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### DOCTOR OF PHILOSOPHY

### Facilitation and biodiversity in the marine benthos

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That's the problem with science; you've got a bunch of empiricists trying to describe things of unimaginable wonder.

Calvin (and Hobbes)

# Facilitation and biodiversity in the marine benthos

A thesis presented to Bangor University for the degree of Doctor of Philosophy

by

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### Summary

Rapid loss of global biodiversity compels us to understand how natural systems respond to disturbance. A major factor influencing this process is how species interact within these systems. Positive species interactions (facilitation) may be able to buffer disturbance and influence how ecosystems respond to species loss. An understanding of the circumstances under which interactions change would not only contribute to many core issues in contemporary community ecology, but would allow us to apply such knowledge to inform new and innovative conservation strategies.

Facilitation through bioengineered stress amelioration is predicted to be particularly important in habitats of high environmental stress (the stress gradient hypothesis (SGH)). This is because the stress reduction is essential for certain associated species to survive. Conversely, the same mechanism of facilitation will not be required under benign conditions. This is the central tenet of the facilitation-stress hypothesis: that facilitation becomes relatively more important as abiotic stress increases. As such, this study aimed to measure the strength of positive interactions in intertidal benthic systems across environmental stress gradients.

The study focused on two common intertidal species with different mechanisms of bioengineering: 1) lugworms (*Arenicola marina*) that oxygenate the sediment through bioturbation and 2) sand masons (*Lanice conchilega*) that stabilise the sediment through construction of biogenic tubes. Through observational studies, field manipulations and mesocosm experiments, my research aimed to find whether the SGH was generally applicable to multiple systems and mechanisms of facilitation.

An initial observational study was undertaken to investigate whether interactions of adjacent species with *A. marina* varied between shores of low and high ambient hypoxic stress (AHS). Lugworm density was found to positively relate to both the depth of the oxic-anoxic chemocline and associated species richness at sites of high AHS. At sites of low AHS, lugworms had no effect. Results suggested that lugworm depressed the apparent redox potential discontinuity depth (aRPD) and increased species richness at sites of high AHS though no trends were found with specific species or within functional groupings.

A second, manipulative study of lugworm was conducted at sites of high and low AHS to test if patterns of aRPD depth and species richness observed in the descriptive study were driven by lugworm density. This study attempted to add to my observational study by establishing causality. Lugworms were excluded from the sediment and effects compared to procedural controls and ambient plots. Lugworms significantly reduced sulphide concentrations at the deepest depths (18 cm) at all high AHS sites, but also at one of the low AHS sites. In contrast to the observational study, lugworms had no effect on species richness. However, *Corophium* spp. were always negatively affected by lugworm; when present, *Bathyporeia* spp. always benefited from lugworms; and *Scoloplos armiger* showed significant, but highly variable, responses at a site level. I suggest that there was an insufficient difference in AHS between sites to detect any differential effects of bioturbation on species' distributions. Furthermore, we propose that effects of lugworms on species' densities that were recorded occurred because of mechanisms of bioengineering other than stress amelioration e.g. funnel and cast formation. As these interactions did not occur because of stress amelioration, they would have operated independently of the stress gradient.

Field manipulations were undertaken on *L. conchilega* to see whether, in line with the SGH, adjacent species interacting with the tubeworms benefited from mimics at high, compared to low stress (current speed). Effects of different densities of tubeworm mimic on an associated infauna community were studied at relatively higher and lower current speed, modified *in situ* by Venturi flumes. Results indicated that *L. conchilega* tubes increased sediment shear strength and maintained species richness as current speed increased possibly as a result of buffered erosion. Small, surface-dwelling organisms appeared to be promoted preferentially. Evidence suggested that sediment-stabilising effects of tubeworms supported the general SGH.

Mesocosm studies were used to further investigate how interactions with L. conchilega mimics shifted over a more complete stress gradient whilst mitigating against confounding effects. In contrast to my field manipulations, the mesocosm experiment aimed to show how effects changed across multiple current speeds in order to find when facilitation manifests and the shape of the facilitation-stress relationship i.e. whether it is accelerating, asymptotic, or hump-backed. Effects of increasing current speeds on a representative community were recorded with and without mimics present. Interactions were measured as a change in live biomass for each species. The only species found to significantly benefit was Corophium volutator, whose mortality was buffered from flow-associated disturbances at current speeds of 9 cm.s<sup>-1</sup>. My experiment was limited by not generating high enough current speeds and I hypothesise that, had I been able to generate these higher speeds, I would have seen effects on more of the species. This has implications for how pair-wise facilitation manifests at a community level at different stress levels.

My findings suggest that facilitation is an idiosyncratic, though important, process determining how communities respond to changing environments. They indicate that the basic SGH may be too simple to apply to all natural systems and I suggest that future research be directed in finding which systems the SGH applies to in order to refine and develop new conceptual models that are more representative of real communities and environmental conditions. As it stands, individual site-specific knowledge is required in order to use facilitation in conservation and restoration projects.

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	Glossary
Biodiversity	the variety of life in a given space
Bioengineer	see ecosystem engineer
Commensal	+/0 species interaction
Competition	-/- species interaction
Disturbance	a temporary change in average environmental conditions that causes a pronounced change in an ecosystem
Ecosystem engineer	an organism that creates, modifies or maintains habitat (also bioengineer)
Ecosystem function	resources and processes that result from natural systems
Facilitation	any species interactions where one species benefits and none are harmed i.e. mutualisms and commensalisms
Foundation species	a species whose presence is required for the foundation of a biological community
Interspecific	between species
Intraspecific	within population
Keystone species	a species that has a disproportionate effect on its environment relative to its abundance
Mutualism	+/+ species interaction
Species richness	the number of species in a system
Stress	any fitness reduction caused by external physical, chemical or biological factors
DEM	dynamic equilibrium model
IDH	intermediate disturbance hypothesis
SGH	stress gradient hypothesis
SPI	sediment profile imagery
RPD	redox potential discontinuity

## **Chapter 1**

**1** General introduction

### 1.1 Types of species interaction

Darwin (1859) believed that natural communities were "bound together by a web of complex relations". Today we understand that no organism lives unconnectedly in nature; all occur as components of intricate interactive assemblages (Grimm 1995). Whenever they interact, there are either neutral, positive or negative effects on the organisms involved. Interactions may be inter- (between-species) or intraspecific (within-species) and are often simplified to neutralism (0/0), competition (-/-), predation/parasitism (+/-), commensalism (+/0) and mutualism (+/+). Organisms will typically exhibit a range of these interactions with different components of their community (e.g. Travis et al. 2006). In reality, interactions are rarely wholly positive or negative, but combinations of positive and negative components (Callaway & Walker 1997, Brooker & Callaghan 1998).

Interspecific facilitation is any interaction that positively affects one of the species, but that does not negatively affect any of the other species involved (i.e. commensalism and mutualism) (Bronstein 1994). Facilitation includes all forms of positive interaction, from "tightly coevolved, mutually obligate relationships to loosely facultative relationships" (Stachowicz 2001). Facilitation can affect marine systems at all levels of organisation, from individual organisms (e.g. specific Symbiodinium spp. endosymbiont dinoflagellates in corals, LaJeunesse et al. 2003) to the formation of large biogenic structures by foundation species that facilitate whole biotopes (e.g. Zostera japonica sea grass beds, Lee et al. 2001). At a population level, facilitation can increase growth and recruitment, reduce mortality of associated species, and/or ameliorate physical and/or consumer pressure (Choler et al. 2001, Callaway et al. 2002). At a community level, facilitation can drive succession (Crain & Bertness 2006) and boost biodiversity, productivity and stability (Stachowicz et al. 2002, Ives & Carpenter 2007, Wall et al. 2008). Understandably then, facilitators have been the focus of much theoretical and empirical study.

### 1.2 Categorisation of facilitators and mechanisms of facilitation

Facilitation can occur through trophic links, but is commonly caused by some form of habitat modification. In this study, I only consider species that facilitate by modification of their habitat. Historically, species that facilitate have been placed in various categories, including 1) foundation (Dayton 1971, Dayton & Hessler 1972) and 2) keystone species (Paine 1969, Grimm 1995) and 3) ecosystem engineers (Jones et al. 1994). These terms are not synonyms, but many species fit into all three categories (Table 1.1).

Table 1.1: Categorisation	n and disambiguation	of species'	classifications
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Category	Definition	Marine examples
Foundation species	whose presence or actions facilitate community development	Seagrass beds <sup>1</sup> Mussel banks <sup>2</sup> Reef-building corals
Keystone species	whose contribution to ecosystem function is unique and whose removal would lead to significant changes in community structure; keystone-community links may be trophic or via habitat modification	Sea stars <sup>3</sup> Sea otter <sup>4</sup>
Ecosystem engineers (bioengineers)	that create, modify, or maintain habitat; autogenic engineers change their environment via their physical presence and allogenic engineers by converting material from one physical state to another	<i>Autogenic –</i> Seagrass beds <sup>1</sup> <i>Allogenic –</i> Polychaete tube worms <sup>5</sup>

1 Edgar 1999b, a; 2 Ragnarsson and Raffaelli 1999; 3 Navarrete and Menge 1996; 4 Estes and Palmisan.Jf 1974; 5 Jones and Jago 1993

To elucidate the differences: foundation species are bioengineers that specifically drive development of a new community; keystone species may act through trophic links as well as via biomodification; and ecosystem engineers are any organism that modifies their habitat in some way – if unusual, they can be termed keystone engineers. For example, the seastar *Pisaster ochraceus* is a keystone predator. Without the seastar preying on intertidal mussels *Mytilus californianus* and *M. trossulus*, a diverse assemblage of low intertidal algae and invertebrates would shift to a monoculture of the competitively dominant mussel *M. californianus* (Paine 1966). However, as the seastar does not modify habitat in any way, it is not

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### Chapter 1

considered an ecosystem engineer. In contrast, bioturbating lugworms (*Arenicola marina*) rework surface sediment and so are bioengineers (Reise 2002). However, other species including bivalves such as cockles, *Cerastoderma edule*, and polychaetes, such as ragworm, *Nereis diversicolor*, also rework sediment. A lugworms role is therefore not unique, hence not keystone.

Other terms have been used to describe the same processes. "Niche construction" refers to any influence that organisms have on their environment (Odling-Smee 1988, Odling-Smee et al. 2003) and can be considered synonymous with ecosystem engineering. However, this term is more commonly used in evolutionary biology, while ecosystem engineering is linked to ecology (Boogert et al. 2006).

It is possible to identify broad mechanisms of bioengineered facilitation that are germane to all biomes (Bruno & Bertness 2001): these include provision of a refuge (Fuentes et al. 1986), increased resource flow (Callaway et al. 1991), amelioration of environmental stress and propagule retention (Bertness & Hacker 1994, Bruno 2000) (Table 1.2). All of these mechanisms may create hotspots of diversity and productivity (Crooks 2002, Bruno et al. 2003).

Mechanism	Notes	Examples
Habitat creation	Habitats may be created by a single foundation species or a guild of species Increases general habitat heterogeneity Provides a surface for attachment	Reef-building corals <sup>1</sup> Oysters and mussels <sup>2</sup> Seagrass beds <sup>3</sup>
Provision of a refuge	Transforming a two-dimensional structure into a three-dimensional structure provides an environment where prey is able to shelter from potential predators Relic structures remaining once species are dead can facilitate long after organisms are gone (Aronson & Precht 2001, Munksby et al. 2002)	Reef-building corals <sup>1</sup> Oysters and mussels <sup>2</sup> Seagrass beds <sup>3</sup> Polychaete worms <sup>4</sup>
Hydrodynamic modification	Decreases flow velocity and increases sedimentation (Luckenbach 1986) Facilitates by increasing sediment stability (Bruno & Kennedy 2000), improving food delivery (Worcester 1995, Shashar et al. 1996) and by increasing propagule retention (Eckman 1983, Bruno 2000)	Reef-building corals <sup>1</sup> Oysters and mussels <sup>2</sup> Seagrass beds <sup>3</sup> Polychaete worms <sup>4</sup>
Amelioration of desiccation stress	Intertidal organisms retain moisture at low tide, shade substrate and provide moisture for associated species (Thompson et al. 1996, Bertness et al. 1999b) Allows species to extend their realised niche (Bertness et al. 1999b)	Fucoid algae <sup>5</sup>
Amelioration of anoxic stress	Through bioturbation and bioirrigation, organisms cause the oxic-anoxic chemocline to move downwards in sediment thus facilitating by reducing anoxic stress Commonly caused by bioturbating and bioirrigating infauna (Pearson 2001, Rosenberg 2001, Meysman et al. 2006)	Lugworms <sup>6</sup> Ghost shrimps <sup>7</sup> Stingray <sup>8</sup>
Enhanced propagule retention	Increases the supply or retention of agents of reproduction	Reef-building corals <sup>1</sup> Oysters and mussels <sup>2</sup> Seagrass beds <sup>3</sup> Polychaete worms <sup>4</sup> Kelp <sup>9</sup>

Table 1.2: Modes of facilitation in the marine benthos. These broad mechanisms of facilitation are common to all biomes.

1 Luckhurst and Luckhurst 1978, Jones and Syms 1998, Syms and Jones 2000, 2004; 2 Tolley and Volety 2005; 3 Dean et al. 2000, Lee and Berejikian 2009; 4 Schwindt and Iribarne 2000, Dame et al. 2001, Schwindt et al. 2004a, Schwindt et al. 2004b; 5 Kappner et al. 2000; 6 Reise 1981b, a, Reise and Volkenborn 2004, Volkenborn et al. 2007, Volkenborn and Reise 2007, Volkenborn et al. 2009; 7 Berkenbusch et al. 2000, Berkenbusch et al. 2007; 8 Valentine et al. 1994; 9 Estes and Palmisan.Jf 1974, Carr 1989

### 1.3 Why is it important that we understand how facilitation operates?

Here I discuss how facilitation relates to two core issues in contemporary community ecology: 1) how biodiversity relates to ecosystem processes (1.4.1) and 2) how the extinction order of species influences this relationship (1.4.2).

### 1.1.1 Biological diversity and ecosystem function

Biodiversity was officially defined as the "variability among living organisms from all sources, including terrestrial, marine, and aquatic ecosystems, and the ecological complexes of which they are part, including diversity within and between species and of ecosystems" at the United Nations Convention on Biological Diversity (1992). Simply, biodiversity is considered to be the range of genetic, taxonomic and/or ecological diversity over different spatial and temporal scales (Harper & Hawksworth 1994). Biodiversity is important for ecological, biogeochemical, socio-economic and psychological reasons (Costanza et al. 1997). Biodiversity values have previously been calculated in an attempt to provide a rationale for conservation of biodiversity (e.g. Costanza et al. 1989, Costanza et al. 1997). For a full typology that classifies and evaluates ecosystem services, goods and functions, see de Groot et al. (2002).

Ecosystem functions can be defined simply as any ecosystem-level processes such as production, element cycling and trophic transfer (Tilman 1999, Duffy & Stachowicz 2006). Relationships between biodiversity and ecosystem functions (B-EF) change contextually. As a result, graphical summaries of the relationships were developed for use as heuristic devices (Vitousek & Hooper 1993, Schlapfer & Schmid 1999, Schlapfer et al. 1999). All graphs share two common fixed points; one where there is no biodiversity and hence no ecosystem function, and another point where there is a natural level of biodiversity and an associated amount of functioning (Vitousek & Hooper 1993). Extensive lists of hypotheses exist to explain the shape of the relationship between these points (Figure 1.1).



Figure 1.1: Heuristic devices describing hypothetical relationships between species richness and ecosystem functioning. Shown are the a) linear, b) redundancy, c) keystone and d) idiosyncratic hypotheses (after Naeem 1998)

The simplest relationship is a linear one, where for every species added or removed an equal amount of function is gained or lost (Figure 1.1a). However, this is considered unrealistic in nature, as it is improbable that species will contribute equal function. Remaining hypotheses can be grouped into three classes: where species' roles are replicated and therefore partially redundant (1.1b); where roles are singular (1.1c); or where effects are idiosyncratic and unpredictable (1.1d).

Whether any of these theoretical models can be successfully applied to natural systems is a controversial issue. Typically, richer systems do have higher levels of ecosystem function and are more stable (reviews in Schwartz et al. 2000, Loreau et al. 2001, Bond & Chase 2002, though see Stachowicz et al. 2002).

This is believed to be due to a number of reasons, including:

- niche complimentarity where species or functional groups occupy functionally distinct niches in an ecosystem and use resources in a complementary way (Naeem et al. 1994, Wojdak & Mittelbach 2007)
- insurance effects where richer communities buffer fluctuations in function caused by species loss (Walker 1992, Naeem 1998, Yachi & Loreau 1999)

 sampling effects an increased probability of highly productive dominant species in more diverse communities (Aarssen 1997, Huston 1997, Tilman et al. 1997)

However, richer systems are not always more stable than species poor systems (Stachowicz et al. 2002), and it is thought that our current understanding is an oversimplification, neglecting factors such as community evenness (Wittebolle et al. 2009) and the mode and strength of species interactions (Doak et al. 1998, Cardinale et al. 2002, Ives & Carpenter 2007, though see Mulder et al. 2001).

Based on conceptual models, species that are important facilitators would generate a 'keystone' richness-function relationship (e.g. Naeem 1998). In general, the difference between B-EF models is driven by a change in the functional magnitude of particular species i.e. a shift from functional redundancy to keystone. From this, one could suggest that the mode and strength of species interactions through biomodification are central in determining the shape of the B-EF relationship as they determine the extent to which organism influence each other. By finding out when interactions shift, it would inform our understanding of when these B-EF relationships occur in nature.

### 1.3.1 Species interactions influence extinction orders

Many B-EF studies test how ecosystem processes respond to extinctions in order to predict how global species loss will impair ecosystem functioning (Ehrlich & Wilson 1991, Schulze & Mooney 1993, Chapin et al. 1997, Vitousek et al. 1997). However, extinctions rarely occur randomly and so it is important that (a) extinction order (Petchey & Gaston 2002) and (b) differences in functional contribution (Symstad et al. 2003) are considered. One suggestion is that two species-specific traits determine how extinctions affect ecosystem function (e.g. Solan et al. 2004) (Figure 1.2): effect traits

that determine functional contributions and response traits that determine sensitivity to disturbance.



Figure 1.2: a) Relationships between species response and effect traits and b) resulting richness–function relationships (after Larsen et al. 2005).

In order to predict how real systems will respond to extinction pressures it is necessary to understand how the roles of functionally important species vary with stress. Crucially, Larsen et al. admit that in systems where species interactions influence function, richness-function relationships would be very different to those they found (also see Tilman 1999). By including facilitation, a number of additional factors are introduced that negate the assumptions of Larsen et al.:

- Functional importance of facilitators would vary independently of species composition and diversity.
- Facilitation could reduce extinction pressure for particular species, thus changing the extinction order.

 Loss of a facilitator could result in a cascade of further extinctions of other species that rely on its stress amelioration in order to survive.

In order to predict how the shift in importance of species interactions alters extinction orders and richness function relationships in it is necessary to first understand the environmental circumstances under which they change.

# 1.4 How the role of facilitation is expected to vary with environmental stress

Species interactions and their influence on biodiversity are expected to vary predictably with stress/disturbance (Bertness & Callaway 1994, Bruno et al. 2003). In this section, I discuss theories relating to the interrelatedness of biodiversity, disturbance and facilitation. Here, ecological stress (any fitness reduction caused by external physical, chemical or biological factors) and disturbance (any process that removes biomass from a community (Grime 1977) will be considered analogous.

