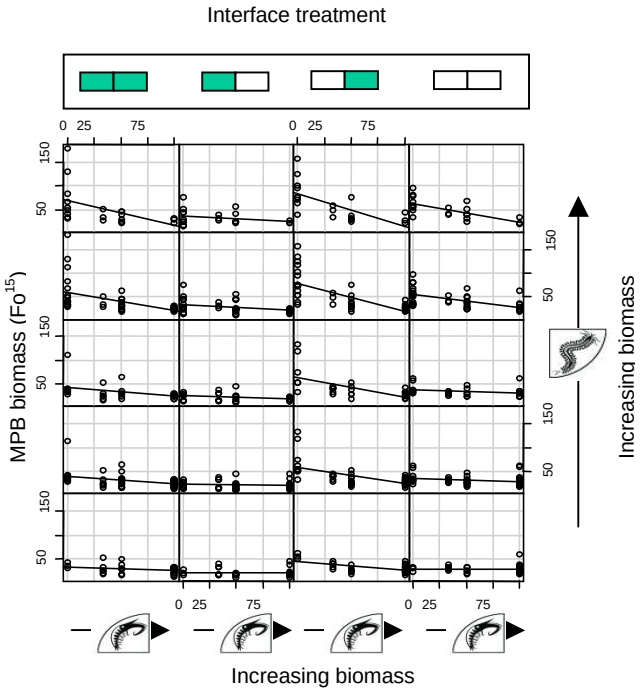


Fig 5.6: Graphical representation of the three-way interaction term, interface × *C. volutator* biomass × *H. diversicolor* biomass. Interface treatment is represented by a diagrammatic plan of the mesocosm divided into two patches where E is an enriched patch (green) and NE is non-enriched patch (white). Species identity is represented by diagrammatic pictures, *Hediste diversicolor* () and *Corophium volutator* (). Species density is a percentage of the natural densities found in the Ythan estuary. Lines represent the linear relationship between variables with model adjustments; data points are present but have not been model adjusted, these points must be used with caution.

The significant three-way interaction terms within the model were: Interface treatment × *Hediste diversicolor* biomass × *Corophium volutator* biomass (L-ratio = 13.78, AIC = 2117.59, d.f. = 37, p = <0.01; Fig 5.6), Interface treatment × *Hydrobia ulvae* biomass × *Corophium volutator* biomass (L-ratio = 15.92, AIC = 2119.74, d.f. = 37 p = <0.01; Fig 5.7), Interface treatment biomass × *Macoma balthica* biomass × *Corophium volutator* biomass (L-ratio = 13.62, AIC = 2117.44, d.f. = 34 p<0.01; Fig 5.8), *Hediste diversicolor* biomass × *Hydrobia ulvae* biomass × *Macoma balthica* biomass (L-ratio = 4.70, AIC = 2112.51, d.f. = 39, p = <0.05; Fig 5.9) and *Hediste diversicolor* biomass × *Corophium volutator* biomass × *Macoma balthica* biomass (L-ratio = 12.52, AIC = 2120.33, d.f. = 39, p = <0.001; Fig 5.10).

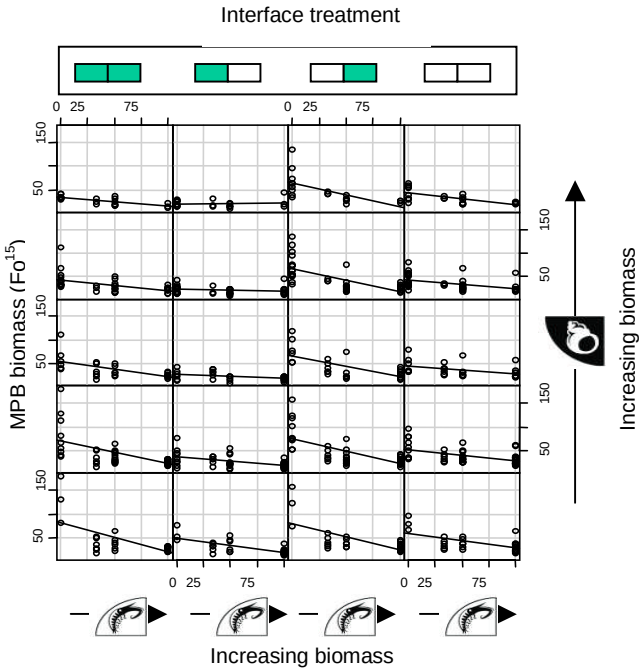


Fig 5.7: Graphical representation of the three-way interaction term, interface × *Corophium volutator* biomass × *Hydobia ulvae* biomass. Interface treatment is represented by a diagrammatic plan of the mesocosm divided into two patches where E is an enriched patch (green) and NE is non-enriched patch (white). Species identity is represented by diagrammatic pictures, *Hydrobia ulvae* ( ) and *Corophium volutator* ( ). Species density is a percentage of the natural densities found in the Ythan estuary. Lines represent the linear relationship between variables with model adjustments; data points are present but have not been model adjusted, these points must be used with caution.

This interface model had similar interaction terms as for the algal model but with two additional three-way interactions. The macrofaunal interaction responses had the same patterns, where *Hediste diversicolor* and *Macoma balthica* enhanced MPB biomass levels and *Hydrobia ulvae* and *Corophium volutator* reduced MPB biomass levels. There was a consistent pattern in that all three significant interactions that include interface always contained *Corophium volutator* biomass as a factor plus another species (Fig 5.6, 5.7 & 5.8). The coefficient values show that the interface term driving this interaction is NE|E.

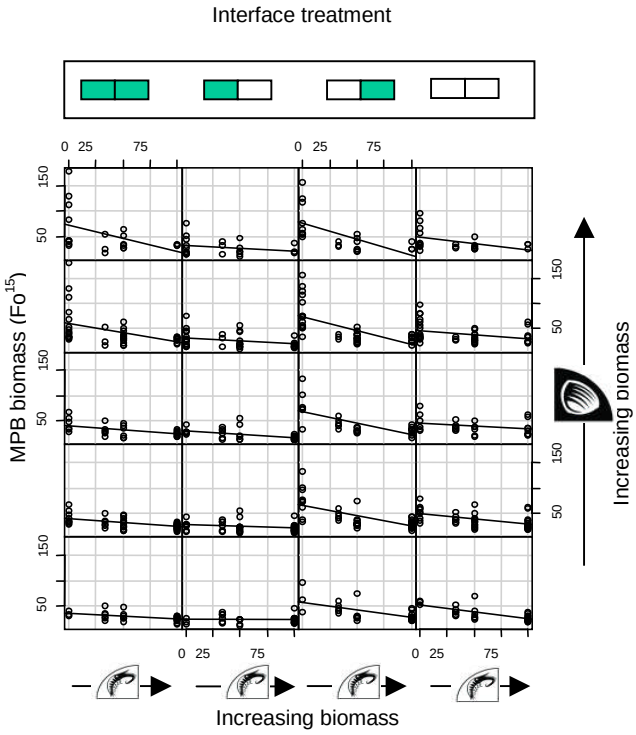


Fig 5.8: Graphical representation of the three-way interaction term, interface × *Corophium volutator* biomass × *Macoma balthica* biomass. Interface treatment is represented by a diagrammatic plan of the mesocosm divided into two patches where E is an enriched patch (green) and NE is non-enriched patch (white). Species identity is represented by diagrammatic pictures, *Macoma balthica* () and *Corophium volutator* (). Species density is a percentage of the natural densities found in the Ythan estuary. Lines represent the linear relationship between variables with model adjustments; data points are present but have not been model adjusted, these points must be used with caution.

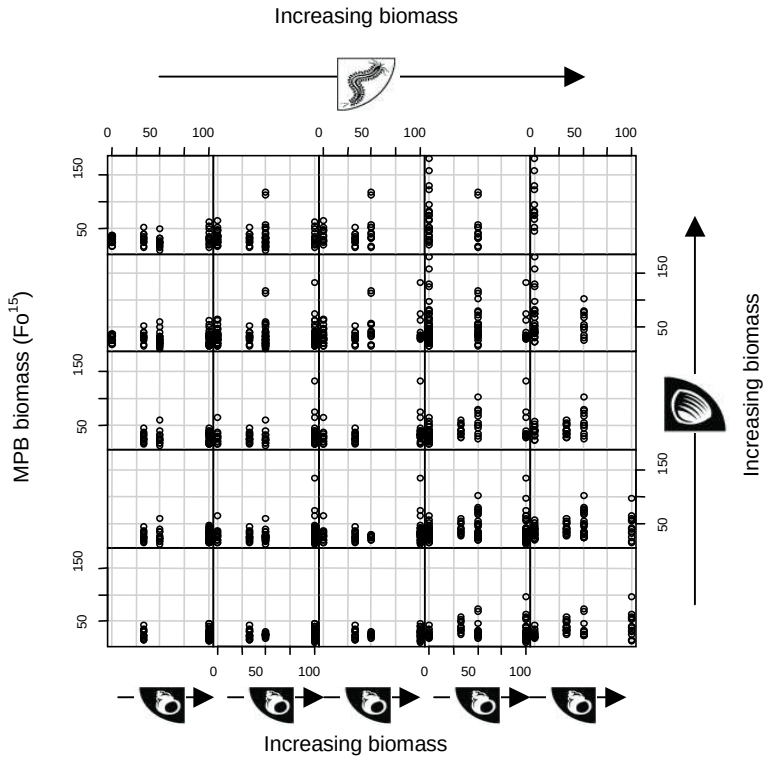


Fig 5.9: Graphical representation of the three-way interaction term, *Hydrobia ulvae* biomass × *Macoma balthica* biomass × *Hediste diversicolor* biomass. Species identity is represented by diagrammatic pictures, *Hediste diversicolor* (), *Macoma balthica* () and *Hydrobia ulvae* (). Species density is a percentage of the natural densities found in the Ythan estuary.

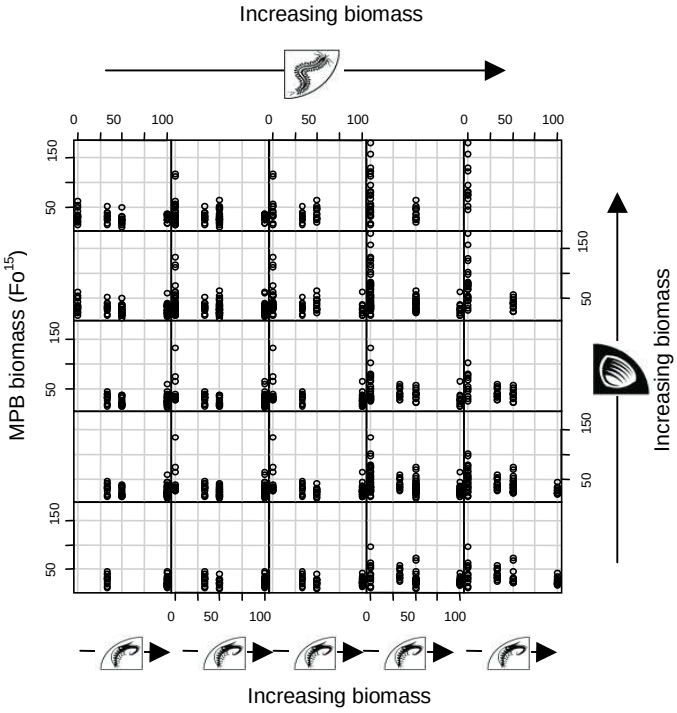


Fig 5.10: Graphical representation of the three-way interaction term, *Corophium volutator* biomass × *Macoma balthica* biomass × *Hediste diversicolor* biomass. Species identity is represented by diagrammatic pictures, *Hediste diversicolor* (), *Macoma balthica* () and *Corophium volutator* (). Species density is a percentage of the natural densities found in the Ythan estuary.

5.3.3: Net movement model for Hediste diversicolor

This model was a linear regression with a GLS extension (allowing for unequal variance within *Hediste diversicolor* biomass factor) incorporating four single factors and one two-way interaction term (AIC = 2154.87, d.f. = 7; Table 5.3).

Table 5.3 Significant interaction terms for net movement of *Hediste diversicolor*

Term type	Significant factors
Single factors	*H. ulvae* biomass
	M. balthica biomass
	C. volutator biomass
Two-way interactions	*H. ulvae* biomass × *M. balthica* biomass
Variance-covariate term	*H. ulvae* biomass

Macoma balthica biomass was the single factor that has the greatest influence on the model (L-ratio = 87.94, AIC = 2238.81, d.f. = 5, $p<0.0001$), followed by *Hydrobia ulvae* biomass (L-ratio = 72.82, AIC = 2223.69, d.f. = 5, $p<0.0001$) and *Corophium volutator* biomass (L-ratio = 70.97, AIC = 2223.84, d.f. = 6, $p<0.0001$).

The significant two-way interaction term within the model was *Hediste diversicolor* biomass × *Macoma balthica* biomass (L-ratio = 7.91, AIC = 2160.78, d.f. = 6, $p = <0.01$; Fig 5.11). The significant interaction term showed that net movement of *Hediste diversicolor* is less likely when there are higher levels of both *Hydrobia ulvae* and *Macoma balthica*.

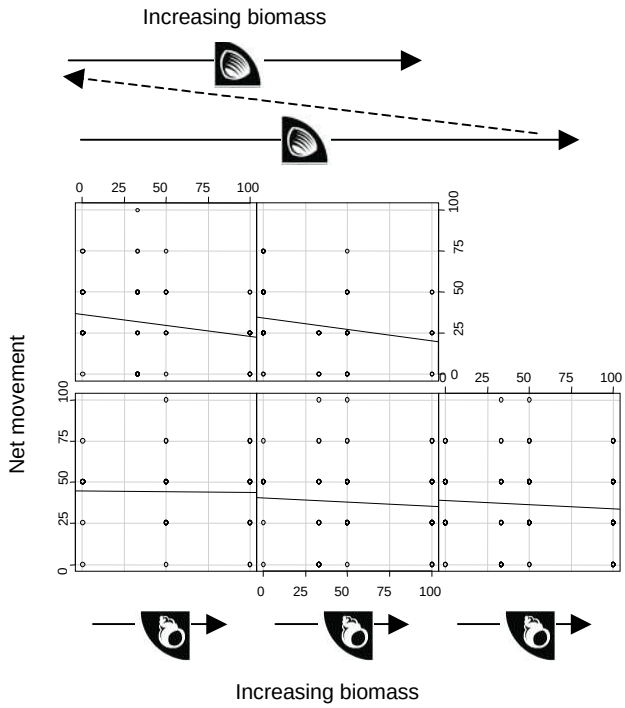


Fig 5.11: Graphical representation of the significant two-way interaction *Macoma balthica* × *Hydrobia ulvae* taken from the net movement model for *Hediste diversicolor*. Net movement was defined as the movement away from the focus patch (left) to the neighbouring patch (right) within the 10 day test period. Species identity is represented by diagrammatic pictures, *Macoma balthica* ( ) and *Hydrobia ulvae* ( ). Species density is a percentage of the natural densities found in the Ythan estuary. Lines represent the linear relationship between variables with model adjustments; data points are present but have not been model adjusted, these points must be used with caution.

5.3.4: Net movement model for Hydrobia ulvae

This model was a linear regression with a GLS extension (allowing for unequal variance for all fitted terms) incorporating four single factors, six two-way interaction terms and two three-way interaction terms (AIC = 1828.49, d.f. = 25; Table 5.4).

Table 5.4 Significant interaction terms for net movement of *Hydrobia ulvae*

Term type	Significant factors
Single factors	Interface treatment
	H. diversicolor biomass
	M. balthica biomass
	C. volutator biomass
Two-way interactions	Interface × *H. diversicolor* biomass
	Interface × *M. balthica* biomass
	Interface × *C. volutator* biomass
	C. volutator biomass × *H. diversicolor* biomass
	C. volutator biomass × *M. balthica* biomass
	H. diversicolor biomass × *M. balthica* biomass
Three-way interaction	*H. diversicolor* biomass × *M. balthica* biomass × *C. volutator* biomass
Variance-covariate term	*H. ulvae* biomass

Corophium volutator biomass was the single factor that has the greatest influence on the model (L-ratio = 286.95, AIC = 2095.44, d.f. = 15, $p<0.0001$), followed by *Macoma balthica* biomass (L-ratio = 282.26, AIC = 2096.75, d.f. = 18, $p<0.0001$), *Hediste diversicolor* biomass (L-ratio = 281.35, AIC = 2089.84, d.f. = 15, $p<0.0001$) and Interface treatment L-ratio = 145.28, AIC = 1943.77, d.f. = 10, $p<0.0001$).

The two significant three-way interaction terms within the model were: *Hediste diversicolor* biomass × *Macoma balthica* biomass × *Corophium volutator* biomass (L-ratio = 16.11, AIC = 1842.60, d.f. = 24, $p<0.001$; Fig 5.12) and Interface treatment × *Corophium volutator* biomass × *Hediste diversicolor* biomass (L-ratio = 8.24, AIC = 1830.73, d.f. = 22, p = <0.05; Fig 5.13). Generally there was greater net movement of *Hydrobia ulvae* with higher densities of *Corophium volutator* (Fig 5.12 & 5.13).

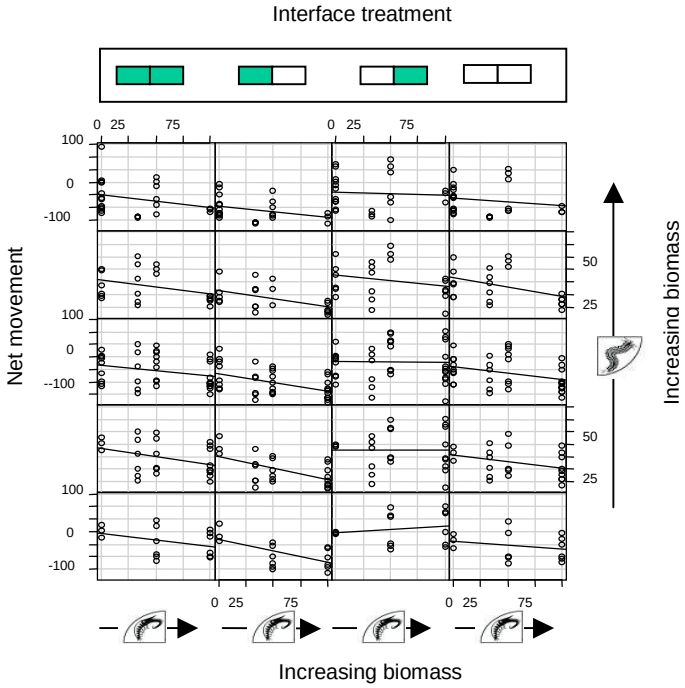


Fig 5.12: Graphical representation of the significant three-way interaction Interface × *Hediste diversicolor* × *Corophium volutator* taken from the net movement model for *Hydrobia ulvae*. Net movement was defined as the movement away from the focus patch (left) to the neighbouring patch (right) within the 10 day test period. Interface treatment is represented by a diagrammatic plan of the mesocosm divided into two patches where E is an enriched patch (green) and NE is non-enriched patch (white). Species identity is represented by diagrammatic pictures, *Hediste diversicolor* () and *Corophium volutator* (). Species density is a percentage of the natural densities found in the Ythan estuary. Lines represent the linear relationship between variables with model adjustments; data points are present but have not been model adjusted, these points must be used with caution.

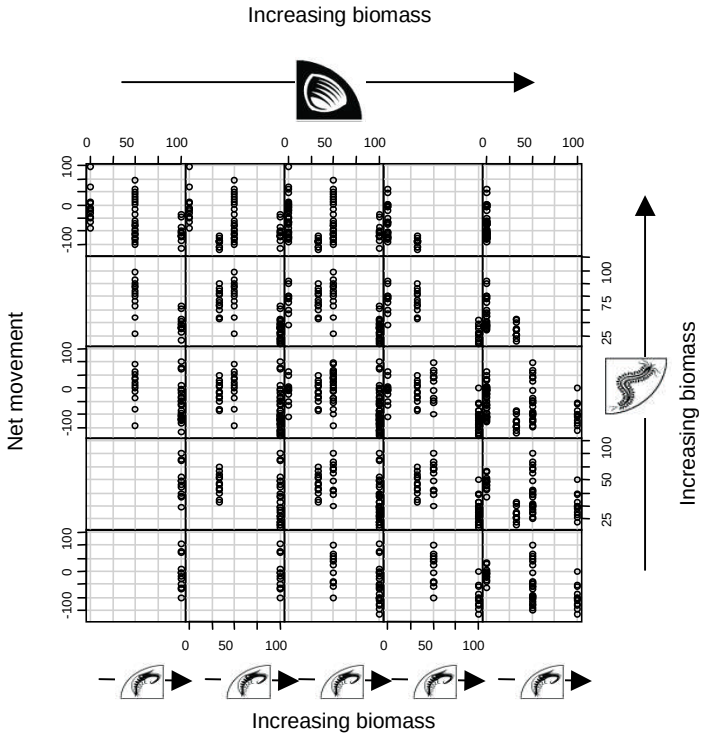


Fig 5.13: Graphical representation of the significant three-way interaction *Macoma balthica* × *Hediste diversicolor* × *Corophium volutator* taken from the net movement model for *Hydrobia ulvae*. Net movement was defined as the movement away from the focus patch (left) to the neighbouring patch (right) within the 10 day test period. Species identity is represented by diagrammatic pictures, *Macoma balthica* (), *Hediste diversicolor* () and *Corophium volutator* (). Species density is a percentage of the natural densities found in the Ythan estuary.

5.3.5 Net movement model for Corophium volutator

This model was a linear regression with a GLS extension (allowing for unequal variance for interface treatment and *Corophium volutator* biomass factor) incorporating four single factors, four two-way interaction terms and one three-way interaction term (AIC = 1813.02, d.f. = 19; Table 5.5).

Table 5.5 Significant interaction terms for net movement of *Corophium volutator*

Term type	Significant factors
Single factors	Interface
	H. diversicolor biomass
	M. balthica biomass
	H. ulvae biomass
Two-way interactions	Interface × *Macoma balthica* biomass
	H. ulvae biomass × *H. diversicolor* biomass
	H. ulvae biomass × *M. balthica* biomass
	H. diversicolor biomass × *M. balthica* biomass
Three-way interaction	*H. diversicolor* biomass × *M. balthica* biomass × *H. ulvae* biomass
Variance-covariate terms	Interface × *C. volutator* biomass

Hydrobia ulvae biomass was the single factor that had the greatest influence on the model (L-ratio = 262.77, AIC = 2067.79, d.f. = 15, $p<0.0001$), followed by *Macoma balthica* biomass (L-ratio = 258.48, AIC = 2057.50, d.f. = 12, $p<0.0001$), *Hediste diversicolor* biomass (L-ratio = 242.19, AIC = 2047.20, d.f. = 15, $p<0.0001$) and Interface treatment L-ratio = 99.50, AIC = 1900.52, d.f. = 13, $p<0.0001$).

The significant three-way interaction term within the model was *Hediste diversicolor* biomass × *Macoma balthica* biomass × *Hydrobia ulvae* biomass (L-ratio = 19.94, AIC = 1830.96, d.f. = 18, $p<0.0001$; Fig 5.14). Increasing the biomass of *Hediste diversicolor* within the system increases net movement of *Corophium volutator*. *Hediste diversicolor* had an optimal standardised density at 50% that caused an increase in net *Corophium volutator* movement.

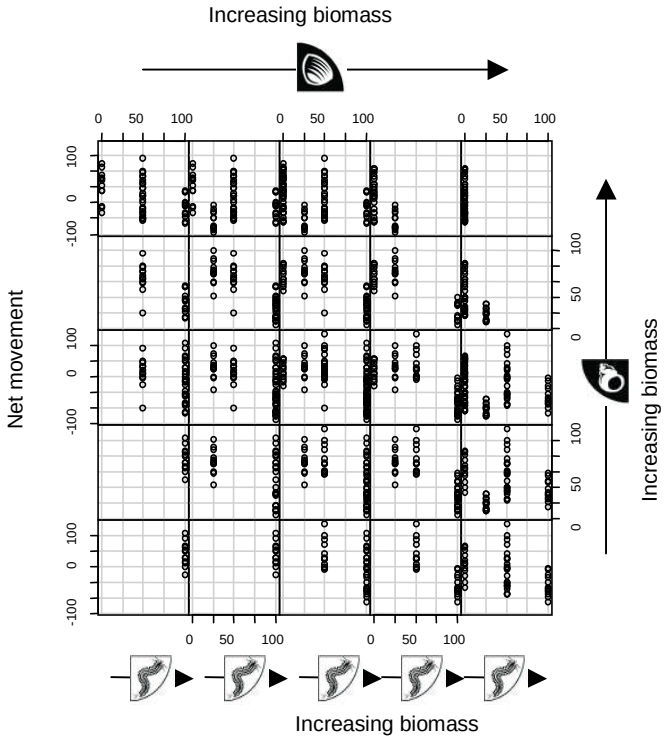


Fig 5.14: Graphical representation of the significant three-way interaction *Macoma balthica* × *Hediste diversicolor* × *Hydrobia ulvae* taken from the net movement model for *Corophium volutator*. Net movement was defined as the movement away from the focus patch (left) to the neighbouring patch (right) within the 10 day test period. Species identity is represented by diagrammatic pictures, *Macoma balthica* (), *Hydrobia ulvae* (). and *Hediste diversicolor* (). Species density is a percentage of the natural densities found in the Ythan estuary.

5.4: Discussion

5.4.1: Species richness

The actual values of ecosystem function as determined through the potential provided by MPB as compared to the predicted values were all under yielding. That is to say those combinations of species produce less effect than each species on it own at the same relative biomass. It is possible that all species combinations in this experiment were having a negative effect on MPB biomass and therefore ecosystem function performance was inhibited by species richness. However, it is more probable that the seasonal variation in MPB biomass in the Ythan estuary, as seen in the subsequent chapters (5 & 6), was having the greatest impact on these results and was therefore an experimental design fault. The single species data (Chapter 3) and the multi species data were collected in the same year. The single species experiments were, however, carried out in early April while the multi species experiments were carried out three months later in July. The seasonal trend (Fig 4.15) demonstrated in Chapter 6 was found over the four months summer sampling period (2005), and would approximate to comparing run 2 with run 5. Clearly, it is not possible to compare these two data sets without further adjustment to compensate for the dramatic reduction on MPB biomass found in the Ythan estuary between April and July.

Despite the lower than predicted MPB biomass, all interface treatments had elevated levels of MPB biomass when *Macoma balthica* was present in the species combinations. This outcome was 'predicted' from bootstrapping the single species data when comparing within the two-way or three-way interactions. However, because the seasonal effects were not corrected, it is not possible to tell if the increase in MPB biomass in the actual data would have caused over yielding.

The interface treatments indicate that the influence of enriching patches within the mesocosm system had an affect on MPB biomass. Enriching the sediment allows the release of nutrients, probably enhanced by macrofaunal bioturbation, from the sediment, which then become available for the MPB, therefore increasing MPB biomass (Bulling et al. submitted, Dyson et al. 2007).

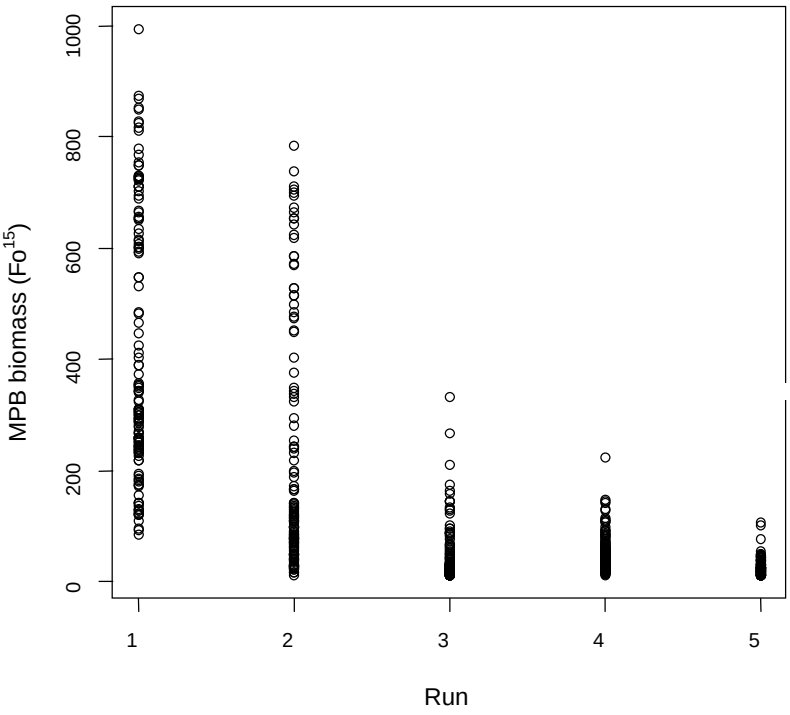


Fig 4.15: Seasonal trends between 'runs' in multi patch measurements (Chapter 6).