### 1.4.1 <u>Diversity-disturbance relationships in the intermediate disturbance</u> <u>hypothesis (IDH)</u>

The IDH (Connell 1978) predicts that intermediate frequencies/intensities of disturbance maximise species richness of sessile, space-holding species (e.g. corals, seagrasses). At low disturbance, competitive dominants monopolise resources leading to lower diversity and, at higher levels of disturbance, only colonising species can establish. Evidence has been found to both support (e.g. Shea et al. 2004) and refute (e.g. Mackey & Currie 2001) IDH patterns. Inconsistency in empirical evidence implies that there are confounding factors affecting the relationship.

In a review of 197 IDH studies (that measured species richness, biodiversity and evenness), most were found to show no significant relationship and peaked responses were shown rarely (Mackey & Currie 2001). From this, the authors suggested that disturbance was not an important factor controlling biodiversity in nature. A number of studies have offered alternative opinions, often including additional predictors of diversity such as productivity (Kondoh 2001) and/or species interactions (Caswell & Etter 1999, Bruno et al. 2003).

### 1.4.2 How facilitation influences diversity-disturbance relationships

By including facilitation in ecological theory, the idea of a 'competitive dominant' is turned on its head. Facilitators are often primary space-holders, but their net effect on species richness is positive (Bruno et al. 2003). This is contrary to the idea of competitive dominants who exhibit strong negative As such, theories such as IDH shift when interspecific interactions. facilitation is considered (after Bruno et al. 2003). Habitat-formers tend to dominate when disturbance is low and, subsequently, species richness of secondary space holders is highest here. Diversity of secondary space holders may demonstrate a negative relationship with disturbance (Dean & Connell 1987), whilst primary space holders would still conform to IDH predictions (Sousa 1979). This is because smaller species often rely on larger habitat-forming species who are more prevalent when frequency/intensity of disturbance/predation is low. The authors suggest that relationships are scale-dependent and that species interactions may help explain experimental contradictions. Furthermore, evidence from metaanalyses suggest that the relationships between biodiversity of sessile and motile organisms and disturbance are different and that negative relationships are more common than peaked ones (Mackey & Currie 2001). For example, intertidal mussels are dominant primary space holders and displace other primary space holding species such as seaweed and barnacles (Paine 1966, 1974). For this reason, the diversity of primary space holders is greater when mussels are not present. However, mussels also support a greater diversity of invertebrate secondary space holders than these other primary space holders do. Thus, the diversity of secondary space holders is greatest whenever mussels are present. Consequently, by considering facilitation, then long-held paradigms such as the IDH can be reimagined and alter how we view the interrelatedness of biodiversity and disturbance.

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### 1.4.3 <u>How facilitation, biodiversity and environmental stress are interrelated</u> The original stress-gradient hypothesis (SGH) predicts that competitive effects will be stronger and more important in low-stress environments, whereas facilitative effects will more important in high-stress environments (Bertness & Callaway 1994) (Figure 1.3).



Figure 1.3: Stress-gradient hypothesis (SGH) (after Bertness & Callaway 1994, Bruno & Bertness 2001) (Fig a). Bruno and Bertness (2001) suggest that if species interactions are de-coupled then the strength of competition will actually remain constant over the stress gradient (Fig b,c).

The SGH is based around the assumption that facilitation will become relatively more important as environmental stress overtakes biological stress as the dominant stressor. The model relies on the supposition that environmental stress and consumer pressure are inversely related as an assumption is made that mobile consumers are more sensitive to stress than their prey and that high environmental stress will reduce predator densities and foraging efficiencies (Menge 1978, Witman & Grange 1998). However, the assumption breaks down when predators are highly mobile and are able to shelter in less stressful refuges (Menge & Olson 1990), potentially provided by habitat modifying facilitators (Eckman & Duggins 1991, Bertness et al. 1999b). With the assumption broken, the relationship shifts so that stress and predation are positively related (Pennings & Bertness 2001).

### 1.4.4 Facilitation in predator-stress models

More complex models divide stress into individual pressures (Menge & Sutherland 1987, Bruno & Bertness 2001) (Figure 1.4). Here, the role of facilitation is also predicted to change with types of stress (Crain & Bertness 2005). In abiotically stressful environments, a facilitator's role in the community is essential; they ameliorate stress and allow organisms to survive in habitats they would otherwise have found intolerable. In benign environments, facilitators act instead by providing a refuge from predation.



Environmental stress

Figure 1.4: Facilitation introduced to the Menge-Sutherland environmental stress model (after Bruno & Bertness 2001)

Facilitators predicted to influence communities most are those that modify limiting resources or confining variables (Crain & Bertness 2006). This is exemplified by interactions in rocky intertidal communities, where competitive interactions shape lower intertidal species distributions and facilitative interactions are prevalent on the upper shore. For example, *Ascophyllum nodosum* shades substrate on the high shore and reduces desiccation stress (Bertness 1989, Bertness & Leonard 1997). Experiments show that maximum daily rock temperatures can be 5-10°C lower and evaporative water loss an order of magnitude less under the canopy compared to canopy removal plots (Bertness et al. 1999b). In general, this causes an increase in growth, recruitment and survival of understory organisms.

As facilitators often create hotspots of diversity and productivity, the fact that they only manifest at high stress has implications for facilitator-diversity relationships. Much of the conceptual framework describing the relationship was proposed by Bruno and Bertness (2001). They proposed that there was a link between facilitator traits, such as density or biomass, and biodiversity and that this relationship shifted with environmental stress (the trait-richness hypothesis). This is because as a facilitator trait increases in a benign habitat, it is unlikely to have any effect on associated species because the facilitator is not required (Figure 1.5a) (Callaway 2007). Conversely, in more stressful environments, species richness is likely to be low if facilitator traits are too low to ameliorate the stress (Heck & Wetstone 1977). The facilitation-richness paradigm shifts when competitive exclusion is considered, as at medium to high stress, competition will manifest once the stress is fully ameliorated (Figure 1.5b).



Figure 1.5: Abiotic stress is predicted to alter the shape of the relationship between facilitator trait and associated species richness whether competitive exclusion is considered (b) or not (a).

### 1.4.5 Empirical testing of models

At present, there is conflicting evidence as to whether the SGH applies to natural systems. Most studies have been conducted in plant communities, where there are examples of positive (Armas & Pugnaire 2005, Crain & Bertness 2005, Sthultz et al. 2007), negative (meta-analysis in Maestre et al.

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2005) and neutral (Casper 1996) relationships between facilitation and abiotic stress. However, the negative result from the meta-analysis has subsequently been disputed and discredited (Lortie & Callaway 2006) and the majority of empirical plant studies support the SGH.

Facilitation-stress studies from the marine environment are scarcer and similarly contradictory. The majority contend a positive facilitation-stress relationship (Bertness et al. 1999a, Bertness et al. 1999b, Menge 2000), but some have found the relationship to be negative (Menge 1976, Norkko et al. 2006). Most of these studies come from rocky shore systems and examine similar mechanisms of facilitation i.e. amelioration of desiccation stress. In order to prove the generality of the hypothesis, empirical evidence for different mechanisms of facilitation and different stressors is ultimately In intertidal systems, facilitation is particularly common as the needed. habitats have steep physico-chemical gradients and primary spaceholders are capable of ameliorating potentially limiting stressors (Bertness & Leonard 1997). In soft-sediment intertidal systems, habitat-mediated species interactions typically occur because of stabilising and destabilising activities (Reise 2002) and should be a focus of future facilitation-stress studies. In addition, if facilitation-stress theory is going to be utilised for any applied function e.g. conservation and/or restoration, then the scientific community need to find which facilitators can be used and why there is inconsistency in experimental and descriptive evidence.

### 1.5 Using facilitation in conservation, restoration and management of ecosystems

Loss of functionally important species can result in cascading effects on other fauna and has significant consequences for the ecosystem as a whole (Coleman & Williams 2002). Ecosystem engineers typify these strongly interacting species (Soulé et al. 2005). Targeting such species, under various pseudonyms, for conservation has been proposed by many previous studies (Mills et al. 1993, Schwartz et al. 2000, Odling-Smee 2005, Soulé et al. 2005, Boogert et al. 2006, Byers et al. 2006, Crain & Bertness 2006, Halpern et al. 2007). By targeting such species for conservation, then

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associated species and functions would also be protected (Crain & Bertness 2006). Due to their role in promoting ecosystem structure and function, their potential use in restoration projects has also been explored (Maestre et al. 2001, Castro et al. 2004, Gómez-Aparicio et al. 2004, Halpern et al. 2007, Hastings et al. 2007, Brooker et al. 2008, Funk et al. 2008). However, the need to identify exactly how the role of these functionally important species vary across environmental gradients has been recognised (Crain & Bertness 2006). How effects of ecosystem engineering manifest (i.e. positive/negative community-level effects) is expected to change in line with the SGH and it is necessary to be able to predict when this switch will occur in order to avoid undesirable outcomes (Halpern et al. 2007). Mechanisms of facilitation are predicted to shift from a supply of predator and competitor refuge at benign and intermediate stress, to stress amelioration at high stress (Crain & Bertness 2006).

As an example of how facilitation can be utilised in management, one can consider saltmarsh restoration. Here, there is enough evidence to formulate a facilitation-based management strategy for the habitat. Research suggests that restoration success requires (Halpern et al. 2007):

- Facilitator marsh grasses to be of sufficient height and density to be used by key marsh species.
- Soil grain size to be appropriate for nitrogen retention to promote plant growth.
- Key marsh predators (e.g. blue crabs) to be common enough to suppress herbivorous snails that can kill marsh grass.
- 4) Culms (stems of grass) to be planted close together in the harsh anoxic mud, to facilitate shading and aeration of the soil.
- Mycorrhizal fungi, which are crucial for plant growth, to be artificially added, since they can take a long time to recruit naturally (Boyer & Zedler 1998, Bologna & Heck 2002).

The whole approach is well informed and utilises a number of different theoretical concepts including species interactions and density dependency.

Despite this sophisticated knowledge, in practice marsh restoration efforts still typically involve simply re-planting small (< 5 cm in diameter) culms of grass on mudflats at evenly spaced intervals to avoid, theoretically, the negative impacts of inter-culm competition (Halpern et al. 2007). Evidently, dissemination of scientific research to managers and policy makers is still not effective. In order to apply the SGH to conservation biology, it is necessary to understand how, when and where the role of facilitation is critical in maintaining ecosystem structure and function. Invariably, this will require the generality of the hypothesis to be tested for different mechanisms of facilitation in multiple systems.

### 1.6 CASE STUDIES

Most facilitation-stress studies are conducted on terrestrial grass systems and, of those from the marine environment, most relate to rocky shore communities. Intertidal soft-sediments are dynamic habitats that offer a 3dimensional refuge from predation, retain water at low tide, accumulate organic matter and at high tide the sediment buffers against changes in salinity, temperature and pH that occur within the water column. However, coastal marine sediments are dynamic and easily moved by tides and currents and the sedimentary environment quickly becomes anoxic with depth. Organisms that form biogenic structures are thus important in coastal communities as they can increase sediment stability (Pearson 2001, Reise 2002) and bioturbators help ameliorate against chemical stress in the form of H<sub>2</sub>S (Mermillod-Blondin & Rosenberg 2006). As such, sedimentary marine systems are ideal for conducting studies into facilitation.

### 1.6.1 Arenicola marina

The lugworm *Arenicola marina* is an important benthic constituent of shallow coastal and intertidal systems in Europe and northeast America. In flats of the Dutch Wadden Sea its biomass exceeds that of all other worm species combined and accounts for 20% of total macrobenthic mass (Beukema et al. 1978). Through bioturbation and bioirrigation, lugworms alter sediment

chemistry (Krantzberg 1985), ecology (Riisgård & Banta 1998), rheology (Jones & Jago 1993) and geology (McIlroy et al. 2003).

*A. marina* occupy sediments from 40 m below high water spring to low water spring tides on exposed, medium-energy shores, with maximum numbers (> 200 m<sup>-2</sup>) around mid-tide level (Retraubun et al. 1996b). Lugworms inhabit 10-25 cm deep J-shaped semi-permanent burrows in a head-down orientation (Reise 2002) (Figure 1.6).

Lugworms are non-selective deposit feeders and ingest quantities of sediment at depth (1-2 ml h<sup>-1</sup> individual<sup>-1</sup>, (Riisgård & Banta 1998). Sediment falls from the feeding pocket and any organics contained in the sediment are digested. A string of undigested sediment is then defecated as coiled casts onto the surface sediment above the tail shaft of the burrow (Turner & Bishop 2006). Pits and mounds created by feeding increase surface roughness, alter current flow and affect particulate matter distribution (Hüttel et al. 1996, Lackschewitz & Reise 1998).



Figure 1.6: Cross-sectional diagram of an *Arenicola marina* burrow showing movement of water due to bioirrigation.

### Bioirrigation mechanisms of lugworm A. marina

As sediment is ingested, piston-like movements in a tail-to-head direction pump overlying water through the burrow and oxygenate the gills (Riisgård & Banta 1998). The tail shaft is surrounded by a 2 mm envelope of oxic sediment, and sulphide concentrations are low within a distance of 6 mm (Wetzel et al. 1995). Thirty lugworms m<sup>-3</sup> are able to pump 31 m<sup>2</sup> h<sup>-1</sup> of seawater through anoxic sediment (Baumfalk 1979). Mechanisms of bioirrigation vary with sediment permeability; in muds, water cannot penetrate into the sediment, so bioirrigation is driven by diffusional transfer across the burrow wall and in sand, ventilation flows penetrate the surrounding sediment via dead end burrows (Meysman et al. 2006).

Burrow irrigation by *A. marina* affects porewater nutrient profiles (Huettel 1990), attracting meio- and macrofauna species to particular sections of the burrow (Reise 1987, Lackschewitz & Reise 1998, Riisgård & Banta 1998). Infauna species form close associations with parts of the burrow due to reduced anoxic stress and provision of a refuge and food.

### Lugworm bioturbation

Oxygenation of hypoxic and anoxic sediment by bioturbators is a major mechanism of facilitation (Levinton & Lopez 1977). Bioturbators are foundation species in many particulate habitats, oxygenating the sediment so that species intolerant of hypoxia can survive (Rhoads et al. 1978) and boosting species richness (Mermillod-Blondin et al. 2003). Bioturbation:

- 1) Loosens and fluidises sediment
- 2) Resuspends sediment into the water column
- 3) Changes seabed topography and thus alters hydrodynamics
- Produces features that provide a basic refuge e.g. burrows and galleries
- 5) Alters particle distribution by bringing fine material to the surface and coarse material downwards or vice versa (conveyor and reverse conveyer belt)

- Increases flux rates of dissolved nutrients and other substances between sediment and water
- Changes sediment chemistry e.g. causing the oxic-anoxic chemocline to become irregular and to shift downward

Bioturbation is likely to negatively affect at least some components of the associated community as it often completely alters the physico-chemistry of the sediment. Dense populations of deposit feeders can also exclude other infauna species competing for space and resources. Many organisms cannot survive in the modified habitat, with unstable, watery sediment and increased turbidity at the sediment-water interface (Woodin 1974, Peterson & Andre 1980). This occurrence has come to be known as 'trophic amensalism', despite being an archetypal example of non-trophic allogenic engineering (Rhoads & Young 1970).

Arenicola marina is an extremely important bioengineer in coastal sediments owing to the large volume of habitat it can modify (Jones et al. 1994, Reise & Volkenborn 2004): 30 lugworms m<sup>-2</sup> can turn over a sediment layer of 15 cm vr<sup>-1</sup> (Rijsgård & Banta 1998) and 85 lugworms m<sup>-2</sup> can rework 400 cm<sup>-3</sup> sediment yr<sup>-1</sup> (equivalent to a 40cm layer of sediment) (Cadée 1976). Biomodified sediment has a lower sulphide concentration and higher concentrations of oxygen and bacteria (Hüttel et al. 1996). This has a facilitating effect on many organisms, but disturbances caused by lugworm activity can have a negative effects on other species (Volkenborn & Reise Negatively impacted groups include tube worms (e.g. Pygospio 2006). elegans), surface deposit feeding worms (e.g. Nereis diversicolor), subsurface deposit feeding worms (e.g. Scoloplos armiger) (Brey 1991, Flach 1992a) and Zostera noltii (Philippart 1994). It is an archetypal example of a trophic group amensalism, when a bioturbating deposit feeder inhibits a suspension feeder (Rhoads & Young 1970). Landscape effects are similar to those observed with single lugworms i.e. lower abundances of sedentary species when lugworms are present (Flach 1992a, Volkenborn & Reise 2006) corresponding to the mobility group hypothesis based on small-scale disturbance by a large bioturbator (Brenchley 1981, Wilson 1981). Despite

having a negative effect on parts of the community, lugworms often have an overall facilitatory effect (Volkenborn & Reise 2006). The SGH predicts that at least some of these interactions are context-dependent and may be driven by environmental stress.

### 1.6.2 Lanice conchilega

The terebellid polychaete *Lanice conchilega* (Pallas 1766) (sand mason) is another common component of soft-sediment shores (Holthe 1978). Sublittoral densities of up to 20,000 individuals m<sup>-2</sup> (Ropert 1996) and intertidal densities up to 10,000 individuals m<sup>-2</sup> (Ziegelmeier 1952) have been recorded. The sand mason is capable of switching between deposit and suspension feeding and favours sandy beaches with moderate wave exposure (Buhr 1976). The worm is distributed along coasts of Europe and the Pacific, but has not been found in Arctic waters (Holthe 1978).

### Tube construction

*L. conchilega* occupies a characteristic tube, composed of cemented terrigenous and biogenic coarse sand grains with the anterior end of the tube topped with a fringe of tentacles projecting 1-4 cm from the sediment. Tubes may be I-, U- or W-shaped (Ziegelmeier 1969). Tubes of adults typically have a diameter of 0.5 cm and can be as long as 65 cm (Ziegelmeier 1952).

### **Bioengineer**

*L. conchilega* is an important bioengineer in sedimentary systems. Sand masons alter sediment properties (Jones & Jago 1993) and oxygen distribution (Forster & Graf 1995) in construction of their sand tubes (Zühlke 2001). A solitary projecting worm tube has a destabilising effect on the sediment, but a concentration of such worm tubes may have a stabilising effect (Graf & Rosenberg 1997) (Figure 1.7). At high densities, tubes may stabilise sediment in perturbed habitats by increasing shear strength and reducing erosion (Jones & Jago 1993). Furthermore, at intermediate densities, tubes promote turbulence, causing deposition of fine particles, and promoting growth of sediment-binding microbes (Eckman 1985).
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Previous work has implicitly examined how the density of worm tubes influences water currents close to the sea bed (Bolam & Fernandes 2002). Friedrichs (2004) found that, for structures similar to *L.conchilega* tubes, roughness density (RD; the percentage of total surface covered) strongly influences the mode of bioengineering. Low numbers of tubes had a destabilising effect due to the generation of turbulence. At a critical density, interactions in turbulence interact to become a skimming flow; effectively creating a secondary benthic boundary layer above the epibenthic structures until water interacts with the tubes as if they were a solid structure (Friedrichs et al. 2000) (Figure 1.7). A switch from destabilising to stabilising conditions occurred at RD 2-4.5% (872-1961 tubes.m<sup>-2</sup>) and pure skimming flow was established at RD 4.5-8.8% (1961-3836 tubes.m<sup>-2</sup>). Natural beds of *L. conchilega* can reach 20,000 n.m<sup>-2</sup> subtidally (Ropert & Dauvin 2000) and 10,000 n.m<sup>-2</sup> intertidally (Ziegelmeier 1952).



Figure 1.7: Bioengineering effects of tubeworms switch from destabilising to stabilising as density increases and turbulence interacts to create a skimming flow. At a critical threshold density, a secondary boundary layer is created above the tubes (Friedrichs et al. 2000).

When *L. conchilega* forms dense reef-like aggregations they can support a more diverse community than tube-free sediments (Zühlke 2001, Rees et al. 2005), but even small groups can have positive effects on nearby communities and populations (Callaway 2006).

### Communities found in association with L. conchilega

As with other bioengineers, *L. conchilega* has both positive and negative effects on different components of its associated community. Taxa found in association with *L. conchilega* include certain polychaetes (*Phyllodoce mucosa, Harmothoe lunulata* and *Eumida sanguinea*), crustaceans (*Gammarus locusta* and *Microprotopus maculates*), juvenile bivalves (*Mya arenaria* and *Mytilus edulis*) and juvenile polychaetes (*Hediste diversicolor*). *H. lunulata* is often found inside the worm tube and *E. sanguinea* among the sand fringe. In contrast, species like polychaetes *Aphelochaeta marioni, Neanthes virens* and *Spio filicornis* and the gastropod *Hydrobia ulvae* are only found when worm densities are very low (Zühlke 2001). Despite negative effects, species richness and abundance are generally higher in *L. conchilega* aggregations compared to *Lanice*-free sediment (Callaway 2006).

### Effects of imitation sand mason tubes

Imitation *L. conchilega* tubes are being used increasingly in field studies as they are easier to manipulate than live worms. Imitation tubes are more rigid, solid rather than hollow, lack a tentacle fringe, and are more persistent over time (Callaway 2003b). Mimics are particularly good at imitating the increased shear strength and reduced erosion associated with real worm tubes. In some studies, imitation tubes affect *more* species than real *L. conchilega* tubes due to the rigidity of imitation tubes having a more pronounced effect on hydrodynamic properties (Callaway 2003b). Although imitation tubes only mimic certain facilitory mechanisms, they are useful tools for partitioning specific mechanisms of facilitation.

### 1.7 Summary

Conceptual models and empirical studies suggest that facilitation increases in strength and importance with environmental stress. However, evidence comes from a limited number of natural systems and more field studies are needed from different systems and for different mechanisms of facilitation to prove the generality of the hypothesis. Furthermore, there is currently a distinct lack of information on how the change in interactions will affect associated biodiversity. Interspecific interactions influence ecosystem-level

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processes in nature and through considering facilitation, some long-held paradigms have recently changed. By appreciating how interactions shift in importance, we could add to our understanding of relationships between biodiversity and disturbance, biodiversity and stability, and biodiversity and ecosystem functioning. Ultimately, this could lead to more effective conservation and restoration strategies being developed.

### 1.8 Aims of this work

The overarching aim of this study is to investigate how interspecific interactions shift along abiotic stress gradients in intertidal systems. I have identified two primary facilitators on whom I wish to focus as they represent the two main functional groups in soft-sediments: bioturbators and sediment-stabilisers. Independent of mechanism, both are expected to conform to the SGH. As such, there are two broad hypotheses that underpin this work:

- That positive effects of lugworm bioturbation on infauna are greater in hypoxic, rather than normoxic, intertidal sediments.
- That positive effects of tubeworm sediment stabilisation on infauna are greater at high, rather than low, current speeds.

There are also a number of additional objectives:

- 1) To describe the relationship between *A. marina* and common associated species across a sedimentary hypoxic stress gradient.
- To describe the relationship between *A. marina* and biodiversity across a sedimentary hypoxic stress gradient.
- To describe the relationship between *L. conchilega* and common associated species across a stress gradient of current speed.
- 4) To describe the relationship between *L. conchilega* and biodiversity across a stress gradient of current speed.

The structure of the thesis is as follows:

Chapter 2.	Concerns particular methodologies and instruments employed in the study and describes the geographical area where the work was carried.
Chapter 3.	An initial broad scale descriptive study, identifying infauna bioengineers in the study area and examining the relationship between their density, infauna biodiversity, and abiotic stress. Lugworms, <i>Arenicola marina</i> , were identified as the dominant engineer and so the study focuses on their changing role with sedimentary hypoxia.
Chapter 4.	Field manipulations to show that speculative relationships described in Chapter 1 were attributable to lugworm bioengineering. In this study, the vertical distribution of toxic hydrogen sulphide in the sediment is also related to lugworm activity.
Chapter 5.	A manipulative study examining the interrelatedness of sediment stabilising tubeworms, <i>Lanice conchilega</i> , community organisation and flow speed. This field experiment used tube mimics to study density effects at relatively low and high current speeds.
Chapter 6.	Laboratory work carried out in specially constructed benthic chambers. Here, bioengineers and associated benthic communities were exposed to a range of stresses to observe how interactions change across a stress gradient. The benthic chambers minimised confounding factors that may have influenced field studies.
Chapter 7.	A general discussion on all of the work covered in the Thesis.

## **Chapter 2**

## 2 Materials and methods

## 2.1 Field sites around North Wales



Figure 2.1: Overview of field sites in N Wales used for observational and *in situ* manipulative studies that tested the applicability of the SGH to lugworms and tubeworms.

## 2.1.1 Traeth Melynog (Traeth Abermenai)

Traeth Melynog (53°08'05"N 4°20'28"W) is located on the southernmost tip of Anglesey (Figure 2.1). It is characterised by muddy-sand biotopes, which are dominated by lugworm, *Arenicola marina,* and cockles, *Cerastoderma edule*. It is relatively sheltered as it is protected by the spit of land known as Abermenai Point (Figure 2.2).



Figure 2.2: Traeth Melynog (Abermenai) field site

## 2.1.2 Rhosneigr

Rhosneigr (53°13'05"N 4°31'31"W) is found on the Eastern coast of Anglesey (Figure 2.1). It is a relatively exposed site, as it is partially open to the Celtic Sea and ultimately the Atlantic Ocean. It is characterised by coarse sediment and outcropping rock (Figure 2.3).



## Figure 2.3: Rhosneigr field site

## 2.1.3 Inland Sea (Beddmanarch Bay and Traeth y Gribin)

The Inland Sea divides Holy Island from the main body of Anglesey (Figure 2.1). It is part of the Cymyran (Beddmanarch) SSSI, designated for ornithological and botanical interest. There are large areas of sandbank, mudflat and saltmarsh, as well as two strands of dune heath. Beddmanarch Bay forms the east portion of the bay and Traeth y Gribin the west. Both sites are sheltered from strong currents from the Irish Sea by Holy Island.



Figure 2.4: Beddmanarch Bay and Traeth y Gribin field sites

## 2.1.4 Church Bay

On the exposed north coast of Anglesey, is a sedimentary embayment amongst the cliffs called Church Bay (53°22'26"N 4°33'24"W) (Figure 2.5). It is composed primarily of sand and sandy mud.



Figure 2.5: Church Bay field site located on the exposed north coast of Anglesey.

## 2.1.5 Church Island

Church Island (53°13'17"N 4°10'07"W) is a small land-mass on the northern bank of the Menai Strait surrounded by muddy sand. The landward side of the island is sheltered from currents by the main rocky body of the island.



Figure 2.6: Church Island field site sheltered by elevated topography and outcropping rocks

## 2.1.6 Moel-y-don

Moel-y-don (53°11'03"N 4°13'22"W) is a sandbank on the northern bank of the Menai Strait. The landward side of the bank is sheltered and is composed of muddy sediments, whilst the outer flank is swept by tidal currents and is composed of sandy sediments.



Figure 2.7: Moel y don field site located along the Menai Strait

## 2.1.7 Foryd Estuary

At the southernmost end of the Menai Strait on the mainland side, the river Gwyrfai forms the Foryd Estuary (53°07'06"N 4°18'53"W). The estuary contains sandy mud to muddy habitats.



Figure 2.8: The Foryd Estuary is found at the southern tip of the Menai Strait

## 2.1.8 Hell's Mouth

At the end of the Llyn Peninsula is an exposed beach called Hell's Mouth (52°48'35"N 4°33'11"W). It is a moderately gentle shelving beach with large patches of sand from medium to low tide levels.



Figure 2.9: Hell's Mouth field site is one of the most exposed stretches of coastline in N Wales

## 2.2 Sediment profile imagery (SPI)

During the study, it was necessary to measure sediment oxygen profiles and the depth of the oxic-anoxic chemocline. In order to do this, I utilised sediment profile imagery (SPI), a relatively new technique that allows crosssectional images of the seabed to be taken through a prism that is punched into the sediment (Figure 2.10).

SPI was employed to estimate apparent redox potential discontinuity depth (aRPD), the depth at which conditions change from oxidising to reducing (Solan et al. 2002). The aRPD is marked by a marked change in the colour of the sediment from lighter shades of yellow, orange and brown to darker shades of brown, grey and black. SPI was conducted using a manually deployed Ocean Imaging Systems Model 3731-D L/W Sediment Profiling Camera. The SPI camera consisted of a prism-shaped imaging module that penetrated the sediment and took high resolution, in situ photographs of the sediment-water interface.



Figure 2.10: Sediment profile imager set up for intertidal use with details of the internals inside the waterproof housing. Indicated are the prism filled with distilled water (p), battery (b) and DSLR (d) camera.

The module housed a mirror mounted at 45° that reflected the image up to a Nikon D80 DSLR camera with a 35mm, f2.0 lens (52.5 mm 35 mm equivalent) (Rhoads & Cande 1971). The camera was set with an f10 aperture, a shutter-speed of 1/60 sec and an effective film speed of ISO 400. Images were saved in RGB (red-green-blue) colour with JPEG (Joint Photographic Experts Group) compression. Using ImageJ software (Version 1.5.0\_9), each image was converted into an RGB stack and the red slice selected. The sediment-water interface was then manually traced using a polygon tool and the water portion of the image removed from analysis. A custom-made, semi-automated macro was then used to save time. The macro converted each image into bitmap using adaptive thresholds of image pixel intensity and distribution. Typically, a histogram of pixel intensities was created and the valley point was chosen from a bimodal distribution to represent the split into relatively lighter and darker sediment. The number of pixels representing oxic sediment was then converted into aRPD depth.

## 2.3 Venturi flumes



Figure 2.11: Venturi flume set up at Traeth Melynog. Unidirectional current flow is indicated by an arrow and an eroded channel with coarser sediment is visible in the flume. Mimic straws were deployed into the flume and controls using a device constructed of nails. Above is a high density mimic treatment after being deployed.

Venturi flumes were used to increase current speed over experimental plots (Figure 2.11). Such flumes have been utilised in a number of previous studies for a similar purpose (Judge et al. 1992, Zühlke & Reise 1994, Powers & Peterson 2000), but my flume design was modified from one developed by Zühlke and Reise (1994).

A racetrack consisting of two pieces of marine plywood of 50 cm depth and 200 cm length was anchored into the sediment using 1-metre steel stakes. Boards were placed at a distance of 70 cm from one another, allowing room for a 50 x 50 cm experimental plot in the centre. At either end of the racetrack were attached funnels constructed from UPVC oyster netting. This allowed currents to be directed into the racetrack without creating excessive drag on the funnels.

## **Chapter 3**

# 3 Descriptive study of intertidal benthic bioengineers in northwest Wales

### 3.1 Abstract

Rapid global biodiversity loss compels us to try and understand how natural communities respond to disturbance. Facilitator species have the potential to buffer extinctions brought about by disturbance and their relative importance is predicted to increase with stress. Here, I tested whether facilitation through amelioration of anoxic stress by the lugworm, Arenicola marina, was relatively more important under abiotically stressful conditions. An observational study was conducted that examined how intertidal lugworm density related to benthic species richness and apparent redox potential discontinuity (aRPD) depth at sites of relatively low and high ambient hypoxic stress (AHS). I found a significant positive relationship between facilitator density and species richness at sites of high AHS that corresponded to a positive relationship between facilitator density and aRPD depth. I suggest that this is due to lugworm activity oxygenating the sediment, resulting in an increase in species richness. This theory was supported by hierarchical partitioning of potential predictors where facilitator density best explained species richness (58.2%) and aRPD depth (44.2%) at sites of high AHS, but had least effect at those of low AHS. These results suggest that facilitation through bioturbation is relatively more common in environments of high AHS compared to relatively benign systems. My work provides further justification for the use of facilitators in conservation and restoration projects that aim to maintain and promote biodiversity.

### 3.2 Introduction

Rapid loss of global biodiversity requires that we understand how natural systems respond to disturbance so that we can develop more effective conservation strategies (Solan et al. 2004; Larsen et al. 2005; Hughes et al. 2007). Amelioration of stress by facilitator species allows adjacent species to proliferate in environments they would not otherwise have been able to tolerate (Bertness & Callaway 1994; Callaway 2007). Furthermore, such facilitation could prevent extinctions and maintain species richness if conditions within a system became excessively stressful (Bruno & Bertness 2001). Knowledge of how and when these species interact may help inform new approaches to conservation and management (Halpern et al. 2007).

At present, our understanding of how interspecific interactions vary contextually is incomplete. Species interactions are ephemeral, yet are expected to vary predictably with disturbance regime (Bruno et al. 2003). Specifically, facilitation through bioengineered stress amelioration is expected to increase in relative importance with environmental stress (SGH) (Bertness & Callaway 1994). This is because, when abiotic stress is high. some species can only persist in the presence of a facilitator, compared to benign habitats where facilitation is not required (Bruno & Bertness 2001). This trend has been described for facilitators such as cushion plants (solar and wind stress) (Arroyo et al. 2003), nurse shrubs (drought stress) (Kitzberger et al. 2000), cordgrass (wave exposure) (Altieri et al. 2007), fucoid algae (desiccation stress) (Bertness et al. 1999) and mussel species (desiccation stress) (Bertness & Leonard 1997). There is, however, a lack of information on: 1) how common these interactions are in natural systems; 2) how interactions vary on a large spatial scale; 3) how density dependence influences interactions; and 4) how the shift in interactions alters community structure.

Relationships between facilitator traits, such as facilitator size or density, and species richness are also predicted to change with anoxic stress (trait-richness hypothesis) (Bruno & Bertness 2001). In benign systems, stress alleviation is not required and so richness is unaffected as facilitator traits

increase. However, in stressed systems, as facilitators reduce the stress, then richness will increase with benefactor density. This is predicted to occur until a threshold where stress is effectively nullified and competition between facilitator and adjacent species reduces richness.

Here, I explore how these concepts relate to the lugworm, Arenicola marina. Lugworms are a capitellid worm species that construct J-shaped burrows 10-25 cm deep on sedimentary shores (Reise 2002). The worms are nonselective deposit feeders that ingest sediment at depth that they then eject onto the surface sediment in casts of pseudofaeces. In construction and bioirrigation of these burrows, lugworms oxygenate the sediment and lower the apparent redox potential discontinuity (RPD) depth - the depth that marks a transition from chemically oxidative to reducing processes (Retraubun et al. 1996). The role of lugworms in intertidal communities has been well-studied such that it is a model species upon which to focus studies of interspecific relationships as it exhibits both strong facilitative and competitive interactions (Reise 1981; Flach 1992; Volkenborn & Reise 2006). Commensal species are attracted to particular parts of the lugworm burrow where they benefit from the association: the polychaete worms Pholoe minuta and Streptosylis websteri resist desiccation and profit from deposition of organic-rich fine particles in the funnel of the burrow; polychaetes Microphthalmus spp. and Capitella spp., oligochaete Tubificidae, and the crustacean amphipod Urothoe poseidonis benefit from oxygenation of the sediment in the feeding pocket; the polychaete Harmothoe sarsi resides in the oxic tailshaft; and the amphipod Bathyporeia sarsi is attracted to particles deposited by turbidity created by the cast (Reise 1981; Wetzel et al. 1995). Several negative associations have also been described: polychaetes Pygospio elegans, Nereis diversicolor and Scoloplos armiger, the seagrass Zostera noltii, and amphipod Corophium spp. are all excluded by sediment reworking and fluidisation (Rhoads & Young 1970, Reise 2002). Facilitationstress theory suggests that some of these interactions may vary contextually yet predictably with abiotic stress (Bertness & Callaway 1994). Associations that occur because of sediment oxygenation will be relatively more important in anoxic, compared to oxic, sediment as stress amelioration is required for

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susceptible species to survive. By ameliorating anoxic stress, adjacent species can proliferate that otherwise would have found the anoxic conditions intolerable (Reise 1981, 1983; Lackschewitz & Reise 1998; Volkenborn & Reise 2006).

I conducted a large-scale, observational study describing how lugworm density (facilitator trait) relates to sediment oxygenation and species richness at sites of low and high ambient hypoxic stress (AHS). I predicted that lugworm activity would only influence oxidation of the sediment at sites of high AHS. Here, facilitative buffering of stress would become relatively more important than disturbance caused by sediment reworking. I hypothesised that this would result in a positive benefactor density-richness relationship at anoxic sites. At sites of low AHS, I predicted that lugworms would have no effect on aRPD depth and thus sediment reworking would have only negative effects on species richness.

### 3.3 Materials and methods

### 3.3.1 Site selection

Intertidal sites with lugworm beds were selected to reflect relatively low and high ambient hypoxic stress (AHS) levels. In intertidal systems, AHS of the sediment is determined by dissolved oxygen in the overlying water (Nilsson & Rosenberg 1997), phytoplankton blooms (Webb & Montagna 1993), overlying vegetation (Valiela et al. 1997), biological oxygen demand within the sediment (Rhoads & Germano 1982) and exposure of the shore (Incera et al. 2003). Here, I chose coastal field sites that were similar, but that differed in wave exposure. Hence, AHS was related directly to exposure, as more exposed sites had a deeper aRPD depth linked to wave action (Incera et al. 2003). Calculation of exposure was based on the Thomas Index, which utilises fetch length and direction measured from GIS (ArcView<sup>™</sup> v.3.2.) and meteorological data to approximate wave energy (Thomas 1986; Ekebom et al. 2002). Meteorological data was obtained from R.A.F. Valley, Anglesey. Then, for each site, fetch and wind energy were measured at consecutive vectors separated by an angle of 7.5°, with a potential maximum fetch of 100 miles. Exposure E was subsequently calculated as:

$$E = \sum_{i=7.5^{\circ}}^{360^{\circ}} \log W_i \times \log \left[ 1 + \frac{F_i}{CS_i + 0.1DS_i} \right]$$

W = Wind energy; calculated as (percentage of time wind blows in a sector/100) x (wind speed [kn])

*F* = Fetch in nautical miles (MAX. 100)

CS = Extent in nautical miles of water < 6 m deep adjoining the shore

DS = Extent in nautical miles of water within the fetch < 6 m deep but not adjoining the shore

Seven sites were selected: Church Bay (CB), Hell's Mouth (HM), and Rhosneigr (RN) represented low AHS and Church Island (CI), the Inland Sea (IS), Moel-y-don (MD), and Traeth Melynog (TM) were of high AHS. Conditions were otherwise similar, with sediment that ranged from fine to medium sand (S1). At each site, a number of stations (3-6) were sampled based on shore heterogeneity and variance of lugworm density (n=34). Due to issues of site accessibility it was not possible to sample the same number of stations at every site. There was a maximum distance of 100 m between stations within a site. At every station, the same community and physico-chemical sampling strategies were employed.