The effect of species richness has previously been explored in the marine environment (Emmerson et al. 2001, Raffaelli et al. 2003a). These studies found that biodiversity had an effect on ecosystem function however it was hard to distinguish between species richness and functional traits. Due to the estuarine system being naturally depauperate of macrofaunal species, the problem of distinguishing between species richness and functional traits still persists. It would be interesting to design an experiment that examined ecosystem function of a species, whereby manipulating the surround environment would alter feeding behaviours and therefore change the measurable functional traits. It is known that *Hediste diversicolor* exhibits a range of different trophic behaviours e.g. deposit feeder, active predator and suspension feeder. Under controlled conditions the environment could be manipulated to induce a specific feeding behaviour and the differences in behaviour would be reflected in the ecosystem function measurements.

5.4.2: Species identity

Species identity had varying effects on MPB biomass, such that some species had a positive and some a negative effect. The single species experiments (Chapter 3; Dyson et al. 2007) demonstrated that *Corophium volutator* and *Hydrobia ulvae* had a negative effect on MBP biomass, *Hediste diversicolor* had a positive effect and *Macoma balthica* had no effect on MPB biomass. With the exception of *Macoma balthica* all species in the multi species combinations had the same effect on MPB biomass as species in monoculture.

Macoma balthica in combination with both *Hydrobia ulvae* and *Hediste diversicolor* had a positive effect on the MPB biomass at both the mesocosm and patch levels. During the single species experiments *Macoma balthica* produced MPB biomass levels that were not significantly different to the control (no macrofauna) mesocosms (Dyson et al. 2007). There were no visual observations of *Macoma balthica* behaving differently in these experiments compared to the single species (Chapter 3) and there was no net movement of this species. Due to the changes in MPB biomass but no notable changes to the observed behaviour of the species involved, it would seem that there is a species interaction but the mechanism for increased MPB biomass is unknown.

Hediste diversicolor in combination with all other species had a positive effect on MPB biomass at both mesocosms and patch level. Increased *Hediste diversicolor* biomass also increased the MPB biomass, this was most probably due to the effects of increased bioturbation. The observed behaviour of *Hediste diversicolor* was again not obviously different than in the single species mesocosms, where they were highly active, deposit feeding and burrowed at depth (Magni and Montani 2006). This activity causes the release of nutrients from the sediment that then becomes available to be utilised by primary producers (McLusky1981).

Hydrobia ulvae in combination with any other species had a negative effect on MPB biomass at both mesocosm and patch levels. Increasing *Hydrobia ulvae* biomass had a negative effect on MPB biomass, this would be expected from a species that is a known consumer of diatoms (Hagerthey *et al* 2002) increasing the number of animals exploiting MPB would decrease the expected biomass. The behaviour of *Hydrobia*

ulvae was previously discussed in the single species experiments, it was thought that the greatest impact on ecosystem function was grazing primary producers at the sediment surface. Grazing by *Hydrobia ulvae* causes limited bioturbation to occur in the top few mm of the sediment surface and releases nutrients that become available for MPB. However the balance between the nutrient release and effects this would have had on MPB biomass during bioturbation is out weighted in this mesocosm experiment by grazing.

Corophium volutator in combination with any other species had a negative effect on MPB biomass at both the mesocosm and patch levels. Increased *Corophium volutator* biomass decreased MPB biomass, this was probably due to its behavioural traits. *Corophium volutator* is highly active and re-suspends particulate material (Smith *et al* 1996, Hagerthey *et al* 2002), burrow maintenance causes the water column to become very turbid. This reduces the amount of light that can penetrate the sediment surface and therefore the amount available for photosynthesis and MPB production.

5.4.3: Algal enrichment

As expected from the results of the single species experiments (Dyson et al. 2007), algal treatments had a significant effect on MPB biomass. Fully enriched treatments (E|E) had a higher MPB biomass than half enriched (E|NE), and non-enriched treatment (NE|NE) had the lowest MPB biomass. This indicates that the addition of powdered *Enteromorpha intestinalis* was having the desired effect and creating a heterogeneous environment. However, in mesocosms where macrofauna were absent or only present at low numbers, there was no difference in MPB biomass in algal treatments. This is probably because of the limited bioturbation under these circumstances. The presence of *Corophium volutator* in the experimental systems had a negative impact on MPB biomass in all algal treatments and this resulted in there being no difference between MPB biomass. This was most probably due to the behavioural traits of *Corophium volutator* as discussed in paragraph 5.4.2.

5.4.4: Interface enrichment

Interface treatment had a significant effect on MPB biomass at the three-way interaction level. Surprisingly, all three-way interface interactions included *Corophium volutator* as one of the other two factors. The relationship between

interface and MPB biomass was different compared with Chapter 3 (single species). Here, MPB biomass was highest in the E|E and NE|E mesocosms, and lowest in NE|NE then E|NE, this was unexpected as previously patches containing enrichment (E|E & E|NE) had the highest MPB values. The surprising change is possibly due to the presence and strong influence of *Corophium volutator* in the three-way interactions. Field studies have shown that *Corophium volutator* moves away from enrichment (Raffaelli 1999, Lawrie et al 2000); therefore it is possible that E|NE patches had surprisingly low MPB biomass because *Corophium volutator* had migrating away from the enriched patch so bioturbation and associated nutrient release would not take place. However at high densities of *Corophium volutator* all interface treatments have similarly low levels of MPB biomass probably due to the increased *Corophium volutator* biomass and associated behavioural traits affecting the turbidity of the water column and reducing photosynthesis of MPB.

5.4.5: Movement

The net movement of *Hediste diversicolor* was influenced by *Hydrobia ulvae* and *Macoma balthica*. In field experiments (Hull 1987, Raffaelli 2000, Cardoso *et al* 2004) and the single species experiments (Bulling *et al* submitted), *Hediste diversicolor* showed movement towards enriched patches. Unexpectedly, interface was not significant within this experiment. Increased levels of *Macoma balthica* reduced net movement of *Hediste diversicolor*, it is possible that *Hediste diversicolor* benefits from being in close proximity to *Macoma balthica*. The predicted values of MPB biomass, from the single species experiments expect *Hediste diversicolor* and *Macoma balthica* to have the highest values within the two-way and three-way interaction terms, however, due to the problems with the seasonal effect on the 'actual' data, it is not possible to determine if this species combination would over-yield and therefore benefit the macrofauna in the system.

The net movement of *Hydrobia ulvae* was influenced by interface treatment, interacting with *Corophium volutator* and *Hediste diversicolor* in a three-way term. The expectations from the single species experiments (Bulling *et al* Submitted) and field experiments (Raffaelli *et al* 2000, Cardoso *et al* 2004) showed that net movement of *Hydrobia ulvae* was towards enriched patches. At low densities the influence of *Corophium volutator* in the system would be minimal and *Hydrobia*

ulvae show a small amount of movement towards enriched patches as previously seen in the single species experiments. However as *Corophium volutator* biomass increases, so does the net movement of *Hydrobia ulvae*, because this was not seen in the single species experiments the most likely reason for this behaviour would be to gain space and to be separated from each other. These two species share the same space (sediment surface) but utilised the resources differently, the burrows made by *Corophium volutator* would cause disturbance to the sediment surface and therefore the availability of MPB as a source of food, this would present a conflict of interest.

Net movement of *Corophium volutator* was influenced by *Hediste diversicolor*, *Hydrobia ulvae* and *Macoma balthica*. In field experiments (Raffaelli 1999, Lawrie *et al* 2000) and the single species experiments (Bulling *et al* submitted), there was a strong response, where movement was away from enriched patches. Unexpectedly interface was not significant within this experiment.

5.5: Conclusion

Species richness, heterogeneity, and species density all influenced the MPB biomass distribution. The experimental design was based on a tried and tested methodology (Ieno *et al* 2006, Dyson *et al* 2007, Bulling *et al* Submitted) and all statistical models followed appropriate procedures (Pinheiro & Bates 2006, West *et al* 2007). Heterogeneity was successfully achieved in the mesocosms by algal enrichment (*Enteromorpha intestinalis*), which caused an increase in MPB biomass.

Comparing species combinations with monoculture was not possible due the seasonal affects on MPB biomass (Paragraph 5.4.1) it was possible, however, to analysis relationships between multi species combinations. Species richness combinations influenced the MPB biomass, macrofaunal combinations both increased and decreased MPB biomass but this was dependent on the combination of species. The species that had lower MPB biomass levels than the control in the monocultures continued to have low MBP biomass in combinations, and vice versa. If species combination were composed of both higher and lower MPB biomass levels, compared to controls in monocultures, then the joint MPB biomass appeared to be the mean.

The most unexpected result was for *Macoma balthica*. This species had a positive interactive effect on all the other macrofauna in the system. It appeared that *Macoma balthica* produced a combined MPB biomass that was greater than the additive values of the combined species. In monoculture and in combination with other species *Macoma balthica* had no net movement so bioturbation effects were negligible. However a possible mechanism for the unexpected increase in MPB biomass may be the result of the waste products (i.e. ammonia) that are excreted by *Macoma balthica* being released from the sediment by other species in the system. The increase in nutrients from the waste products would have a positive effect on the MPB biomass, the release of nutrients and subsequence effects on MPB biomass would not be possible in monoculture.

6. Ecosystem function for multiple patches in static mesocosms

6.1 Introduction

Previous investigative studies (Lovett *et al* 2005) and evidence from this thesis (chapter 4) have demonstrated that habitat heterogeneity is an important factor in the proper understanding of ecosystem functioning (Bulling *et al* submitted, Dyson *et al* In press). However, these experiments have only considered two patches, whereas the natural environment is clearly much more varied. Spatial heterogeneity has many facets, abiotic and biotic that should be considered for a holistic understanding. Abiotic factors included climate, topography and substrata, and biotic factors such as species assemblage, disturbance events and the activities of humans (Chipin *et al* 1996). Heterogeneity can also to be considered over spatial scales, from the vast continental variations of ice, desert and vegetation through a range of smaller scales, landscapes that are broken up with crops, woodland, lakes and urban developments, to the smallest of microhabitats.

Turner & Chapin (2005) suggested distinguishing between two general classes of ecosystem process when considering ecosystem function in heterogeneous landscapes. They described the first as 'Point processes' that represent rates measured at a particular "point" or location, the second is the 'Lateral transfers' that describe the flow of materials, energy or information from one location to another. In my study, measurements of net primary production are taken at a point and are patch specific. Spatial heterogeneity can be considered for both the drivers and the ecosystem response variables and it is possible drivers in one area may influence a response in another. The spatial heterogeneity in this case is generated by sediment enrichment (driver) that causes increased MPB biomass (response). The nature of this response may be driven by local change (sediment nutrient) or might have more extensive influence (water column).

The experimental design included multi patch mesocosms (22 patches) with two different patch arrangements that were selected to represent two possible natural habitats. The first design was the simplest, the mesocosm was effectively split in half where one half was enriched and the other was not enriched, this was similar in design

to that utilised in Chapter 3-5 but on a larger scale (x11). The second design represented a more fragmented habitat with smaller, more widely spread patches of enrichment. The numbers of enriched and non-enriched patches was balanced only the pattern was altered, and from this the null hypothesis that ecosystem function will not be affected by global enrichment (mesocosm design) or local enrichment (patch treatment) can be examined

6.2: Materials and methods

6.2.1: Sediment and macrofauna collection

The collection and treatment of sediment and macrofauna are described in Chapter 2 (Materials and methods)

6.2.2: Experimental design

The multi patch mesocosms were design so that each patch covered the same surface area and had the same volume of sediment as one patch from the other experiments (Chapter 3-5), the patches tessellated in the most efficient manner, hence each patch was a hexagon shape. The mesocosms had 2 different patch arrangements, this was referred to as 'global enrichment', fig 6.1 and is the 'simple' design and fig 6.1b is the 'fragmented' design. Both fig 6.1a & 6.1b show enriched patches in green and non-enriched patches in white, this is referred to as 'local enrichment'. The experiments were run over a period of time (6 d), each run (n = 5) had the two globally enriched mesocosm designs for each of the 3 species tested (5 × 2 × 3 = 30; Fig. 6.2). The three macrofaunal species used in these experiments were, *Hediste diversicolor*, *Hydrobia ulvae* and *Corophium volutator*. At the start of experimentation standardised densities (100% carrying capacity for the Ythan estuary) were placed in every hexagon. The measured patch under consideration was designated as the 'focus patch', and the patches surrounding the focus patch were the 'neighbouring' patches.

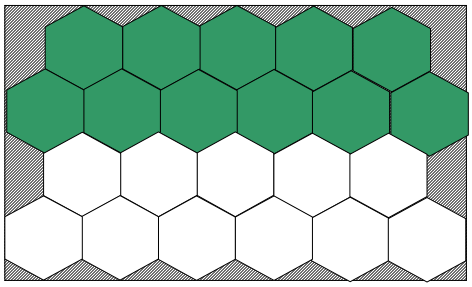


Fig 6.1a Global enrichment; basic design

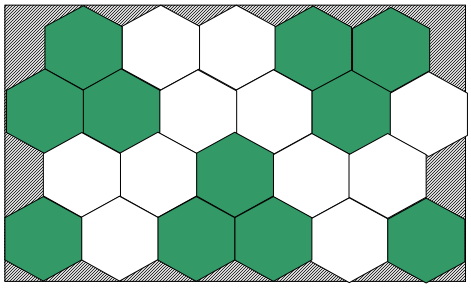


Fig 6.1b Global enrichment; fragmented design

Other factors inherent within this experimental design were also included within the statistically model; patches were classified as either having an outside edge or contained entirely within the hexagon matrix. The number of neighbouring patches for any focus patch were counted and coded for enrichment and non-enrichment, allowing for the influence of neighbouring patches to be included into the statistical model. Other random factors that were considered within this model included experimental run (n = 5) and the mesocosm number (position) within each run. Auto-correlation effects across the hexagons within each mesocosm were also considered and corrected.

6.2.3: Fluorescence measurements

Fluorescence readings were taken on day 6, an appropriate length of time that best captures the changes caused by experimental effects without the fluorescence levels dropping below a reliable limit as a consequence of the laboratory conditions, as discussed by Defew *et al*, (2002b). The measurable output of the PAM fluorescence meter was Fo^{15} (ratio, no units) which represents the chlorophyll *a* biomass present at the sediment surface (Serôdio *et al* 2001, Honeywill *et al* 2002) and can therefore be used as a proxy for primary production potential (Consalvey *et al* 2004b). One fluorescence reading was taken per patch, enabling the MPB biomass to be assessed at a patch level.

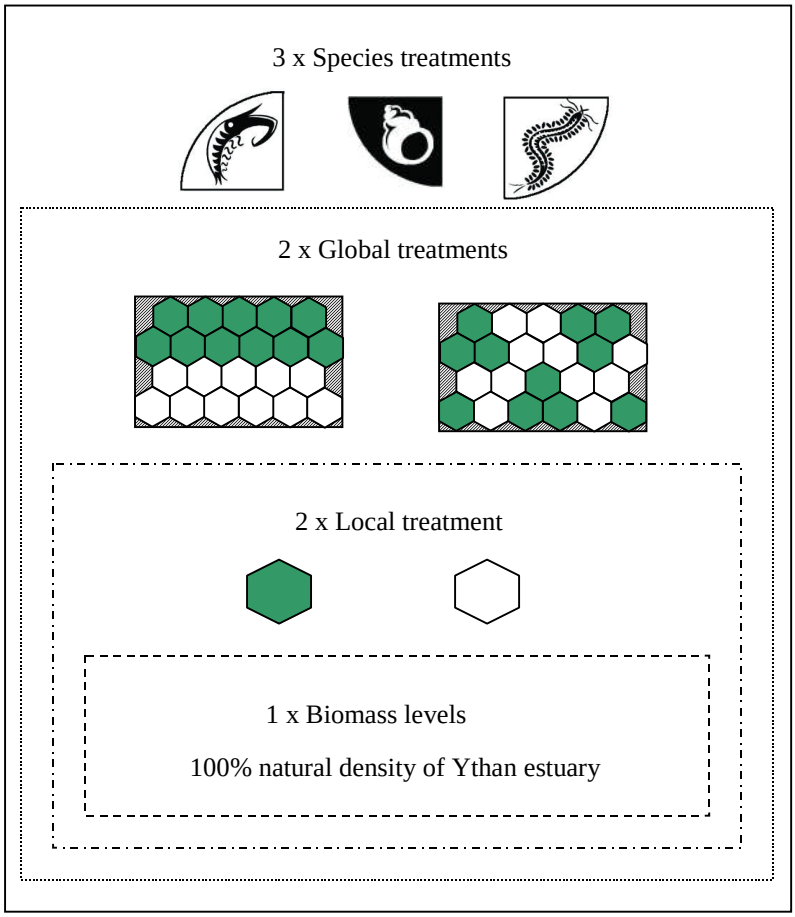


Fig 6.2: Overview of the experimental design. Species biomass was established at the start of the experiment, using the relative levels of the 100% natural density at the study site. This combination was used for each of the two mesocosm designs, basic ( ) and fragmented ( ) (enriched is shaded and non-enriched is green). Local enrichment was considered for each hexagon, enriched (green) and non-enriched (white). Every species density-interface combination was used for each of the four species (*Corophium volutator* ( ), *Hydrobia ulvae* ( ), *Macoma balthica* ( ) and *Hediste diversicolor* ( )) and all combinations were treated under either flow or static conditions.

6.2.4: Macrofaunal movement measurements

Macrofaunal movement measurements were taken on day 10. Each patch was isolated using the Perpex divider, the mud was collected and sieved and the macrofauna within counted (as described in Chapter 2). Net movement was calculated by subtracting the initial macrofaunal density from the final macrofaunal density. A positive (+) movement response was designated as emigration into the focus patch and a negative (–) movement response was designated as migration away from the focus patch.

6.2.5: Data analysis

All data were analysed using a linear mixed effect model, with a generalized least squares (GLS) extention to allow for heteroscedasticity within model. The random effect, mesocosm, and auto-correlation between hexagons were artefacts of the experimental design and were taken into account in the model. A description of linear mixed effect model is provided (Materials and methods, Chapter 2). To explain the two models;

- Model 1; $Fo^{15} \sim f$ (species identity, global enrichment, local enrichment, number of neighbours, enrichment of neighbours, edge effect, run)
- Model 2; Movement $\sim f$ (species identity, global enrichment, local enrichment, number of neighbours, enrichment of neighbours, edge effect, run)

Where:

Species identity: macrofauna species (*Hediste diversicolor*, *Hydrobia ulvae* and *Corophium volutator*)

Global enrichment: design of patch arrangement, two possible combinations, (0 = simple design (Fig 6.1a) and 1 = fragmented design (Fig 6.1b))

Local enrichment: enrichment treatment of focus hexagonal patch (enriched hexagon patches are green, non-enriched hexagon patches are white, Fig 6.1a & b).

Number of neighbours: the count of hexagonal patches surrounding focus patch (2 - 6).

Enrichment of neighbours: neighbouring hexagonal patches were coded for enrichment (non-enriched = 0, enriched = 1).

Edge effect: Hexagonal patches at the edge of the mesocosm were coded (edge patch = 1, non-edge patch = 0)

Run: the number of the experimental batches taken over time (n = 5).

6.3: Results

6.3.1: Microphytobenthos model

The optimal model was a linear mixed effect regression with a GLS extension incorporating one two-way interaction term and three single terms. Single factors were species identity, local enrichment, and run. The two-way interaction was species identity × run. There were no significant 3-way interactions. The variance-covariate terms were species identity, and run, and the random effect was mesocosm (Table 6.1).

Table 6.1 Significant interaction and variance-covariate terms for MPB model.

Term type	Significant factors
Single factors	Species identity
	Local enrichment
	Run
Two-way interactions	Species identity × run
Random effect	Mesocosm
Variance-covariate terms	Species identity × Run

Run was the single factor that had the greatest influence on MPB biomass (L-ratio = 168.03, d.f. = 22, $p<0.0001$), followed by species identity (L-ratio = 148.12, d.f. = 24, $p<0.0001$), and local enrichment (L-ratio = 17.56, d.f. = 33, $p<0.0001$).

The significant two-way interaction term was species identity × run (L-ratio = 130.24, d.f. = 26, p<0.0001, Fig 6.3). *Hediste diversicolor* had the weakest effect in terms of MPB response (highest MPB biomass) followed by *Hydrobia ulvae,* and *Corophium volutator* (lowest MPB biomass). There was a consistent pattern, run 1 maintained the highest levels of MPB biomass, and this declined for all subsequent runs (Fig 6.3). *Hediste diversicolor* caused a linear decline in MPB biomass from run 1 to 5 whereas both *Corophium volutator* and *Hydrobia ulvae* caused an exponential decline in MPB biomass over runs 1-3 but showed a slight increase by run 4 but decreased again in run 5.

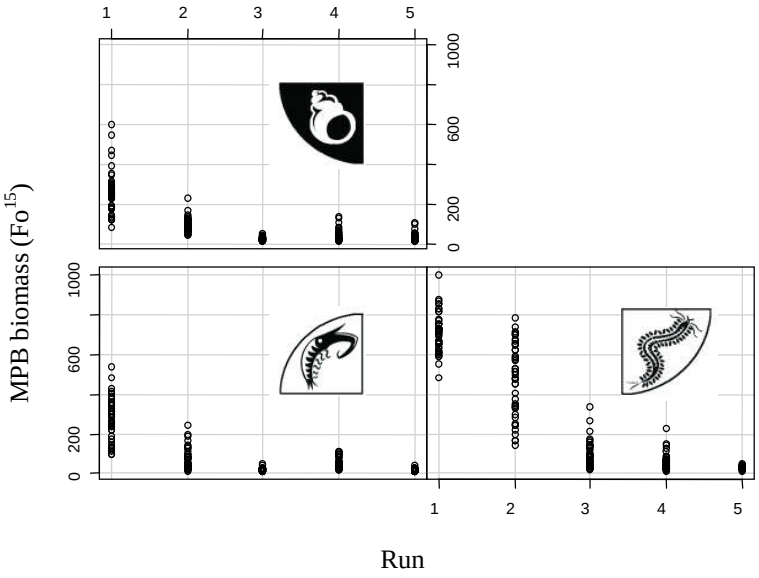


Fig 6.3: Graphical representation of the two-way interaction term, species identity × run. Species identity is represented by diagrammatic pictures, *Hydrobia ulvae* ( ), *Hediste diversicolor* ( ) and *Corophium volutator* ( ). The five repeated experiments were run over time (1 to 5).

6.3.2: Movement model

The optimal model was a linear regression with a GLS extension incorporating two, two-way interaction terms and three single terms. Single factors were species identity, local enrichment, and neighbours. Two-way interaction terms were species

identity × local enrichment, and species identity × neighbours. There were no significant 3-way interactions. The variance-covariate terms were species identity, and local enrichment (Table 6.2).

Table 6.2 Significant interaction and variance-covariate terms for net movement model.

Term type	Significant factors
Single factors	Species identity
	Neighbours
	Local enrichment
Two-way interactions	Species identity × local enrichment
	Species identity × neighbours
Variance-covariate terms	Species identity × local enrichment

The significant two-way interaction terms were species identity × local enrichment (L-ratio = 132.32, d.f. = 15, $p<0.0001$, Fig 6.4), and species identity × neighbours (L-ratio = 12.97, d.f. = 15, $p<0.01$, Fig 6.5). The interaction species identity × local enrichment showed that *Hydrobia ulvae* had the greatest effect in terms of movement response followed by *Corophium volutator,* and *Hediste diversicolor*. *Hydrobia ulvae* had a strong response, moving towards enriched patches, *Corophium volutator* had a weak response but moved away from enriched patches and *Hediste diversicolor* showed no movement in response to local enrichment.

The interaction species identity × neighbours (Fig 6.5) showed a weak movement response, *Hediste diversicolor* showed greater movement into patches that had a higher number of neighbouring patches. *Corophium volutator* had the opposite movement response and had greatest movement away from patches that had a higher number of neighbouring patches. *Hydrobia ulvae* had a mixed movement response to the number of neighbouring patches but overall there was no net movement.

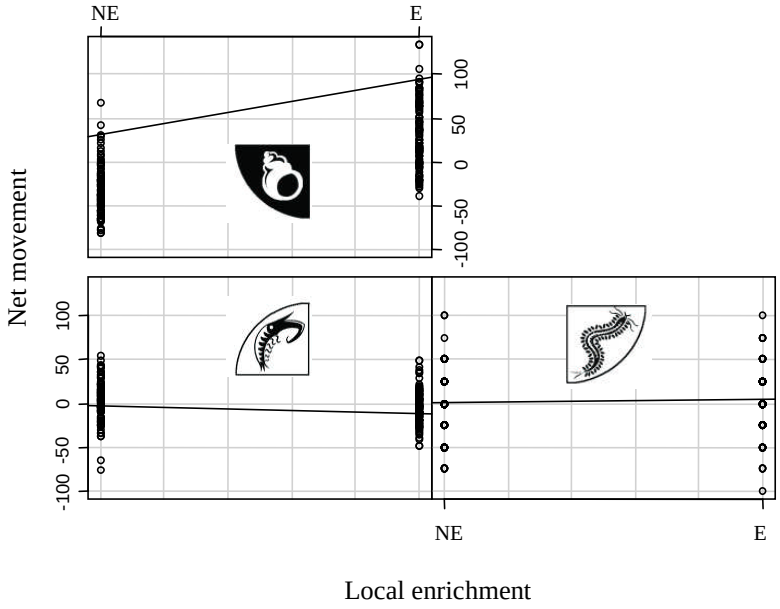


Fig 6.4: Graphical representation of the two-way interaction term, species identity × local enrichment. Net movement was defined as the movement away from the focus patch (left) within the 10 day test period. Local enrichment is represented by NE (non-enriched) and E (enriched) and species identity is represented by diagrammatic pictures, *Hydrobia ulvae* (), *Hediste diversicolor* () and *Corophium volutator* (). Lines represent the linear relationship between variables with model adjustments; data points are present but have not been model adjusted, these points must be used with caution.

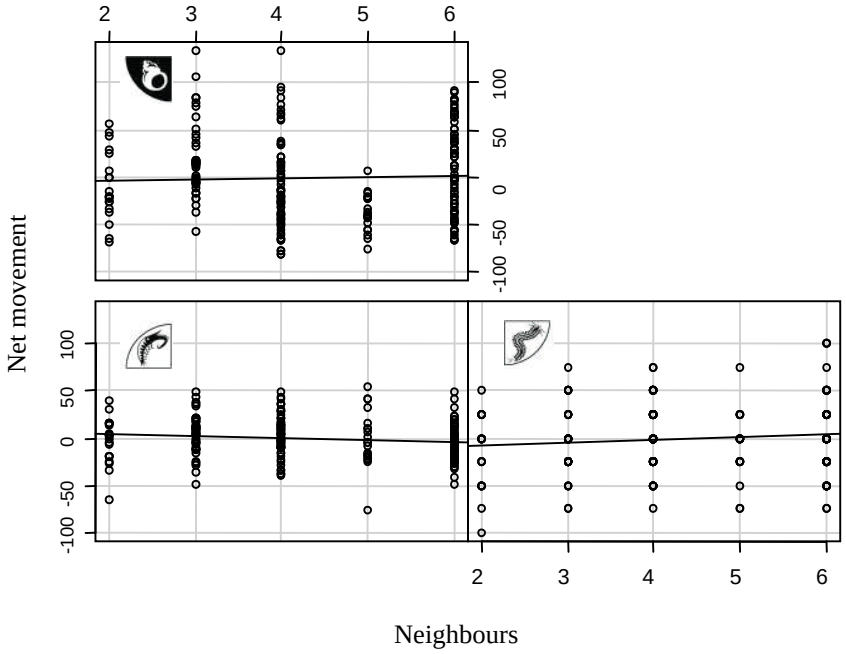


Fig 6.5: Graphical representation of the two-way interaction term species identity × neighbours. Net movement was defined as the movement away from the focus patch (left) within the 10 day test period. Species identity is represented by diagrammatic pictures, *Hydrobia ulvae* (), *Hediste diversicolor* () and *Corophium volutator* (). Neighbours are the number of other patches surrounding the focus patch (2 to 6). Lines represent the linear relationship between variables with model adjustments; data points are present but have not been model adjusted, these points must be used with caution.