### 3.3.2 Community sampling

Intertidal macrofauna communities were sampled to a depth of 15 cm using a PVC suction-corer (123 mm internal diameter). Due to the relative scarcity of fauna in intertidal sediment and based on pilot studies, five cores were taken at each station at each site and their contents combined to make a single sample. Samples were sieved in situ through a 500 µm sieve and any macrofauna retained in the sieve were fixed in 4% buffered formalin. Lugworm density was estimated by counting cast numbers (Volkenborn & Reise 2006). A quadrat (33 cm x 33 cm) was haphazardly thrown onto the sediment at each station and the number of worm casts per unit area was recorded. The quadrat was thrown six times at each station and an average density taken to account for patchiness and to best estimate how many lugworm burrows passed through the core samples and SPI images. During subsequent laboratory analysis, all macrofauna organisms were identified to the lowest taxonomic classification possible and enumerated. Biomass was calculated by blotting dry the organisms on absorbent paper before weighing them.

### 3.3.3 Physico-chemical sampling

At each station, sediment grain size, porosity, organics and sediment shear strength were measured to examine which abiotic factors influenced aRPD. Sediment shear strength was measured *in situ* using a TORVANE. Six measurements were taken at each station and a mean calculated to account for patchiness. Additional sediment was also taken for subsequent granulometric analysis. In the laboratory, *c*. 15 g wet sediment from each sample was weighed, dried at 80 °C in an oven for 24 h, and reweighed to

determine water content. These dry samples were then combusted at 650 °C for 24 h, and reweighed to establish organic content. The combusted samples were then sieved to remove the > 2 mm fraction and the smaller fraction was analysed by laser diffraction in a Malvern Mastersizer 2000 particle sizer. Each sample was measured three times by the instrument and a mean grain size calculated.

#### 3.3.4 Sediment profile imaging

Sediment profile imagery (SPI) was employed at each site to estimate aRPD (Solan et al. 2002). SPI was conducted using a manually deployed Ocean Imaging Systems Model 3731-D L/W Sediment Profiling Camera. Six images were taken at each station to account for patchiness. Using ImageJ software (Version 1.5.0\_9), each image was converted into a red slice. The sediment-water interface was manually traced and the water portion of the image removed. Each image was converted into a bitmap using adaptive thresholding of image pixel intensity and distribution. Typically, a histogram of pixel intensities was created and the valley point chosen from a bimodal distribution representing lighter and darker sediment. Pixels representing oxic sediment were converted into aRPD depth.

#### 3.3.5 Data analyses

To examine how lugworm effects changed contextually, generalised ANOVAs were used with lugworm density and AHS as predictors and both species richness and aRPD depth as response variables. Lugworm density was considered a covariate and the interaction between the factors was tested to see whether lugworm effects were modified by AHS.

Biological traits were used as an additional response variable. Each species was linked to be appropriate trait using the Biological Traits Information Catalogue (BIOTIC) developed by the Marine Life Information Network (MarLIN) ( <u>www.marlin.ac.uk/biotic/</u> ). Then the organismal biomass associated with each trait was summed to give a community estimate of that trait.

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To test whether lugworm density was the dominant influence on response variables, hierarchical partitioning (HP) was used. HP uses a hierarchical approach to decompose  $R^2$  through incremental partitioning. All predictors were used so that the average independent contribution of a predictor could be obtained. This allowed us to quantify the relative contribution of predictors to the total explained variance of a model, for all possible candidate models (after Cheven & Sutherland 1991). Other predictors included mean sediment grain size, sediment water content, organic content, shear strength and exposure. Where there was a significant lugworm influence, effects were investigated by carrying out regressions on lugworm density and both species' density and biomass of traits (e.g. feeding mode).

Multivariate analyses were conducted to examine lugworm effects on community organisation using PRIMER 6 (Clarke 1993; Clarke & Gorley 2006). Species abundances were initially  $\sqrt{\sqrt{-}}$ -transformed to downweight contributions of very abundant species (Clarke & Gorley 2006). Intersample similarities were then calculated using the Bray–Curtis similarity coefficient (Bray & Curtis 1957) and ordinated using non-metric multidimensional scaling (MDS). MDS ordinations were also created for traits based on log(X+0.1) biomass. Overlaying lugworm density as bubble size and trajectory allowed any effect of *A. marina* to be illustrated.

### 3.4 Results

There was a significant positive relationship between lugworm density (facilitator trait) and species richness (Figure 3.1a; Table 3.1). Furthermore, AHS had no influence on species richness and did not modify the effect of lugworm density. However, the degree to which lugworm density explained variability in species richness varied markedly between low and high AHS (Figure 3.1b hierarchical partitioning). At anoxic sites, variability was best explained by lugworm density (58.2% of effects), but at more oxic sites variation was best explained by a combination of sediment properties, including sediment water content (%) (35.5%), mean grain size (22.1%), and sediment shear strength (N.m<sup>-2</sup>) (21.0%). In these oxygen-rich environments, lugworm had a relatively small influence (8.0%).

Table 3.1: Effects of lugworm density (n.m<sup>-2</sup>) on species richness in sediment of high and low AHS ( $R^2$ =53.01%)

Factor	df	Seq SS	Adj SS	F	р
Lugworm density	1	75.769	35.999	8.25	0.007
High / low AHS	1	57.542	0.008	<0.01	0.965
Interaction	1	14.329	14.329	3.28	0.080
Error	30	130.860	130.860		
Total	33	278.500			

There was an overall significant negative relationship between lugworm density and aRPD depth (Figure 3.2a; Table 3.2). Apparent RPD was also significantly deeper at sites of low AHS. However, there was a significant interaction between these predictors which contributed to the overall negative trait-aRPD relationship. At sites of high AHS, there was a significant positive interaction between lugworm density and oxygen depth compared to no discernible effect of lugworms at oxic sites. This corresponds to hierarchical partitioning (HP) results that showed lugworm to have the greatest influence of measured predictors on aRPD at high AHS sites (44.2%), but to have the smallest at low AHS sites (2.2%) where variability was explained best by mean sediment grain size (39.6%) (Figure 3.2b).



Figure 3.1: a) Relationship between lugworm density and species richness at sites of low ( $\circ$ ) and high ( $\bullet$ ) AHS and b) from hierarchical partitioning, how lugworm density (ARE) contributes to the variance of species richness relative to other predictors including mean sediment grain size (µm) (GRA), sediment shear strength (N.m<sup>-2</sup>) (SHE), sediment water content (%) (WAT), sediment organic content (%) (ORG), and site exposure (EXP) at the AHS levels. There are overlapping data points on Fig 3.1a.



Figure 3.2: a) Relationship between lugworm density and aRPD depth at sites of low ( $\circ$ ) and high ( $\bullet$ ) AHS and b) from hierarchical partitioning, how lugworm density (ARE) contributes to the variance of aRPD depth relative to other predictors including mean sediment grain size (µm) (GRA), sediment shear strength (N.m<sup>-2</sup>) (SHE), sediment water content (%) (WAT), sediment organic content (%) (ORG), and site exposure (EXP) at the AHS levels.

Factor	df	Seq SS	Adj SS	F	р
Lugworm density	1	75.25	58.62	11.25	0.002
High / Iow AHS	1	791.82	411.18	78.90	<0.001
Interaction	1	69.92	69.92	13.42	0.001
Error	30	156.34	156.34		
Total	33	1093.34			

Table 3.2: Effects of lugworm density  $(n.m^{-2})$  on aRPD depth (cm) in sediment of high and low AHS (R<sup>2</sup>=85.70%)

Within high AHS sites, there were no trends between lugworm density and individual species' densities (S2). Neither were there trends between lugworm density and most biological traits. There were however significant negative relationships between lugworm density and both medium sized and immotile organisms (Table 3.3).

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Trait		$R^2$	p
Individual size (relative weight)	Small	<0.01	0.963
	Small-medium	0.67	0.423
	Medium	8.63	0.009
	Medium-large	1.70	0.210
Adult longevity	2 to 5 years	0.22	0.647
	>5 years	1.02	0.327
Reproductive technique	Sexual (broadcast spawner)	1.24	0.282
	Sexual (planktonic larvae)	0.04	0.848
Adult motility	Low	6.83	0.018
	Medium	1.24	0.281
Adult life habit	Sessile	0.02	0.889
	Swim	2.64	0.122
	Crawl	<0.01	0.963
	Burrow	1.21	0.286
Body flexibility	>45°	0.84	0.372
	<10°	0.01	0.925
Feeding habit	Filter/suspension	1.07	0.315
	Opportunist/scavenger	1.69	0.211
	Predator	1.47	0.243

Table 3.3: Relationships between lugworm density (n.m<sup>-2</sup>) and biological traits (g) in adjacent communities in hypoxic sediments.

Multivariate analysis showed that there were no relationships between increasing lugworm density and taxonomic or trait community structure at sites of high AHS (Figure 3.3).



Figure 3.3: Multi-dimensional scaling ordinations showing there is no pattern of effects of lugworm density on A) taxonomic ( $\sqrt{\sqrt{-transformed}}$ ) and B) trait (log(X+0.1)) community structure at sites of high AHS; lugworm density (n.m<sup>-2</sup>) is overlain as bubble size and trajectory.

### 3.5 Discussion

### 3.5.1 Facilitation by lugworms increases with abiotic stress

Through an observational study, I found a significant positive relationship between mimic density and species richness in intertidal sites of high hypoxic anoxic stress (AHS). This corresponded with a linear relationship between mimic density and aRPD depth. I propose that, in these anoxic sediments, lugworms were depressing aRPD depth through bioturbation and bioirrigation and that this increased species richness. Amelioration of AHS by lugworms is a common phenomenon in intertidal sediments (Reise 1981; Lackschewitz & Reise 1998; Riisgård & Banta 1998). Conversely, I found no relationships between lugworm density and either species richness and aRPD in sites of low AHS. At low AHS sites, sediment composition better explained species richness and aRPD variance compared to lugworm density. It is known that the bioengineering effects of lugworm activity vary contextually (Volkenborn et al. 2007a; Volkenborn et al. 2007b). What this study adds is a link between field studies that describe individual interactions (e.g. Volkenborn & Reise 2006) and facilitation-stress theory (Bertness & Callaway 1994).

In my study, the positive trait-richness relationship at high AHS sites was not explained by any particular interspecific interactions as there were no significant relationships between lugworm and individual species' densities. It is likely that site-specific confounding effects concealed any interspecific relationships over a landscape scale as community composition was different between shores. Neither was the trait-richness relationship explained by any organismal traits as there were no significant positive relationships between lugworm density and biomass of traits. However, there were negative relationships between lugworm density and both medium sized and immotile organisms at sites of high AHS. This indicates that a form of trophic group amensalism may have been occurring, where one trophic group is excluded by another, due to environmental modification by the second trophic group (Rhoads & Young 1970). An amensalism between organisms that rework and fluidise the sediment, such as *A. marina*, and sessile organisms that can stabilise it is common in natural systems (e.g. Volkenborn et al. 2009).

Densities of lugworms here were relatively high (up to 28.8 n.m<sup>-2</sup>), although much higher densities have been recorded in the literature (e.g. 85 n.m<sup>-2</sup> Cadée 1976). It is possible that at very high densities, lugworms would have driven community structure to one dominated by bioturbating infauna due to the exclusion of stabilising species (Callaway 2006; Volkenborn et al. 2009). Explicit studies examining which traits are promoted by facilitators are extremely important as they would allow us to understand the direction in which facilitators drive particular communities as stress increases.

### 3.5.2 Removal of lugworms through bait digging

Lugworms are harvested from foreshores in large numbers in the UK for use as bait for fishing (Olive 1993). Based on evidence from this study, if lugworms were removed in large numbers from sites of high AHS, then aRPD depth could decrease, causing a cascade of extinctions. However, removal of lugworms may also allow proliferation of otherwise excluded species (Volkenborn & Reise 2006, 2007; Volkenborn et al. 2009). Potentially, our improved understanding of the role of lugworms in structuring intertidal communities could be used when managing bait-digging to maintain local diversity and ecosystem function (Crain & Bertness 2006).

### 3.5.3 Facilitators as habitat restorers

Anoxic and hypoxic events associated with eutrophication are a growing problem in global coastal waters (Rosenberg et al. 1990; Karlson et al. 2002). Bioturbators have the potential to facilitate recovery after anoxic events by priming sediment for recolonisation (Norkko et al. 2002). This capacity of facilitators in general to buffer stress and regenerate habitats obviously has much more widespread implications in terms of conservation (Halpern et al. 2007). Most empirical research to date has been conducted in plant communities (e.g. Arroyo et al. 2003; le Roux & McGeoch 2008), but information from marine systems is growing (Kawai & Tokeshi 2007; van Wesenbeeck et al. 2007a). Facilitators are already being utilised in a few conservation and restoration projects, but as potential management tools their usage could be even more widespread, particularly in marine systems

(Crain & Bertness 2006; Halpern et al. 2007; Brooker et al. 2008; Funk et al. 2008).

## 3.6 Conclusion

This observational study found a positive relationship between lugworm density and aRPD, suggesting that dense beds of lugworms could generate deeper oxic layers. Correspondingly, a positive relationship between lugworms and species richness could be driven by this increase in oxic layer. More studies are needed to show whether lugworms are driving these patterns or other confounding factors are causing them.

## Appendix



Appendix 3.1: Mean sediment grain site across the field sites varied from fine to medium sand. Sites were all located in NW Wales and included Moel-y-don (MD n=5), Church Island (CI 5), the Inland Sea (IS 4), Church Bay (CB 6), Traeth Melynog (TM 6), Rhosneigr (RN 4) and Hell's Mouth (HM 5).

Appendix 3.2: Relationships between lugworm density and species' densities and biological traits in sites of high AHS

Hydrobia ulvae (R <sup>2</sup> =0	.004)			
	df	MS	F	р
Regression	1	1070	0.07	0.796
Res error	17	15527		
Total	18			
Tubificaidaa banadii (	$D^2 - 0.059$			
Tubilicoldes beliedir	K -0.000)	MC	-	
Pogrossion	1	10201	1.04	p
Reserror	17	9804	1.04	0.322
Total	18	3004		
10101	10			
Y627 6 167 167.2				
<i>Pygospio elegans</i> (R	<sup>2</sup> <0.001)			
	df	MS	F	р
Regression	1	0.6	<0.01	0.975
Res error	17	627.5		
lotal	18			
Scoloplos armiger (R	<sup>2</sup> =0.005)			
	df	MS	F	D
Regression	1	25.7	0.08	0.784
Res error	17	330.5		
Total	18			
	2			
Corophium spp. ( $R^2$ =	0.132)			
	df	MS	F	р
Regression	1	1572.1	2.59	0.126
Res error	17	607.4		
Total	18			
•				
Small organisms (R <sup>2</sup>	=<0.001)			
	df	MS	F	р
Regression	1	0.06	<0.01	0.963
Res error	17	26.57		
Total	18			
Small-medium organi	$sms (R^2 = 0.038)$	)		
onian modiant organi	df	/MS	F	n
Regression	1	17.25	0.67	0 423
Res error	17	25.56	0.01	0.120
Total	18	- 0 · 0 z		
Medium organisms (F	R <sup>2</sup> =0.337)			
	df	MS	F	a
Regression	1	152.11	8.63	0.009
Res error	17	17.63		
Total	18	sect. Constructs of		

Medium-large organi	sms (R <sup>2</sup> =0.091)			
	df	MS	F	q
Regression	1	41.01	1.70	0.210
Res error	17	24.16		
Total	18	(data Dilama te Facader)		
	0			
Medium longevity (R	-=0.013)			
	df	MS	F	р
Regression	1	5.69	0.22	0.647
Res error	17	26.24		
I Otal	18			
Long longevity (R <sup>2</sup> =0	0.057)			
	df	MS	F	р
Regression	1	25.58	1.02	0.327
Res error	17	25.07		
Total	18			
Broadcast spawners	(R <sup>2</sup> =0.068)			
	df	MS	F	р
Regression	1	30.62	1.24	0.282
Res error	17	24.78		
Total	18			
Egg laver/brooder –	nlanktonic larvae	$(R^2=0.002)$		
Lgg layon brooder	df	MS	F	n
Regression	1	1.00	0.04	0.848
Res error	17	26.52		0.0.0
Total	18	Annual T. S. Andréador (15)		
Low adult motility (R	==0.287)			
	df	MS	F	р
Regression	1	129.54	6.83	0.018
Res error	1/	18.96		
I OTAI	18			
Medium adult motility	(R <sup>2</sup> =0.068)			
	df	MS	F	p
Regression	1	30.66	1.24	0.281
Res error	17	24.77		
Total	18			
Sassila organisme (E	$R^2 = 0.001$			
Cessile Organisms (r	df	MS	F	n
Regression	1	0.53	0.02	0.889
Res error	17	26.55		0.000
Total	18	and the second sec		
Swimmers (R <sup>2</sup> =0.135	5)		2 Minute 17	
	df	MS	F	р
Regression	1	60.81	2.64	0.122
Res error	1/	23.00		
TOTAL	10			

## Crawlers (R<sup>2</sup><0.001)

0141013 (11 -0.001)				
, , , , , , , , , , , , , , , , , , , ,	df	MS	F	p
Regression	1	0.06	< 0.01	0.963
Res error	17	26.57		
Total	18			
- Total	10			
Burrowers (R <sup>2</sup> =0.067)				
	df	MS	F	p
Regression	1	30.12	1.21	0.286
Res error	17	24.80		
Total	18			
>45° flexibility (R <sup>2</sup> =0.0)	05)			
	df	MS	F	p
Regression	1	2.12	0.08	0.781
Res error	17	26.45		
Total	18			
$<10^{\circ}$ flexibility ( $P^2 = 0.0$	17)			
	+/)df	MS	F	r
Regression	1	21.29	0.94	0.270
Regiession Regiession	47	21.20	0.84	0.372
Res error	17	25.33		
Deposit leeders (R =0	.001) df	MS	F	
Regression	1	0.24	0.01	0.025
Pos orror	17	26 56	0.01	0.920
Total	10	20.50		
Total	16			
Filter feeders (R <sup>2</sup> =0.05	9)			
	df	MS	F	P
Regression	1	26.77	1.07	0.315
Res error	17	25.00		
Total	18	1 (1949) - Contractor (1975)		
Opportunists $(R^2=0.09)$	0)			
	df	MS	F	r
Regression	1	40.82	1 69	0.211
Reserror	17	24 18	1.00	0.21
Total	10	24.10		
IUIdi	10			
Predators (R <sup>2</sup> =0.079)				
· /	df	MS	F	l.
Regression	1	35.86	1.47	0.243
Res error	17	24.47		
Total	18	10000 1 500500 A51752		
TUIAI	1 ( )			

4 Differential effects of lugworm exclusion on porewater sulphide profiles and associated benthic community structure with varying sedimentary hypoxia
# 4.1 Abstract

According to the stress gradient hypothesis (SGH), facilitation is more important in stressed systems, compared to benign ones. Descriptive studies suggest that effects of lugworms (*Arenicola marina*) may vary with ambient hypoxic stress (AHS) of the sediment (Chapter 1). Here, I excluded lugworms from foreshores of low and high AHS and examined effects on H<sub>2</sub>S profiles and community organisation in order to test if lugworms were the driving force between any observed patterns. I predicted that positive effects of lugworms would be more important at high compared to low AHS.

Most lugworms were excluded and there were significantly fewer lugworms in exclusion plots compared to ambient and controls. In contrast to predictions, lugworms were significantly reducing H<sub>2</sub>S concentrations at 18 cm depth at both hypoxic sites, but also one of the normoxic sites. H<sub>2</sub>S profiles showed that my sites may not have been sufficiently different in oxygen status (i.e. at different ends of a sedimentary hypoxia stress gradient) to conform to the SGH. There may also have been a lack of power associated with the limited number of sites I used.

Lugworms had no effect on species richness, abundance or biomass. Lugworms did have significant effects on particular species though none supported the SGH: *Corophium* spp. were always negatively affected by lugworm activities; when present, *Bathyporeia* spp. always benefitted from lugworms; and *Scoloplos armiger* showed highly variable responses at a site level.

I suggest that interactions within lugworm-dominated ecosystems are more complex than anticipated due to multiple mechanisms of bioengineering resulting in strong negative and positive interactions simultaneously and that species interactions with lugworms will therefore not conform to the basic SGH. Future studies will need to try and fit different bioengineers to the SGH to see whether it is a useful tool that could be used in conservation and restoration projects or too much of an oversimplification of natural systems

# 4.2 Introduction

# 4.2.1 The stress gradient hypothesis (SGH)

Species interactions are important drivers of ecosystem structure and function (Hooper et al. 2005), yet our understanding of how and when they occur is incomplete (Bruno et al. 2003). Positive interactions resulting from stress amelioration may become increasingly important in abiotically stressed systems (the stress gradient hypothesis (SGH)) (Bertness & Callaway 1994, Callaway & Walker 1997, Brooker & Callaghan 1998), but the generality of this theory needs to be tested in multiple systems (Crain & Bertness 2006). If facilitator species maintain and promote more diverse communities as environmental stress increases, then they could be utilised in conservation and restoration of natural systems (Odling-Smee 2005, Boogert et al. 2006, Halpern et al. 2007).

Most empirical research testing the SGH comes from terrestrial plant and saltmarsh communities (review in Brooker et al. 2008), with a few studies from rocky shores (e.g. Bertness & Leonard 1997, e.g. Bertness et al. 1999a). Yet coastal sedimentary habitats are ideal systems within which to study facilitation as they contain diverse communities whose organisation is driven by species interactions and environmental forces (Crain & Bertness 2006). Furthermore, many marine habitats are formed by ecosystem engineers (Reise 2002), whose role could shift according to the SGH.

# 4.2.2 Lugworms as ecosystem engineers

Lugworms (*Arenicola marina*) are cosmopolitan components of particulate European foreshores (e.g. Beukema 1979). *A. marina* is a conspicuous bioengineer and, when present in high densities, can transform intertidal landscapes by bioturbation (Hüttel 1990, Wetzel et al. 1995, Retraubun et al. 1996b, Riisgård & Banta 1998, van Wesenbeeck et al. 2007b, Volkenborn et al. 2007b). Through sediment reworking, they both benefit and disturb nearby infauna (e.g. Volkenborn & Reise 2006). Lugworm activity can increase habitat heterogeneity (Cadée 1976), improve food supply (Reichardt 1988), provide refugia at low tide (Reise 1981b) and oxygenate the sediment, decreasing toxic sulphide levels (Wetzel et al. 1995). One observational study suggests that, in line with the SGH, facilitation through amelioration of hypoxic sedimentary stress by lugworms is more important in sites of high AHS (Chapter 1). However, descriptive studies can only indicate possible relationships; they cannot infer true causal relationships by ruling out alternative explanations. In this study, we experimentally excluded lugworms at sites of low and high AHS in order to find out whether interactions with lugworms do change from predominately negative to positive with increasing sedimentary hypoxia as predicted by the SGH. In doing so, I aim to find out whether lugworms were actually driving patterns in aRPD and biodiversity seen in Chapter 1 or whether it was due to confounding factors.

Removing lugworms from intertidal sediments leads to a reduction in sediment permeability, causing higher concentrations of ammonium, phosphate, silicate and sulphide in the porewater (Volkenborn et al. 2007a). Effects of lugworms on porewater chemistry are more pronounced in fine diffuse sediments, compared to coarser, advective sedimentary systems. This suggests that changes in effects of lugworm bioengineering are mediated by environmental factors. Here, I attempt to explain this change in bioengineering impact using the framework of the SGH.

#### 4.2.3 Hydrogen sulphide

Lugworms live in deeper anoxic sediment compared to most intertidal infauna, but obtain oxygen by actively pumping oxygenated water down through the burrow and over their gills (Hüttel 1990, Riisgård & Banta 1998). At low tide, when there is no supply of oxygenated water, lugworm switch from a normal aerobic metabolism to anaerobiosis (Zebe & Schiedek 1996). The metabolic rate drops and the worms are able to survive several days of anoxia (Groenendaal 1980, 1981). Hydrogen sulphide that is associated with sediment hypoxia is highly toxic to many benthic organisms as it inhibits cytochrome c oxidase, thus preventing aerobic metabolism (Vismann 1991, Bagarinao 1992, Giere 1992). However, lugworm density is inversely related to H<sub>2</sub>S concentration at the depth of their burrows (Groenendaal 1979) as lugworms oxygenate and detoxify the sulphide (Zebe & Schiedek 1996).

Consequently, lugworms are able to facilitate nearby organisms by ameliorating stress associated with H<sub>2</sub>S.

In this study, I exclude lugworms from different habitats in order to test whether the positive relationship observed between species richness and lugworm density found in Chapter 1 is driven by lugworm activity increasing the aRPD depth and, in doing so, test that sediment-mediated interactions with lugworms conform to the SGH. To do this, I test to see whether sulphide remediation by lugworm varies with AHS and what subsequent effect this has on the infauna community. I predict that when lugworms are excluded from sites of low AHS, there will be no effect on H<sub>2</sub>S distribution as there is already sufficient oxygen to oxidise sulphides and levels will be low. Subsequently, lugworms will have no effect on species richness at these low AHS sites because sulphide is not a dominant controlling factor. Conversely, I hypothesise that by excluding lugworms at sites of high AHS, that H<sub>2</sub>S concentrations will increase due to a lack sediment reworking and species richness will fall as infauna emigrate away from the toxic sulphides. In doing this, I hope to test the generality of the SGH by applying it to a new system, the sedimentary intertidal, and a new form of bioengineer, bioturbators.

### 4.3 Methods

A manipulative field study was conducted to test whether patterns with sediment oxygenation and species richness were attributable to habitat modification by lugworms and whether this was driven by changes in ambient hypoxic stress (AHS). Lugworms were excluded from shores of relatively low or high AHS in order that changes in H<sub>2</sub>S distribution and community structure could be attributed to their activities. This would add to my earlier work that suggested a relationship was present, but that did not prove causality (Chapter 1).

#### 4.3.1 Field sites and treatments

Experiments were conducted at four intertidal sites: Beddmanarach Bay and Traeth y Gribin (both normoxic and near the Inland Sea from Chapter 1), Traeth Melynog and Moel-y-don (both hypoxic). These sites were chosen as they were similar in most aspects - such as grain size and infauna communities - but differed in sedimentary oxygen status. Sedimentary hypoxia is influenced by many factors, including dissolved oxygen in the overlying water (Nilsson & Rosenberg 1997), phytoplankton blooms (Webb & Montagna 1993), biological oxygen demand within the sediment (Rhoads & Germano 1982) and exposure of the shore (Incera et al. 2003). I chose coastal field sites that were similar, but that differed in wave exposure. Hence, oxygen state was assumed to be directly related to exposure, as more exposed sites had a deeper aRPD depth linked to wave action (Incera et al. 2003). Calculation of exposure was based on the Thomas Index, which utilises fetch length and direction measured from GIS (ArcView<sup>™</sup> v.3.2.) and meteorological data to approximate wave energy (Thomas 1986; Ekebom, Laihonen & Suominen 2002) (Figure 4.1). Traeth Melynog and Moel-y-don are relatively sheltered and therefore more hypoxic, whereas Beddmanarach Bay and Traeth y Gribin are normoxic due to particle mixing caused by wave exposure (see Figure 2.1).



Figure 4.1. Wind rose for Valley meteorological station based on hourly data from 1992-2004. Percentage calm is given at the centre of the rose. Pers comm. Simon Neill.

At each site, three block locations were chosen that were similar in lugworm density and tidal height and that were a minimum of 10 m apart (Figure 4.2). Within each block, three treatments were applied, measuring 1 x 1 m and at a minimum distance of 1 m apart.



Figure 4.2: Experimental set-up to investigate differential effects of sedimentmediated lugworm interactions. Three blocks of three treatments were used per site: an ambient treatment that was left untouched, an exclusion treatment where mesh was dug 10 cm into the sediment, and an experimental control where sediment was dug over and then left. Experiments were set up at two normoxic sediment and two hypoxic sedimentary sites around N Wales and left for 10 months.

An exclusion treatment was created where a fine mesh (250  $\mu$ m) was dug 10 cm into the sediment and covered over. Exclusion of lugworms in this

manner is common practice in intertidal experiments in order to attribute changes to their influence (Flach 1992a, Volkenborn & Reise 2006). The two other treatments consisted of an experimental (procedural) control which was initially dug over to replicate disturbance caused by the exclusion treatment of digging in mesh, but that was then left untouched to control for the effects of digging, and an ambient treatment that was not altered in any manner. Treatments were left for ten months which should have been ample time for any changes to occur and then infauna and porewater were sampled.

#### 4.3.2 Porewater sampling and analysis

In order to sample porewater for hydrogen sulphide analysis, I used a multiple porewater sampler designed by Hüttel (1990) (Figure 4.3). The sampler allows porewater to be extracted from five depths simultaneously whilst maintaining its anaerobic properties.

The instrument consisted of a lance with a series of adjacent holes at depths I wished to sample: 2, 4, 7, 12 and 18 cm deep. Each hole was attached to a T-piece connector with opposing ends covered with 40  $\mu$ m gauze to prevent sediment particles being sampled. The T-piece was in turn attached to a hose (internal  $\phi$  1mm) which ran up the lance to a 50 ml syringe. Five hoses corresponding to the five depths were attached to syringes which were connected in parallel so that all samples could be drawn simultaneously. At the tip of each syringe was another T-piece and a non-return valve. This enabled porewater samples to be drawn and transferred into sample bottles whilst minimising contact with air that would have oxidised the H<sub>2</sub>S.



Figure 4.3: Porewater sampling lance (after Hüttel 1990, Volkenborn et al. 2007a). Gauze-covered holes allowed sampling at depths of 2, 4, 7, 12 and 18 cm. By attachment of a 1-way valve (circled), water was siphoned off into smaller syringes for sample storage without samples coming into contact with the air.

Porewater samples were taken from the middle of the treatment plot (c 50 x 50 cm) to minimise edge effects. From four random positions within this area 5 ml samples of porewater were taken and pooled. Sample syringes were stored in a cool-box with lid and icepacks in order to minimise oxygenation. Once back in the laboratory, they were immediately frozen until use.

In order to measure sulphide concentrations in the porewater I used the method described by Cline (1969). In brief, a reaction with N,N-dimethyl-*p*-phenylenediamine sulphate and ferric chloride was used in order to spectrophotometrically determine sulphide concentrations using a Jenway 6300 Visible Range Spectrophotometer. The spectrophotometer was calibrated using known concentrations of sodium sulphide.

### 4.3.3 Community sampling

In August 2009, intertidal macrofauna communities were sampled to a depth of 10 cm using a PVC suction-corer (123 mm  $\phi$ ). I sampled to a depth of 10 cm because only the upper layer could be sampled at exclusion sites due to

the net at 10 cm depth. Due to the relative scarcity of fauna in intertidal sediment, six cores were taken at each station at each site and their contents pooled in order to sample a representative portion of the benthic community. Once again, samples were taken from the central 50 x 50 cm portion of the plot in order to minimise edge effects. Samples were sieved *in situ* through a 500 µm sieve and any macrofauna retained were fixed in 4% buffered formalin. Lugworm density was estimated by counting casts in treatment plots (after Volkenborn & Reise 2006). During subsequent laboratory analysis, all macrofauna organisms were identified to the lowest taxonomic classification possible and enumerated. Biomass was calculated by blotting dry organisms on absorbent paper before weighing.

#### 4.3.4 Data analyses

Differential effects of lugworms with changing sedimentary hypoxia were tested for using a three-factor nested block ANOVA. Factors included oxygen status (normoxic/hypoxic), site (nested with oxygen status), and treatment (exclusion/control/ambient). Any changes in the effect of lugworm activity with hypoxia would be seen as a significant interaction between treatment and oxygen status (AHS). Response variables included species richness, total abundance and total biomass. The same ANOVA model was used to test whether lugworms had variable effects on individual organismal abundances.

Multivariate community effects were investigated using PRIMER 6 (Clarke 1993; Clarke & Gorley 2006). Species abundances were  $\sqrt{\sqrt{-}}$ transformed to downweight contributions of very abundant species (Clarke & Gorley 2006). Intersample similarities were calculated using the Bray-Curtis similarity coefficient (Bray & Curtis 1957) and ordinated using non-metric multidimensional scaling (MDS).

# 4.4 Results

# 4.4.1 Lugworm exclusion

Lugworm exclusions were successful in that the number of casts inside the exclusion plots was significantly lower than both ambient and control plots ( $F_{2,35} = 86.09$ ; p = <0.001) (Figure 4.4). However, a few full size casts and pits were evident inside the very edge of the treatment plot and smaller burrow structures were present in the centre. Besides treatment, no other factor had a significant effect. Lugworm densities were thus equivalent across sites.



Figure 4.4: Effectiveness of lugworm exclusion ( $\Box$ ) on lugworm density compared to experimental controls ( $\blacksquare$ ) and ambient treatments ( $\blacksquare$ ) at four intertidal sites in N Wales: Moel-y-don (MD), Traeth Melynog (TM), Beddmanarch Bay (BB) and Traeth y Gribin (TyG). Bars represent mean  $\pm$  SD (n = 3).

# 4.4.2 Hydrogen sulphide distribution

Profiles of H<sub>2</sub>S revealed that lugworms were altering sulphide distributions (Figure 4.5). Up to 18 cm depth, there were no differences in H<sub>2</sub>S concentration between treatments. Concentrations of hydrogen sulphide were significantly higher at 18 cm depth in exclusion plots at the hypoxic Moel-y-don ( $F_{2,8} = 8.81$ ; p = 0.016) and Traeth Melynog ( $F_{2,8} = 12.41$ ; p = 0.007) and normoxic Beddmanarch Bay ( $F_{2,8} = 6.60$ ; p = 0.031) compared to control and ambient treatments, but not at Traeth y Gribin.



Figure 4.5: Lugworm exclusion differentially influences sulphide porewater profiles. Shown are means and SD (n = 3). Lugworm exclusion compared to ambient sediment and experimental controls at Moel-y-don (MD), Traeth Melynog (TM), Beddmanarch Bay (BB) and Traeth y Gribin (TyG). Significant differences in treatments at depths are highlighted (\*). Samples were taken from surface water to represent sulphide concentrations in the overlying water (depth +1 cm). Dashed line indicates depth of mesh.

# 4.4.3 Community effects of lugworm exclusion

No lugworm effects (i.e. facilitation or competition) were independently evident at a community level where lugworm exclusion had no significant effects on species richness, abundance, or biomass (Table 4.1; Figure 4.6).

Had lugworms been driving patterns in these response variables, exclusion treatments would have been significantly different to control and ambient treatments. Furthermore, in contrast to my hypotheses, they were no significant interactions between hypoxia and lugworms for species richness, abundance or biomass (Table 4.1).

However, the largest effects on community structure were universally caused by changes in oxygen status and site-specific effects. Species richness and biomass were significantly affected by site and abundance by oxygen status (Table 4.1). Communities from normoxic sites were significantly more abundant than those from hypoxic sites.

Multivariate analysis using ANOSIM similarly showed that there was no effect of lugworms on community structure at either low or high AHS (Global R = -0.014; p = 0.564). Graphical summaries again showed that assemblage structure varied significantly based predominately on site location (Figure 4.7). However, there was noticeably more variability within the hypoxic sites (Beddmanarch Bay and Traeth y Gribin) shown by lower overall similarity perhaps caused by lugworms.

Table	4.1:	Effects	of	lugworm	exclusion	on	benthic	community	parameters	at
normox	kic an	nd hypox	ic s	ites in N V						

		Species richness					
Source	df	MS	F	p	F versus		
Oxygen status	1	81	1.09	0.407	Si(Ox)		
Site(Oxy status)	2	74.5	22.73	<0.001	RES		
Treatment	2	1.1944	0.22	0.809	TrXSi(Ox)		
Oxy statusXTreatment	2	10.5833	1.98	0.252	TrXSi(Ox)		
TrXSi(Ox)	4	5.3333	1.63	0.200	RES		
RESIDUALS	24	3.2778					
		Abundance					
A	df	MS	F	p	F versus		
Oxygen status	1	2807859	23.09	0.041	Si(Ox)		
Site(Oxy status)	2	121582.3	2.63	0.093	RES		
Treatment	2	11656.58	0.38	0.710	TrXSi(Ox)		
Oxy statusXTreatment	2	7837.028	0.25	0.789	TrXSi(Ox)		
TrXSi(Ox)	4	31072.03	0.67	0.619	RES		
RESIDUALS	24	46309.31					
		Biomass (Ln(n))					
	df	MS	F	р	F versus		
Oxygen status	1	10.6066	0.94	0.435	Si(Ox)		
Site(Oxy status)	2	11.3227	11.21	<0.001	RES		
Treatment	2	0.1871	0.17	0.849	TrXSi(Ox)		
Oxy statusXTreatment	2	0.0183	0.02	0.983	TrXSi(Ox)		
TrXSi(Ox)	4	1.094	1.08	0.387	RES		
RESIDUALS	24	1.0105					



Figure 4.6: Effects of lugworm exclusion on infauna assemblages at normoxic and hypoxic sites around N Wales. Bars represent ( $\Box$ ) exclusion treatments where mesh was dug 10 cm into the sediment to exclude lugworms; ( $\blacksquare$ ) experimental controls where sediment was dug over and then left; and ( $\blacksquare$ ) ambient treatments that were left untouched ( $\pm$  SD). Treatments were left for 10 months and sampled in August 2009.



Figure 4.7: MDS plot showing that lugworm effects (exclusion (E); ambient (A); and control (C)) are negligible compared to those of location. Variability within hypoxic sites (Moel-y-don and Traeth Melynog) is greater than normoxic sites (Beddmanarch Bay and Traeth y Gribin). Clusters are significant splits based on Simprof tests.

### 4.4.4 Species interactions with lugworms

Across the survey, the commonest species (mean abundance  $(n.m^{-2}) \pm SD$ ) were *Hydrobia ulvae* (2716 ± 3262), *Corophium* spp. (a mixture of *C. arenarium* and *C. volutator*) (1134 ± 1106), *Pygospio elegans* (812 ± 700), *Macoma balthica* (588 ± 672), *Bathyporeia* spp. (392 ± 672), *Retusa obtusa* (98 ± 109.2), *Scoloplos armiger* (54.6 ± 72.8), *Cerastoderma edule* (26.6 ± 25.2), *Nephtys* spp. (18.2 ± 21) and *Hediste diversicolor* (9.8 ± 14).