6.4: Discussion

6.4.1: Enrichment effects on MPB

A heterogeneous environment was created using dried and powdered *Enteromorpha intestinalis*. Previous experiments showed that higher MPB biomass was found in enriched patches and lowest in non-enriched patches. These differences were further compounded by the individual characteristics and behaviour of the macrofaunal species (Dyson *et al* 2007). Consistent with previous experiment, it was found that MPB biomass was influenced by local enrichment. However, there was no global difference between mesocosms, the arrangement of enriched and non-enriched patches within the hexagon mesocosms made no difference to average MPB biomass. This would imply that local sources of nutrients are more import to the system as a whole than the arrangement of those nutrient sources. To put this in terms of the natural environment, if a wrack of decomposing macro algae became marooned on an estuarine mudflat, affecting the nutrient composition of the sediment, this would only impact the ecosystem function of the immediate area. However, in the experiment the scale at which algae patches are present in the system is equal with patches non-enriched, therefore if the amount of decomposing macro algae marooned on the estuarine mudflat disrupted this balance, this could then have an affect on ecosystem functioning of the estuary as a whole in addition to localised effects.

6.4.2: Species identity effects on MPB

Species identity significantly affected MPB biomass; this was expected considering previous experiments within this (Dyson *et al* 2007, Bulling *et al* Submitted) and other studies (Emmerson *et al* 2001, Raffaelli *et al* 2003a). Throughout all runs *Hediste diversicolor* had the least impact on MPB biomass (highest levels), this is thought to be due to behavioural characteristics. *Hediste diversicolor* is a highly active sub-surface deposit feeder and this activity creates deep bioturbation that aerates the sediment and increases the potential for nutrients to be released, this then becomes available for the MPB to utilise, and while *Hediste diversicolor* has potential to act as a surface deposit feeder there was no evidence of this behaviour under the current experimental regime. It would be interesting to see how one species feeding behaviour could affect ecosystem function; *Hediste diversicolor* is known to feeding in different way under varying environmental conditions. To manipulate the

environment and induce the different feeding behaviours would allow the variations in ecosystem function to be measured.

Hydrobia ulvae is a known consumer of MPB (Defew *et al* 2002a, Hagerthey *et al* 2002), and if consumption exceeds growth MPB biomass would decrease. However, being a surfical bioturbator, the top few cm of sediment are aerated by *Hydrobia ulvae* and the nutrient release could increase MPB biomass. In this experiment it was found that *Hydrobia ulvae* had a negative impact on MPB biomass, so it is likely that the consumption rate of MPB must be greater than growth. The positive effects of bioturbation within this system are not great enough to compensate for the loss. It is assumed that all other possible factors effecting growth rates (e.g. light and temperature) are controlled in the experimental set up such that relative differences between runs can only be a result of starting condition and infaunal behaviour.

Corophium volutator had the greatest impact on MPB biomass, reducing it to the lowest levels throughout all the runs. The behavioural characteristics of this species have two negative impacts on MPB biomass. Firstly, *Corophium volutator* is a known consumer of MPB that would decrease biomass through grazing, and secondly *Corophium volutator* is a habitat engineer creating 'U' shaped burrows that need continual maintenance and aeration. Hagerthey *et al* (2002) showed that both *Corophium volutator* and *Hydrobia ulvae* select for different diatoms but consume relatively equal amounts. Here, the MPB biomass remains higher within *Hydrobia ulvae* mesocosm compared to *Corophium volutator* so another explanation for the reduced MPB biomass is needed. This could be explained by *Corophium volutator* behavioural activity that causes fine particulate matter to be ejected from the burrows into the water column; this activity reduces light attenuation within the mesocosm and the benthic photosynthesis of MPB (Dyson *et al* 2007). Studies by Emmerson *et al* (2001) show *Corophium volutator* releasing relative high levels of ammonia compared to other species, it is possible that the benefits of ammonia for the functioning and growth of MPB are being suppressed by the reduced light and the impact this has on photosynthesis.

6.4.3: Seasonal trends in MPB

The parameter 'run' in this experiment reflects the season trends in MPB biomass, the decline shown here are not unusual (Admirall *et al* 1982). A bloom of MPB usually appears after winter as warmer weather and longer day light hours prevail, during this time the conditions are ideal for MPB growth, in addition consumption by macrofauna is minimal due to limited numbers yet active on the estuary. This initial bloom ends when macrofaunal species consume the MPB, causing their levels to decrease (Admiraal *et al* 1984). A final MPB bloom may take place towards the end of the summer when macrofaunal densities decrease resulting in reduced grazing pressure (Kromkamp et al 2006).

Within the scope of this experiment 'run' was an important factor that needed to be introduced into the statistical model framework, but it was not necessary for it to be expressed as an output. However due to the limitations of linear mixed effect model it was not possible to include two random factors.

6.4.4: Neighbouring patches

As expected from previous experiments, neighbouring patches had no influence on the MPB biomass of the focus patch. Nutrients were obtained from a bottom up mechanism (Dyson *et al* 2007), therefore the type of sediment within a patch influences MPB biomass, and the transfer of nutrient around the mesocosm through the water column was insignificant. Therefore a neighbouring patch would have no influence on MPB in the focus patch.

6.4.5: Movement

Local enrichment within each mesocosm was significant whereas the global enrichment was not. This is consistent with the MPB biomass response, indicating that both responses could be linked. Therefore macrofaunal movement might be due to availability of a food resource (Rosenburg 1995) or nutrient levels (Raffaelli 1999; Rossi and Underwood 2002). A larger implication for the study is that it does not matter how patchy the environment becomes, ecosystem function will be sustained. However, in our system all patches were interconnected, with the passage between them being maintained, the balance between enriched and non-enriched patches was equal and all patches were a consistent size. In the natural environment none of these

assumptions can be guaranteed (Lovett et al 2005) which may effect the prediction of continued ecosystem function.

Hydrobia ulvae and *Hediste diversicolor* had a strong net movement response towards enriched patches and away from non-enriched patches. This response was expected from previous experiment (Hagethey *et al* 2002, Bulling *et al* Submitted) and field experiments (Cardoso *et al* 2004). *Hydrobia ulvae* are highly motile and have been shown to feed on MPB, specifically diatoms (Defew *et al* 2002a). Enriched patches have previously been shown to have higher MPB biomass levels compared to non-enriched patches. Therefore *Hydrobia ulvae* will spend more time grazing in these enriched patches and net movement will be toward them.

The amount of movement associated with *Corophium volutator* was not as great as was expected. Previous experiments (Bulling *et al* Submitted) and field studies (Raffaelli 1999) show *Corophium volutator* to have a strong, net movement, response away from enriched patches and toward non-enriched patches, in this study there was only a weak net movement response away from enriched patches.

6.5: Conclusion

Spatial heterogeneity within the experimental system played an important role determining MPB biomass and clear interaction with macrofaunal species identity. Algal treatment was shown to be an important factor where patches of enrichment were created causing elevated MPB biomass. This difference in patch treatment was thought to have caused the net movement observed. The experimental regime was based on a tried and tested methodology (Ieno *et al* 2006, Dyson *et al* 2007, Bulling *et al* Submitted) and all statistical models followed appropriate procedures (Pinheiro & Bates 2006, West *et al* 2007).

Local enrichment was an important factor influencing both MPB biomass and net movement. However, global enrichment, the patterns of enriched and non-enriched patches in the mesocosm was not a significant factor for either MPB biomass or net movement. Therefore, on the basis of this study it can be surmised that only the

conditions of the immediate vicinity had an impact on local ecosystem function, the surrounding area was of less importance.

Although these are the first empirical studies to determining the importance of spatial heterogeneity on ecosystem function, many questions still remain unanswered. This study showed that the specific heterogeneity created by enriching patches was an important factor in ecosystem function. Therefore heterogeneity in general should be included into future ecosystem function experimentation. However, there are many different factors that make up a heterogeneous landscape (abiotic and biotic) so it is import to consider these within the context of each ecosystem studied. Mesocosm designs are useful tools for manipulating the environment by limiting the amount of factors that can effecting the response however these systems can only represent a small proportion of possible natural habitat interactions. Future experiments should include species richness combinations with this multi patches mesocosm design and validated under field conditions.

7. Discussion and conclusion

7.1: Justification of approach

7.1.1: Review of objectives

The objectives of this thesis were successfully achieved, enabling each chapter to build on the previous work. Monocultures of macrofauna (Chapter 3) were initially investigated, and each of the selected macrofaunal species was tested to gain an understanding of the baseline level of their relative ecosystem functioning. The complexity of the monocultural analysis was increased by experimental manipulation of macrofaunal densities and spatial heterogeneity. The net movement of the macrofauna was also considered as an important factor and was therefore measured. It has been shown in field studies that high levels of macroalgae influence the distribution of macrofaunal populations (Raffaelli 2000, Lawrie et al 2000, Rossi and Underwood 2002), therefore it was considered that in the experimental systems, heterogeneity in organic detritus (dried algae) may influence the distribution and behaviour of macrofauna and influence ecosystem function. Flow was introduced in Chapter 4, as an additional variable to those already included in Chapter 3 (species identity, macrofaunal density and spatial heterogeneity). Water flow is an unavoidable feature for biota in estuarine systems and has been shown to have an effect on macrofaunal behaviour (Vogel, 1994). The ecosystem function measurements in Chapter 5, from the monoculture experiments were used to predict the additive effects of each species combination, for each heterogeneous treatment. Species richness (biodiversity) experiments then enabled the 'predicted' values to be compared to the 'actual' values to determine if species richness affects ecosystem functioning in a heterogeneous system. Finally, having found that heterogeneity was important to ecosystem functioning in the experimental systems (2 patches over dm scale), it was investigated at a larger scale (22 patches over m scale). Scaling up the investigation enabled a distinction to be made in terms of the distribution of heterogeneity in the system. At the smaller scale only neighbouring conditions were possible while at a larger scale each patch might have up to 6 neighbours in either of the conditions. Essentially this study examined if it matters what type of house you live in and do the types of houses your neighbours have influence you too?

7.1.2: MPB biomass as ecosystem function measurement

The response variable selected for this work was the biomass of the microphytobenthos (MPB) that inhabit intertidal sediment systems. MPB serve an important functional role in the estuarine system, contributing up to 50% of primary production (Brotas *et al*. 1995, Underwood and Krompkamp 1999), providing an important resource for grazers (Middleburg *et al*. 2000) and contributing to the stabilisation of the sediment (Paterson 1989). Previous workers have exploited MPB in mesocosm experiments (Defew et al. 2002, Dyson et al 2007) and the relationship between MPB biomass and primary productivity is well-known (Pinckney & Zingmark 1993). This close relationship can be exploited to allow MPB biomass to be used as a proxy for potential primary productivity of the system (Dyson et al 2007). Quantifying the amount of MPB biomass has traditionally been by chlorophyll *a* extraction, which destroys the sediment surface. More recently a new method, pulse amplitude modulated (PAM) fluorometry has been used to quantify MPB biomass at the sediment surface (Honeywill et al 2002). An advantage of this approach is that measurement of MPB biomass is non-invasive and preserves the structure of the sediment (Consalvey et al. 2004b). The classical approach in estuarine systems of measuring nutrient flux as a proxy for ecosystem functioning (Emmerson et al 2001, Biles et al 2003, Raffaelli et al 2004, Ieno et al 2006) is of no use when considering landscape heterogeneity. The nutrient affects are integrated at the water column level whereas the localization of PAM fluorescence allows the variation of MPB biomass between patches to be measured.

Although MPB biomass has been successfully used as a measure of ecosystem function (Dyson *et al*. 2007); there are some considerations to take into account when designing this type of ecosystem function experiment. There is little spatial correlation of MPB in the field above the 2 cm scale (Jesus *et al*. 2005). Thus there is likely to be spatial heterogeneity of MPB distribution within the mesocosms and even within their “homogeneous” patches. The resolution for this was to take multiple MPB biomass reading and gain an average measurement thus enabling any differences between algal heterogeneity within patches to be discounted. However this thesis has demonstrated spatial heterogeneity at the patch level to be important. Herein lies a problem, at what scale and in what circumstances should heterogeneity be included into experimentation? This problem is probably ubiquitous since

heterogeneity is a part of the natural ecosystem; it is therefore important when designing biodiversity and ecosystem functioning experiments that spatial heterogeneity is considered throughout the design.

Using MPB biomass in experimental systems as a proxy for an ecosystem function measure revealed a problem with seasonal variation. Any experiment that was too large to achieve in a single run was divided up into several runs, the duration of each run was 10 days and the testing period lasted from April until August. Over a period of months MPB biomass varies considerably and may affect the result of experimentation. This problem was dealt with in chapter 6 by including the term "run" as a random factor in the model. This allowed temporal variation, and the difference between runs to be explicitly included in the design. However, it was not possible to compare the multi species data with the single species due to the huge variation between the measurements. Careful consideration is needed in the future if data is going to be compared over a long (season) period of time. One possible solution to this problem would be to culture MPB in laboratory conditions and 'top up' the natural levels over the course of the testing period pre-selected experimental amounts.

7.1.3: Heterogeneity

It was established that the addition of powdered algae, *Enteromorpha intestinalis*, provided a suitable mechanism to induce system heterogeneity. In the experimental systems algal enriched patches had the highest MPB biomass readings compared to non-enrichment. Decaying macro-algae within an estuarine system can increase the organic content and under the right conditions increase nutrients within the sediment in the immediate vicinity (Raffaelli 2000). MPB utilises these limited resources during photosynthesis and elevated levels of nutrients increase the growth and reproductive capability of the MPB. The differences between algal enriched and non-enriched patches are recognised by the levels of MPB biomass, these differences influenced the movement of macrofauna in the experimental systems. Movement was species specific and was predicted due to field investigations that showed localised enrichment events would induce a macrofaunal response. The field studies showed that *Hediste diversicolor* and *Hydrobia ulvae* move towards enrichment whereas *Corophium volutator* move away (Raffaelli *et al*. 1991, Lawrie *et al*. 2000, Raffaelli

2000). Bioturbation caused by the behavioural traits of macrofauna and the potential for the sediment to release nutrient provide a mechanism by which heterogeneity influences ecosystem functioning.

7.1.4: Species identity

The macrofaunal species selected for this work were known to be consumers of diatoms and therefore there was an *a priori* reason to expect an effect on MPB biomass, both through the direct effects of grazing and through the indirect influence of nutrient release through sediment bioturbation (Biles *et al*. 2001, Emmerson *et al*. 2001). Estuaries are depauperate systems, containing relatively few species and therefore limiting the choice of macrofauna that can be used in experimental systems. The species used in these experiments must therefore represent a large proportion of the macrofaunal composition of the Ythan estuary, which will add to the realism and provide a closer resemblance to the natural ecosystem function measurement with regard to estuarine systems.

7.1.5: Flow

The experimental approach was firmly based on previous studies (Emmerson et al. 2001, Raffaelli et al. 2003, Ieno et al. 2006), the flow variation and subsequent mesocosms experiments were a progression from these initial experiments (Biles et al. 2003). The control (no macrofauna) mesocosms for the flow and static treatments were not significantly different, therefore any changes in ecosystem function were due to the direct influence of flow on macrofauna and the possible effect that would have on ecosystem function in a heterogeneous environment. It was expected that flow would change the feeding behaviour of macrofauna and this would affect ecosystem function (Biles et al 2003). It was not the aim of this study to investigate the environmental drivers that influence the change in macrofaunal behaviour, however it would be interesting to evoke a number of different feeding methods by one species to find if there is an effect of trophic behaviour on ecosystem function.

7.2: Accomplishing the objectives

7.2.1: Objective 1 single species experiments

It was shown that MPB biomass loss due to macrofaunal feeding was balanced by macrofaunal bioturbation and the ultimate release of nutrients (Fig 8.1). The species that produced the lowest MPB biomass readings in the systems were *Corophium volutator* and *Hydrobia ulvae,* both of these species have been shown to consume high volumes of MPB (Smith et al 1996, Defew et al 2002, Hagerthey et al 2002). *Hydrobia ulvae* is a shallow bioturbator and this reduces the relative amounts of nutrient released, therefore the overall balance is net loss of MPB biomass compared to the controls (Fig 8.1a). It was also thought that other behaviour traits beyond feeding also had an effect on MPB biomass. Burrow maintenance and aeration by *Corophium volutator* caused the overlying water to become turbid; this reduced the amount of light penetrating the sediment surface, in turn reducing MPB photosynthesis and over time MPB biomass. Although *Corophium volutator* is a highly active bioturbator (Emmerson et al. 2001, Raffaelli et al 2003) the loss of MPB through behavioural traits seemed dominant; therefore the overall balance was a net loss of MPB biomass compared to the controls (Fig 8.1b). The only species to have a net gain of MPB biomass was *Hediste diversicolor*, which was a highly active bioturbator that fed at depth (Fig 8.1c). The biomass of MPB with *Macoma balthic* was not significantly different to the controls, therefore the net balance of MPB biomass loss and gain were equal (Fig 8.1d).

Heterogeneity was a significant factor that influenced the movement of macrofauna. The influence was species specific with movement either toward or away from enriched patches. However, the amount of net movement measured could not be simply related to bioturbation effects as reflected by the MPB biomass. For example, macrofaunal movement was only determined by migration between patches, the movement of individuals within patches was not considered. In addition, *Corophium volutator* showed the greatest net movement (and hence bioturbation) but had the lowest MPB biomass levels. Also movement does not relate strictly to bioturbatory activity, some organisms create more turnover of the sediment by their movement than others.

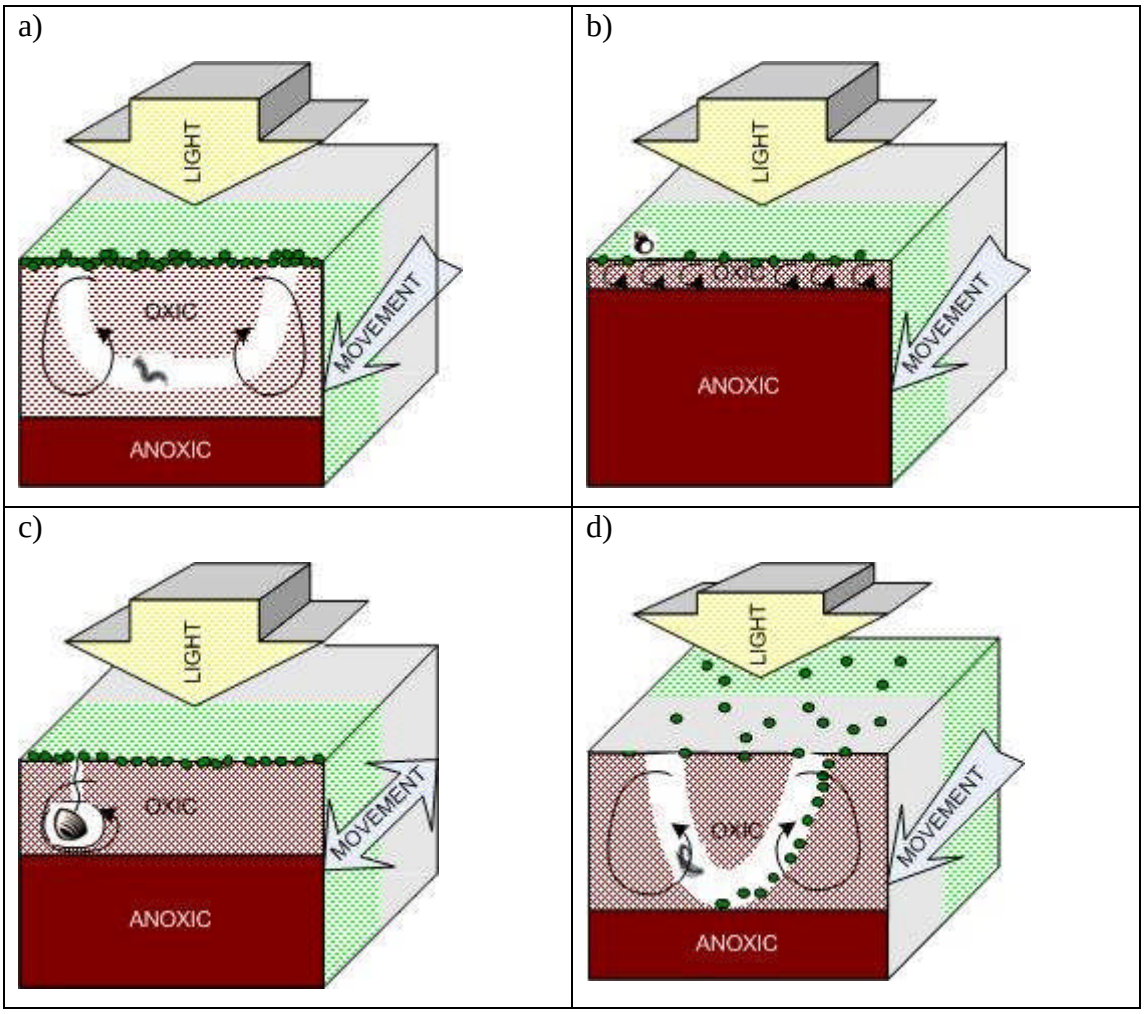


Fig 8.1: Conceptual diagram of the experimental macrofaunal species in monoculture. a) *Hediste diversicolor* created deep burrows which improved the oxygenation of the sediments, b) *Hydrobia ulvae* is a consumer of MPB reducing the biomass, c) *Macoma balthica* did not move and had MPB biomass level similar to the controls, and d) *Corophium volutator* increased the turbidity in the water column reducing light attenuation. Net macrofaunal movement between heterogeneous treatments (➡) where the green half was enriched and the grey half was non-enriched. MPB were present at the sediment surface (●), the net gain of MPB biomass was associated with the effect of bioturbation (○) in the oxic sediment. The release of nutrient through bioturbation enhances MPB growth. Net loss of MPB biomass from the system was through macrofaunal consumption.

7.2.2: Objective 2, flow experiments

Flow had a significant affect on ecosystem function, it was thought that species behaviour changed under flow conditions and this was likely to be the mechanism (Biles et al. 2003). The differences between flow and static conditions were species specific. Both *Corophium volutator* and *Hydrobia ulvae* increased MPB biomass, whereas *Hediste diversicolor* deceased MPB biomass (Fig 8.2) under flow conditions. It is thought that the change in the behaviour of *Corophium volutator* induced by flow reduced the need for manual aeration as the flow in the system contributed to the water movement through the burrow. This resulted in the overlying water being less turbid under flow, allowing photosynthesis, and ultimately an increase in MPB biomass compared to the static mesocosms. Flow did not seem to influence movement or heterogeneity.

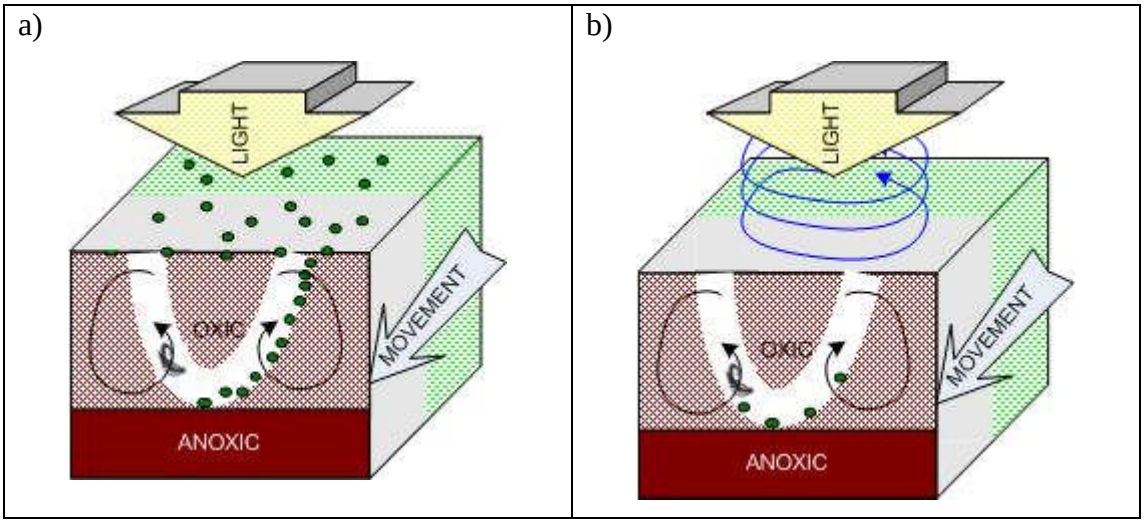


Fig 8.2: Conceptual diagram of the affects of flow ( ) on *Corophium volutator* in monoculture under, a) static treatment where particulate matter ejected for the *Corophium volutator* burrows caused a reduction in light attenuation, and b) flow treatment that reduced the affects of turbidity increasing light attenuation and MPB biomass. Net macrofaunal movement between heterogeneous treatments ( ) where the green half was enriched and the grey half was non-enriched. MPB were present at the sediment surface ( ), the net gain of MPB biomass was associated with the effect of bioturbation ( ) in the oxic sediment. Net loss of MPB biomass from the system was through macrofaunal consumption.

7.2.3: Objective 3, Multi species (species richness) experiments

Some species combinations produced higher MPB biomass levels than expected. *Macoma balthica* had a significant effect in combination with all other species, and the combined effect of *Hediste diversicolor* and *Macoma balthica* produced the highest MPB biomass. Considering that *Macoma balthica* showed no movement and produced MPB biomass levels that were the same as the control mesocosms, it was surprising that this species in combination was having such a significant effect. A possible mechanism for *Macoma balthica* in combination with other species causing an effect in increasing ecosystem function could be due to the release of waste products. In both the monoculture experiments and the multi species combinations *Macoma balthica* was shown to be sedentary, any waste from metabolic activity would remain in the sediment. However, in multi species systems this metabolic waste (i.e. ammonia) could be released from the sediment by the bioturbatory activities of other species. Once released from the sediment could fuel the growth of MPB, increasing biomass beyond the expected yield.

7.2.4: Objective 4, multi patch experiments

The large scale multi patch experiments showed, for every species tested, that the nature of the surrounding patches did not affect ecosystem function. Rather, it was the type of patch that macrofauna inhabited that was important for ecosystem function. Net movement was specific for each species and was consistent with the single species (Bulling et al Submitted), multi species experiments (Chapter 5) and field observations (Raffaelli 2000, Lawrie et al 2000, Rossi 2006). Increasing macrofaunal biomass also had the same effect of reducing MPB biomass as shown in the single species (Dyson et al 2007) and multi-species experiments (Chapter 5).

7.3: General overview

7.3.1: Synthetic systems

The construction of synthetic assemblages in microcosm system is open to criticism (Carpenter 1996) but allows an empirical approach to be tested. However, it cannot be claimed that such system are an accurate representation of natural systems but rather they allow the development of theory that can be later tested under more natural

conditions. As the theory and practise of BEF research develops this allows for better statistical models to be constructed (Loreau *et al*. 2001, Naeem & Wright 2003, Balvanera *et al*. 2006, Raffaelli 2006).

7.3.2: Sampling site

With the development of large studies such as this, the impact of intensively using the sample site could have its own adverse effects on the ecosystem functioning of the site. For this project, over a period of two years a total of 1,226 *Hediste diversicolor*, 617g *Hydrobia ulvae*, 765 *Macoma balthic* and 314g *Corophium volutator* were used. Some of the macrofauna were returned to the estuary after experimentation; however the impact of removal and re-establishment is unknown. This study was also one of three that were running concurrently, for every sampling effort that took place there would have been a trampling effect on the estuary as an average of eight people collected the macrofauna (Fig 8.3), again the impact of this on both fauna and flora in the immediate and surrounding is a relevant source of disturbance for the conservation and management of mudflats (Rossi *et al* 2007). Sediment was also collect on a large scale removing large quantities of MPB, which is the food source of many macrofauna. Again, it is not known if this had a direct impact on macrofaunal biomass or if this further impacted the food chain.

Fig 8.3: Trampling due to sampling on the Ythan estuary.

7.3.3: Methods of measuring ecosystem function

During this study, two ecosystem function measurements were taken, NH_4 and MPB biomass. Although the NH_4 data has not been presented in this thesis, it has become apparent that some species interactions are valued differently depending on which measure of ecosystem function was used; MPB biomass or nutrients (Bulling *et al*. submitted, Dyson *et al*. 2007). In experiments where NH_4 has been used as the measure of ecosystem function *Corophium volutator* has the highest affect, and was recognised as a key contributor. However when MPB biomass was the measure of ecosystem function *Corophium volutator* had a very low influence and was found to contribute very little to the functioning of the ecosystem. It was assumed that the behaviour of *Corophium volutator* that resulted in the overlaying water becoming turbid was preventing light from penetrating the sediment surface and consequently dramatically reducing photosynthesis. Although this affect is not likely to be reproduced in a natural environment due to tidal movement, however the amount of faith that is put on ecosystem function measurement are only ever as good as the experimental design (Hector and Bagchi 2007). The ability to transpose these results into the natural system will depend on system knowledge but will never be able to truly replicate it.

7.3.4: Length of experimentation

The length of most of the experiments was 10 days; this was selected based of previous studies (Defew et al 2002a, Emmerson et al 2001), as it was considered that this period of time was enough to achieve normal macrofaunal behaviour and nutrient flux levels to be greater than the detectable threshold. However MPB biomass greatly declines over the duration of a 10 day test, this could effect the macrofaunal movement as this might be based on food availability. On day 10 MPB biomass was very low in all algal treatments, maximum movement is likely to be achieved during the period when there is the greatest differential between algal treatments. This may mean that the maximum net macrofauna movement has been missed, further investigations are needed to find the balance between maximum net movement, nutrients and MPB biomass.

7.4: Conclusion

Throughout this thesis, heterogeneity has been shown to play an important role and should be considered in future biodiversity and ecosystem function experiments. The spatial scale at which heterogeneity should be considered requires careful thought, as it is not always possible to account for all scales. It has been shown in this thesis that heterogeneity can occur, all be it at a micro-scale, in patches that were considered homogeneous at a macro-scale.

This thesis has also touched on the effects of what Hector and Bagchi (2007) are calling multifunctionality. All ecosystems are managed and valued for several ecosystem services, yet to-date most the ecosystem function experiments have only been tested using one. Although the nutrients data has not been presented in the thesis, it has become evident that using different methods to measure ecosystem function changes the order of macrofauna that give most to enhance ecosystem services. Until further research is done to test this theory, the best solution would be to ensure no further loss of biodiversity. Thereby any differences in functional measurements along with changes in environmental variables will buffer ecosystem function (Yachi and Loreau 1999) to provide future generations with the ecosystem services and the continued functioning of the biosphere.

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Influence of macrofaunal assemblages and environmental heterogeneity on microphytobenthic production in experimental systems

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Influence of macrofaunal assemblages and environmental heterogeneity on microphytobenthic production in experimental systems

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Abstract

Despite the complexity of natural systems, heterogeneity caused by the fragmentation of habitats has seldom been considered when investigating ecosystem processes. Empirical approaches that have included the influence of heterogeneity tend to be biased towards terrestrial habitats; yet marine systems offer opportunities by virtue of their relative ease of manipulation, rapid response times and the well-understood effects of macrofauna on sediment processes. Here, the influence of heterogeneity on microphytobenthic production in synthetic estuarine assemblages is examined. Heterogeneity was created by enriching patches of sediment with detrital algae (*Enteromorpha intestinalis*) to provide a source of allochthonous organic matter. A gradient of species density for four numerically dominant intertidal macrofauna (*Hediste diversicolor, Hydrobia ulvae, Corophium volutator, Macoma balthica*) was constructed and microphytobenthic biomass at the sediment surface was measured. Statistical analysis using generalised least squares regression (GLS) indicated that heterogeneity within our system was a significant driving factor that interacted with

macrofaunal density and species identity. Microphytobenthic biomass was highest in enriched patches, suggesting that nutrients were obtained locally from the sediment-water interface and not from the water column. Our findings demonstrate that organic enrichment can cause the development of heterogeneity which influences infaunal bioturbation and consequent nutrient generation, a driver of microphytobenthic production.

Keywords: habitat heterogeneity, ecosystem function, microphytobenthos, mesocosm, marine, benthic.

1. INTRODUCTION

It is rare in nature to find an entirely uniform habitat or for the distribution of organisms to be completely regular. Most organisms exhibit a patchy distribution reflecting the heterogeneous nature of the environment (Tilman *et al.* 1994, Williams *et al.* 2006). Therefore, it is surprising that the natural heterogeneity of ecosystems has rarely featured in the experimental analysis of ecosystem processes (Cheng *et al.* 2007, Holzschuh *et al.* 2007). Heterogeneity has functionally important consequences for productivity and other ecosystem services provided by an ecosystem, particularly if the transmission of material and resources between patches is slow or restricted (Strayer 2005). Heterogeneity is also known to be important in the maintenance of species diversity (Sommer 2000), habitat (Levinton and Kelaher 2004) and material and energy flow (Franklin 2005), such as nutrient cycling (Bengtson *et al.* 2006). It is clear that both local processes (Levinton and Kelaher 2004) and the landscape matrix, which they form, are important in determining habitat quality (Williams *et al.* 2006).

If we are to fully understand the role of species in mediating ecosystem processes, particularly at larger scales, it is essential to integrate heterogeneity effects when considering overall habitat performance. Investigation of the spatial distribution of specific populations is common (Noren and Lindegarth 2005, Bengtson *et al.* 2006, Grenyer *et al.* 2006, Jones *et al.* 2006, Condeso and Meentemeyer 2007) and some studies now include links to related functional attributes. For example, Jesus *et al.* (2005) provided a detailed analysis of microphytobenthos (MPB) distribution on the surface of an estuarine mudflat and linked it to the photosynthetic functionality at a cm scale. However, the inclusion of spatial distribution patterns has not yet been incorporated as a treatment in studies of biodiversity and ecosystem functioning.

Coastal zones and estuarine ecosystems have proven to be valuable sites for the investigation of relationships between biodiversity and ecosystem function (BEF) (for review, see Covich *et al.*, 2004). Different attributes of the marine environment have been incorporated into experimental systems to test empirical relationships (e.g. flow, Biles *et al.* 2003; regional attributes, Emmerson *et al.* 2001; grazers, Duffy 2006, Hagerthey *et al* 2002) using an approach (Raffaelli *et al.* 2003) that is analogous to those used in other systems (Schmid *et al.*, 2002). In many instances, the rate or flux of nutrients has been used as a measure of ecosystem function (e.g. Ieno *et al.*, 2006) and, for such point processes, spatial heterogeneity becomes important when considering nutrient cycling at larger scales.

In intertidal areas, one natural and reproducible element of heterogeneity is the patchiness of macroalgae (Hagerthey *et al.* 2003) and the associated physicochemical variability of the sediment bed (Raffaelli 2000). Buried algae decays rapidly providing resources for infaunal organisms (Rossi and Underwood 2002) but may also lead to sediment anoxia, thus the overall effect on organisms may be positive or

negative. This may lead to opposing organisational forces (localized detrital input versus mobility of consumers) in deposit-feeding marine communities that exert structural control at the landscape scale (Levinton and Kelaher 2004). The major primary producers in mudflat systems are the MPB (Paterson and Hagerthey 2001) and it is known that their distribution can be patchy, varying over spatial scales of <1 cm (Jones *et al.* 2006), in response to environmental variables and macrofaunal composition (Christie et al 2000; Hagerthey *et al.* 2002). The biomass of MPB can be assessed by non-destructive pulse amplitude modulated (PAM) fluorescence techniques (Honeywill *et al.* 2002, Consalvey *et al.* 2004a, Jesus *et al.* 2006b), which allows repeated measurements over restricted spatial and temporal scales (Jesus *et al.* 2005).

Here, we manipulated the spatial heterogeneity within mesocosm systems by the selective addition of detrital algal material to a defined region of sediment. The influence of this induced heterogeneity on ecosystem function was assessed using MPB biomass distribution as a proxy for photosynthetic capacity (Honeywill *et al.* 2002, Consalvey *et al.* 2004b, Jesus *et al.* 2006a). The factorial experiment was designed to examine the influence of species identity, species density and algal enrichment (as a mechanism for inducing heterogeneity) on microphytobenthic primary production. We hypothesised that (1) macrofaunal distribution (identity and biomass) would influence production capacity, but that (2) this would be influenced by the patchiness created in the experimental system.

2. MATERIALS AND METHODS

Sediment was collected from the Ythan estuary, Aberdeenshire, Scotland, UK (N 57º 20.085´, W 02º 0.206´) and sieved (500μm) in seawater to remove unwanted

macrofauna, and left to settle for 24 h to retain the fine fraction (<63 μm). Excess water was removed, the sediment slurry homogenised and distributed between mesocosms (opaque aquaria, 21 × 15 × 14 cm). Sediment was added to each mesocosm to a depth of 3 cm. Enrichment was achieved by the addition of dried and ground *Enteromorpha intestinalis* collected from the Ythan Estuary. Perspex sheets were used to divide the mesocosms into equal halves, 1g of algae was added to enrich selected patches (equivalent to 126 g m^{-2}, within levels found naturally, Raffaelli, 2000). Mesocosms were initially filled with 2.5 L seawater (UV-sterilised, 10 μm pre-filtered, salinity ≈ 33), left for 24 h, and refilled with seawater to eliminate nutrient pulses associated with assembly (Ieno *et al.* 2006). Mesocosms were placed in a controlled temperature room (11°C ±2°C), aerated and the photoperiod was set to a 12 h light-dark cycle (26 mm Ø white fluorescent tubes, model GE F36W/35; 36W, 3500ºK). The experiment ran for 10 days.

396 mesocosms were established, divided randomly and equally between two experimental runs, to determine the effects of macrofaunal species identity, macrofaunal species biomass and algal enrichment on MPB biomass. Two patches were established in each mesocosm. The deposit-feeder *Hediste diversicolor* (Polychaeta), the surficial grazer *Hydrobia ulvae* (Gastropoda), the regenerator *Corophium volutator* (Crustacea) and the suspension/deposit-feeding bivalve *Macoma balthica* were added on day 0. Macrofauna were confined to their initial patches for 24 h using perspex dividers. Combinations of macrofaunal biomass (0, 25, 50 and 100% of natural density in both the left and right patches. i.e. 16 possible combinations) were established for all possible interface combinations of patch arrangements (E|E, E|NE, NE|E and NE|NE where '|' represents the interface and E = enriched and NE = non-enriched; the measured patch is on the left of '|' and a

neighbouring patch is on the right of '|') for each of the 4 macrofaunal species (Figure 1). Each configuration was replicated 3 times (n=396). For *M. balthica* and *H. diversicolor*, whole individuals were counted and 4 individuals patch^{-1} was taken as analogous to the natural density on the Ythan estuary. For *H. ulvae* and *C. volutator*, the natural wet weight biomass was determined (2g and 1g per patch respectively) and appropriate proportional wet weights added to the mesocosms. In addition, replicate (n=3) control mesocosms containing no macrofauna were established for each interface configuration (n=12) to determine the effect of the presence of macrofauna, irrespective of identity.

An emergent property of the experimental design allowed the analysis of the influence of the difference between the initial and final biomass of the macrofauna set in adjacent patches. The initial difference was expressed numerically as the difference in biomass between the measured patch and the adjacent patch, such that: +4 = all macrofauna (at maximum biomass) were in the measurement patch; 0 = equal distribution in each patch; and -4 = all macrofauna (at maximum biomass) were in the adjacent (non-measured) patch.

Measurements of MPB biomass were taken on day 6 based on pulse-amplitude modulated (PAM) fluorescence (Consalvey *et al.*, 2004a) using a Hansatech™ FMS2 meter. A 6 day interval was appropriate because this was the combination for optimum MPB biomass and species activity (Defew *et al.* 2002). Mesocosms were dark-adapted for 15 min to optimise MPB biomass estimates from the Fo^{15} output, which is a proxy for Chl *a* content (Honeywill *et al.* 2002). To reduce variability, two measurements of Fo^{15} were taken from each patch and averaged, and three replicate mesocosms were measured for each treatment (n =3).

A GLS (Generalised Least Squares) (Pinheiro & Bates, 2001) statistical mixed modelling approach was used to assess the two experimental hypotheses. A GLS framework is preferential over linear regression using transformed data because it retains the structure of the data whilst accounting for unequal variance in the variance-covariate terms. As a first step, a linear regression model was fitted. Model validation was applied to verify that underlying statistical assumptions were not violated; normality of residuals was assessed by plotting theoretical quantiles versus standardised residuals (Q-Q plots), homogeneity of variance was evaluated by plotting residuals versus fitted values, and influential data points were identified using Cook's distance method (Quinn and Keough 2002). The validation procedure showed that there was no evidence of non-linearity but there was evidence of unequal variance among the explanatory variables. The GLS model was refined by manual backwards stepwise selection using maximum likelihood (ML) to remove insignificant terms, and the final model was presented using restricted maximum likelihood (REML) (West *et al.* 2007). The highest potential level of interaction that was assessed was the three-way interaction terms. The statistical outputs of these models are based on the comparisons of the first level within each term with all other levels; no other within level comparison is made. To assess the importance of individual independent variables, a likelihood ratio test was used to compare the full minimal adequate model with models in which the independent variable, and all the interaction terms it was involved with, was omitted. As a complementary indicator of the importance of these individual variables, in each case we calculated the decrease in the adjusted R^2 value for the model without that variable as compared with the full model. Analyses were performed using the 'R' statistical and programming

environment (R Development Core Team 2005) and the 'nlme'package (Linear and nonlinear mixed effects models; (Pinheiro *et al.* 2006).

3. RESULTS

The minimal adequate model was a linear regression with a GLS extension incorporating four two-way interaction terms and four single terms (adjusted R-squared = 0.49). Single factors were species identity, interface, species density, and initial density difference. Two-way interactions were species identity × interface, species identity × species density, interface × species density, and species density × initial density difference. There were no significant 3-way interactions. The variance-covariate terms were species identity, interface, and species density.

a. Independent terms

Species identity had the greatest influence on MPB biomass (L-ratio = 755.18, d.f. = 15, $p<0.0001$, decrease in adjusted R-squared (R^2_{dec}) = 0.38), followed by interface type (L-ratio = 425.78, d.f. = 15, $p<0.0001$, R^2_{dec} = 0.16), species density (L-ratio = 218.34, d.f. = 33, $p<0.0001$, R^2_{dec} = 0.08) and initial density difference (L-ratio = 9.78, d.f. = 2, $p<0.005$, $R^2_{dec} < 0.001$). As the source of nutrients fuelling MPB growth can either originate from bottom up (sediment) or top down (water column) processes, we compared MPB biomass in non-macrofaunal control mesocosms. These analyses showed that the focus patches (left) had a significant effect on MPB biomass, whilst neighbouring patches (right) had no significant effect (Two-way ANOVA: Left patch, F = 5.93, d.f. = 1, p = <0.05; Right patch, F = 0.26, d.f. = 1, p = 0.627), indicating that bottom-up processes were determining MPB biomass.

b. Two-way interaction terms

The significant two-way interaction terms, in order of importance, were species identity × interface (L-ratio = 39.18, d.f. = 33, p<0.0001; Figure 2), species identity × species density (L-ratio = 38.13, d.f. = 39 p<0.0001; Figure 3), species density × interface (L-ratio = 24.15, d.f. = 39 p<0.0001; Figure 4), and species density × initial density difference (L-ratio = 4.42, d.f. = 41, p = 0.036; Figure 5). *H. diversicolor* had the weakest affect in terms of MPB response (highest MPB biomass) followed by *M. balthica, H. ulvae,* and *C. volutator* (lowest MPB biomass). There was a consistent pattern in that enriched patches (E) maintained higher levels of MPB biomass than non-enriched (NE) patches (Figure 2). However, while the fully enriched condition (E|E) maintained the highest biomass of MPB for *H. diversicolor* and *H. ulvae* this was not the case for *M. balthica* or *C. volutator* where the heterogeneous condition (E|NE) maintained the highest level of biomass. Within the interaction species identity × interface, *M. balthica* × E|NE (p = 0.019, coefficient (95% confidence intervals, CI) = 70.26 (11.54 – 129.00)) and *C. volutator* × E|NE (p = 0.017, coefficient (95% CI) = 56.11 (10.29 – 101.94)) and *C. volutator* × NE|E (p = 0.027, coefficient (95% CI) = 43.80 (4.93 – 82.66)) were significant compared with *H. diversicolor* × E|E. The nature of the interaction was to increase the influence of the E|NE condition (Figure 2), so that for these two species, the interface condition positively influenced MPB biomass.

For each species, there was an overall reduction in MPB biomass with increasing density (Figure 3). At low density levels, *H. diversicolor* had least effect (highest MPB biomass), *M. balthica* and *H. ulvae* had similar effects, and *C. volutator* had the greatest effect on MPB biomass (lowest MPB biomass). As species density increased, the rate of decline in MPB biomass was similar for all species with the

exception of *M. balthica*, for which it was less pronounced (p = 0.0033, coefficient (95% CI) = 11.48 (3.82 – 19.15)).

The interaction species density × interface showed an overall reduction in MPB biomass as the density of each species increased in all treatments except for NE|NE (Figure 4). The rate of change in MPB biomass was similar between the three declining treatments. At low densities, MPB biomass varied with interface treatment, with the highest biomass associated with E|NE followed by E|E, NE|E and NE|NE.

The interaction species density × initial density difference was also significant, but very weak (L-ratio = 4.42, d.f. = 41, p = 0.036). Model visualisation (Figure 5) indicates that the level of MPB biomass declined as species density increased. The rate of decline was greatest in mesocosms with the maximum biomass in the focus patch and zero biomass in the neighbouring patch, followed by treatments with initial densities evenly distributed between patches, and mesocosms with the maximum biomass in the neighbouring patch and zero biomass in the focus patch.

4. DISCUSSION

The mesocosm experiments were designed to examine the influence of spatial heterogeneity on MPB production. It was established that the addition of powdered algae, *Enteromorpha intestinalis*, provided a suitable mechanism to induce system heterogeneity. The highest MPB biomass was recorded from enriched (E|E) mesocosms and the lowest in mesocosms that had not been enriched (NE|NE). The macrofaunal species selected for this work were known to be consumers of diatoms and therefore there was an *a priori* reason to expect an effect on MPB biomass, both through the direct effects of grazing and through the indirect influence of nutrient release through sediment bioturbation. However the macrofauna used have different

bioturbatory characteristics, and these are variable depending on environmental conditions (Biles *et al.*, 2003). Effects of species density on MPB biomass will therefore reflect the behaviour of individual species.

To-date, BEF effort has included studies on species identity, diversity, biomass and functionality but without reference to the inherent natural variability of habitat. While the impact of spatial heterogeneity on ecosystem function has been considered (Lovett *et al.* 2005), empirical data is largely lacking. This contribution represents an initial empirical step to consider the role of spatial heterogeneity. It should be noted, however, that the classical mesocosm approach to measure nutrients as a proxy for ecosystem functioning (Raffaelli *et al.* 2003) when considering spatial heterogeneity, is of limited value in marine benthic systems since the measured effects are integrated at the water column level and a local contribution cannot be ascertained. The localization capability of PAM fluorescence allows the variation of MPB biomass between patches to be measured conveniently at a range of spatial scales.

The current experimental approach was firmly based on previous studies (Emmerson *et al.* 2001, Raffaelli *et al.* 2003, Ieno *et al.* 2006), but we acknowledge that the construction of synthetic assemblages in a mesocosm system is open to criticism (Carpenter 1996). It is important to reiterate here that, despite the apparent limitations of mesocosm systems, they allow theory to be tested and global-scale environmental problems to become amenable to experimental endeavour (Benton *et al.*, in press). Such systems are not an accurate representation of natural systems, rather they allow the development of theory that can be later tested under more natural conditions as the

theory and practice of BEF research develops (Loreau *et al.* 2001, Naeem and Wright 2003, Balvanera *et al.* 2006, Raffaelli 2006).

The statistical model indicated that species identity, type of interface (heterogeneity) and species density were the strongest determinants of ecosystem response. The influence of species identity and density is unsurprising and consistent with numerous studies (for review see Covich et al, 2004). Of particular significance, however, is that the macrofaunal species used in this study represent varied functional attributes and have clear trophic connections with the response variable, yet heterogeneity (interface type) was a driver for two of the three strongest interaction terms in the model. It is clear that spatial heterogeneity is of absolute importance and that point measurements of function may lead to qualitatively different and scale-dependent interpretations that are not relevant when considering processes at an ecosystem scale.

Decaying macroalgae within an estuarine system can increase the organic content and, under the right conditions, increase nutrient levels within the sediment in the immediate vicinity (Raffaelli 2000). MPB can also utilise these resources during photosynthesis to enhance production and levels of biomass. Localised enrichment has also been shown to influence macrofaunal behaviour (Levinton and Kelaher 2004). Previous work found that *H. diversicolor* and *H. ulvae* move towards enrichment, whereas *C. volutator* moves away (Lawrie *et al.* 2000, Raffaelli, 2000), and *M. balthica* shows very little movement (de Goeij & Luttilchuizen, 1998). Here, *H. diversicolor* had a positive effect on MPB biomass compared with the other species, although this did decrease with increasing biomass. This positive effect was possibly due to its relatively large size and bioirrigatory capacity (Magni and Montani

2006) increasing nutrient turnover, as well as stimulating microbial activity (Hansen and Kristensen 1997). In contrast, *H. ulvae,* although highly active, had limited impact on sediment nutrient turnover (consistent with Ieno *et al.* 2006; Orvain 2006) whilst the behaviour of *M. balthica*, which tends to siphon feed in still water (Kamermans 1994; de Goeij & Luttikhuizen, 1998), is unlikely to impact on MPB biomass. Although *C. volutator* has been shown to be highly active and mediate the release of comparatively large quantities of nutrient (NH_4-N) (Emmerson *et al.* 2001), the low MPB biomass levels found in *C. volutator* treatments appear to be influenced by a secondary effect caused by the behaviour of this species. Sediment resuspension during burrow maintenance causes the water column to become turbid, attenuating light and reducing photosynthesis at the sediment surface (de Deckere *et al.*, 2000).

Microphytobenthos can utilise nutrients generated in the enriched sediments at the sediment-water interface or from nutrients previously released into the water column. Nutrients released into the water column become available for the whole mesocosm and any response is likely to be effected over the entire system. It follows, therefore, that if the MPB obtain nutrients locally from the sediment-water interface, any observed responses in our experimental system would only occur in algal-enriched sediment. Overall, the highest MPB biomass was in enriched patches and the lowest MPB biomass was in non-enriched patches, irrespective of the neighbouring patch type. It is clear, therefore, that the source of nutrients for MPB is derived locally from the sediment-water interface rather than the water column itself and that sediment heterogeneity is an important determinant of MPB production.

Heterogeneity was induced by the addition of allochthonous carbon that may have both direct and indirect effects on the functional response of the system. The principal direct effect was expected through the release of resources (nutrients) that enhance MPB biomass at the sediment surface. In addition, the presence of organic material will influence the behaviour and migration of the macrofauna (Raffaelli *et al.* 1991, Rossi 2006) with a consequent feedback on MPB biomass (Hagerthey *et al.* 2002). This feedback is difficult to predict, as the effect may be positive (bioturbation releasing nutrients) or negative (grazing of MPB). Our results suggest that the important independent variables for MPB, in order of greatest effect, are macrofaunal species identity, the nature of the interface between two patches, macrofaunal density and the gradient in macrofaunal biomass between two patches. Although the interactions between these factors were more complex, the influence of system heterogeneity is clearly a significant factor for MPB performance, particularly in the case of *C. volutator* and *M. balthica*. When these species are present, the statistical model indicated that the functionality was higher than expected, suggesting that any negative effect of the species (direct grazing) was more than compensated for by the positive effects of bioturbation, such as increased nutrient turnover. This point is not trivial, as it has important ecological consequences since growth may be enhanced sufficiently to compensate for grazing pressure and result in increased standing stock (production). This suggests that the landscape matrix is more important than local ecosystem structure in determining MPB production (Williams *et al.* 2006) and may, in the longer term, have consequences for macrofaunal fitness and reproductive capacity. The model does not allow for more specific determination of interaction terms (suitable post-hoc analyses are not possible) but it does highlight the overall importance of the interface. Elucidating the mechanistic effect requires further work

but is likely to be a combination of species movement expressed through bioturbation, grazing and nutrient recycling.

5. CONCLUSIONS

Spatial heterogeneity plays an important role in determining MPB production, interacting with both macrofaunal species identity and density, even at the restricted level of patches within our experimental mesocosms. In nature, these effects are likely to be widespread. Attention must now be given to the development of novel methodologies capable of incorporating these interactions, to further elucidate the nature of the relationship between habitat heterogeneity and ecosystem function and the mechanisms underlying them, as well as the consequences for the conservation of biodiversity and ecosystem services in changing environments.

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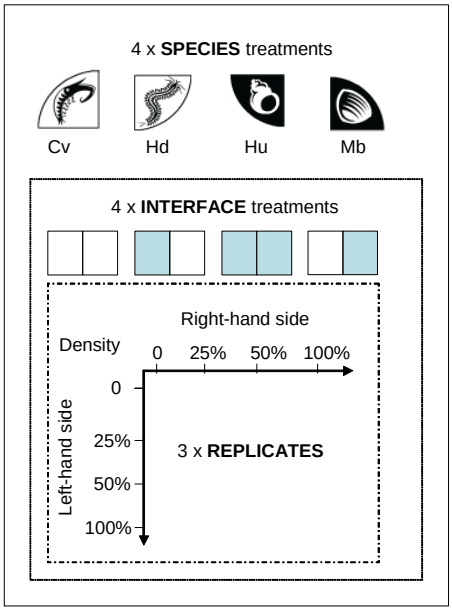
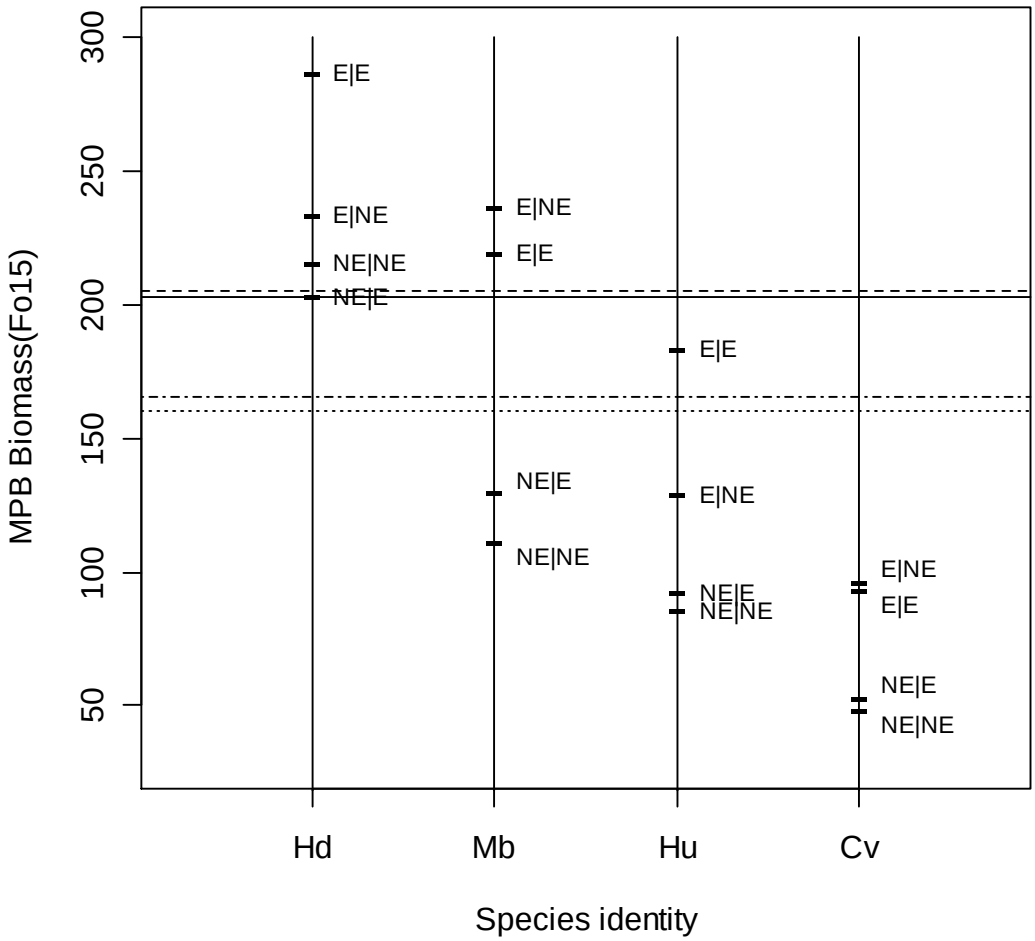
Fig 1


4 x **SPECIES** treatments
Cv
Hd
Hu
Mb
4 x **INTERFACE** treatments
Right-hand side
Density
0
25%
50%
100%
Left-hand side
0
25%
50%
100%
3 x **REPLICATES**

1 Fig 2


MPB Biomass(Fo15)
300
250
200
150
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E|E
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NE|NE
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Hd
Mb
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Cv
Species identity