Of these species, there were only significant lugworm interactions with *Corophium* spp., and *Bathyporeia* spp. (Table 4.2; Figure 4.8). *Corophium* spp. were always more abundant in exclusion plots indicating that they were consistently inhibited by lugworms regardless of oxygen status. Unfortunately, as *Bathyporeia* spp. were only present at hypoxic sites I could not test for an effect of oxygen status, but where present *Bathyporeia* spp. were repeatedly facilitated by lugworms.

In general, oxygen status had the greatest effect on these populations relative to treatments and site-specific effects (Table 4.2; Figure 4.8). *H. ulvae, P. elegans, M. balthica* and *R. obtusa* were all more abundant in normoxic sites. Furthermore, *Bathyporeia* spp. and *S. armiger* were only present in hypoxic sites and *H. diversicolor* was absent from most hypoxic treatments.

Table 4.2: Effects of lugworm exclusion on the density of common infauna species abundances at normoxic and hypoxic sites around N Wales. Note that tests for *Bathyporeia* spp and *Scoloplos armiger* are from within the hypoxic sites as they were not present at the normoxic ones. %V = variance explained (SS<sub>factor</sub>/SS<sub>total</sub>).

	Hydrobia ulvae				Corophium spp. (In.x)					
Source	DF	MS	F	Р	MS	F	Р	F versus		
Ox	1	1344054	62.2	0.016	69.712	8.37	0.102	Si(Ox)		
Si(Ox)	2	21609.44	1.2	0.318	8.326	39.91	<0.001	RES		
Tr	2	13899.53	2.92	0.165	1.086	40.83	0.002	TrXSi(Ox)		
OxXTr	2	14394.69	3.03	0.158	0.142	5.32	0.075	TrXSi(Ox)		
TrXSi(Ox)	4	4752.28	0.26	0.898	0.027	0.13	0.971	RES		
RES	24	17956.33			0.209					
		<b>.</b> .	,				<i>a</i>			
0	<b>DF</b>	Pygospio	elegans		Macoma	balthica	(ln.x+1)			
Source	DF	MS	F	P		F	P	F versus		
Ox	1	62333.44	138.45	0.007	111.985	842.37	0.001	Si(Ox)		
Si(Ox)	2	450.22	0.6	0.559	0.133	0.37	0.694	RES		
Tr	2	1217.25	4	0.111	0.009	0.05	0.954	TrXSi(Ox)		
OxXTr	2	596.86	1.96	0.255	0.843	4.28	0.101	TrXSi(Ox)		
TrXSi(Ox)	4	304.06	0.4	0.805	0.197	0.55	0.702	RES		
RES	24	754.44			0.359					
8		Potusa ob	tusa							
Sourco										
	1	1344.44	71.6	0.014						
	ו ר	1044.44	0.96	0.014						
JI(UX)	2	51 59	1 47	0.455						
	2	22.60	4.47	0.095						
	7	11 53	0.53	0.234						
RES	24	21.81	0.00	0.710						
NLO	24	21.01								
Anno 1997 - 1997 - 1997 - 1997 - 1997 - 1997 - 1997 - 1997 - 1997 - 1997 - 1997 - 1997 - 1997 - 1997 - 1997 - 1										
		Bathypore	<i>ia</i> spp. (li	1.x+1)	Scoloplos armiger					
Source	DF	MS	F	P	MS	F	Р	F versus		
Si	1	1.591	3.11	0.103	98.000	8.48	0.013	RES		
Tr	2	2.282	4.47	0.036	7.389	0.64	0.545	RES		
SiXTr	2	0.077	0.15	0.862	62.169	5.38	0.022	RES		
RES	12	0.511			11.556					
0	Hediste diversicolor									
Source	DF	MS	F	P						
SI	1	0.056	0.06	0.813						
lr	2	0.389	0.41	0.672						
SiXTr	2	0.722	0.76	0.487						
RES	12	0.944								



Figure 4.8: Effects of lugworm exclusion on common infauna species from normoxic and hypoxic sites in N Wales. Bars represent ( $\Box$ ) exclusion treatments where mesh was dug 10 cm into the sediment to exclude lugworms; (**■**) experimental controls where sediment was dug over and then left (**■**); and ambient treatments that were left untouched. Treatments were left for 10 months and sampled in August 2009.

# 4.5 Discussion

#### 4.5.1 Testing the stress-gradient hypothesis (SGH)

The results of this study provide no community-level support of the SGH. There was no significant interaction between treatment and oxygen status on species richness, abundance or biomass. Total abundance varied significantly with oxygen status, but species richness and organismal biomass only varied significantly between sites. Any patterns of lugworm effects were overwhelmed by much larger site-specific effects and effects of sedimentary hypoxia. This is in contrast to my earlier descriptive study that suggested there was a relationship between lugworm, sedimentary hypoxia and community structure. These results could have occurred because either 1) the patterns in my observational study were not driven by lugworms but by other confounding factors and lugworm-dominated systems do not conform to the SGH; or 2) the experimental design I used here was not sufficient to partition differential effects of lugworm engineering with sedimentary hypoxia. H<sub>2</sub>S concentrations were similar, though more variable, to those found in other lugworm exclusion studies (Volkenborn et al. 2007a). Amelioration of toxic H<sub>2</sub>S by lugworms at depth was evident at Traeth Melynog, Moel-y-don and Beddmanarch Bay (Figure 4.5). As sulphide reduction was evident at one of my presumed normoxic sites, it indicates that my definition of hypoxic and normoxic sediments may have been unsuitable or that my choice of sites was inappropriate. These two limitations are the most obvious weak points in the study.

Furthermore, there was no support for the SGH from individual interactions. There were no significant interactions between treatment and oxygen status for the abundance of any of the most common species, though lugworms affected amphipods *Corophium* spp. and *Bathyporeia* spp. and the orbiniid polychaete *Scoloplos* cf. *armiger* independently of oxygen status. *Corophium* spp. was negatively affected by lugworm activities regardless of site or oxygen status; *Bathyporeia* spp. benefitted from the presence of lugworms, but was only present at hypoxic sites; and the effect on *S. armiger* varied with site. At Moel-y-don, lugworms appeared to be having a beneficial

effect as S. cf. armiger was more abundant in exclusion plots compared to ambient sediment though not procedural controls, indicating that sediment mixing is having an effect regardless of whether it was shovel- or lugwormbased. In contrast, at Traeth Melynog, there was no significant effect of treatment on S. cf. armiger. Associations between lugworms and these three species are relatively well understood and have been described in a number of previous studies: Bathyporeia spp. benefit from cast-associated turbidity (Hüttel et al. 1996, Lackschewitz & Reise 1998, Reise 2002), Corophium spp. are disturbed by sediment reworking and are exposed to predation when they leave the sediment (Flach 1992a, 1992b, 1993, Flach & Debruin 1993, 1994), and S. cf. armiger are thought to benefit from low sulphide concentrations near tail shafts (Hüttel 1990, Volkenborn & Reise 2006). An unexpected effect of lugworm exclusion was the response of the opisthobranch Retusa obtusa (the Arctic barrel-bubble). At hypoxic sites, they were only recorded when lugworm were also present. This interaction has not been described before to my knowledge, though their presence in lugworm beds has been recorded (Lackschewitz & Reise 1998). R. obtusa lives in the top 3.5 cm of surface sediment (Smith 1967a, 1967b) and because it lives so high in the sediment, I suggest that it is being facilitated through a mechanism other than sediment oxygenation; maybe through the cast in a similar manner to Bathyporeia spp. (Lackschewitz & Reise 1998).

#### 4.5.2 Lack of lugworm effects

Fewer lugworm interactions were recorded compared to previous studies (Volkenborn & Reise 2006, 2007, Volkenborn et al. 2009). The lack of any major effects could have been caused by a number of factors: no effect of lugworm exclusion or a lack of statistical power and/or poor experimental design.

Previous lugworm exclusion experiments have observed both strong positive and negative sediment-mediated interactions not observed here: negative effects on *Hediste diversicolor*, *Nephtys hombergii*, *Pygospio elegans*, *Capitella capitata* and *Cerastoderma edule* (e.g. Reise 1981b, Brey 1991, e.g. Flach 1992a); and positive associations with platyhelminthes,

foraminifera, *Microphthalmus* spp., *Capitella* sp., tubifid oligochaetes, *Urothoe poseidonis* and *Harmothoe sarsi* (Reise 1985, 1987, Lackschewitz & Reise 1998, Reise 2002). Most negative interactions are a result of disturbance caused by sediment reworking by lugworm, but the positive interactions come from a number of commensalisms within the lugworm burrow (summary in Reise 2002). I predicted that interactions occurring because of oxygenation of sulphide would shift along a hypoxic stress gradient, but it may be that the suite of sediment-mediated lugworm interactions is too complex to fit to the SGH. Interactions resulting from modifications other than sediment oxygenation – including increasing sediment roughness, creation of refugia, and fluidisation of sediment (Reise & Ax 1979, Reise 1981b, 1981a, Reichardt 1988, Wetzel et al. 1995, Riisgård & Banta 1998) – would not change with oxygen status and may have been why I recorded no net community effects.

Alternatively, my experiment could have been of an inappropriate design. However, my design was based around previous studies that used a similar degree of replication and found significant effects (see Volkenborn & Reise 2007). Using mesh to exclude lugworms is common practise in intertidal field studies (e.g. Volkenborn & Reise 2006). Some of these studies have excluded lugworms from much larger areas of foreshore (e.g. 400 m<sup>2</sup> in Volkenborn and Reise 2006), and it may be that 1 m<sup>2</sup> exclusions were not sufficient to change sediment porewater chemistry. Larger areas of exclusion are more likely to change chemistry on a landscape scale. Yet other studies have found significance using similar size lugworm manipulations (Flach 1992a) and I detected a change in sulphide distribution here. Another possibility is that I was unable to detect changes in the very rare species here due to their patchy distribution. In order to allow time for the sedimentology and community to change, I let the experiment run for 10 months and sampled when lugworm activity was optimal. Effects were seen within this period in other studies (Volkenborn & Reise 2006, 2007, Volkenborn et al. 2009). An alternative explanation is that my sites differed in some way from those other study sites. My experiment assumes that sediments are relatively more or less hypoxic or normoxic. Evidence from

observational studies suggests that sediment-mediated lugworm effects change between these locations. However, sulphide profiles suggest that there may not have been a sufficient gap in oxygen status to detect a shift in interactions with lugworms. One possible solution would be to look at truly hypoxic and oxic sediments, but then grain size would change dramatically and associated communities with it. I chose to try and keep grain size constant in order to remove its potentially confounding effects.

### 4.5.3 <u>Scale</u>

Scale is always an important consideration in benthic ecology. Lugworms are known to facilitate on a microscale, due to commensalisms with regions of their burrows (Reise 2002). However, many of these associations were not observed here and this may have been due to the small scale of my experiment. For example, *Harmothoe* spp. are known to reside in lugworm burrows, but at relatively low densities; one worm in every two or three burrows (Beukema 1979, Lackschewitz & Reise 1998). Some of these interactions may have been missed due to the scale of sampling. Furthermore, exclusions were carried out within a landscape wholly modified by dense lugworm beds, thus the larger species pool would have been already altered by their activity. Thus, although a relative change in interactions could be observed, the experimental design did not allow for true immigration of different species.

### 4.6 Conclusion

Lugworm interactions were found to be similar to those observed in other studies (i.e. promotion of *Corophium spp*. and exclusion of *Bathyporeia spp*.). However, these interactions were not found to vary with AHS, either because there was genuinely no effect or because of limitations with the experimental design. I suggest that lugworm-dominated systems may be too complex to fit to the SGH, but more studies are needed that partition different sediment-mediated lugworm interactions in order to test this theory. Specifically, mimics could be used to imitate sulphide amelioration and pit and mound construction in order to isolate specific interactions.

# 5 Effects of sediment stabilisation by tubeworms on adjacent species vary with flow speed

# 5.1 Abstract

High levels of abiotic stress typically reduce biodiversity in natural communities. However, stress-ameliorating facilitation is predicted to increase in relative importance in line with abiotic stress and may buffer this loss of diversity, hence maintaining species richness. Using field manipulations, I examined how benthic species richness associated with mimics of the tubeworm, Lanice conchilega, varied with tube density and current speed on a muddy-sand shore. Four densities of mimic (0%, 0.1%, 1%, and 10% roughness density, RD) were inserted into the sediment at low (control) and high (disturbance) current speeds augmented by experimental Venturi flumes. Mimic density had no effect on species richness in the controls, but there was a significant positive density-richness relationship in the flumes. As RD approached 10%, species numbers increased in flumes to control levels. Tubeworms were facilitating small organisms that live at the sediment-water interface, Pygospio elegans and Corophium spp.. Here, community benefits correlated with a significant increase in sediment shear strength, suggesting that mimics were preventing erosion. Mv results suggest that facilitators are able to buffer increasing stress and maintain biodiversity and moreover provide further evidence that facilitators could be a useful tool in conservation and management.

# 5.2 Introduction

Interactions between species are often ephemeral, varying circumstantially with parameters including population density and abiotic stress (Brooker & Callaghan 1998). The idea that these interactions vary contextually but predictably is central to community ecology, yet our understanding of when exactly interactions change is incomplete (e.g. Bertness & Callaway 1994, Rodriguez 2006, e.g. Brooker et al. 2008). Here, I consider how the pervasiveness of facilitation (i.e. positive species interactions caused by trophic links and/or habitat modification) may influence biodiversity and determine how systems respond to abiotic stress. By understanding how interspecific interactions relate to biodiversity and environmental drivers, I could inform new strategies for conservation of ecosystem structure and function.

Though facilitation can occur through a number of different mechanisms, most research has concentrated on facilitation through environmental stress Here, facilitator species buffer abiotic stress and allow amelioration. organisms to survive in environments they would not otherwise have been able to tolerate e.g. algal species extending the vertical distribution of understory species by reducing desiccation stress (Bertness et al. 1999b). Facilitation via stress amelioration is expected to be more important in abiotically stressed, rather than benign, habitats where its role is essential for maintaining ecosystem structure and function (facilitation-stress hypothesis, Bertness & Callaway 1994). In addition, the shape of the relationship between facilitator traits (such as density) and species richness is also predicted to shift with abiotic stress (the stress gradient hypothesis (SGH), Bruno & Bertness 2001). This is because as a facilitator trait increases in a benign habitat, it is unlikely to have any positive effect on associated species because the facilitator is not required (Callaway 2007). Conversely, in more stressful environments, species richness is likely to be low until facilitator traits reach a level where stress amelioration occurs (Heck & Wetstone 1977). The facilitation-richness paradigm shifts if competitive exclusion is considered, as at medium to high stress, competition will manifest once the stress is fully ameliorated and species richness will peak at intermediate levels of facilitator trait.

Field studies supporting facilitation-stress theory come from a limited number of natural systems. Most empirical evidence comes from terrestrial plant (e.g. Sthultz et al. 2007, Brooker et al. 2008) and saltmarsh (e.g. van Wesenbeeck et al. 2007a, e.g. Crain 2008) communities, with fewer studies from marine rocky shores (Bertness et al. 1999b). In order to formulate broad conceptual models of the interrelatedness of facilitation, biodiversity and environmental drivers we require empirical evidence from multiple systems. Currently, there is limited evidence from sedimentary, intertidal systems. Such habitats are ideal for testing facilitation-stress theories as they are amongst the most productive and diverse systems on Earth and experience sharp temporal and spatial gradients of stress (Suchanek 1994).

The sand mason, Lanice conchilega, is a bioengineering polychaete worm that inhabits intertidal and subtidal sediments (Callaway 2006, Rabaut et al. 2007, Van Hoey et al. 2008). L. conchilega constructs a characteristic tube, composed of cemented coarse sand grains, with the anterior end of the tube topped with a fringe of tentacles projecting 1-4 cm from the sediment (Zühlke 2001). Tubeworm tubes may be as long as 65 cm and are typically 0.5 cm diameter (Ziegelmeier 1952, 1969). Tubes facilitate by providing a refuge from predation, oxygenating the sediment at depth and by promoting sediment deposition (Van Hoey et al. 2008). Perhaps the most conspicuous method of tubeworm bioengineering though is stabilisation/destabilisation of surrounding sediment. At high densities, tubes can prevent sediment erosion by decelerating current flow and increasing sediment shear strength, a sediment's maximum resistance to shearing stresses (Jones & Jago 1993, Friedrichs et al. 2000). Through this mechanism of sediment stabilisation, tubeworms are able to buffer benthic species from disturbances associated with increasing current speeds (Callaway 2006).

Bed stabilisation by tubeworms is both density and diameter dependent (Eckman et al. 1981, Friedrichs et al. 2000, Bolam & Fernandes 2002,

Friedrichs 2004). As roughness density (RD; the percentage of total surface covered) increases, a new benthic boundary layer is created above the epibenthic structures until water interacts with the tubes as if they were a solid structure. Friedrichs et al. (2000) found that for tubes similar to a sand mason's, a switch from destabilising to stabilising conditions occurred at RD 2-4.5% (872-1.961 tubes.m<sup>-2</sup>) and pure skimming flow (sensu Morris 1955) was established at RD 4.5-8.8% (1,961-3,836 tubes.m<sup>-2</sup>). In skimming flow, maximum turbulent kinetic energy and shear stress production occur above the bed (Eckman et al. 1981). Such numbers are not uncommon, as aggregations of L. conchilega can reach 20,000 n.m<sup>-2</sup> subtidally (Ropert & Dauvin 2000) and 10.000 n.m<sup>-2</sup> intertidally (Ziegelmeier 1952). Any change in flow regime will have a large impact on how benthic species between the structures are influenced (Biles et al. 2003), but no empirical study has specifically examined how interactions and diversity associated with tubeworms vary with flow regime. Here, I use mimic sand mason tube to effectively partition and isolate this specific mechanism of facilitation as other interactions, such as effects of the fringe or influence of the worms feeding, are not imitated (Woodin 1978, Friedrichs et al. 2000, Callaway 2006).

This study does not attempt to examine how tubeworms influence adjacent fauna as a number of mechanistic studies have already examined sedimentmediated effects of *L. conchilega* (e.g. Callaway 2003a, 2006, e.g. Rabaut et al. 2007). Rather, I hope to use contemporary theory to explain how the importance and prevalence of sediment stabilisation by the worms changes with environmental drivers and aim to prove the generality of the SGH.

I hypothesize that increasing densities of mimics will increase sediment shear strength independently of current speed. Furthermore, I hypothesise that this increase in shearing strength in flow-dominated systems will have positive effects on the associated community, increasing species richness in line with the SGH. This is based on the assumption that as erosion decreases and skimming flow moves turbulence away from the substratum, the physical disturbance to benthic species will be buffered. Conversely, in benign, low current systems, I expect tube density to have no effects on communities.

# 5.3 Methods

Through field manipulations, I studied the effects of tubeworm mimic density on benthic species richness and sediment shear strength at low and high current speed.

# 5.3.1 Site selection

Experiments were carried out within a single biotope at Traeth Melynog on Anglesey, NW Wales. Current speed is naturally low here and so was left as a control. High current speeds were generated by the use of experimental Venturi flumes (based on Judge et al. 1992, Zühlke & Reise 1994, Powers & Peterson 2000). Flumes consisted of two boards of marine plywood (50 cm x 200 cm) fixed into the sediment 70 cm apart using steel stakes (Figure 5.1a). Previous work has mapped the effects of such flumes on currents in detail and so variation in hydrodynamics within the flume with tidal movement were known (Judge et al. 1992, Powers & Peterson 2000). Pilot studies showed that the racetrack part of the flumes had no effect on infauna on their own (Appendix p95).



Figure 5.1: a) Venturi flume designed to increase current speed over a treatment area b) deployment device to implant tubeworm mimics in the sediment at a high density treatment (4358 tubes.m<sup>-2</sup>).

Four sections of oyster netting (50 cm x 200 cm) were attached to the ends and staked out to form funnels. Current speed was measured 10 cm above the sediment in flumes and controls using a flow meter on an ebbing spring tide. Speeds were consistently higher in flumes (21.49 cm.s<sup>-1</sup>  $\pm$  2.48) compared to controls (11.70 cm.s<sup>-1</sup>  $\pm$  1.86) (Appendix 1).

Eight treatment plots (30 cm x 30 cm) were randomly designated in the study area, representing four densities of L. conchilega mimic at relatively low and high current speed. Mimics were used preferentially over live worms in order to isolate a specific mechanism of tubeworm bioengineering - that of sediment stabilisation. Mimics consisted of straws of length 15 cm and 0.5 cm ø, inserted to a depth of 12 cm (Figure 5.1b). Densities were based on previous studies to increase the likelihood of a skimming flow (Friedrichs et al. 2000): 0%, 0.1%, 1% and 10% RD. Mimics were deployed using a rack of nails, onto which the straws were placed (Figure 5.1b). The rack was then flipped and the nails and straws forced into the sediment. On removal of the rack, only the straws remained in the sediment. I used this device on all treatments to ensure continuity i.e. for controls, an empty rack was forced into the sediment. I thus had two treatments: low (no flume) and high (flume) current speeds, both with four densities of mimic. Plots were always > 10 m from each other and treatments within flumes were placed 20 cm away from the adjacent boards in order to minimise edge effects.

One month after setup, the benthic community was sampled and sediment shear strength was measured to see if mimics had altered it. Sediment shear strength was measured using a torvane. Six measurements were taken per plot at the sediment surface and a mean calculated to account for patchiness. The flumes were then moved and reinserted into the sediment at a minimum distance of five metres from any previous treatments. The experiment was repeated five times to provide replication from April 11<sup>th</sup> to May 14<sup>th</sup>, May 14<sup>th</sup> to June 13<sup>th</sup>, June 13<sup>th</sup> to July 18<sup>th</sup>, October 2<sup>nd</sup> to 31<sup>st</sup> (all 2008) and December 8<sup>th</sup> 2008 to January 6<sup>th</sup> 2009.

### 5.3.2 Community analysis

Intertidal macrofauna communities were sampled to a depth of 15 cm using a PVC suction-corer (123 mm internal diameter) after the treatments. Seven cores were taken in each treatment plot - as this was the maximum the size allowed - and their contents combined to ensure that a sufficient amount of fauna was sampled. Samples were then sieved *in situ* through a 500 µm sieve and any macrofauna retained in the sieve were fixed in 4% buffered formalin. During subsequent laboratory analysis, all macrofauna organisms were identified to the lowest taxonomic classification possible and enumerated.

# 5.3.3 Statistical analysis

General linear models (GLM) were used to test whether current or RD significantly influenced species richness or shear strength independently. The interaction between current and RD was also calculated to see whether any effect of RD was significantly modified by current. A series of GLMs were used to test for effects of current and RD on abundance of the ten commonest species (log(n+0.1)). A Bonferroni correction was used to maintain the familywise error rate by testing each hypothesis against a higher statistical significance ( $\alpha/n$ ). Multivariate analyses were conducted using PRIMER 6 to see whether facilitated communities differed in structure from controls (Clarke 1993, Clarke & Gorley 2006). Species abundances were  $\sqrt{\sqrt{-}}$  transformed to down-weight contributions of outliers (Clarke & Gorley 2006). Intersample similarities were calculated using the Bray–Curtis similarity coefficient (Bray & Curtis 1957) and ordinated using non-metric MDS (e.g. Kruskall & Wish 1978).

# 5.4 Results

# 5.4.1 Species richness

Species richness varied significantly with flow speed and tube density, and the interaction between these two factors (Table 5.1; Figure 5.2). In flumes, species richness increased significantly with tube density, whereas in controls, species richness was unaffected.

Table 5.1: General linear model of the effects of tubeworm density (0-10% RD) and current regime (low/ high speed) on associated benthic species richness ( $R^2 = 66.57\%$ )

Source	df	F	р
log(RD+0.1)	3	5.56	0.003
Flow	1	16.67	<0.001
Interaction	3	10.13	<0.001

# 5.4.2 Physico-chemical changes

Sediment shear strength varied significantly with tube density alone (Table 5.2; Figure 5.2). Shear strength increased with mimic density regardless of flow.

Table 5.2: General linear model showing the effects of tubeworm density (0-10% RD) and current regime (low/ high speed) on associated sediment shear strength ( $R^2 = 51.12\%$ )

Source	df	F	р
log(RD+0.1)	3	9.71	<0.001
Flow	1	3.07	0.090
Interaction	3	0.43	0.734



Figure 5.2: Differential effects of sediment-mediated tubeworm engineering. Relationships between tubeworm mimic density (log (n+0.1)) and a) associated species richness and b) sediment shear strength (N.m<sup>-2</sup>) at low ( $\circ$ ) and high current speed (x). There are overlapping data points in both figures.

#### 5.4.3 Species responses to tubeworm mimics

Of the ten most common species, the density of six varied strongly with flow regime and/ or RD (Table 5.3; Figure 5.3). *Hydrobia ulvae* and Oligochaeta were less abundant in flumes compared to controls and were unaffected by tubeworm mimics. *Capitella capitata* were less abundant in flumes, but increased in density with mimics regardless of flow. *Pygospio elegans* and *Corophium spp*. were less abundant in flumes and decreased with tubeworm mimics in controls, but increased in density with them in flumes. *Macoma balthica* was the only species to decrease in density as mimic density increased regardless of flow. However, only *H. ulvae, P. elegans* and

*Corophium spp.* varied significantly after the Bonferroni correction estimated the critical significance level at 0.005.

# 5.4.4 Community changes

Sampled communities were largely similar between controls and formed two closely related, but distinct clusters with flume communities associated with RDs of 1-10% (A and B) (Figure 5.4). In general, communities in flumes with lower densities of mimic formed two separate assemblages (C and D). As RD increased in flumes the communities typically became more similar to controls.

Seasonal effects did not influence how density-richness relationships varied with stress here (see Appendix p95).



Figure 5.3: Differential effects of increasing tubeworm density on common intertidal species. Relationship between roughness density (log (n+0.1)) and species abundance at two levels of abiotic stress, low ( $\circ$ ) and high current speed (x) for the ten commonest species in field study sites. Some data points overlap; n=5 for all treatments at all densities.

Table 5.3: GLMs of the effects of tubeworm mimic density (RD 0-10%) on species abundance under different hydrodynamic conditions (control/ high current speed); species trait information from www.marlin.ac.uk/biotic; critical significance level = 0.005.

				Mimic density (log(RD+0.1))		Flow speed		Interaction	
Taxon	Habit	Feeding method	R <sup>2</sup>	F	р	F	р	F	р
Hydrobia ulvae	Free-living	Surface deposit	0.359	1.64	0.200	11.66	0.002	0.44	0.724
OLIGOCHAETA	Free-living	Detritivore	0.216	0.69	0.566	5.76	0.022	0.33	0.803
Scoloplos armiger	Burrow- dwelling	Sub-surface deposit	0.280	2.74	0.059	1.66	0.207	0.85	0.478
Pygospio elegans	Tube-dwelling	Surface deposit	0.931	33.45	*<0.001	160.45	*<0.001	57.69	*<0.001
		Suspension feeder							
Cerastoderma edule	Free-living	Suspension feeder	0.117	0.99	0.408	0.86	0.361	0.14	0.936
Eteone cf. longa	Free-living	Predator	0.121	0.65	0.587	1.65	0.208	0.27	0.849
		Scavenger							
Bathyporeia spp.	Free-living	Scavenger	0.188	0.78	0.513	0.73	0.400	1.45	0.247
		Omnivore							
Corophium spp.	Burrow-dwelling	Surface deposit	0.728	4.87	*0.007	24.12	*<0.001	15.68	*<0.001
		Suspension feeder							
Macoma balthica	Burrow-dwelling	Surface deposit	0.335	3.05	0.042	1.67	0.206	1.77	0.172
		Suspension feeder							
Capitella capitata	Free-living	Sub-surface deposit	0.386	3.45	0.028	4.66	0.039	1.69	0.188



Figure 5.4: MDS ordination showing the effects of tubeworm density (RD 0-10%) on community structure ( $\sqrt{\sqrt{-transformed}}$ ) under different hydrodynamic conditions (control/ high current speed); dashed lines represent significant clusters at a 68% similarity level (SIMPROF). In general, clusters A and B denote control communities and flume communities with 1-10% RD, whilst clusters C and D represent flume communities with 0-1% RD.
#### 5.5 Discussion

By examining interactions between tubeworm mimic density, current speed and species richness, I showed for the first time that the stress-gradient hypothesis applies to tubeworms that stabilise sediment. My mimic tubes increased sediment shear strength and appeared to buffer erosion of the sediment (Friedrichs et al. 2000, Callaway 2006). Under normal flow conditions, this increase in sediment cohesion had no effect on community structure, but at high flow speeds, species richness was maintained relative to controls. Moreover, as abiotic stress increased, the bioengineering role of the tubeworm increased in importance, seemingly buffering mortality.

I also demonstrated that there is a positive relationship between benefactor density and beneficiary density (Bruno & Bertness 2001). As mimic density increased at low stress, it had no effect on species richness; the current was not sufficient to disturb the community and facilitation was therefore not required to buffer the disturbance. At higher current speeds, the relationship shifted so that there was a significant positive relationship between mimic density and species richness. It is likely that, as density increased even further, competitive exclusion would have begun to operate as space became limited (Bruno & Bertness 2001). Furthermore, competitive exclusion would be stronger in nature as here mimics only benignly competed for space, not other resources. Additional studies using a broader range of RDs could be used to better observe the shape of this relationship.

From my regression, in high stress systems, the richest communities are predicted to occur as RD approaches 10%. Previous studies have shown that skimming flow is achieved at RD 4.5-8.8% for a current speed of 5 cm.s<sup>-1</sup> (Friedrichs et al. 2000). Here, I demonstrated that benefits to the community can be detected at much lower densities. Commensalisms with real *L. conchilega* tubes are common in nature, but I did not expect them to operate here due to the lack of a sand fringe and realistic surface for attachment (Callaway 2003b, 2003a, 2006). Thus, low densities of mimic may simply have been facilitating by increasing habitat heterogeneity and providing some basic form of refuge from predation (Van Hoey et al. 2008).

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#### 5.5.1 Trophic group amensalism

*L. conchilega* promoted specific organisms (*Corophium spp.* and *Pygospio elegans*) and I suggest this was due to the biological traits of these species. Both *Corophium spp.* (burrow-dwelling amphipods) and *P. elegans* (a tube-dwelling spionid) are small, surface-dwelling organisms found predominately in sheltered environments, where tidal flow is weak (<0.5 m.s<sup>-1</sup>) (Jensen 1985, Hughes 1988, Bolam 2004). They are hence particularly susceptible to any kind of hydrodynamic disturbance. The maximum current speed recorded in my flumes was only 0.23 m.s<sup>-1</sup>, but this was enough to have a negative effect on both species. Unfortunately, it was not possible to record current speed between the tubes in the field, otherwise I could have quantified the extent of current deceleration.

Multivariate analysis showed that tubes of RD 1-10% within flumes maintained a community structure similar to controls. However, though not observed here, it is possible that at even higher stresses, species such as *Corophium spp.* and *P. elegans* could be promoted by facilitation to the detriment of other species (Rhoads & Young 1970, Snelgrove & Butman 1994). Both of these species are known to increase sediment shear strength in natural systems and an amensalism between stabilising and destabilising species has been described previously (Meadows & Tait 1989). The amensalism suggests that at very high densities of tubeworm, deposit feeding species such as *A. marina* and *C. edule* may be negatively impacted (Rhoads & Young 1970, Volkenborn & Reise 2006).

#### 5.6 Conclusion

Tubeworm mimics increase sediment shear strength independently of overlying current speed. This increase in sediment stability may be causing an increase in species richness at high current speeds where erosion and turbulence can be buffered. Additional studies are need to show which organisms benefit from this mechanism and at what speeds facilitation begins to manifest.

#### 5.7 Appendix



Appendix 5.1: Pilot study of effects of flume racetrack (control) versus ambient sediment. Racetracks were left in place for one month and then sampled in the same position as the treatments in the flumes. At the same time, faunal samples were taken from adjacent sediment. Both sets of samples were analysed and the communities compared. Communities were found to be equivalent.



Appendix 5.2: Current speeds in experimental Venturi flumes (n = 4 for each) and controls (n = 10). Measurements were taken using a flow meter during the ebbing of a spring tide to record maximum tidal velocities. The number of replicates reflects the most measurements that the tide allowed.

□ None

0.1% RD

■ 1% RD

🗖 10% RD





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Appendix 5.3: Seasonal variations in effects of tubeworm mimic density on benthic species richness at low and high current speeds; RDs (roughness density) are the percentage of surface sediment covered by the structures.

# 6 How interactions with tubeworm mimics shift along a gradient of current speed – a mesocosm study

#### 6.1 Abstract

Few experimental studies testing the stress gradient hypothesis (SGH) are able to determine at what level of stress facilitation begins or to describe the shape of the relationship between stress and facilitation along the complete stress gradient i.e. determining whether it is accelerating, linear, asymptotic or hump-backed. Chapter 4 showed in a field experiment that positive effects of sediment stabilisation by tubeworms *Lanice conchilega* were relatively more important for associated species at higher compared to lower current speeds. However, in order to utilise benefits associated with facilitation in conservation and management of natural systems, we first need to understand precisely how they vary in importance with environmental drivers.

In this study, I used benthic mesocosms to set a stress gradient of current speeds and examined the response of benthic communities with and without tubeworm mimics to buffer the stress. Communities consisted of *Hediste diversicolor, Corophium* spp., *Macoma balthica, Cerastoderma edule* and *Hydrobia ulvae*. Flow speed around tubes was slower than controls without tubes and there was increased turbulence compared to controls. Yet, neither current speed nor mimics had a significant effect on biomass of *C. edule, H. diversicolor, H. ulvae* or *M. balthica*. The only positive support of the SGH occurred at the highest current speeds, where significantly more *C. volutator* died in controls compared to mimic treatments indicating that facilitation was essential for their survival.

I conclude that, at current speeds approaching *c*. 9 cm.s<sup>-1</sup>, the importance of sediment-stabilising biomodification by *L. conchilega* becomes more important for one out of 5 species only, probably because it is small, surface-dwelling species. The importance of facilitation for this one species increased suddenly when stress increased above a threshold instead of gradually increasing as stress increased. However, due to limitations of the benthic chambers I was unable to generate flow as high as observed in my flume study and the range of current speeds in the flume study was insufficient to describe the shape of the facilitation-stress relationship over an entire stress gradient.

#### 6.2 Introduction

Species interactions are predicted to shift in mode and importance with environmental stress (Bertness & Callaway 1994, Bertness & Hacker 1994). Specifically, interactions resulting from habitat modification are expected to change in relative importance (Bruno & Bertness 2001). In stressed systems, positive interactions caused by stress amelioration are predicted to be relatively more important and may be able to negate and overtake negative effects associated with competitive exclusion (the stress gradient hypothesis (SGH)) (Bertness & Callaway 1994). This is based on assumptions that facilitating bioengineers (benefactors) will ameliorate stress and thus allow associated species (beneficiaries) to survive in environments they would otherwise have found intolerable (extend their realised niche).

Empirical evidence generally supports the theory that facilitation is more important in stressed compared to benign systems (e.g. Bertness et al. 1999b) but descriptions of the shape of the facilitation-stress relationship over a stress gradient are scarcer and it is not known at what level of stress that stress amelioration begins. Conceptual models predict that facilitation begins to increase in importance at intermediate levels of stress, where stress begins to overtake competition and predation in relative importance. Facilitation is anticipated to be most important at medium-high stress levels, before the stress becomes too great to buffer (e.g. Bruno & Bertness 2001).



Figure 6.1: Conceptual model of how species interactions may change in importance with abiotic stress (from Bruno & Bertness 2001).

Along an environmental stress gradient, mechanisms of facilitation are also predicted to change (Bruno & Bertness 2001) (Figure 6.1). At low levels of stress, facilitation of beneficiary species by a bioengineering benefactor can occur through the provision of associational defences (i.e. protection from predators/competitors), but under these low stress circumstances the role of associational defences is not as essential for the survival of organisms as it is when stress is high (Crain & Bertness 2006). However, in stressed systems the role of stress amelioration is thought to be essential for the survival of some species. It has been suggested that knowledge of facilitation could be utilised in conservation and management to target these benefactor species (Odling-Smee 2005, Crain & Bertness 2006, Halpern et al. 2007). However, if benefactor species are to be used in restoration and conservation projects it is first important to understand how their role varies with environmental drivers (Halpern et al. 2007). This is because at certain levels of stress they may actually have negative effects on associated species due to competitive interactions and their protection or promotion may lead to negative impacts on ecosystem structure and function (Bruno & Bertness 2001).

Sedimentary, intertidal systems, are ideal environments to study the SGH as they are naturally exposed to sharp physico-chemical gradients. In such systems, ecosystem engineering by infauna can either stabilise or destabilise the sediment (Reise 2002). The tubeworm Lanice conchilega is a ubiquitous component of European sedimentary shores (e.g. Degraer et al. 2008) and facilitates by providing a hard structure in soft sediment systems, offering refugia from predation, altering near-bed currents, oxygenating sediment (though to a much lesser extent than A. marina), and by stabilising the sediment by increasing shear strength and decreasing erosion (Zühlke 2001, Callaway 2006, Rabaut et al. 2007, Van Hoey et al. 2008). Evidence suggests that facilitation through sediment stabilisation is more important at low, compared to higher, current speeds and thus conforms to the SGH (Chapter 5). What is not known is at what current speed sediment stabilisation begins to benefit infauna species and how the relative importance changes over a gradient of current speeds.

In this chapter, I examined the effect of tubeworm mimics on an artificially assembled community over a current speed gradient in order to test the hypothesis that positive interactions are most important at medium-high levels of environmental stress and to find out at what current speed facilitation begins to act. The experimental community was composed of species that commonly co-occur with L. conchilega in nature: Cerastoderma edule, Macoma balthica, Hediste diversicolor, Hydrobia ulvae and Corophium spp. (Zühlke 2001). These species were chosen to represent a range of functional groups and sensitivities to increasing water flow (Table 6.1). Here, in methodology adapted from plant ecology, interactions were measured as a difference in live biomass before and after interactions for each associated species (Armas et al 2004). My methodology assumed that organisms would die or decrease in biomass within the duration of the experiment due to stress of increasing current speeds. Negative effects of tube worms on biomass would be indicated by a lower biomass when in the presence of the tubeworm mimics and positive interactions by a relatively higher one at high currents but not at low (e.g. Armas et al. 2004).