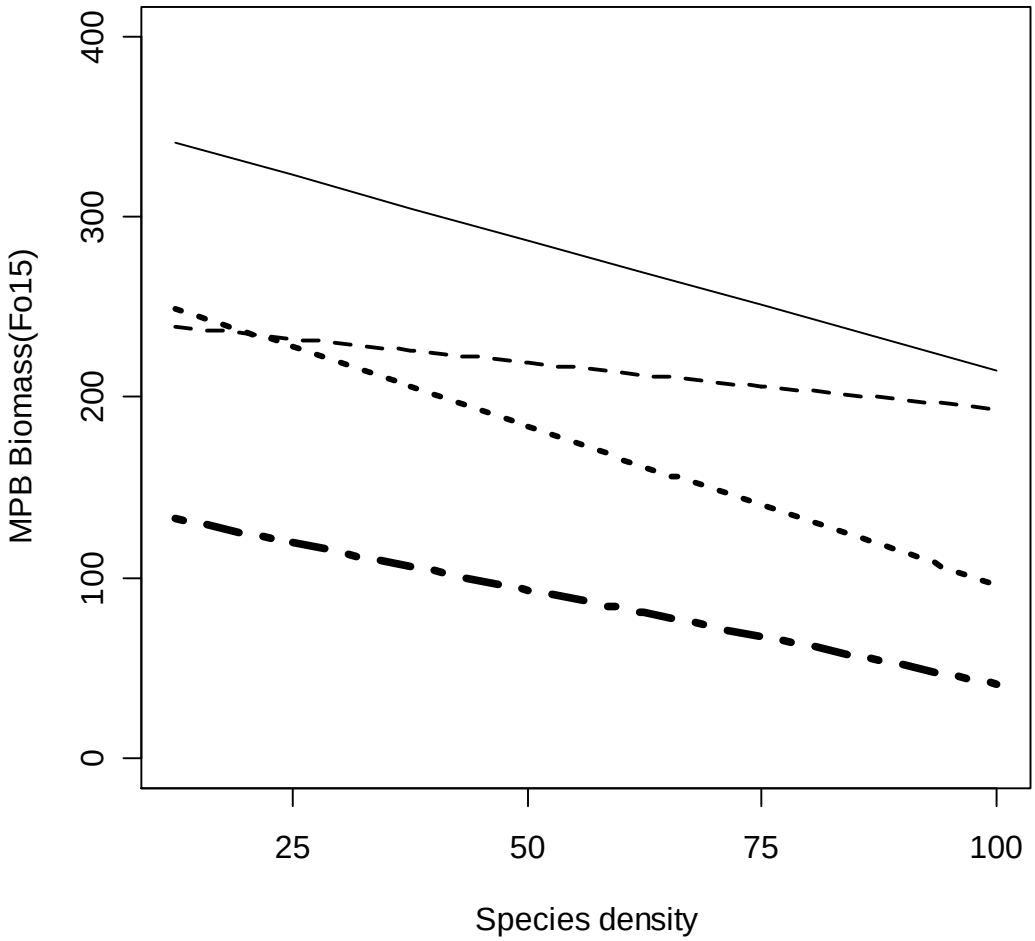
2

3

4

1 Fig 3

2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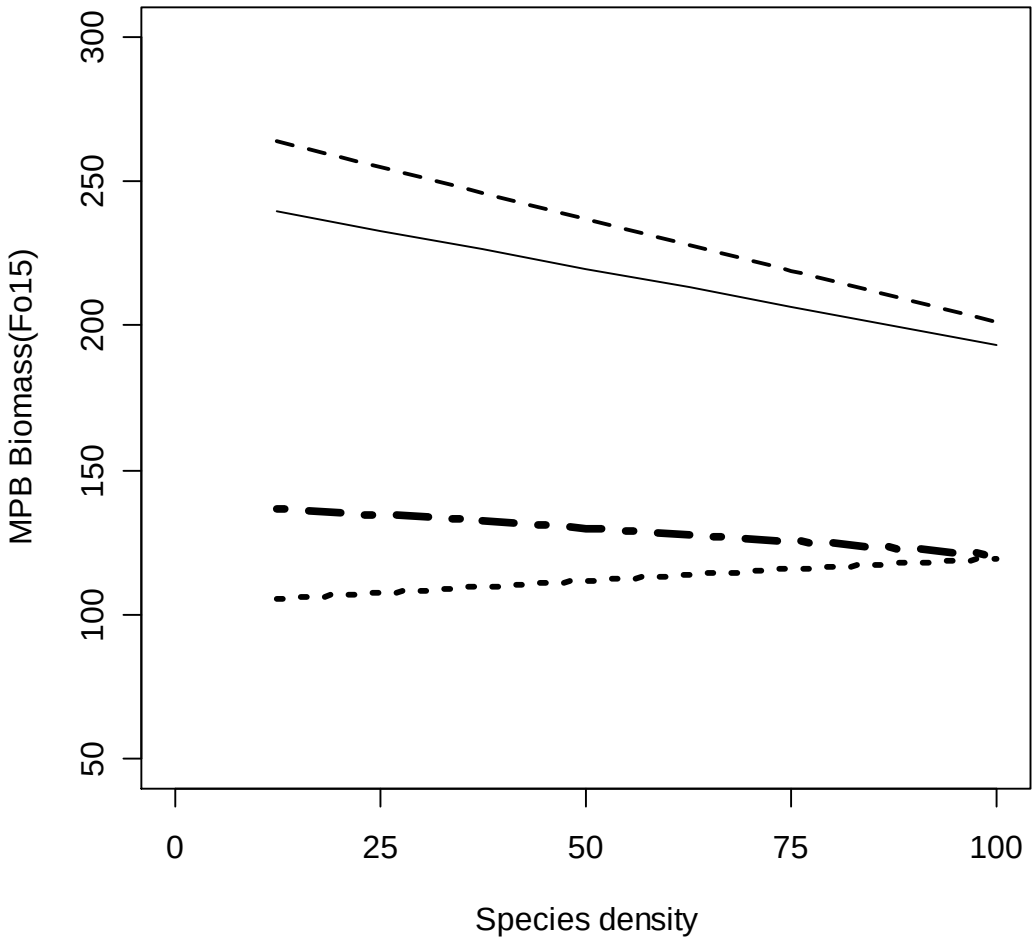
100
200
300
400
25
50
75
100
Species density

3

1 Fig 4


MPB Biomass(Fo15)
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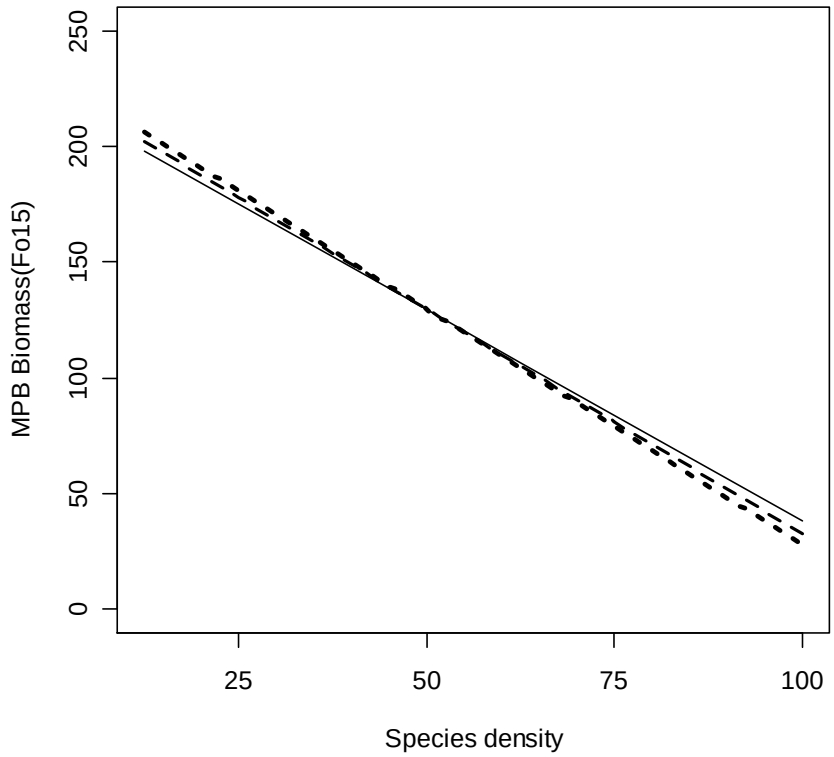
250
200
150
100
50
0
25
50
75
100
Species density

2

3

1 Fig 5

2


MPB Biomass(Fo15)
0
50
100
150
200
250
25
50
75
100
Species density

3

4

Figure 1: Overview of experimental design. The species density gradients across the patch interface were established at the start of the experiment, using the relative levels of 0%, 25%, , 50% and 100% natural density at the study site. These combinations were used for each of the four interface treatments (enriched is shaded and non-enriched is not shaded), and every species density-interface combination was used for each of the four species (*Corophium volutator* (Cv), *Hydrobia ulvae* (Hu), *Macoma balthica* (Mb) and *Hediste diversicolor* (Hd)).

Figure 2: Graphical representation of the effect of the two-way interaction term species identity × interface. Vertical lines represent species identity: *Hediste diversicolor* (1); *Macoma balthica* (2); *Hydrobia ulvae* (3); and *Corophium volutator* (4). Horizontal bars represent predicted values from the optimal regression model for each heterogeneity treatment, 'patches' are represented by the expression on the left of '|' while neighbouring patches are on the right. The two horizontal lines are the averaged for control mesocosms (containing no macrofauna) at interface treatment E|E (——), E|NE (- - -), NE|E (—-) and NE|NE (·····). As the GLS framework allows for different spread in the data, individual data points are omitted to prevent misinterpretation.

Figure 3: Graphical representation of the effect of the two-way interaction term species identity × species density. Lines represent species identity: *Hediste diversicolor* (——); *Macoma balthica* (– – –); *Hydrobia ulvae* (· - - - -); and *Corophium volutator* (—— –). Species density is expressed as a percentage of the natural densities at the study site. As the GLS framework allows for different spread in the data, individual data points are omitted to prevent misinterpretation.

Figure 4: Graphical representation of the effect of the two-way interaction term interface × species density. Lines represent heterogeneity: E|E (——); E|NE (– – –); NE|NE (·– · – ·); and NE|E (—— –), where E is an enriched patch, NE is a non-enriched patch and "|" is the interface between each patch. Analysis is based on the left patch and coded for neighbouring patch on the right. Species density is a percentage of the natural densities found at the study site. As the GLS framework allows for different spread in the data, individual data points are omitted to prevent misinterpretation.

Figure 5: Graphical representation of the effect of the two-way interaction term species density × initial density difference Lines represent the initial density difference: -4 (——); 0 (– – –); 4 (· - - - -), where initial density difference ranges from a maximum density in the right-hand patch and no macrofauna in the left-hand patch (-4) to a maximum density in the left hand patch and no macrofauna in the right-hand patch (4). Species density is a percentage of the natural densities found at the study site. As the GLS framework allows for different spread in the data, individual data points are omitted to prevent misinterpretation.

Species effects on ecosystem processes are modified by faunal responses to habitat composition

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Species effects on ecosystem processes are modified by faunal responses to habitat composition

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Running title: Faunal responses to habitat composition affect ecosystem processes

Abstract

Heterogeneity is a well-recognized feature of natural environments, and the spatial distribution and movement of individual species is primarily driven by resource requirements. Recent research has found that species composition can be critical in determining levels of ecosystem processes. As most small experimental systems are spatially homogeneous, however, the importance of the link between environmental heterogeneity, the redistribution of species, and ecosystem process has not been fully explored. Here, we used a mesocosm system to investigate the relationship between habitat composition, species movement and sediment nutrient release for four species of benthic invertebrate macrofauna. Various habitat configurations were generated by selectively enriching patches of sediment with macroalgae, a natural source of spatial variability in intertidal mudflats. We found that the direction and extent of faunal movement between patches differs with species identity, density and habitat composition. These relationships are complex, dynamic and demonstrate that no single factor drives the spatial dynamics of benthic communities. Combinations of these interactions lead to concomitant changes in nutrient release, such that habitat composition effects are modified by species identity (in the case of NH_4-N) and by species density (in the case of PO_4-P). These findings suggest that natural patterns of spatial heterogeneity need to be accommodated in future biodiversity-ecosystem function studies, rather than rigorously controlled for, and that failure to do so may result in an incomplete understanding of system behaviour.

Keywords: Ecosystem function, species identity, biodiversity, habitat heterogeneity, patch dynamics

Introduction

The potential relationships between the rate of ecosystem processes and biodiversity have been the focus of a considerable research effort over the past 15 years and have been given additional impetus through the conclusions of the Millennium Ecosystem Assessment (2005). Experimental approaches continue to be successful in defining these relationships, and in unravelling the underlying biological mechanisms, but the high degree of experimental control employed often means that the environmental variation seen in nature is not acknowledged or welcomed in most experiments. One source of variation is spatial heterogeneity which can, in turn, have significant effects on ecosystem processes (Hovel & Lipcius 2001), including ecosystem productivity (Benedetti-Cecchi 2005).

In the marine benthic environment, heterogeneity in both biodiversity and ecosystem processes is well-recognized at a variety of scales and is known to be generated by microbial activity (mm^2 – seconds scale), the behaviour and bioturbatory activities of infaunal invertebrates (cm^2-day), the feeding activities of epibenthic vertebrate predators (m^2-months) and storms and anoxic events (km^2-years) (Hall et al. 1994; Teixido et al. 2002; Parry et al. 2003; Noren & Lindegarth 2005). The dominant explanatory paradigm for this pattern is that patches are in different successional stages that reflect the time for re-assembly following disturbance (Johnson, 1972; Pearson & Rosenberg, 1978; Rhoads et al., 1978), and that the seafloor is essentially a dynamic mosaic of microbial and invertebrate patches (Zajac, 2001), together with their associated sediment biogeochemical processes, operating over a range of different spatial and temporal scales (Noren & Lindegarth 2005, Ellingsen & Gray

2002; Sanvicente-Anorve et al. 2002; Goodsell & Connell 2002, Hewitt et al. 2002). For instance, hollows in the sediment generated by mammal, bird or fish feeding behaviour (Cadée, 2001) provide low-flow traps for organic matter, thereby enhancing local food quality for mobile deposit feeders, and these patches will only later be colonised by less mobile or suspension feeding species when the organic matter has been depleted.

Central to the dynamics of these mosaics is the movement of the different types of benthic organisms as they respond to, deplete and then move away from resource patches (Kelaher & Levinton, 2003; Levinton & Kelaher, 2004). Movement through and over the sediment surface constitutes bioturbation which is additional to, and perhaps qualitatively distinct from, the bioturbation normally recorded in micro- and mesocosm studies, which generally control for heterogeneity in habitat quality. The effect of habitat heterogeneity on organism movement, and the impact of that movement on rates of ecosystem processes, remains largely unquantified (except Dyson et al 2007) and has not, to date, been an explicit feature of experiments on the relationships between biodiversity and ecosystem function (Covich et al., 2004; Balvanera et al 2006; Cardinale et al., 2006), including those carried out for marine benthic systems (Emmerson et al. 2001; Raffaelli et al. 2003; Covich et al., 2004, Waldbusser et al. 2004; Bulling et al 2006; Ieno et al., 2006). Incorporating heterogeneity into biodiversity-sediment process experiments will add greater realism and, hence, predictive power with respect to functional relationships, in particular in relation to the importance of compositional (relative abundance) rather than species richness effects.

Here, we quantify the effects of habitat quality (organic matter content of sediment) on the movement of benthic species in a mesocosm environment and measure the consequences of such activity for ecosystem processes (nutrient release to the overlying water column). This allowed us to test the hypothesis that the movement of benthic invertebrates, and the effects this may have on nutrient release, will differ between homogeneous (characteristic of mesocosm experiments) and heterogeneous (typical of natural sediments) environments.

Materials and Methods

Sediment, algal and faunal collection

All sediment, the algal material used for manipulating habitat quality, and invertebrates were collected from mud flats in the Ythan estuary, Aberdeenshire, Scotland, UK (57° 20.085'N, 02° 0.206' W). The sediment is muddy sand (mean particle size = 50.0 μm, silt content 60.0%), with an organic carbon content of c. 4.0%. Prior to establishment of the mesocosms (see below), sediment was sieved over a 0.5 mm mesh in a seawater bath to remove macrofauna and then allowed to settle for 24 h to retain the fine fraction (<63 μm). Excess water was removed and the settled sediment was homogenised to slurry to facilitate distribution between the mesocosms.

Four macrofaunal species were added to the mesocosms at different biomass levels (Figure 1): the suspension/deposit-feeding polychaete *Hediste diversicolor*, the surficial grazing gastropod *Hydrobia ulvae*, the deposit-feeding shrimp *Corophium volutator* and the suspension/deposit-feeding bivalve *Macoma balthica*. These are the most significant bioturbators regularly encountered at the study site (Biles et al., 2003; Raffaelli et al., 2003).

Mesocosms and experimental design

Mesocosms (non-transparent plastic aquaria, 21 × 15 × 14 cm) containing sediment to a depth of 3 cm and seawater (2.5 l) were held in a constant-temperature room (11 ± 2.0 ºC) with a 12:12 h light-dark regime (1 × 26 mm Ø white fluorescent tube per 8 mesocosms, model GE F36W/35; 36W, 3500ºK). Spatial differences in temperature within the cold room were < 1ºC. Sediment and seawater (UV-sterilised, 10μm pre-filtered, salinity ≈ 33) were added to each mesocosm 24 h prior to species addition. Seawater was siphoned off and replaced after 24 h to remove excess nutrients associated with sediment disturbance during assembly. All mesocosms were continually aerated and ran for 10 d.

We assembled 414 mesocosms, split equally between two runs, to determine the effects, for each species, on nutrient (NH_4-N and PO_4-P) release from the sediment due to infaunal movement in response to habitat heterogeneity and local density. To incorporate heterogeneity into our model system, each mesocosm was subdivided into two equal halves (henceforth each half is termed a 'patch') by establishing a cross-sectional interface (Figure 1). Each patch consisted of either non-enriched sediment (NE) or sediment that was enriched (E) with 1 g of dried and powdered *Ulva intestinalis*, so that 4 interface types can be recognised (Figure 1). *U. intestinalis* is the dominant form of organic input to the Ythan estuary in the summer (Raffaelli 2000). The addition of 1g of *U. intestinalis* as a powder in our mesocosms allows a significant enrichment of the sediment, but is insufficient to generate a hostile physico-chemical environment (Raffaelli 2000).

For each of the 4 macrofaunal species, we also manipulated species density (4 levels; 0%, 25%, 50% and 100% natural density in the left and right hand patches, i.e. 16 possible combinations) and across 4 interface types (E|E, E|NE, NE|E and NE|NE, where '|' represents the interface between the two patches) (Figure 1), in order to generate initial density differences between different patch types, a likely driver of movement. The maximum species densities used in the mesocosms were similar to those at the study site (Biles et al., 2003), equivalent to 1g and 2g wet weight patch^{-1} for *Corophium volutator* and *Hydrobia ulvae* respectively, and 4 individuals patch^{-1} for *Macoma balthica* and *Hediste diversicolor*. Each configuration was replicated 3 times which, after the removal of redundancy due to mirror-image equivalence (including the entire treatment block NE|E), gave a total of 396 mesocosms (Figure 1). We also assembled treatments containing no macrofauna (controls; n = 3) for each of the 3 levels of enrichment (E|E, E|NE, NE|NE) for both experimental runs (an additional 18 mesocosms), in order to assess the effects of algal addition on nutrient flux.

Net movement was estimated for each species as the difference in abundance between patches at the end of an experimental run. Macrofauna were confined to an initial patch for the first 24 h using a perspex insert prior to burrow establishment. Following removal of the insert, the net movement of individuals across the patch interface was recorded after 10 d by recovering the fauna from each patch and counting the number of individuals present. To avoid error caused by variability in wet weight analyses, we counted individuals at the end of the experiment and converted these numbers back to biomass by multiplying the proportion of individuals in each patch by the total starting biomass. To facilitate comparison between species, the relative change in

macrofaunal biomass within a given patch was expressed on a scale of -100% (out of) to 100% (into) the patch.

In addition to creating habitat heterogeneity to drive animal movement, the experimental set-up also allowed us to test the effects of organic enrichment by re-coding the mesocosms according to the three levels of total mesocosm enrichment (NE|NE, NE|E and E|NE, E|E; Figure 1).

Pre-filtered (Nalgene, 0.45μm) water samples were taken on the final day of the experiment. Ammonical-nitrogen (NH_4-N) and phosphate-phosphorus (PO_4-P) concentrations were determined using standard protocols with a modular flow injection auto-analyser (FIA Star 5010 series) using an artificial seawater carrier solution.

Data analysis

Statistical models were developed (following Zuur et al., 2007) for the dependent variables (nutrient release and movement), and the independent variables species identity, species density and enrichment (nutrient models) or interface type (movement models) (Figure 1). Because we expected that differences in species density between patches within a mesocosm at the start of the experiment might influence species movement, we added this term as a fourth explanatory variable (starting density difference).

Graphical exploratory techniques were used to check for outliers. As a first step we fitted a linear regression. A model validation was applied to check that underlying

statistical assumptions were not violated; normality was assessed by plotting theoretical quantiles versus standardised residuals (quantile-quantile plots), homogeneity of variance was evaluated by plotting residuals versus fitted values, non-linearity was evaluated by plotting residuals versus explanatory variables, and influential data points were identified using Cook's distance (Quinn & Keough 2002). The validatory procedure gave no indication of non-linearity but revealed strong heteroscedasticity in some of our models. Where there was evidence of unequal variance in the residuals, we used linear regression with the generalized least squares (GLS) estimation procedure (Pinheiro & Bates, 2000; Faraway, 2006; West et al., 2006; Zuur et al., 2007). A GLS framework is preferential to Poisson regression because nutrient concentration is a continuous variable and therefore the Poisson distribution is less appropriate. Furthermore, GLS models are based on linear relationships, whereas the Poisson regression imposes exponential relationships which are less suitable for our data. A data transformation to stabilise the variance is not necessary when using GLS models because the use of variance-covariate terms allows for unequal variance (Pinheiro & Bates, 2000), allowing us to retain the original variance structure of the data.

Model selection. To find the minimal adequate model, we adopted the approach outlined by Verbeke & Molenberghs (2000) and Diggle et al. (2002). In the first step of this approach, the optimal structure in terms of random components is determined using REML (Restricted Maximum Likelihood) estimation and, in the second step, the optimal fixed effects structure is determined using ML (Maximum Likelihood) estimation. The optimal random structure was determined by starting with a model without any variance-covariate terms (equivalent to linear regression) and comparing

this model with subsequent GLS models that contained specific variance structures (i.e. different spread per stratum for each nominal variable, an increase of spread along a continuous variable using various mathematical forms, or a combination; see Table 5.2 in Pinheiro & Bates, 2000). To find the optimal random structure, we used the AIC (Akaike Information Criteria; Sakamoto et al., 1986; Burnham & Anderson 2002), likelihood ratio tests and plots of residuals versus fitted values. The optimal fixed structure was established by applying a backward selection using the likelihood ratio test obtained by ML estimation. The importance of each explanatory factor in the minimum adequate model was assessed by comparing a reduced model (with all terms involving the factor of interest removed) with the full model, using the likelihood ratio test. The numerical output of the optimal model was obtained using REML estimation (West et al., 2006). All analyses were performed using the 'nlme' package (v. 3.1, Pinheiro et al., 2006) in the 'R' statistical and programming environment (R Development Core Team, 2005).

Results

Overall, significant effects were detected for species identity, species density, nutrient enrichment, interface type and starting density difference, but these effects were mediated through interaction terms. Following Underwood (1997), we only interpret the highest order significant interaction term(s) as interpretation of lower order terms is unreliable, unless they are not nested within the higher order terms.

Movement Model

We modelled net movement using a linear regression with a GLS extension to allow for unequal variance within species identity and interface type. Our minimal adequate

model incorporated four single factors, six two-way interaction terms and three three-way interaction terms (Model S1). By comparing the minimal adequate model with models in which each variable in turn was dropped, we found that starting density difference had the greatest influence on the model (L-ratio = 436.42, d.f. = 49, $p<0.0001$), followed by species identity (L-ratio = 283.58, d.f. = 30, $p<0.0001$), interface type (L-ratio = 204.12, d.f. = 33, $p<0.0001$) and species density (L-ratio = 86.33, d.f. = 49, $p<0.0001$). The highest order interaction terms that were significant were the three-way interactions species identity × interface type × species density (Figure 2), species identity × interface type × starting density difference (Figure 3), and species identity × species density × starting density difference (Figure 4).

H. diversicolor and *H. ulvae* both moved towards enriched patches (Figure 2a, 2b), whereas *M. balthica* and *C. volutator* both moved away from enriched patches (Figure 2c, 2d). All movements increased with density, but these density effects were most pronounced for *C. volutator* and, to a lesser extent, *H. diversicolor* (Figure 2). The estimated regression parameters for the first interaction term indicated that the responses displayed by *H. diversicolor* and *C. volutator* were significantly different (Coefficient Table S1). *H. diversicolor*, *H. ulvae* and *C. volutator* all showed strong movement responses to starting density difference and interface type (Figure 3). For *H. diversicolor* and *H. ulvae*, responses to starting density difference strongly influenced responses to interface type (Figure 3a, 3b). Both species showed similar patterns of response, moving out from higher starting density areas, although the responses were stronger for *H. diversicolor* (Figure 3a). *C. volutator*, in contrast, responded more strongly to interface type, moving out of enriched areas, although this species also showed a slight density response, since the out-movements occurred at a

greater rate when higher density coincided with a high level of enrichment (Figure 3d). *M. balthica* demonstrated low rates of movement with weak starting density difference and interface type effects (Figure 3c).

H. diversicolor and *C. volutator* showed strong density dependence, moving away from high density patches (Figure 4a, 4d). However, where there was high density in the right hand patch and low density in the left hand patch (solid lines), this effect became more pronounced with *H. diversicolor* (Figure 4a) and less pronounced with *C. volutator* (Figure 4d). This increase in effect was confirmed in the reciprocal starting density treatment (dotted lines) for *H. diversicolor*, but not for *C. volutator*. *H. ulvae* showed similar directional density dependence effects, but there was little interaction with increasing species density (Figure 4b). In contrast, *M. balthica* showed no obvious response to either species density or starting density difference (Figure 4c).

Nutrient model

As for our movement model, our minimal adequate model for both NH_4-N and PO_4-P was a linear regression with a GLS extension (allowing for unequal variance within species identity, enrichment and species density). For NH_4-N, the model incorporated four single factors, four two-way interaction terms and one three-way interaction term (Model S2, Coefficient Table S2). By comparing the minimal adequate model with models in which each variable in turn was dropped, we found that species identity had the greatest influence (L-ratio = 386.38, d.f. = 19, $p<0.0001$), followed by species density (L-ratio = 107.25, d.f. = 29, $p<0.0001$), enrichment (L-ratio = 73.64, d.f. = 29, $p<0.0001$) and starting density difference (L-ratio =20.34, d.f. = 29, $p<0.001$). The

highest significant interaction terms were the three-way term species identity × species density × starting density difference (L-ratio = 7.76, d.f. = 34, p = 0.05; Figure 5), and the two-way term, species identity × enrichment (L-ratio = 73.64, d.f. = 31, p = <0.0001; Figure 6).

The three-way term, species identity × species density × starting density difference, indicated that for *H. ulvae*, and especially *C. volutator*, a high starting density difference and a high species density in the left-hand patch resulted in a greater effect on NH_4-N compared to *H. diversicolor*, although this term was only marginally significant (Figure 5b, 5c). These effects were not necessarily reciprocated in the right-hand patch, suggesting that the effects of starting density difference can be variable (Figure 5a). Increasing species density and starting density difference in treatments containing *M. balthica* had minimal effects on NH_4-N (Figure 5d).

The overall degree of sediment enrichment had little effect on the patterns of NH_4-N concentration, but there were clear differences between species contributions, with greatest NH_4-N concentrations associated with *H. diversicolor* and the least with *M. balthica* (Figure 6). Interestingly, treatments with the highest (or lowest) level of enrichment were not always associated with the highest (or lowest) NH_4-N concentrations.

For PO_4-P, the minimal adequate model incorporated three single factors, three two-way interaction terms and one three-way interaction term (Model S3). We found that enrichment (L-ratio = 609.39, d.f. = 20, p<0.0001) had by far the greatest influence on the model, followed by species identity (L-ratio = 108.41, d.f. = 18, p<0.0001) and

species density (L-ratio = 92.48, d.f. = 24, $p<0.0001$). The most significant interaction term was the three-way term species identity × enrichment × species density (L-ratio = 25.66, d.f. = 30, $p<0.001$; Figure 7).

In contrast to NH_4-N, there was a consistent pattern of increasing PO_4-P with increasing enrichment (compare Figure 7a-c), irrespective of species identity. None of the species showed strong density trends in PO_4-P release at low levels of sediment enrichment (Figure 7a). As sediment enrichment increased, increasing densities of *H. diversicolor, H. ulvae* and *C. volutator* had a negative impact on PO_4-P release, although for *M. balthica*, there was no such effect (Figure 7b, 7c). The regression parameters (Coefficient Table S3) indicated that, for *C. volutator, H. ulvae* and *H. diversicolor*, PO_4-P release at intermediate levels of enrichment (NE|E and E|NE) with the lowest species densities was equivalent to that released from fully enriched sediment (E|E) with high species densities.

Discussion

In natural environments, the behaviour of individual species is driven by resource requirements which are distributed heterogeneously in space and time. Habitat heterogeneity is reflected in the dispersion patterns (local density variation) of invertebrates in mud flats (Hall et al., 1994) and local density is also likely to be a driver of movement. In addition, movement rates and patterns of local density variation are likely to be species-dependent. Yet, experimental approaches (Schmid et al., 2002; Raffaelli et al., 2003) examining the contribution of species to ecosystem processes have largely taken place over short time scales and in small experimental mesocosms that are spatially homogeneous (for reviews, see Balvanera et al., 2006;

Cardinale et al., 2006). In contrast to previous methodology, we used a two patch mesocosm system that allowed for variable habitat configurations whilst maintaining the experimental control necessary to unambiguously interpret responses. The latter required consideration of the inherent heteroscedasticity in the data caused by the markedly different functional behaviour of the species (achieved here using a GLS modelling framework). In the broadest terms, our results demonstrate that habitat composition has clear effects on both infaunal movement and nutrient release, but that these effects were species specific and/or modified by differences in density, both within and between patches.

Within different enrichment configurations, the direction and extent of faunal movement between patches differed with species. Both *Hediste diversicolor* and *Hydrobia ulvae* moved towards enriched patches (i.e. away from natural, non-enriched patches), whilst the reverse was true for *Corophium volutator*. These responses are consistent with the lifestyle traits of the organisms; *H. diversicolor* and *H. ulvae* are deposit feeders and *C. volutator* appears to be less tolerant to the reduced conditions generated by enrichment (Raffaelli et al 1991; Raffaelli 1999; Norrko, 1998). In the case of *M. balthica*, however, the lack of movement could be interpreted to mean that the species does not respond to localised resource heterogeneity or, alternatively, that the species exhibits a behavioural response other than movement. It is known, for example, that *M. balthica* is more vulnerable to the more hostile sediment conditions associated with algal-mats than the other species in this study (Norkko and Bonsdorff, 1996a, 1996b; Norkko, 1998) and that the toxic effects of sulphide are avoided by temporary valve closure rather than active migration (Jahn et al., 1997).

The creation of patches rich in organic material, the movement of different species in and out of patches and the subsequent depletion of those patches, is a dynamic and continuing process that drives movement in, across and within surficial sediments leading to spatial variation in species density (Kelaher and Levinton 2003). Our experiments demonstrated density dependence effects on movement, such that individuals of all species, with the exception of *M. balthica*, tended to favour patches containing lower densities. These effects were not isolated but formed an important component of interaction terms modifying the effects of species and habitat composition discussed above. Thus, heterogeneity, species movement and species density appear to all be modified by each other, and no single, unambiguous factor can be said to drive the system.

We found that habitat composition was also a key driver of nutrient release, but as in the movement models, this effect was modified by species identity for NH_4-N, and by both species identity and density for PO_4-P. These findings are qualitatively consistent with previous mesocosm experiments (Emmerson et al 2001; Raffaelli et al 2003; Mermillod-Blondin et al. 2005). *H. diversicolor* had the greatest impact on NH_4-N and *M. balthica* the least, whilst the relative contribution of species to PO_4-P release was much less distinct. However, there were very clear differences in the levels of PO_4-P release between enrichment treatments. Interestingly, in other studies which have controlled for heterogeneity, *H. ulvae* has generally been found to have only a weak effect on nutrient release. The greater importance of *H. ulvae* in the present study may be attributable to its increased activity in a heterogeneous environment.

It is important to consider the applicability of our mesocosm-based results to large-scale natural systems (Benton et al., 2007). In previous experiments at the study site, the abundances of all four species used here have been measured at several biomass levels of *U. intestinalis* and reveal similar patterns of dispersion in relation to enrichment (Hull 1987; Raffaelli, 1999). We are therefore confident that the algal enrichment treatments used here generated effects comparable to those which would occur under natural conditions in the field. Furthermore, the local density variations (initial density treatment) we employed in the present experiments span the range of densities normally encountered in the field due to the patchy distribution of these species. On the Ythan estuary, this patchiness occurs typically on a scale of 3-6cm (Lawrie, 1996), a similar spatial scale to the dimensions of the patches within our mesocosms.

The implications for manipulative experiments aimed at assessing the effects of species richness on ecosystem processes, such as nutrient release, that are intimately linked to sediment bioturbation, are clear: experiments which do not incorporate the heterogeneity found in nature at these smaller (<10cm) scales are likely to underestimate both the absolute magnitude of nutrient release and effects generated by species responding to local heterogeneity in different ways. In the present experimental set-up, it was not possible to measure this effect quantitatively, because the effects of enrichment and the way in which fauna respond to heterogeneity could not be clearly separated. Nevertheless, the findings of the present study imply that natural patterns of spatial heterogeneity need to be accommodated, rather than rigorously controlled for, within experiments which attempt to quantify the contribution of species to ecosystem processes. Larger scale marine benthic

mesocosm experiments that capture very large areas of sediment intact could preserve such heterogeneity, but this is often destroyed when the sediment is sieved in order to provide a standardised sediment lacking macrofauna. Whilst it may remain impractical to capture natural heterogeneity within experimental systems, it is clear that failure to include such effects in consideration of landscape-scale biodiversity-ecosystem process research may result in an incomplete understanding of system behaviour.

Acknowledgements

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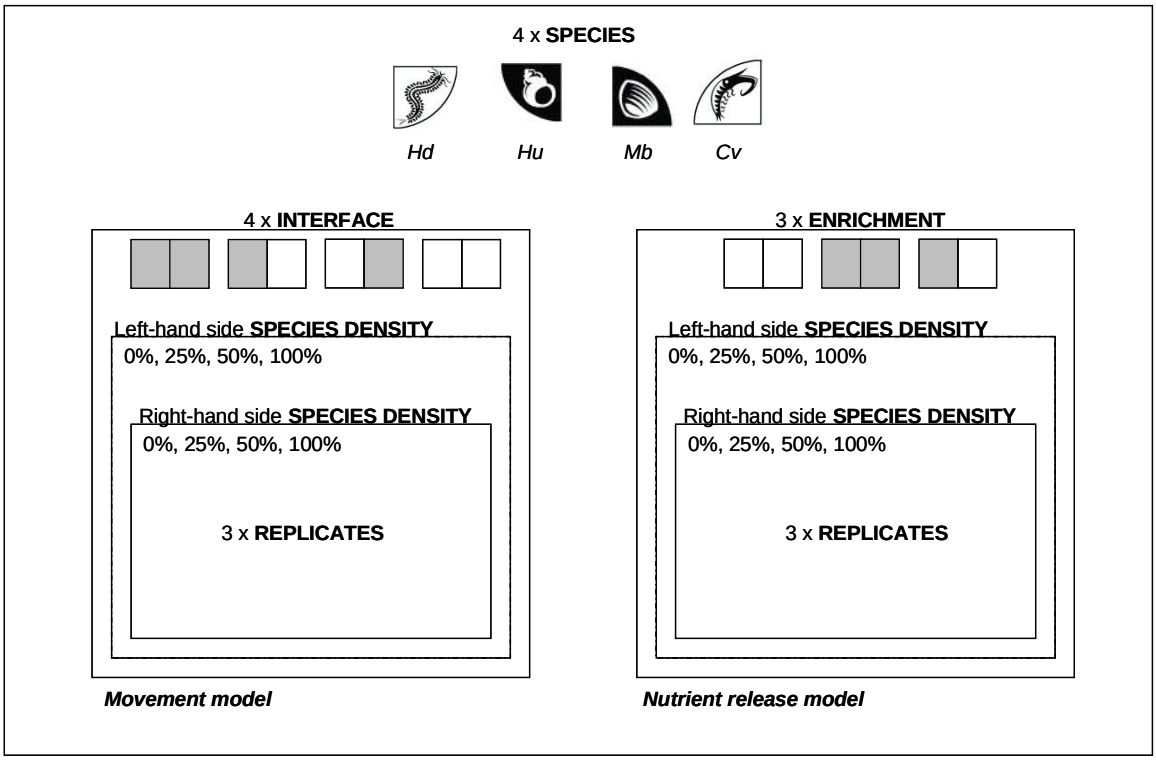
Figure 1


4 x SPECIES
Hd
Hu
Mb
Cv
4 x INTERFACE
Left-hand side SPECIES DENSITY
0%, 25%, 50%, 100%
Right-hand side SPECIES DENSITY
0%, 25%, 50%, 100%
3 x REPLICATES
Movement model
3 x ENRICHMENT
Left-hand side SPECIES DENSITY
0%, 25%, 50%, 100%
Right-hand side SPECIES DENSITY
0%, 25%, 50%, 100%
3 x REPLICATES
Nutrient release model

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3 Figure 2

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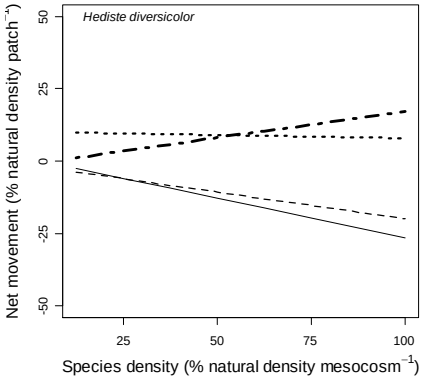
(a)



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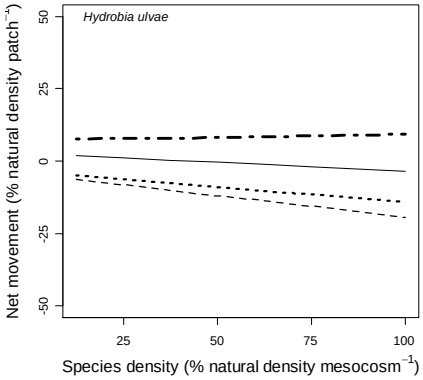

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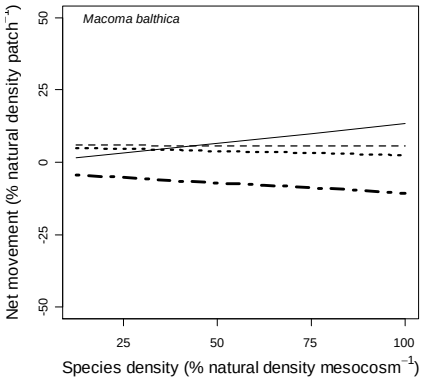

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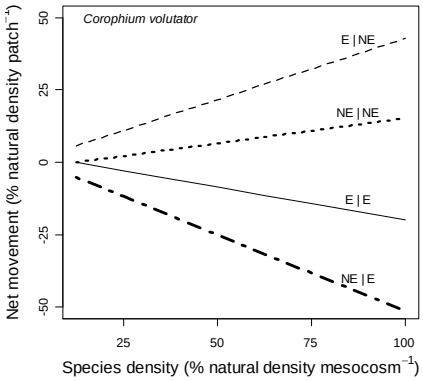

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1 Figure 3

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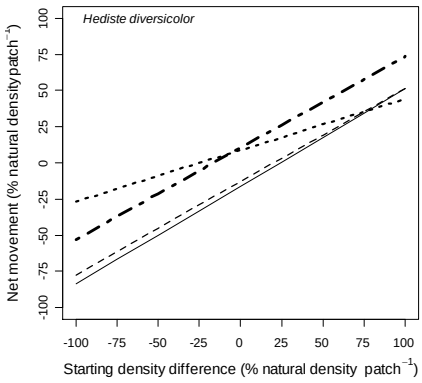

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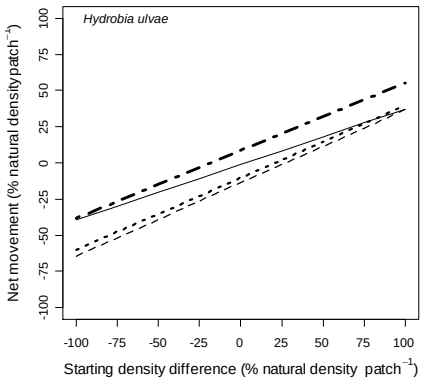

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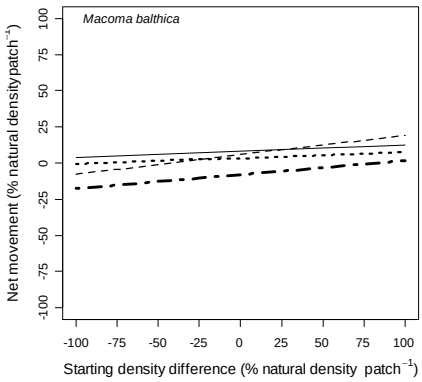

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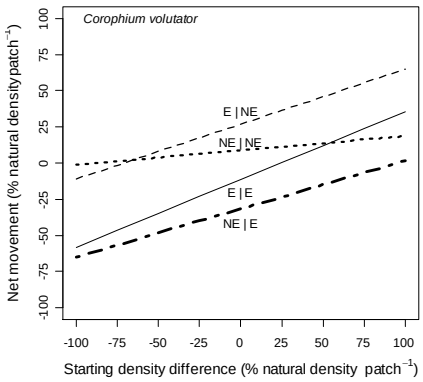

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1 Figure 4

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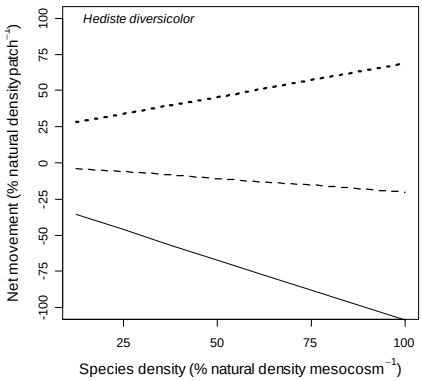

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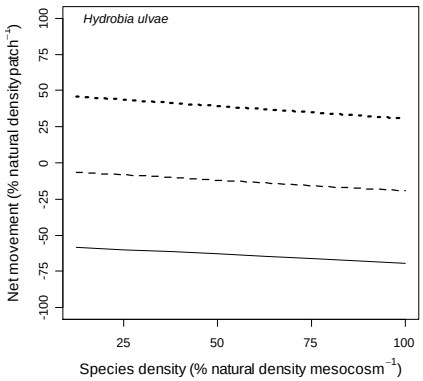

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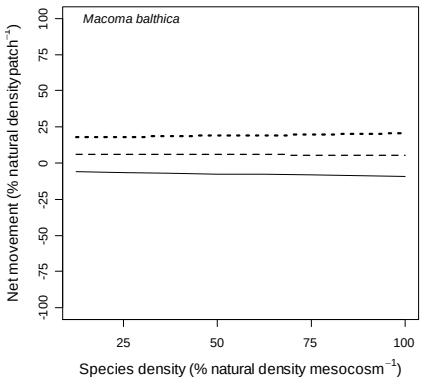
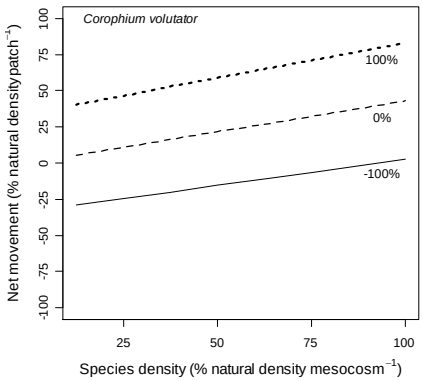


(d)



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1 Figure 5

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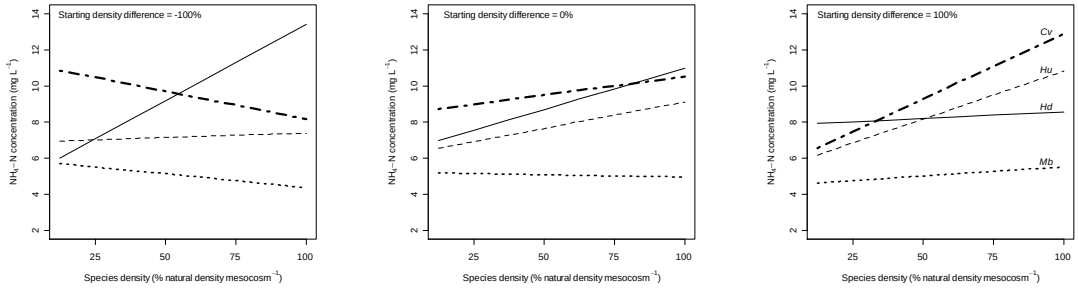
(a) (b) (c)



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1 Figure 6

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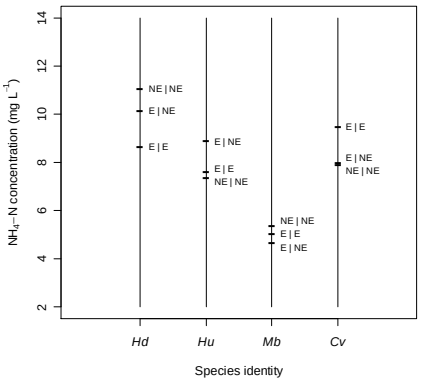

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1 Figure 7

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(a) (b) (c)

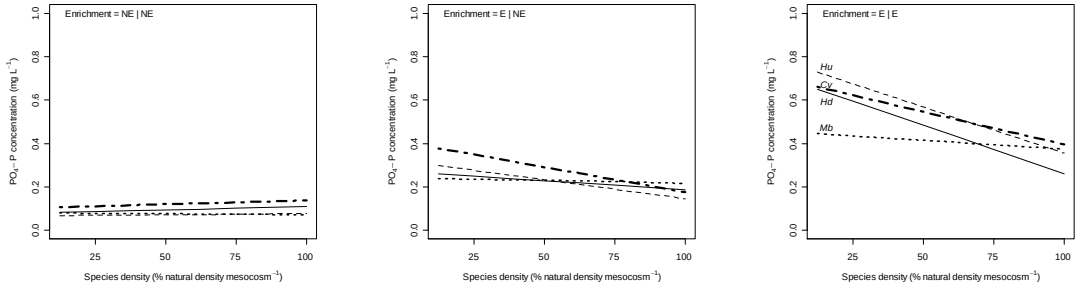

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Table and Figure Legends

Figure 1: Overview of experimental design and model structure. For each of four species (*Hediste diversicolor*, *Hd*; *Hydrobia ulvae*, *Hu*; *Macoma balthica*, *Mb*; and *Corophium volutator*, *Cv*) a species density gradient was established across a patch interface at the start of the experiment using the relative levels of 0%, 25%, 50% and 100% of natural density. These combinations were used for each of four interface treatments (E|E, E|NE, NE|E, NE|NE) composed of mixtures of enriched (E) and non-enriched (NE) patches. Two model structures were used in the analyses, one investigated faunal activity (movement model) and the other nutrient generation (nutrient release model). For the latter, nutrient release could not be measured at the patch scale so E|NE and NE|E were treated as equivalent.

Figure 2: Graphical representation of the effect of the three-way interaction term species identity × interface type × species density on net macrofaunal movement for (a) *Hediste diversicolor*, (b) *Hydrobia ulvae*, (c) *Macoma balthica,* and (d) *Corophium volutator*. Lines represent predicted values from the minimal adequate regression model for interface treatments (indicated in panel (d)) where: both patches were enriched E|E (solid line); only a single patch on the left was enriched E|NE (dashed line); no patches were enriched NE|NE (dotted line); or only a single patch on the right was enriched, NE|E (dot-dashed line). Species densities ranged from no macrofauna (0%) to natural density (100%) per mesocosm. For net movement, positive values indicate a directional migration from the left patch to the right patch whilst negative values indicate the reciprocal. As the GLS framework allows for

different spread in the data, individual data points are omitted to avoid misinterpretation.

Figure 3: Graphical representation of the effect of the three-way interaction term species identity × interface type × starting density difference on net macrofaunal movement for (a) *Hediste diversicolor*, (b) *Hydrobia ulvae*, (c) *Macoma balthica*, and (d) *Corophium volutator*. Lines represent predicted values from the optimal regression model for interface treatments (indicated in panel (d)) where: both patches were enriched, E|E (solid line); only a single patch on the left was enriched, E|NE (dashed line); no patches were enriched, NE|NE (dotted line); or only a single patch on the right was enriched, NE|E (dot-dashed line). Starting density difference ranges from a density equivalent to natural density in the right-hand patch and no macrofauna in the left-hand patch (-100%) to a density equivalent to natural density in the left hand patch and no macrofauna in the right-hand patch (100%). For net movement, positive values indicate a directional migration from the left patch to the right patch whilst negative values indicate the reciprocal. As the GLS framework allows for different spread in the data, individual data points are omitted to prevent misinterpretation.

Figure 4: Graphical representation of the effect of the three-way interaction term species identity × species density × starting density difference on net macrofaunal movement for (a) *Hediste diversicolor*, (b) *Hydrobia ulvae*, (c) *Macoma balthica*, and (d) *Corophium volutator*. Lines represent predicted values from the optimal regression model for selected starting density differences (indicated in panel (d)): maximum density in the right-hand patch and no macrofauna in the left-hand patch (solid line); no difference in density between patches (dashed line); and maximum

density in the left hand patch and no macrofauna in the right-hand patch (dotted line). Species densities ranged from no macrofauna (0%) to natural density (100%) per mesocosm. For net movement, positive values indicate a directional migration from the left patch to the right patch whilst negative values indicate the reciprocal. As the GLS framework allows for different spread in the data, individual data points are omitted to prevent misinterpretation.

Figure 5: Graphical representation of the effect of the three-way interaction term species identity × species density × starting density difference on NH_4-N concentration. Selected representations are shown for a starting density difference of (a) natural density in the right-hand patch and no macrofauna in the left-hand patch (-100%), (b) equal densities in each patch (0%) and (c) natural density in the left-hand patch and no macrofauna in the right-hand patch (100%). Lines represent predicted values from the optimal regression model for each species (indicated in panel (c)): *Hediste diversicolor*, *Hd* (solid line); *Hydrobia ulvae*, *Hu* (dashed line); *Macoma balthica*, *Mb* (dotted line); and *Corophium volutator*, *Cv* (dot-dashed line). Species densities ranged from no macrofauna (0%) to natural density (100%) per mesocosm. As the GLS framework allows for different spread in the data, individual data points are omitted to avoid misinterpretation.

Figure 6: Graphical representation of the effect of the two-way interaction term species identity × enrichment. Vertical lines locate species identity *Hediste diversicolor* (*Hd*), *Hydrobia ulvae* (*Hu*), *Macoma balthica* (*Mb*) and *Corophium volutator* (*Cv*). Horizontal bars represent predicted values from the optimal regression model for each enrichment treatment composed of mixtures of enriched (E)

and non-enriched (NE) patches (indicated as E|E, E|NE, NE|NE). As the GLS framework allows for different spread in the data, individual data points are omitted to avoid misinterpretation.

Figure 7: Graphical representation of the effect of the three-way interaction term species identity × enrichment × species density on PO_4-P concentration for enrichment treatments where (a) no patches were enriched, NE|NE; (b) only a single patch was enriched, E|NE; or (c) both patches were enriched, E|E, within a mesocosm. Lines represent predicted values from the optimal regression model for each species: *Hediste diversicolor*; *Hd* (solid line); *Hydrobia ulvae*, *Hu* (dashed line); *Macoma balthica*, *Mb* (dotted line); and *Corophium volutator*, *Cv* (dot-dashed line). Species densities ranged from no macrofauna (0%) to natural density (100%) per mesocosm. As the GLS framework allows for different spread in the data, individual data points are omitted to avoid misinterpretation.

Electronic Supplementary Material

Summary of our statistical analysis. For each of our 3 models, we list the initial linear regression model and the minimal adequate linear regression model with GLS estimation, a comparison of the standardised residuals versus fitted values for the initial and minimal adequate models and a summary of the coefficient table.

For brevity in this document, we use the following abbreviations:

SpeciesID

1 = *Hediste diversicolor*

2 = *Hydrobia ulvae*

3 = *Macoma balthica*

4 = *Corophium volutator*

Enrichment

1 = E|E

2 = E|NE

3 = NE|NE

Interface (left-hand patch is focus patch)

1 = E|E

2 = E|NE

3 = NE|NE

4 = NE|E

Start_Dens_Diff = starting density difference

Model S1

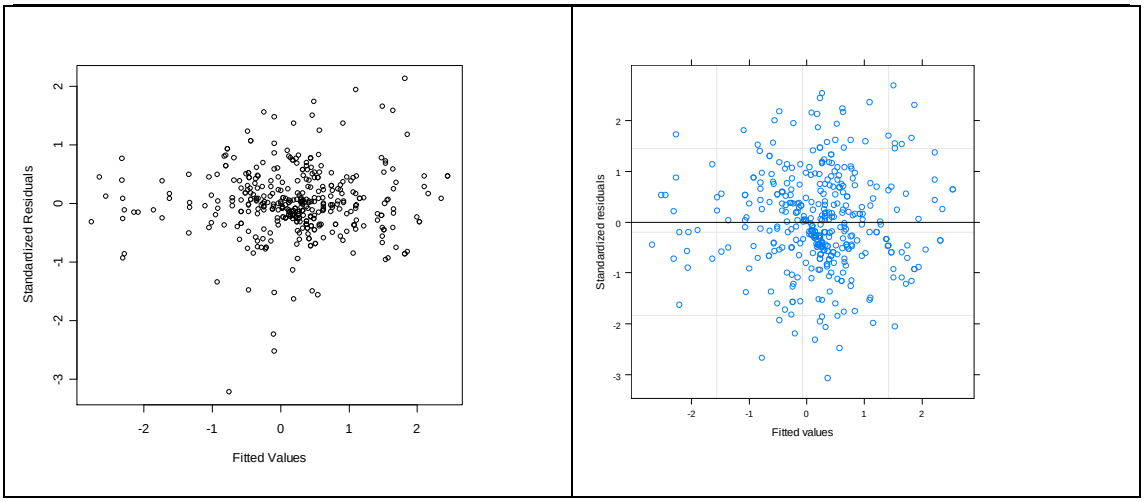
Initial linear regression model:

```
Movement  ~ as.factor(SpeciesID) + as.factor(Interface) + Density + Start_Dens_Diff +
as.factor(SpeciesID):as.factor(Interface) +
as.factor(SpeciesID):Density +
as.factor(SpeciesID):Start_Dens_Diff +
as.factor(Interface):Density +
as.factor(Interface):Start_Dens_Diff +
Density:Start_Dens_Diff +
as.factor(SpeciesID):as.factor(Interface):Density +
as.factor(Interface):Density:Start_Dens_Diff +
as.factor(SpeciesID):as.factor(Interface):Start_Dens_Diff +
as.factor(SpeciesID):Density:Start_Dens_Diff)
```

Minimal adequate model

```
Movement  ~ as.factor(SpeciesID) + as.factor(Interface) + Density + Start_Dens_Diff +
as.factor(SpeciesID):as.factor(Interface) +
as.factor(SpeciesID):Density +
as.factor(SpeciesID):Start_Dens_Diff +
as.factor(Interface):Density +
as.factor(Interface):Start_Dens_Diff +
Density:Start_Dens_Diff +
as.factor(SpeciesID):as.factor(Interface):Density +
as.factor(SpeciesID):as.factor(Interface):Start_Dens_Diff +
as.factor(SpeciesID):Density:Start_Dens_Diff,
weights = varComb(varExp(form = ~Density),
varIdent(form = ~1|as.factor(SpeciesID)*as.factor(Interface))) , method = "REML")
```

Initial linear regression	Minimal adequate model



Coefficient Table S1:

	Value	Std.Error	t-value	p-value
(Intercept)	0.040463	0.338441	0.119558	0.9049
as.factor(SpeciesID)2	0.064564	0.376527	0.171472	0.864
as.factor(SpeciesID)3	-0.04338	0.413835	-0.10482	0.9166
as.factor(SpeciesID)4	0.072709	0.401861	0.18093	0.8565
as.factor(Interface)2	-0.09606	0.444255	-0.21623	0.8289
as.factor(Interface)3	0.365748	0.410317	0.891379	0.3733
as.factor(Interface)4	-0.08495	0.403717	-0.21041	0.8335
Density	-0.13794	0.10948	-1.25993	0.2085
Start_Dens_Diff	0.269638	0.180167	1.496601	0.1354
as.factor(SpeciesID)2:as.factor(Interface)2	-0.1888	0.482863	-0.391	0.696
as.factor(SpeciesID)3:as.factor(Interface)2	0.337572	0.568588	0.593702	0.5531
as.factor(SpeciesID)4:as.factor(Interface)2	-0.00184	0.506924	-0.00362	0.9971
as.factor(SpeciesID)2:as.factor(Interface)3	-0.61942	0.443659	-1.39617	0.1636
as.factor(SpeciesID)3:as.factor(Interface)3	-0.1474	0.502068	-0.29358	0.7693
as.factor(SpeciesID)4:as.factor(Interface)3	-0.56472	0.48066	-1.17488	0.2409
as.factor(SpeciesID)2:as.factor(Interface)4	0.272844	0.449367	0.607174	0.5441
as.factor(SpeciesID)3:as.factor(Interface)4	-0.05167	0.501322	-0.10307	0.918
as.factor(SpeciesID)4:as.factor(Interface)4	0.022911	0.472833	0.048454	0.9614
as.factor(SpeciesID)2:Density	0.107597	0.121935	0.882406	0.3782
as.factor(SpeciesID)3:Density	0.204587	0.133684	1.530381	0.1268
as.factor(SpeciesID)4:Density	0.023961	0.130193	0.184045	0.8541
as.factor(SpeciesID)2:Start_Dens_Diff	0.130127	0.197203	0.659863	0.5098
as.factor(SpeciesID)3:Start_Dens_Diff	-0.24658	0.224684	-1.09745	0.2732
as.factor(SpeciesID)4:Start_Dens_Diff	0.161898	0.209167	0.774015	0.4395
as.factor(Interface)2:Density	0.044812	0.145659	0.307649	0.7585
as.factor(Interface)3:Density	0.127243	0.134249	0.947807	0.3439
as.factor(Interface)4:Density	0.229984	0.132311	1.738205	0.0831
as.factor(Interface)2:Start_Dens_Diff	-0.0311	0.156518	-0.19868	0.8426
as.factor(Interface)3:Start_Dens_Diff	-0.31935	0.178327	-1.79081	0.0742
as.factor(Interface)4:Start_Dens_Diff	-0.04167	0.151548	-0.27499	0.7835
Density:Start_Dens_Diff	0.081121	0.031668	2.561585	0.0108
as.factor(SpeciesID)2:as.factor(Interface)2:Density	-0.08869	0.157945	-0.5615	0.5748

as.factor(SpeciesID)3:as.factor(Interface)2:Density	-0.11316	0.188133	-0.6015	0.5479
as.factor(SpeciesID)4:as.factor(Interface)2:Density	0.282095	0.166225	1.697071	0.0906
as.factor(SpeciesID)2:as.factor(Interface)3:Density	-0.14926	0.145158	-1.02826	0.3045
as.factor(SpeciesID)3:as.factor(Interface)3:Density	-0.20879	0.164269	-1.27102	0.2046
as.factor(SpeciesID)4:as.factor(Interface)3:Density	0.074379	0.157264	0.472953	0.6365
as.factor(SpeciesID)2:as.factor(Interface)4:Density	-0.19014	0.147645	-1.28781	0.1987
as.factor(SpeciesID)3:as.factor(Interface)4:Density	-0.3322	0.163617	-2.03036	0.0431
as.factor(SpeciesID)4:as.factor(Interface)4:Density	-0.37877	0.155064	-2.44267	0.0151
as.factor(SpeciesID)2:as.factor(Interface)2:Start_Dens_Diff	0.154618	0.172939	0.894063	0.3719
as.factor(SpeciesID)3:as.factor(Interface)2:Start_Dens_Diff	0.123076	0.197952	0.621745	0.5345
as.factor(SpeciesID)4:as.factor(Interface)2:Start_Dens_Diff	-0.05942	0.183399	-0.324	0.7461
as.factor(SpeciesID)2:as.factor(Interface)3:Start_Dens_Diff	0.435122	0.192817	2.256658	0.0247
as.factor(SpeciesID)3:as.factor(Interface)3:Start_Dens_Diff	0.317727	0.218202	1.456114	0.1463
as.factor(SpeciesID)4:as.factor(Interface)3:Start_Dens_Diff	-0.05117	0.208898	-0.24495	0.8066
as.factor(SpeciesID)2:as.factor(Interface)4:Start_Dens_Diff	0.126637	0.169304	0.747987	0.455
as.factor(SpeciesID)3:as.factor(Interface)4:Start_Dens_Diff	0.094106	0.184792	0.509253	0.6109
as.factor(SpeciesID)4:as.factor(Interface)4:Start_Dens_Diff	-0.09499	0.179711	-0.52859	0.5974
as.factor(SpeciesID)2:Density:Start_Dens_Diff	-0.08427	0.033496	-2.51591	0.0123
as.factor(SpeciesID)3:Density:Start_Dens_Diff	-0.07692	0.040989	-1.8765	0.0614
as.factor(SpeciesID)4:Density:Start_Dens_Diff	-0.07346	0.035033	-2.0968	0.0367

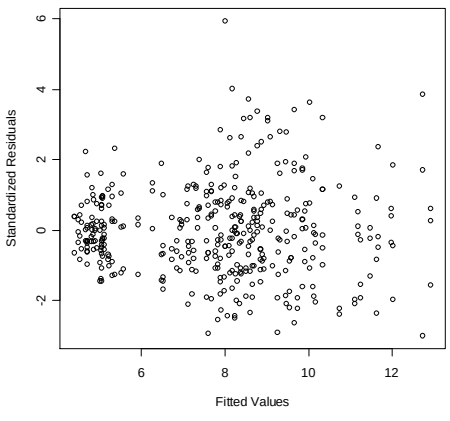
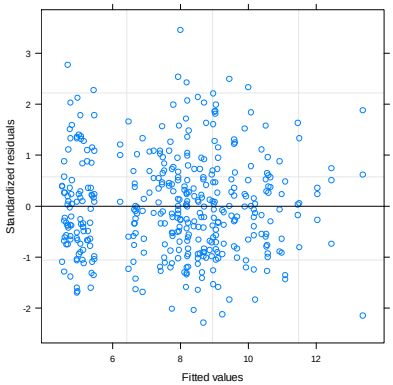
Model S2

Initial linear regression model:

NH4 ~ as.factor(SpeciesID) + as.factor(Enrichment) + Density + Start_Dens_Diff +
as.factor(SpeciesID):as.factor(Enrichment) +
as.factor(SpeciesID):Density +
as.factor(SpeciesID):Start_Dens_Diff +
as.factor(Enrichment):Density +
as.factor(Enrichment):Start_Dens_Diff +
Density:Start_Dens_Diff +
as.factor(SpeciesID):as.factor(Enrichment):Density +
as.factor(Enrichment):Density:Start_Dens_Diff +
as.factor(SpeciesID):as.factor(Enrichment):Start_Dens_Diff)

Minimal adequate model:

NH4 ~ as.factor(SpeciesID) + as.factor(Enrichment) + Density + Start_Dens_Diff +
as.factor(SpeciesID):as.factor(Enrichment) +
as.factor(SpeciesID):Density +
as.factor(SpeciesID):Start_Dens_Diff +
Density:Start_Dens_Diff +
as.factor(SpeciesID):Density:Start_Dens_Diff,
weights = varComb(varIdent(form = ~ 1|as.factor(SpeciesID)*as.factor(Enrichment)),varExp(form = ~Density)), method = "REML"

Initial linear regression	Minimal adequate model
	

Coefficient Table S2:

	Value	Std.Error	t-value	p-value
(Intercept)	6.395281	0.536699	11.91595	0
as.factor(SpeciesID)2	-0.19118	0.629505	-0.3037	0.7615
as.factor(SpeciesID)3	-1.18756	0.581496	-2.04225	0.0418
as.factor(SpeciesID)4	2.052751	0.727289	2.822468	0.005
as.factor(Enrichment)2	1.459579	0.452886	3.22284	0.0014
as.factor(Enrichment)3	2.386932	0.508158	4.697226	0
Density	0.5745	0.089302	6.433206	0
Start_Dens_Diff	0.359237	0.335623	1.070359	0.2852
as.factor(SpeciesID)2:as.factor(Enrichment)2	-0.18739	0.532506	-0.35191	0.7251
as.factor(SpeciesID)3:as.factor(Enrichment)2	-1.82945	0.489037	-3.74092	0.0002
as.factor(SpeciesID)4:as.factor(Enrichment)2	-2.95842	0.628217	-4.70923	0
as.factor(SpeciesID)2:as.factor(Enrichment)3	-2.63936	0.584629	-4.5146	0
as.factor(SpeciesID)3:as.factor(Enrichment)3	-2.06545	0.559877	-3.68912	0.0003
as.factor(SpeciesID)4:as.factor(Enrichment)3	-3.9499	0.646018	-6.11423	0
as.factor(SpeciesID)2:Density	-0.21159	0.10578	-2.00025	0.0462
as.factor(SpeciesID)3:Density	-0.60808	0.097826	-6.21591	0
as.factor(SpeciesID)4:Density	-0.31333	0.120066	-2.60966	0.0094
as.factor(SpeciesID)2:Start_Dens_Diff	-0.53092	0.400165	-1.32675	0.1854
as.factor(SpeciesID)3:Start_Dens_Diff	-0.53457	0.368129	-1.45213	0.1473
as.factor(SpeciesID)4:Start_Dens_Diff	-1.05769	0.459638	-2.30113	0.0219
Density:Start_Dens_Diff	-0.1207	0.075258	-1.60386	0.1096
as.factor(SpeciesID)2:Density:Start_Dens_Diff	0.1958	0.089592	2.185457	0.0295
as.factor(SpeciesID)3:Density:Start_Dens_Diff	0.160698	0.082522	1.947322	0.0522
as.factor(SpeciesID)4:Density:Start_Dens_Diff	0.28175	0.102603	2.746027	0.0063

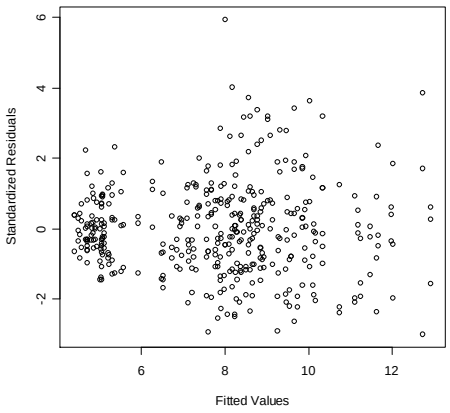
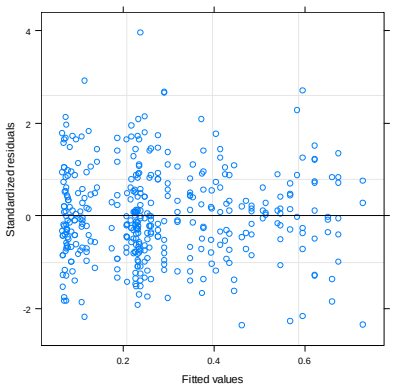
Model S3

Initial linear model:

PO4 ~ as.factor(SpeciesID) + as.factor(Enrichment) + Density + Start_Dens_Diff +
as.factor(SpeciesID):as.factor(Enrichment) +
as.factor(SpeciesID):Density +
as.factor(SpeciesID):Start_Dens_Diff +
as.factor(Enrichment):Density +
as.factor(Enrichment):Start_Dens_Diff +
Density:Start_Dens_Diff +
as.factor(SpeciesID):as.factor(Enrichment):Density +
as.factor(Enrichment):Density:Start_Dens_Diff +
as.factor(SpeciesID):as.factor(Enrichment):Start_Dens_Diff

Minimal adequate model:

PO4 ~ as.factor(SpeciesID) + as.factor(Enrichment) + Density +
as.factor(SpeciesID):as.factor(Enrichment) +
as.factor(SpeciesID):Density +
as.factor(Enrichment):Density +
as.factor(SpeciesID):as.factor(Enrichment):Density,
weights = varIdent(form = ~1|as.factor(Enrichment)* as.factor(SpeciesID)), method = "REML"

Initial linear regression	Minimal adequate model
	

Coefficient Table S3:

	Value	Std.Error	t-value	p-value
(Intercept)	0.70704	0.052307	13.51725	0
as.factor(SpeciesID)2	0.074777	0.08502	0.879517	0.3797
as.factor(SpeciesID)3	-0.25274	0.066773	-3.78499	0.0002
as.factor(SpeciesID)4	-0.00915	0.089606	-0.10214	0.9187
as.factor(Enrichment)2	-0.4384	0.055984	-7.83078	0
as.factor(Enrichment)3	-0.62876	0.052865	-11.8937	0
Density	-0.05578	0.011862	-4.70207	0
as.factor(SpeciesID)2:as.factor(Enrichment)2	-0.02298	0.088496	-0.25972	0.7952
as.factor(SpeciesID)3:as.factor(Enrichment)2	0.224883	0.074029	3.037758	0.0026
as.factor(SpeciesID)4:as.factor(Enrichment)2	0.146518	0.096668	1.515685	0.1304
as.factor(SpeciesID)2:as.factor(Enrichment)3	-0.08892	0.085568	-1.03914	0.2994
as.factor(SpeciesID)3:as.factor(Enrichment)3	0.254786	0.067901	3.752303	0.0002
as.factor(SpeciesID)4:as.factor(Enrichment)3	0.032256	0.091106	0.354051	0.7235
as.factor(SpeciesID)2:Density	0.002357	0.019281	0.122269	0.9028
as.factor(SpeciesID)3:Density	0.045813	0.015143	3.025443	0.0027
as.factor(SpeciesID)4:Density	0.01793	0.020321	0.882342	0.3782
as.factor(Enrichment)2:Density	0.045597	0.012774	3.569596	0.0004
as.factor(Enrichment)3:Density	0.059523	0.011989	4.964987	0
as.factor(SpeciesID)2:as.factor(Enrichment)2:Density	-0.01429	0.020143	-0.70917	0.4787
as.factor(SpeciesID)3:as.factor(Enrichment)2:Density	-0.03857	0.016939	-2.2772	0.0233
as.factor(SpeciesID)4:as.factor(Enrichment)2:Density	-0.03651	0.022071	-1.65442	0.0989
as.factor(SpeciesID)2:as.factor(Enrichment)3:Density	-0.00456	0.019405	-0.23507	0.8143
as.factor(SpeciesID)3:as.factor(Enrichment)3:Density	-0.05074	0.015399	-3.29484	0.0011
as.factor(SpeciesID)4:as.factor(Enrichment)3:Density	-0.01713	0.020661	-0.82888	0.4077

End of Supplementary Material

Chapter 3

- Model 1; Fo^{15} (whole mesocosm) ~ *f* (algae, species identity, species density, starting density differential)

A = Algae (1 = E|E, 2 = E|NE, 3 = NE|NE, 4 = NE|E)

S = Species ID (1 = Hd, 2 = Hu, 3 = Mb, 4 = Cv)

N = Standardised biomass (continuous)

Best model

```
tmp.glsopt6a<- gls(FMS_6_Fo ~ as.factor(S) + as.factor(A) + N +
as.factor(S):as.factor(A) +
as.factor(S):N +
as.factor(A):N,
weights = varComb(varIdent(form = ~ 1|as.factor(S) * as.factor(A)),varPower(form =
~N)), method = "REML")
```

```
Coefficients:
                              Value Std.Error   t-value p-value
(Intercept)                348.6657 27.483127 12.686536  0.0000
as.factor(S)2              -79.9580 30.700678 -2.604436  0.0096
as.factor(S)3              -94.2908 30.994603 -3.042167  0.0025
as.factor(S)4             -198.1663 28.349524 -6.990109  0.0000
as.factor(A)2              -67.3634 25.876980 -2.603215  0.0096
as.factor(A)3             -100.6656 28.108440 -3.581331  0.0004
N                          -17.6753  4.757884 -3.714954  0.0002
as.factor(S)2:as.factor(A)2  -11.6010 25.742160 -0.450662  0.6525
as.factor(S)3:as.factor(A)2   21.0739 27.245799  0.773472  0.4397
as.factor(S)4:as.factor(A)2   37.4235 22.547214  1.659786  0.0978
as.factor(S)2:as.factor(A)3  -30.1172 27.335121 -1.101778  0.2713
as.factor(S)3:as.factor(A)3  -46.0993 27.139622 -1.698598  0.0902
as.factor(S)4:as.factor(A)3   16.9331 25.646265  0.660256  0.5095
as.factor(S)2:N               -4.8231  4.818832 -1.000894  0.3175
as.factor(S)3:N                9.6385  5.005946  1.925408  0.0549
as.factor(S)4:N                3.7875  4.513661  0.839113  0.4019
as.factor(A)2:N                2.1562  3.519397  0.612673  0.5405
as.factor(A)3:N                9.2238  3.200051  2.882405  0.0042
```

Importance of single factors

```
> anova (tmp.glsopt6a, tmp.glsoptS)
             Model df      AIC      BIC    logLik   Test  L.Ratio p-value
tmp.glsopt6a     1 31 4341.970 4465.394 -2139.985
```

```
tmp.glsoptS      2 19 4609.589 4685.236 -2285.794 1 vs 2 291.6189  <.0001
> anova (tmp.glsopt6a, tmp.glsoptA)
             Model df      AIC      BIC    logLik   Test  L.Ratio p-value
tmp.glsopt6a     1 31 4341.970 4465.394 -2139.985
tmp.glsoptA      2 21 4443.281 4526.891 -2200.641 1 vs 2 121.3115  <.0001
> anova (tmp.glsopt6a, tmp.glsoptN)
             Model df      AIC      BIC    logLik   Test  L.Ratio p-value
tmp.glsopt6a     1 31 4341.970 4465.394 -2139.985
tmp.glsoptN      2 25 4437.034 4536.570 -2193.517 1 vs 2 107.0646  <.0001
```

- Model 2; Fo^{15} ($patch^{-1}$) ~ *f* (interface, species identity, species density, starting density differential)

Best model

```
tmp.gls800REML<- gls(Fo15_6 ~ as.factor(SpeciesID) + as.factor(Interface) + Number_all
+ LRDiffstart_all +
as.factor(SpeciesID):as.factor(Interface) +
as.factor(SpeciesID):Number_all +
as.factor(Interface):Number_all +
Number_all:LRDiffstart_all,
weights = varComb(varPower(form =~Number_all),varIdent(form = ~1|as.factor(SpeciesID)
* as.factor(Interface))),
method ="REML")
```

```
Coefficients:
                                              Value Std.Error   t-value p-value
(Intercept)                                359.0073 22.021607 16.302502  0.0000
as.factor(SpeciesID)2                      -87.6371 23.764245 -3.687770  0.0002
as.factor(SpeciesID)3                     -112.7696 24.789995 -4.548995  0.0000
as.factor(SpeciesID)4                     -212.4055 22.272940 -9.536483  0.0000
as.factor(Interface)2                      -43.6318 26.649791 -1.637229  0.1020
as.factor(Interface)3                     -105.1602 21.570116 -4.875271  0.0000
as.factor(Interface)4                     -101.4157 22.963347 -4.416416  0.0000
Number_all                                 -17.9982  3.779748 -4.761744  0.0000
LRDiffstart_all                              1.3648  3.511622  0.388665  0.6976
as.factor(SpeciesID)2:as.factor(Interface)2  -1.6308 25.544093 -0.063842  0.9491
as.factor(SpeciesID)3:as.factor(Interface)2  70.1867 29.669230  2.365641  0.0182
as.factor(SpeciesID)4:as.factor(Interface)2  56.1037 23.289566  2.408961  0.0162
as.factor(SpeciesID)2:as.factor(Interface)3 -27.3638 20.699348 -1.321962  0.1866
as.factor(SpeciesID)3:as.factor(Interface)3 -37.4958 21.281527 -1.761893  0.0785
as.factor(SpeciesID)4:as.factor(Interface)3  25.9748 19.637033  1.322746  0.1863
as.factor(SpeciesID)2:as.factor(Interface)4  -7.3996 23.055331 -0.320950  0.7483
as.factor(SpeciesID)3:as.factor(Interface)4  -6.0155 23.953122 -0.251138  0.8018
as.factor(SpeciesID)4:as.factor(Interface)4  43.6463 19.952226  2.187539  0.0290
as.factor(SpeciesID)2:Number_all             -3.8187  3.668744 -1.040871  0.2983
as.factor(SpeciesID)3:Number_all             11.3951  3.899834  2.921943  0.0036
as.factor(SpeciesID)4:Number_all              4.7885  3.449678  1.388093  0.1655
as.factor(Interface)2:Number_all             -2.3314  3.579597 -0.651295  0.5151
as.factor(Interface)3:Number_all              8.5951  2.612057  3.290541  0.0010
as.factor(Interface)4:Number_all              4.3813  3.151965  1.390033  0.1649
Number_all:LRDiffstart_all                   -0.3263  0.750313 -0.434858  0.6638
```

Significance of 2-way interaction terms

```
> anova(tmp.gls800, tmp.gls801)
           Model df      AIC      BIC    logLik   Test  L.Ratio p-value
tmp.gls800     1 42 8842.478 9038.809 -4379.239
tmp.gls801     2 33 8863.655 9017.915 -4398.827 1 vs 2 39.17714  <.0001
> anova(tmp.gls800, tmp.gls802)
           Model df      AIC      BIC    logLik   Test  L.Ratio p-value
tmp.gls800     1 42 8842.478 9038.809 -4379.239
tmp.gls802     2 39 8874.604 9056.911 -4398.302 1 vs 2 38.12599  <.0001
> anova(tmp.gls800, tmp.gls803)
           Model df      AIC      BIC    logLik   Test  L.Ratio p-value
tmp.gls800     1 42 8842.478 9038.809 -4379.239
tmp.gls803     2 39 8860.623 9042.930 -4391.311 1 vs 2 24.14505  <.0001
> anova(tmp.gls800, tmp.gls804)
           Model df      AIC      BIC    logLik   Test  L.Ratio p-value
tmp.gls800     1 42 8842.478 9038.809 -4379.239
tmp.gls804     2 41 8844.898 9036.555 -4381.449 1 vs 2 4.420354  0.0355
```

- Model 3; Movement ~ *f* (species identity, interface, species density, starting density differential)

Best model

```
tmp.gls700     <-     gls(Unitchange_coded           ~     as.factor(SpeciesID)     +
as.factor(Interface_coded) + Number_all + UnitStdiff_coded +
as.factor(SpeciesID):as.factor(Interface_coded) +
as.factor(SpeciesID):Number_all +
as.factor(SpeciesID):UnitStdiff_coded +
as.factor(Interface_coded):Number_all +
as.factor(Interface_coded):UnitStdiff_coded +
Number_all:UnitStdiff_coded +
as.factor(SpeciesID):as.factor(Interface_coded):Number_all +
as.factor(SpeciesID):as.factor(Interface_coded):UnitStdiff_coded,
weights = varComb(varExp(form = ~Number_all),
varIdent(form = ~1|as.factor(SpeciesID)*as.factor(Interface_coded))) , method = "ML")
```

```
Coefficients:
                                     Value Std.Error   t-value p-value
(Intercept)                      -0.2458475 0.3133891 -0.784480  0.4333
as.factor(S)2                     0.3850453 0.3505429  1.098426  0.2728
as.factor(S)3                     0.2506099 0.3800973  0.659331  0.5101
as.factor(S)4                     0.3506621 0.3755359  0.933765  0.3511
as.factor(In)2                    0.1858225 0.4423433  0.420087  0.6747
as.factor(In)3                    0.3633423 0.4140486  0.877535  0.3808
as.factor(In)4                    0.1993405 0.3854438  0.517171  0.6054
```

N	-0.0530280	0.1019907	-0.519930	0.6034
Undif	0.5469057	0.1384117	3.951296	0.0001
as.factor(S)2:as.factor(In)2	-0.5084893	0.4780982	-1.063567	0.2883
as.factor(S)3:as.factor(In)2	0.0525682	0.5592413	0.093999	0.9252
as.factor(S)4:as.factor(In)2	-0.2750736	0.5006923	-0.549387	0.5831
as.factor(S)2:as.factor(In)3	-0.6175246	0.4472405	-1.380744	0.1682
as.factor(S)3:as.factor(In)3	-0.1479300	0.5051942	-0.292818	0.7698
as.factor(S)4:as.factor(In)3	-0.5623253	0.4839486	-1.161952	0.2461
as.factor(S)2:as.factor(In)4	-0.0468253	0.4302526	-0.108832	0.9134
as.factor(S)3:as.factor(In)4	-0.3438713	0.4771109	-0.720737	0.4716
as.factor(S)4:as.factor(In)4	-0.2527740	0.4525368	-0.558571	0.5768
as.factor(S)2:N	0.0125218	0.1141790	0.109668	0.9127
as.factor(S)3:N	0.1171571	0.1238055	0.946299	0.3447
as.factor(S)4:N	-0.0580682	0.1223198	-0.474725	0.6353
as.factor(S)2:Undif	-0.1799011	0.1519164	-1.184211	0.2371
as.factor(S)3:Undif	-0.5293655	0.1647245	-3.213641	0.0014
as.factor(S)4:Undif	-0.1089656	0.1627477	-0.669537	0.5036
as.factor(In)2:N	-0.0376035	0.1456850	-0.258115	0.7965
as.factor(In)3:N	0.1277674	0.1348642	0.947379	0.3441
as.factor(In)4:N	0.1471244	0.1270014	1.158447	0.2475
as.factor(In)2:Undif	-0.0377208	0.1574450	-0.239581	0.8108
as.factor(In)3:Undif	-0.3185512	0.1794381	-1.775270	0.0767
as.factor(In)4:Undif	-0.0583953	0.1497083	-0.390060	0.6967
N:Undif	0.0057552	0.0080956	0.710902	0.4776
as.factor(S)2:as.factor(In)2:N	0.0044218	0.1571357	0.028140	0.9776
as.factor(S)3:as.factor(In)2:N	-0.0300077	0.1858775	-0.161438	0.8718
as.factor(S)4:as.factor(In)2:N	0.3614169	0.1647581	2.193621	0.0289
as.factor(S)2:as.factor(In)3:N	-0.1495500	0.1456755	-1.026597	0.3053
as.factor(S)3:as.factor(In)3:N	-0.2083651	0.1645522	-1.266256	0.2063
as.factor(S)4:as.factor(In)3:N	0.0737746	0.1576320	0.468018	0.6401
as.factor(S)2:as.factor(In)4:N	-0.0972491	0.1421517	-0.684122	0.4944
as.factor(S)3:as.factor(In)4:N	-0.2467065	0.1565888	-1.575505	0.1161
as.factor(S)4:as.factor(In)4:N	-0.2989648	0.1490197	-2.006210	0.0456
as.factor(S)2:as.factor(In)2:Undif	0.1626294	0.1737614	0.935935	0.3500
as.factor(S)3:as.factor(In)2:Undif	0.1298385	0.1985297	0.654001	0.5135
as.factor(S)4:as.factor(In)2:Undif	-0.0527504	0.1840061	-0.286678	0.7745
as.factor(S)2:as.factor(In)3:Undif	0.4342399	0.1938227	2.240397	0.0257
as.factor(S)3:as.factor(In)3:Undif	0.3172810	0.2189383	1.449180	0.1482
as.factor(S)4:as.factor(In)3:Undif	-0.0517955	0.2097311	-0.246961	0.8051
as.factor(S)2:as.factor(In)4:Undif	0.1447135	0.1677019	0.862921	0.3888
as.factor(S)3:as.factor(In)4:Undif	0.1106364	0.1831439	0.604095	0.5462
as.factor(S)4:as.factor(In)4:Undif	-0.0776978	0.1780320	-0.436426	0.6628

Chapter 4

- Model 1; $Fo^{15} \sim f$ (algae, species identity, species density, flow, run)

SpID = Species Identity (1 = Hd, 2 = Hu, 3 = Cv)

Algae (1 = E|E, 2 =E|NE, 3 = NE|NE)

Density (0 = 0, 1 = low, 4 = high)

Flow (0 = Off, 1 = On)

```
Best model

tmp.mixed400REML<-lme(FMS_D6 ~ as.factor(SpID) + as.factor(Algae) + as.factor(Density)
+ as.factor(Flow) +
as.factor(SpID):as.factor(Density) +
as.factor(SpID):as.factor(Flow) +
as.factor(Algae):as.factor(Density) ,
weights = varIdent(form = ~ 1|as.factor(Run)),
random=~1|factor(Run), method = "REML")

Coefficients:
                                       Value Std.Error DF   t-value p-value
(Intercept)                        156.94122  37.55465 86  4.179009  0.0001
as.factor(SpID)2                   -24.53989   3.88780 86 -6.312028  0.0000
as.factor(SpID)3                   -19.75443   3.24507 86 -6.087521  0.0000
as.factor(Algae)2                   -3.29290   3.09365 86 -1.064405  0.2901
as.factor(Algae)3                    1.07361   2.18279 86  0.491850  0.6241
as.factor(Density)4                -20.19321   3.47585 86 -5.809582  0.0000
as.factor(Flow)1                     5.73688   7.51261 86  0.763634  0.4472
as.factor(SpID)2:as.factor(Density)4  19.29128   4.97817 86  3.875175  0.0002
as.factor(SpID)3:as.factor(Density)4  16.71702   4.26928 86  3.915657  0.0002
as.factor(SpID)2:as.factor(Flow)1     -2.74783   7.97776 86 -0.344436  0.7314
as.factor(SpID)3:as.factor(Flow)1    -16.07351   8.53882 86 -1.882404  0.0632
as.factor(Algae)2:as.factor(Density)4  -0.53550   4.66708 86 -0.114739  0.9089
as.factor(Algae)3:as.factor(Density)4  -2.04829   3.58851 86 -0.570791  0.5696


importance of single factors


> anova (tmp.mixed400, tmp.mixed400Sp)
               Model df      AIC      BIC    logLik   Test L.Ratio p-value
tmp.mixed400       1 24 1098.062 1162.433 -525.0311
tmp.mixed400Sp     2 18 1138.190 1186.469 -551.0953 1 vs 2 52.1284  <.0001
```

```
> anova (tmp.mixed400, tmp.mixed400A)
              Model df      AIC      BIC    logLik   Test  L.Ratio p-value
tmp.mixed400      1 24 1098.062 1162.433 -525.0311
tmp.mixed400A     2 20 1110.201 1163.843 -535.1003 1 vs 2 20.13845   5e-04
> anova (tmp.mixed400, tmp.mixed400D)
              Model df      AIC      BIC    logLik   Test  L.Ratio p-value
tmp.mixed400      1 24 1098.062 1162.433 -525.0311
tmp.mixed400D     2 19 1134.905 1185.865 -548.4524 1 vs 2 46.84272  <.0001
> anova (tmp.mixed400, tmp.mixed400F)
              Model df      AIC      BIC    logLik   Test  L.Ratio p-value
tmp.mixed400      1 24 1098.062 1162.433 -525.0311
tmp.mixed400F     2 21 1112.387 1168.711 -535.1934 1 vs 2 20.32467   1e-04
```

- Model 2; Movement ~ *f* (interface, species identity, species density, flow, run)

Best model

```
tmp.lme800 <- lme(Movement ~ as.factor(SpID) + as.factor(Algae) + as.factor(Density) +
as.factor(SpID):as.factor(Algae) +
as.factor(SpID):as.factor(Density) +
as.factor(Algae):as.factor(Density) +
as.factor(SpID):as.factor(Algae):as.factor(Density),
weights = varIdent(form = ~ 1|as.factor(Density)),
random=~1|factor(Run), method = "ML")
```

Coefficients:

	Value	Std.Error	DF	t-value	p-value
(Intercept)	0.3559595	0.1321054	81	2.6945108	0.0086
as.factor(SpID)Hydrobia	0.1317059	0.1686173	81	0.7810937	0.4370
as.factor(SpID)Nereis	0.0643024	0.1630227	81	0.3944381	0.6943
as.factor(Algae)2	0.0449593	0.1660007	81	0.2708381	0.7872
as.factor(Algae)3	0.0435234	0.1674245	81	0.2599582	0.7956
as.factor(Density)4	0.8432691	0.2766163	81	3.0485153	0.0031
as.factor(SpID)Hydrobia:(Algae)2	0.0514136	0.2472362	81	0.2079533	0.8358
as.factor(SpID)Nereis:(Algae)2	-0.3061338	0.2309148	81	-1.3257435	0.1887
as.factor(SpID)Hydrobia:(Algae)3	-0.1232405	0.2355054	81	-0.5233020	0.6022
as.factor(SpID)Nereis:Algae)3	-0.0836378	0.2371234	81	-0.3527186	0.7252
as.factor(SpID)Hydrobia:(Density)4	0.7289325	0.3937410	81	1.8512993	0.0678
as.factor(SpID)Nereis:(Density)4	0.4808739	0.4046257	81	1.1884413	0.2381
(Algae)2:(Density)4	-0.3542422	0.3888870	81	-0.9109130	0.3650
(Algae)3:as.factor(Density)4	0.2280151	0.3901088	81	0.5844911	0.5605
as.factor(SpID)Hydrobia:(Algae)2:(Density)4	0.6839412	0.5525433	81	1.2378056	0.2194
as.factor(SpID)Nereis:(Algae)2:(Density)4	1.2231569	0.5609555	81	2.1804883	0.0321
as.factor(SpID)Hydrobia:(Algae)3:(Density)4	-0.1467421	0.5531295	81	-0.2652943	0.7915
as.factor(SpID)Nereis:(Algae)3:(Density)4	-0.4463369	0.5579022	81	-0.8000272	0.4260

Importance of individual factors

```
> anova (tmp.lme800, tmp.lme800Sp)
             Model df      AIC      BIC    logLik   Test  L.Ratio p-value
tmp.lme800       1 21 151.7137 208.0384 -54.85682
tmp.lme800Sp     2  9 166.6305 190.7697 -74.31528 1 vs 2 38.91691   1e-04
> anova (tmp.lme800, tmp.lme800Al)
             Model df      AIC      BIC    logLik   Test  L.Ratio p-value
tmp.lme800       1 21 151.7137 208.0384 -54.85682
tmp.lme800Al     2  9 144.7331 168.8723 -63.36654 1 vs 2 17.01943  0.1489
> anova (tmp.lme800, tmp.lme800De)
             Model df      AIC      BIC     logLik   Test  L.Ratio p-value
tmp.lme800       1 21 151.7137 208.0384  -54.85682
tmp.lme800De     2 12 243.3017 275.4873 -109.65087 1 vs 2 109.5881  <.0001
```

Chapter 5

- Model 1; $Fo^{15} \sim f$ (algae, BS *Hediste diversicolor*, BS *Hydrobia ulvae*, BS *Macoma balthica*, BS *Corophium volutator*)

N_BS = Biomass standardised for Hd

H_BS = Biomass standardised for Hu

M_BS = Biomass standardised for Mb

C_BS = Biomass standardised for Cv

Algae (1 = E|E, 2 = E|NE, 3 = NE|NE)

Interface (1 = E|E, 2 = E|NE, 3 = NE|E, 4 = NE|NE)

```
Best model

tmp.gls800<- gls(FMS ~ as.factor(Algae) + N_BS + H_BS + M_BS + C_BS +
as.factor(Algae):N_BS +
as.factor(Algae):H_BS +
as.factor(Algae):M_BS +
as.factor(Algae):C_BS +
N_BS:H_BS +
N_BS:M_BS +
N_BS:C_BS +
H_BS:M_BS +
H_BS:C_BS +
M_BS:C_BS +
as.factor(Algae):M_BS:C_BS +
N_BS:H_BS:M_BS +
N_BS:C_BS:M_BS,
weights = varComb(varExp(form = ~ C_BS), varExp(form = ~ H_BS)), method  = "REML")

Coefficients:
                              Value Std.Error   t-value p-value
(Intercept)                12.284655  43.38017  0.283186  0.7773
as.factor(Algae)2          11.704077  51.65715  0.226572  0.8210
as.factor(Algae)3          21.181527  59.64854  0.355106  0.7228
N_BS                        0.049931   0.05720  0.872862  0.3836
H_BS                        0.016380   0.05790  0.282909  0.7775
M_BS                        0.054413   0.05791  0.939586  0.3484
C_BS                        0.041001   0.05874  0.697996  0.4859
as.factor(Algae)2:N_BS     -0.023546   0.06635 -0.354856  0.7230
as.factor(Algae)3:N_BS     -0.024428   0.07662 -0.318826  0.7501
as.factor(Algae)2:H_BS     -0.012077   0.06585 -0.183398  0.8546
as.factor(Algae)3:H_BS     -0.014826   0.07604 -0.194983  0.8456
as.factor(Algae)2:M_BS     -0.033499   0.06757 -0.495756  0.6205
as.factor(Algae)3:M_BS     -0.079121   0.07802 -1.014049  0.3116
```

```
as.factor(Algae)2:C_BS      -0.004628   0.06579 -0.070340  0.9440
as.factor(Algae)3:C_BS      -0.025986   0.07597 -0.342066  0.7326
N_BS:H_BS                    0.000019   0.00005  0.374283  0.7085
N_BS:M_BS                    0.000388   0.00010  4.027511  0.0001
N_BS:C_BS                   -0.000120   0.00006 -2.121826  0.0349
H_BS:M_BS                    0.000018   0.00005  0.351226  0.7257
H_BS:C_BS                   -0.000100   0.00006 -1.707784  0.0890
M_BS:C_BS                   -0.000073   0.00008 -0.936704  0.3499
as.factor(Algae)2:M_BS:C_BS -0.000054   0.00008 -0.704582  0.4818
as.factor(Algae)3:M_BS:C_BS  0.000155   0.00009  1.765335  0.0788
N_BS:H_BS:M_BS              -0.000001   0.00000 -2.839106  0.0049
N_BS:M_BS:C_BS              -0.000001   0.00000 -3.885717  0.0001


Single factors

> anova (tmp.gls800, tmp.gls800A)
            Model df      AIC      BIC    logLik   Test  L.Ratio p-value
tmp.gls800      1 28 2090.078 2190.205 -1017.039
tmp.gls800A     2 16 2091.250 2148.465 -1029.625 1 vs 2 25.17167   0.014
> anova (tmp.gls800, tmp.gls800N)
            Model df      AIC      BIC    logLik   Test  L.Ratio p-value
tmp.gls800      1 28 2090.078 2190.205 -1017.039
tmp.gls800N     2 20 2111.448 2182.967 -1035.724 1 vs 2 37.36964  <.0001
> anova (tmp.gls800, tmp.gls800H)
            Model df      AIC      BIC    logLik   Test  L.Ratio p-value
tmp.gls800      1 28 2090.078 2190.205 -1017.039
tmp.gls800H     2 21 2085.181 2160.276 -1021.590 1 vs 2 9.102392  0.2454
> anova (tmp.gls800, tmp.gls800M)
            Model df      AIC      BIC    logLik   Test  L.Ratio p-value
tmp.gls800      1 28 2090.078 2190.205 -1017.039
tmp.gls800M     2 18 2106.657 2171.024 -1035.328 1 vs 2 36.57841   1e-04
> anova (tmp.gls800, tmp.gls800C)
            Model df      AIC      BIC    logLik   Test L.Ratio p-value
tmp.gls800      1 28 2090.078 2190.205 -1017.039
tmp.gls800C     2 19 2109.808 2177.751 -1035.904 1 vs 2 37.7298  <.0001
```

- Model 2; $Fo^{15} \sim f$ (interface, BS *Hediste diversicolor*, BS *Hydrobia ulvae*, BS *Macoma balthica*, BS *Corophium volutator*)

```
Best model

tmp.gls600REML<-gls(FMS ~ as.factor(Interface) + N_BS + H_BS + M_BS + C_BS +
as.factor(Interface):N_BS +
as.factor(Interface):H_BS +
as.factor(Interface):M_BS +
as.factor(Interface):C_BS +
```

```
N_BS:H_BS +
N_BS:M_BS +
N_BS:C_BS +
H_BS:M_BS +
H_BS:C_BS +
M_BS:C_BS +
as.factor(Interface):N_BS:C_BS +
as.factor(Interface):H_BS:C_BS +
as.factor(Interface):M_BS:C_BS +
N_BS:H_BS:M_BS +
N_BS:C_BS:M_BS,
weights = varComb(varExp(form = ~ C_BS), varExp(form = ~ H_BS)), method  = "REML" )
```

Coefficients:

	Value	Std.Error	t-value	p-value
(Intercept)	14.832030	49.18690	0.301544	0.7633
as.factor(Interface)2	12.436873	68.91048	0.180479	0.8569
as.factor(Interface)3	3.473108	68.91048	0.050400	0.9598
as.factor(Interface)4	19.900267	68.91048	0.288784	0.7730
N_BS	0.055173	0.06545	0.842954	0.4001
H_BS	0.000350	0.06734	0.005199	0.9959
M_BS	0.043886	0.06545	0.670505	0.5032
C_BS	0.036554	0.07654	0.477606	0.6334
as.factor(Interface)2:N_BS	-0.055742	0.08920	-0.624933	0.5326
as.factor(Interface)3:N_BS	0.022411	0.08920	0.251254	0.8018
as.factor(Interface)4:N_BS	-0.016902	0.08920	-0.189487	0.8499
as.factor(Interface)2:H_BS	-0.016930	0.09304	-0.181953	0.8558
as.factor(Interface)3:H_BS	0.063044	0.09304	0.677565	0.4987
as.factor(Interface)4:H_BS	0.017802	0.09304	0.191327	0.8484
as.factor(Interface)2:M_BS	-0.067148	0.08920	-0.752805	0.4523
as.factor(Interface)3:M_BS	-0.013525	0.08920	-0.151626	0.8796
as.factor(Interface)4:M_BS	-0.094570	0.08920	-1.060245	0.2902
as.factor(Interface)2:C_BS	-0.048432	0.10742	-0.450879	0.6525
as.factor(Interface)3:C_BS	0.093674	0.10742	0.872060	0.3841
as.factor(Interface)4:C_BS	-0.015023	0.10742	-0.139860	0.8889
N_BS:H_BS	-0.000005	0.00005	-0.094007	0.9252
N_BS:M_BS	0.000321	0.00009	3.463303	0.0006
N_BS:C_BS	-0.000151	0.00010	-1.451015	0.1482
H_BS:M_BS	0.000024	0.00005	0.446793	0.6555
H_BS:C_BS	-0.000065	0.00010	-0.643983	0.5202
M_BS:C_BS	-0.000102	0.00010	-0.978193	0.3290
as.factor(Interface)2:N_BS:C_BS	0.000155	0.00013	1.188055	0.2361
as.factor(Interface)3:N_BS:C_BS	-0.000297	0.00013	-2.273453	0.0239
as.factor(Interface)4:N_BS:C_BS	-0.000026	0.00013	-0.201000	0.8409
as.factor(Interface)2:H_BS:C_BS	0.000106	0.00013	0.795147	0.4274
as.factor(Interface)3:H_BS:C_BS	-0.000375	0.00013	-2.796737	0.0056
as.factor(Interface)4:H_BS:C_BS	-0.000112	0.00013	-0.836940	0.4035
as.factor(Interface)2:M_BS:C_BS	0.000131	0.00013	1.006009	0.3155
as.factor(Interface)3:M_BS:C_BS	-0.000229	0.00013	-1.755542	0.0805
as.factor(Interface)4:M_BS:C_BS	0.000194	0.00013	1.483412	0.1394

```
N_BS:H_BS:M_BS                  -0.000001   0.00000 -2.119623  0.0351
N_BS:M_BS:C_BS                  -0.000001   0.00000 -3.449278  0.0007


Single factors

> anova (tmp.gls600, tmp.gls600In)
             Model df      AIC      BIC    logLik   Test  L.Ratio p-value
tmp.gls600       1 40 2109.813 2252.851 -1014.906
tmp.gls600In     2 16 2166.414 2223.629 -1067.207 1 vs 2 104.6012  <.0001
> anova (tmp.gls600, tmp.gls600N)
            Model df      AIC      BIC    logLik   Test  L.Ratio p-value
tmp.gls600      1 40 2109.813 2252.851 -1014.906
tmp.gls600N     2 28 2148.919 2249.046 -1046.460 1 vs 2 63.10692  <.0001
> anova (tmp.gls600, tmp.gls600H)
            Model df      AIC      BIC    logLik   Test  L.Ratio p-value
tmp.gls600      1 40 2109.813 2252.851 -1014.906
tmp.gls600H     2 30 2117.259 2224.537 -1028.629 1 vs 2 27.44625  0.0022
> anova (tmp.gls600, tmp.gls600M)
            Model df      AIC      BIC    logLik   Test  L.Ratio p-value
tmp.gls600      1 40 2109.813 2252.851 -1014.906
tmp.gls600M     2 28 2127.850 2227.977 -1035.925 1 vs 2 42.03768  <.0001
> anova (tmp.gls600, tmp.gls600C)
            Model df      AIC      BIC    logLik   Test  L.Ratio p-value
tmp.gls600      1 40 2109.813 2252.851 -1014.906
tmp.gls600C     2 23 2140.715 2222.962 -1047.357 1 vs 2 64.90228  <.0001
```

- Model 3; *Hediste diversicolor* Movement ~ *f* (interface, BS *Hydrobia ulvae*, BS *Macoma balthica*, BS *Corophium volutator*)

```
Best model

tmp.gls_best <- gls(N_R ~ H_BS + M_BS + C_BS +
H_BS:M_BS,
weights = varExp(form = ~N_BS), method = "REML")


> anova(tmp.gls1100, tmp.gls1101)
            Model df      AIC      BIC    logLik   Test  L.Ratio p-value
tmp.gls1100     1  7 2154.867 2177.633 -1070.434
tmp.gls1101     2  6 2223.835 2243.349 -1105.918 1 vs 2 70.96786  <.0001
> anova(tmp.gls1100, tmp.gls1102)
            Model df      AIC      BIC    logLik   Test  L.Ratio p-value
tmp.gls1100     1  7 2154.867 2177.633 -1070.434
tmp.gls1102     2  6 2160.782 2180.296 -1074.391 1 vs 2 7.914624  0.0049


Coefficients:
               Value Std.Error   t-value p-value
```

```
(Intercept) 354.6262 27.116083 13.078076  0.0000
H_BS         -0.3376  0.064090 -5.267288  0.0000
M_BS         -0.3858  0.064363 -5.994171  0.0000
C_BS         -0.4623  0.050526 -9.149248  0.0000
H_BS:M_BS    -0.0007  0.000267 -2.808197  0.0055


# - drop H_BS
tmp.gls1100_H <- gls(N_R ~ M_BS + C_BS,
weights = varExp(form = ~N_BS), method = "ML")

# - drop M_BS
tmp.gls1100_M <- gls(N_R ~ H_BS + C_BS,
weights = varExp(form = ~N_BS), method = "ML")

# - drop C_BS
tmp.gls1100_C <- gls(N_R ~ H_BS + M_BS +
H_BS:M_BS,
weights = varExp(form = ~N_BS), method = "ML")
```

- Model 4; *Hydrobia ulvae* Movement ~ *ƒ* (interface, BS *Hediste diversicolor*, BS *Macoma balthica*, BS *Corophium volutator*)

```
Best model

tmp.gls_best <- gls(H_R ~ as.factor(Interface) + N_BS + M_BS + C_BS +
as.factor(Interface): N_BS +
as.factor(Interface): M_BS +
as.factor(Interface): C_BS +
C_BS:N_BS +
C_BS:M_BS +
N_BS:M_BS +
N_BS:M_BS:C_BS +
C_BS:as.factor(Interface):N_BS,
weights = varExp(form = ~fitted(.)), method = "REML")


Coefficients:
                                  Value Std.Error   t-value p-value
(Intercept)                    376.9331  33.29885 11.319704  0.0000
as.factor(Interface)2         -108.3066  35.04826 -3.090213  0.0023
as.factor(Interface)3           42.5759  44.91856  0.947847  0.3446
as.factor(Interface)4          -22.5955  39.25592 -0.575596  0.5657
N_BS                            -0.4167   0.08278 -5.033313  0.0000
M_BS                            -0.4209   0.07560 -5.568120  0.0000
C_BS                            -0.4518   0.08214 -5.499964  0.0000
as.factor(Interface)2:N_BS       0.1055   0.07704  1.369028  0.1728
as.factor(Interface)3:N_BS      -0.0478   0.09524 -0.501874  0.6164
```

```
as.factor(Interface)4:N_BS        -0.0175   0.08521 -0.205651  0.8373
as.factor(Interface)2:M_BS         0.1623   0.06054  2.680903  0.0081
as.factor(Interface)3:M_BS        -0.0671   0.07727 -0.868065  0.3866
as.factor(Interface)4:M_BS         0.0609   0.06723  0.905467  0.3665
as.factor(Interface)2:C_BS         0.0627   0.07711  0.813164  0.4173
as.factor(Interface)3:C_BS         0.0552   0.09819  0.562246  0.5747
as.factor(Interface)4:C_BS        -0.0161   0.08555 -0.188082  0.8510
N_BS:C_BS                         -0.0002   0.00029 -0.845006  0.3993
M_BS:C_BS                         -0.0002   0.00021 -0.733478  0.4643
N_BS:M_BS                         -0.0003   0.00022 -1.207390  0.2290
N_BS:M_BS:C_BS                     0.0000   0.00000 -3.845421  0.0002
as.factor(Interface)2:N_BS:C_BS    0.0003   0.00028  1.204674  0.2300
as.factor(Interface)3:N_BS:C_BS   -0.0003   0.00035 -0.890726  0.3744
as.factor(Interface)4:N_BS:C_BS    0.0004   0.00030  1.188625  0.2363


dropping single factors

> anova(tmp.gls300, tmp.gls300_int)
               Model df      AIC      BIC    logLik   Test  L.Ratio p-value
tmp.gls300         1 25 1828.490 1909.665 -889.2449
tmp.gls300_int     2 10 1943.767 1976.238 -961.8837 1 vs 2 145.2776  <.0001
> anova(tmp.gls300, tmp.gls300_N)
             Model df      AIC      BIC     logLik   Test  L.Ratio p-value
tmp.gls300       1 25 1828.490 1909.665  -889.2449
tmp.gls300_N     2 15 2089.836 2138.542 -1029.9183 1 vs 2 281.3467  <.0001
> anova(tmp.gls300, tmp.gls300_M)
             Model df      AIC      BIC     logLik   Test  L.Ratio p-value
tmp.gls300       1 25 1828.490 1909.665  -889.2449
tmp.gls300_M     2 18 2096.745 2155.192 -1030.3726 1 vs 2 282.2553  <.0001
> anova(tmp.gls300, tmp.gls300_C)
             Model df      AIC      BIC     logLik   Test  L.Ratio p-value
tmp.gls300       1 25 1828.490 1909.665  -889.2449
tmp.gls300_C     2 15 2095.441 2144.146 -1032.7206 1 vs 2 286.9514  <.0001
```

- Model 5; *Macoma balthica* Movement ~ *f* (interface, BS *Hediste diversicolor*, BS *Hydrobia ulvae*, BS *Corophium volutator*)

No movement therefore no model needed.

- Model 6; *Corophium volutator* Movement ~ *f* (interface, BS *Hediste diversicolor*, BS *Hydrobia ulvae*, BS *Macoma balthica*)

```
Best model
```

```
tmp.gls600REML <- gls(C_R ~ as.factor(Interface) + N_BS + M_BS + H_BS +
as.factor(Interface): M_BS +
H_BS:N_BS +
H_BS:M_BS +
N_BS:M_BS +
N_BS:M_BS:H_BS,
weights = varComb(varIdent(form = ~1|as.factor(Interface)),varExp(form = ~C_BS)),
method = "REML")


Coefficients:
                             Value Std.Error   t-value p-value
(Intercept)               347.7530 18.771227 18.525853  0.0000
as.factor(Interface)2      53.8448  7.230446  7.446949  0.0000
as.factor(Interface)3     -10.9711  7.267850 -1.509545  0.1329
as.factor(Interface)4      13.9481  9.931214  1.404469  0.1619
N_BS                       -0.4397  0.052332 -8.401961  0.0000
M_BS                       -0.4334  0.056442 -7.677908  0.0000
H_BS                       -0.5179  0.051897 -9.979771  0.0000
as.factor(Interface)2:M_BS -0.0923  0.029954 -3.080645  0.0024
as.factor(Interface)3:M_BS -0.0190  0.030109 -0.631207  0.5287
as.factor(Interface)4:M_BS -0.0329  0.041027 -0.802890  0.4231
N_BS:H_BS                   0.0001  0.000185  0.383521  0.7018
M_BS:H_BS                   0.0001  0.000185  0.594184  0.5531
N_BS:M_BS                   0.0000  0.000186 -0.080694  0.9358
N_BS:M_BS:H_BS              0.0000  0.000001 -4.422794  0.0000


dropping single factors

> anova (tmp.gls600, tmp.gls600Int)
              Model df      AIC      BIC    logLik   Test  L.Ratio p-value
tmp.gls600        1 19 1813.020 1874.813 -887.5102
tmp.gls600Int     2 13 1900.520 1942.800 -937.2602 1 vs 2 99.50003  <.0001
> anova (tmp.gls600, tmp.gls600N)
            Model df      AIC      BIC     logLik   Test  L.Ratio p-value
tmp.gls600      1 19 1813.020 1874.813  -887.5102
tmp.gls600N     2 15 2047.208 2095.992 -1008.6041 1 vs 2 242.1879  <.0001
> anova (tmp.gls600, tmp.gls600H)
            Model df      AIC      BIC     logLik   Test  L.Ratio p-value
tmp.gls600      1 19 1813.020 1874.813  -887.5102
tmp.gls600H     2 15 2067.791 2116.575 -1018.8956 1 vs 2 262.7708  <.0001
> anova (tmp.gls600, tmp.gls600M)
            Model df      AIC      BIC     logLik   Test  L.Ratio p-value
tmp.gls600      1 19 1813.020 1874.813  -887.5102
tmp.gls600M     2 12 2057.501 2096.528 -1016.7506 1 vs 2 258.4808  <.0001
```

Chapter 6

- Model 1; $Fo^{15} \sim f$ (species identity, global enrichment, local enrichment, number of neighbours, enrichment of neighbours, edge effect, run)

Species (1 = Hd, 2 = Hu, 3 = Cv)

Local enrichment (0 = non-enriched, 1 = enriched)

```
Best models REML

tmp.lme80REML<-lme(FMS_D3   ~   as.factor(Species)      +   as.factor(Local_Enrich)   +
as.factor(Run) +
as.factor(Species):as.factor(Run) ,
correlation=scf1,weights = varIdent(form = ~ 1|as.factor(Species) * as.factor(Run)),
random=~1|factor(Mesocosm), control=lmc, method = "REML")


Coefficients:
                                      Value Std.Error  DF   t-value p-value
(Intercept)                         272.4823  28.40814 629  9.591699  0.0000
as.factor(Species)2                 429.0318  44.10279  15  9.727996  0.0000
as.factor(Species)3                 -22.5140  45.46216  15 -0.495226  0.6276
as.factor(Local_Enrich)1              5.2140   1.03452 629  5.040027  0.0000
as.factor(Run)2                    -210.5089  33.31647  15 -6.318463  0.0000
as.factor(Run)3                    -255.0685  28.54295  15 -8.936305  0.0000
as.factor(Run)4                    -224.5862  29.50357  15 -7.612168  0.0000
as.factor(Run)5                    -257.5793  28.44989  15 -9.053791  0.0000
as.factor(Species)2:as.factor(Run)2  -36.6858  71.69130  15 -0.511719  0.6163
as.factor(Species)3:as.factor(Run)2   55.8855  50.04846  15  1.116629  0.2817
as.factor(Species)2:as.factor(Run)3 -353.6613  49.38586  15 -7.161187  0.0000
as.factor(Species)3:as.factor(Run)3   27.8741  45.66493  15  0.610405  0.5507
as.factor(Species)2:as.factor(Run)4 -422.2592  46.46490  15 -9.087702  0.0000
as.factor(Species)3:as.factor(Run)4   12.9030  47.04998  15  0.274240  0.7876
as.factor(Species)2:as.factor(Run)5 -417.9817  44.20125  15 -9.456332  0.0000
as.factor(Species)3:as.factor(Run)5   37.2714  45.98082  15  0.810585  0.4303


Importance of single factors

> anova (tmp.lme80, tmp.lme80Sp)
            Model df      AIC      BIC    logLik   Test  L.Ratio p-value
tmp.lme80       1 34 6500.650 6653.386 -3216.325
tmp.lme80Sp     2 24 6628.758 6736.571 -3290.379 1 vs 2 148.1079  <.0001
> anova (tmp.lme80, tmp.lme80LE)
            Model df     AIC      BIC    logLik   Test  L.Ratio p-value
tmp.lme80       1 34 6500.65 6653.386 -3216.325
tmp.lme80LE     2 33 6516.21 6664.454 -3225.105 1 vs 2 17.56018  <.0001
> anova (tmp.lme80, tmp.lme80R)
```

```
           Model df      AIC      BIC    logLik   Test  L.Ratio p-value
tmp.lme80      1 34 6500.650 6653.386 -3216.325
tmp.lme80R     2 22 6644.679 6743.508 -3300.340 1 vs 2 168.0293  <.0001
```

- Model 2; Movement ~ *f* (species identity, global enrichment, local enrichment, number of neighbours, enrichment of neighbours, edge effect, run)

```
Best model

tmp11_REML<-gls(DBiomass~
  Neighbours+factor(Species)+factor(Local_Enrich)+
  factor(Species)*factor(Local_Enrich)+
  Neighbours*factor(Species),
  correlation=scf3,
  weights=varIdent(form=~1|factor(Species) * factor(Local_Enrich)),method = "REML")


Coefficients:
                                        Value  Std.Error   t-value p-value
(Intercept)                         0.1533312 0.05281023  2.903437  0.0038
Neighbours                         -0.0248481 0.01074429 -2.312679  0.0211
factor(Species)2                   -0.3173547 0.11219077 -2.828706  0.0048
factor(Species)3                   -0.6459748 0.09459632 -6.828752  0.0000
factor(Local_Enrich)1              -0.0894548 0.02990891 -2.990906  0.0029
factor(Species)2:factor(Local_Enrich)1  0.1508094 0.06230922  2.420339  0.0158
factor(Species)3:factor(Local_Enrich)1  0.7277049 0.05462823 13.321040  0.0000
Neighbours:factor(Species)2         0.0540923 0.02315236  2.336362  0.0198
Neighbours:factor(Species)3         0.0641254 0.01980326  3.238122  0.0013


Importance of single factors can’t be done.
```