This added to my previous work by 1) implicitly testing for the level at which stress amelioration begins to act and by 2) describing the change in interactions over multiple stress levels rather than just at low/high. I hypothesised that the mimic's role of sediment stabilisation would become more important for associated species as flow speed increases. In doing so, mimics would ameliorate negative effects of increasing current speed, buffer physiological stress, and facilitate sensitive components of the artificial community. I expected this benefit to be greatest at medium-high current speeds, before the stress become too big for mimics to counteract (Bruno & Bertness 2001). Moreover, I predicted that species benefiting the most would be small, surface-dwelling organisms.

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#### 6.3 Methods

In mesocosms, the response of species to increasing flow was measured with and without tubeworm mimics to investigate whether the role of facilitation by tubeworms increases in importance with increasing stress. Interspecific interactions were measured as a change in living biomass after one week i.e. mimics compared to controls: a positive change would indicate facilitation, a negative change competition.

Imitation *L. conchilega* tubes are more practical to use in field studies as they are easier to manipulate than live worms. Imitation tubes are more rigid, solid rather than hollow, lack a tentacle fringe, and are more persistent over time (Callaway 2003b). Mimics are particularly good at imitating the increased shear strength and reduced erosion associated with real worm tubes that I was interested in for this experiment. Although imitation tubes only mimic certain facilitatory mechanisms, they are particularly useful tools for partitioning specific mechanisms of facilitation.

#### 6.3.1 Benthic mesocosms

Experiment carried out in benthic mesocosms were constructed so that stressors could be manipulated whilst limiting confounding factors (Figure 6.2). Mesocosms were used as they allow confounding factors to be controlled and specific environmental drivers (e.g. current speed) to be manipulated. Mesocosm design was based on benthic chambers developed by Biles (2002). They consisted of Perspex cylinders of 20 cm ( $\phi$ ) x 30 cm (h) sealed with Perspex caps at top and bottom. On the Perspex lid was bolted a motor (RS 440.082) capable of generating 60 rpm that ran off a 12 volt mains controller. A stainless steel spindle ran from the motor, through the lid and into the chamber, where it was attached to an impeller. The impeller consisted of a revolving skirt (*c*. 15 cm  $\phi$ ) that created an annular flow with greatest bottom shear stress towards the chamber wall (Biles 2002). Though this meant that shear was not equal across the sediment surface, it was considered that this was still the most practical means of

generating a current within a benthic chamber of limited size. Through the mains controller, the RPM of the motor and skirt could be adjusted.



Figure 6.2: Benthic chambers with treatments applied: a) Control with low current speed and b) control with high current speed. Note that due to the current in b) there is more suspended sediment and that the sediment topography has changed.

#### 6.3.2 Measuring current speeds within the chambers

A Met-flow Ultrasound Velocity Profile (UVP) Monitor (Model UVP-DUO with software version 3.0) was used to map the currents within the mesocosms so conditions could be related to natural systems (Figure 6.3). The UVP measures a velocity profile in liquid flow along an ultrasonic beam axis by detecting a phase shift of echoed ultrasound as a function of time. The transducer is very small and so could be placed inside the chambers with minimal disturbance to the flow. In use, the transducer was placed parallel to the wall of the chamber at a distance of 4 cm as this was where maximum current velocities would occur (Jaco Baas, pers comms).



Figure 6.3: Current profiles through the benthic chambers recorded using a Met-flow UVP-DUO instrument. Measurements were taken parallel to the side wall and 4-5 cm from the transducer head where current speeds were predicted to be greatest (a). The transducer was placed in the chambers without obstructing the impeller (b).

#### 6.3.3 Mesocosm community

Mesocosms were housed in a temperature-controlled laboratory at a constant temperature of  $15.0 \pm 0.5^{\circ}$ C and were continually aerated. Laboratory lights were left constant and consisted of 3 x 36W fluorescent tube lights.

Sediment for the benthic community was initially frozen to kill any macrofauna present. It was then sieved through a 1000 µm mesh in order to remove coarse particles and any dead macrofauna present. Seawater was added to the mesocosms first and then the sediment added slowly to avoid air bubbles. Sediment was filled to a depth of 20 cm and left for 24 hours before macrofauna was added. The benthic community was chosen to both be representative of local intertidal muddy sand systems and represent a range of functional groups with varying sensitivity to chosen stressors (Table 6.1). Densities were chosen that were typical of local infauna and that were not so high as to have large competitive interactions (based on observations from my descriptive studies): *Hediste diversicolor* (1 per benthic chamber), *Corophium spp.* (5), *Hydrobia ulvae* (10), *Macoma balthica* (2) and *Cerastoderma edule* (1).

#### 6.3.4 Treatments and response variables

To study the ability of tubeworms to buffer increasing current speed, four current speeds were chosen to represent benign to relatively higher stress levels within the benthic chambers: 0, 3, 6, and 9 cm.s<sup>-1</sup>. Previous studies have found that *Corophium* spp. can be impacted at speeds as low as 1 cm.s<sup>-1</sup> (Ford & Paterson 2001) and my upper velocity was set by the capabilities of the system. Experiments with tubeworm mimics at 7.5% RD (roughness density) were compared to controls where there were none. Thus, I had eight treatments - four current speeds x presence/absence facilitator mimic – that were replicated four times. This allowed us to test the effect of increasing current speeds on individual species with and without the presence of tubes mimicking a benefactor species.

Mimic treatments had imitation tubeworm tubes placed in the sediment prior to introduction of the community to avoid direct mortality caused by the insertion of the straws Mimics were plastic drinking straws 10 cm long and were inserted so that 3 cm protruded from the sediment at densities of 7.5% RD. Organisms were blotted dry and weighed prior to introduction to the chamber. They were then introduced carefully without disturbing the sediment. Communities were left for an hour to allow the organisms to establish themselves in the sediment. Motors were then switched on to generate currents within the chambers. Treatments were then left to run for seven days, before sediment was sieved through a 2000 µm mesh overlying a 1000 µm mesh to carefully remove the fauna. The length of time was chosen to provide the maximum number of treatment replicates given my time constraints.

In plant ecology, interactions are measured using a change in biomass (Armas et al. 2004) and in this study I apply this principle to intertidal marine communities. Competition and facilitation are not separated (Goldberg et al. 1999), but the net effect of interactions is calculated, so that a relative change in strength can be observed. Though I use mimics and not live worms, I make the assumption that tube mimics would still compete for space

with the larger organisms. Thus, response was measured as a relative change (%) in living biomass over the duration of the experiment.

#### 6.3.5 Data analysis

Statistical analyses were designed to test the effects of tubeworm mimics at different current speeds. Initially, GLMs were used to detect any effect of mimics on flow near to the sediment surface with increasing current speed. Changes in biomass were then analysed using a GLM of mimic and current speed that also tested for any potential interaction between these two factors.

TAXON	Hediste diversicolor	Corophium volutator	Hydrobia ulvae	Cerastoderma edule	Macoma balthica
PHYLUM	Annelida	Crustacea	Mollusca	Mollusca	Mollusca
CLASS	Polychaeta	Eumalacostraca	Gastropoda	Pelecypoda	Pelecypoda
FOOD	Detritus Plankton Other macrofauna	POM Bacteria Diatoms	Detritus Microalgae	Plankton POM	Diatoms Phytoplankton Detritus
SIZE	Med (11-20 cm)	Small (1-2cm)	V small (<1cm)	Small-med (3-10cm)	Small (1-2cm)
FRAGILITY	Fragile	Intermediate	Robust	Robust	Intermediate
HABIT	Burrow dwelling	Burrow dwelling	Free living	Free living	Burrow dwelling
POSITION	Infaunal	Infaunal	Infaunal	Epifaunal	Infaunal
FEEDING MODE*	SDF ASF Grazer	Scavenger Sc & Omni	SDF & SSDF PSF Sc & Omni	SDF	SDF ASF
MOBILITY	Burrower Crawler Swimmer	Burrower Swimmer	Crawler Burrower Swimmer	Crawler Drifter	Burrower
SUBSTRATUM	Mud to sandy mud	Fine clean sand	Mud to sandy mud	Mud to sandy mud	Mud to sandy mud
FLOW	Negligible to weak (<1 kn)	-	Negligible to weak (<1 kn)	Negligible to moderate (1-3 kn)	Weak to moderate (1-3 kn)
EXPOSURE	Sheltered to ext sheltered		Sheltered to ext sheltered	Ultra Sheltered to sheltered	Sheltered to ext Sheltered

### Table 6.1: Taxonomic and functional classification of taxon used in benthic mesocosms (from www.marlin.ac.uk/biotic)

\*SDF/SSDF=surface/subsurface deposit feeder; P/ASF=passive/active suspension feeder; Sca=scavenger; Omni=omnivore

#### 6.4 Results

#### 6.4.1 Current speeds

My benthic chambers generated a flow of up to c. 10 cm.s<sup>-1</sup>. Tubeworm mimics created conspicuous turbulence and decreased flow speed at the sediment surface compared to controls (Figure 6.4 & 6.5). At the highest current speeds suspended sediment was evident in the whole water column.





Figure 6.4: Effect of tubeworm mimics on turbulent flow in benthic chambers at low ( $\Box$ ), medium ( $\blacksquare$ ) and high flow ( $\blacksquare$ ). Points are mean flow speeds ± SD (n = 4000) measured along a vector from the front of the transducer every 0.02 s for 1 min intervals at the sediment surface (see Figure 6.3a). Mimics reduce the average flow velocity and cause turbulence at the sediment surface, evident from the heterogeneous current flow. Black arrow indicates where flow starts to interact with the wall of the chambers (~7 cm from transducer); red arrows are approximate locations of tubeworm mimics.



Figure 6.5: Effect of tubeworm mimics ( $\blacksquare$ ) on flow velocity compared to controls ( $\blacksquare$ ) close to the sediment surface (at heights of 5 and 15 mm) in benthic mesocosms. Bars are mean speeds  $\pm$  SD (n = 68) taken at 0.75 mm increments along a vector from the transducer head (Figure 6.3a). No statistical tests were conducted as all readings were taken in the same chamber and were not true replicates.

In controls, high speed treatments altered sediment topography, eroding sediment at the edge of the chamber and depositing it in the centre so that a dome shape formed with centre approx. 3 cm higher than edge. In mimic treatments, this change was not evident; instead, small bed forms formed around tubes. Sediment erosion produced dips in the sediment behind the tubes in the direction of flow (Figure 6.6).



Figure 6.6: Small bedforms form around the tubeworm mimics at the highest current speeds (~  $9 \text{ cm.s}^{-1}$ ) due to scour in the direction of flow.

#### 6.4.2 Biomass effects

There was no effect of current speed or mimics on biomass of *Cerastoderma edule, Hediste diversicolor, Hydrobia ulvae* or *Macoma balthica* over the course of the experiment (Table 6.2; Figure 6.7). However, the highest current speeds reduced the numbers of *Corophium volutator*, which led to a significant decrease in live biomass. Dead individuals were found floating in the flow or buried in the sediment. Here, mortality was lower in mimic treatments where any disturbance was buffered, indicated by significant interactions between flow speed and mimics.

Table	6.2:	Differential	effects	of	tubeworm	mimics	on	organismal	biomass	with
varying	g flov	v speeds in I	penthic of	cha	mbers (AN	OVA)				

	Ce	rastoderma edule				
Source	df	MS	F	р		
Mimic	1	0.48	0.04	0.849		
Current speed	3	22.33	1.71	0.192		
Interaction	3	16.26	1.24	0.316		
RESIDUALS	24	13.07				
	Co	rophium spp.				
	df	MS	F	р		
Mimic	1	2116.6	8.26	0.008		
Current speed	3	2455.7	9.58	<0.001		
Interaction	3	863.5	3.37	0.035		
RESIDUALS	24	256.3				
	He	diste diversicolor				
	df	MS	F	р		
Mimic	1	0.447	0.07	0.799		
Current speed	2	0.629	0.09	0.963		
Interaction	2	8.274	1.22	0.323		
RESIDUALS	24	6.762				
	Hy	Hydrobia ulvae				
	df	MS	F	р		
Mimic	1	15.77	0.48	0.497		
Current speed	2	9.00	0.27	0.845		
Interaction	2	20.16	0.61	0.616		
RESIDUALS	24	33.14				
	Ma	acoma balthica				
	df	MS	F	p		
Mimic	1	18.3	0.11	0.741		
Current speed	2	25.0	0.15	0.927		
Interaction	2	186.8	1.14	0.354		
RESIDUALS	24	164.4				



Figure 6.7: The effect of tubeworm mimics on biomass loss caused by increasing current speeds. Points represent means  $\pm$  SD (n = 4).

#### 6.4.3 Behavioural effects

During the experiments I noticed that flow influenced the behaviour of *H. ulvae*. At the end of some treatments, some *H. ulvae* had moved up onto the side walls of the benthic chambers and, when present, onto and even into tube mimics. As I did not start recording *H. ulvae* position until halfway through the study, it was not possible to do statistical tests on the data due to lack of replication on some treatments.



Figure 6.8: Position of *Hydrobia ulvae* in benthic chambers after exposure to different flow speeds. Proportions are locations of ten organisms after one weeks exposure (n = 2-3).

It appears as if more *H. ulvae* are found on tank walls at the highest current speeds compared to lower speeds (Figure 6.8). Furthermore, when mimics are present, *H. ulvae* are also more likely to be found on these at high current speeds. This is significant as it suggests that the mud snails may have been using the mimics as a refuge from stress, but equally they may simply have been seeking elevation.

#### 6.5 Discussion

#### 6.5.1 Evidence for the stress gradient hypothesis (SGH)

There were no effects of tubeworm mimics on *Cerastoderma edule, Hediste diversicolor, Hydrobia ulvae* or *Macoma balthica* at any of the flow speeds. As there were no effects on these species, there was no evidence to support the SGH (Bertness & Callaway 1994, Bruno & Bertness 2001, Crain & Bertness 2006). However, at the highest current speeds, tubeworm mimics reduced the mortality of *Corophium volutator*. Here, positive effects became more important at the highest generated current speeds (*c*. 9 cm.s<sup>-1</sup>) in accordance with the SGH. Therefore, in this study, facilitation began to manifest at this level for one out of five species. In addition, mimics also appeared to provide a refuge and surface for attachment for *H. ulvae* at the highest speeds. In the tubeworm's natural environment there is no alternative hard substratum available as a refuge.

My experiment was based on the assumption that a decrease in organismal fitness caused by increasing current speeds would result in a drop in species' biomass over the course of the experiment. Infauna are naturally disturbed by increasing flow in a number of different ways (Hall 1994):

- Physical damage
- Erosion of sediment dislodging organisms
- Change in sediment distribution
- Risk of smothering from increased suspended sediment
- Disruption to feeding mechanism
- Loss of habitat

In this study, based on my experimental setup and timeframe, the two main factors impacting organisms were likely to have been physical damage and erosion and suspension of the smaller species. At the highest flow speeds, I observed *C. volutator* being eroded from the sediment when the current started and often the current was too great for them to re-establish themselves in the sediment. As they were the only species to be adversely

affected by flow, they were the only species who could possibly benefit from effects of tubeworm mimics. This finding corresponds to the facilitation of C. volutator by tubeworm mimics I observed in my flume studies (Chapter 5). Though a different type of flow (i.e. circular rather than linear), the highest flow speeds generated here were only similar to the controls in that study (i.e. upper speeds of 9 cm.s<sup>-1</sup> in mesocosms compared to 11.7 cm.s<sup>-1</sup> in controls from the flume study). Evidently, facilitation begins at much lower speeds than those I described in my field study and this highlights a reoccurring problem with SGH studies. When such experiments are based around only two or three levels of environmental stress, it does not give us much information about how interactions actually change with stress. Whilst acknowledging that my stress gradient was inadequate to determine the shape of the facilitation-stress relationship, I strongly recommend that future studies make certain their experiments represent a sufficient range of stress in order to achieve this. For this study, it would involve using a mechanism that could generate much higher current speeds; I suggest speeds up to 1 m.s<sup>-1</sup>.

The 'no effects' observed for the other species could have occurred for a number of reasons. Foremost, organisms may simply have been unaffected by tubeworm mimics and water flow. However, the 'no effect' may also have been caused by 1) the experiment not running for a sufficient period of time to detect any changes in biomass of the larger organisms and/or 2) the current speeds generated not being high enough to disturb the larger, deeper-dwelling species. Due to the lack of effect at the higher current speeds, it is not possible to determine the precise shape of the facilitation-stress relationship.

I expected *H. ulvae* to be more strongly affected by increasing current flow than my results suggested as other studies found that increasing flow *restricts* their locomotion and displaces individuals (Schanz et al. 2002). What I observed here was no mortality, but instead a shift in location from the sediment surface to the sides of the benthic chamber and onto and into tubeworm mimics at the highest flow speeds. I speculate that, had the walls

of the chamber not been available as an alternative surface, more *H. ulvae.* individuals would have attached to the mimics. I suggest that I did not see a significant interaction because in controls, *H. ulvae* simply used the walls as an alternative hard substratum. Moreover, *H. ulvae* is known to be particularly abundant in sea grass meadows that are also known reduce flow speed above the seabed in a manner similar to tubeworms (Cordoso et al. 2005). It would be interesting to test whether *H. ulvae* are more abundant in nature when found in association with organisms that typically reduce sediment erosion.

In this study, tubeworm mimics reduced flow speed close to the sediment surface. This reduction in current speed would effectively reduce sediment shear stress and hence erodibility of the sediment. Thus mimics were effectively negating any physical disturbance for epifauna and infauna. Effects of flow reduction by mimics were greater as flow speed increased. However, my results indicate that the experiment would have been more fruitful had the current speeds been much greater. When the original SGH was conceived it related implicitly to plant communities and considered a stress gradient to be one of productivity, the upper stress limit being achieved when productivity was zero (Grime 1977, Bertness & Callaway 1994, Callaway 2007, Maestre et al. 2009). If the same concept could be transferred to marine systems, then I suggest that a complete stress gradient must end when a population is eradicated by the given stressor, which is rarely the case (e.g. Norkko et al. 2006). These are not just weaknesses with this study but are inherent problem with many empirical SGH studies (Maestre et al. 2009).

#### 6.5.2 Disadvantages of using imitation tubeworm tubes

By using mimics, I effectively isolated a specific mechanism of tubeworm bioengineering; that of sediment stabilisation. However, mimics are not identical to real tubes in their effects. Imitation tubes are more rigid, solid rather than hollow, they lack a tentacle fringe, and are more persistent over time (Callaway 2003b, 2003a). Real tubeworms also modify habitat by oxygenating sediment and altering flow via their tentacular fringe, and have

trophic influences that were not imitated here (Zühlke 2001). In the future, studies could use real *L. conchilega* reefs to see if SGH effects observed with mimics are realistic in real systems.

#### 6.5.3 Limitations of benthic chambers compared to real systems

In this study, I used a mesocosm-style setup in order to minimise confounding variables. However, there are a number of drawbacks when using closed systems for community ecology studies.

There is no opportunity for continued sediment erosion or deposition. In my benthic chambers, at the highest flow speeds, sediment was suspended in the water column. In a naturally connected system, finer particles would be washed out of the system when suspended, leaving a much coarser substrate. A change in substrate in this manner is likely to be a major stress for many of the organisms unaffected here.

Also, the flow generated within the chambers was circular; produced from the rotation of the impeller. It is unlikely that flow in real systems would act in this manner. Furthermore, turbulence generated within the straws would have been different as a result and bed forms that formed as a result are very different to those observed with linear currents (Friedrichs et al. 2000, Friedrichs 2004). This may have meant that my results were more conservative, as organisms that were simply swirled around in my chambers would have the opportunity to resettle, whereas under natural conditions they would have been eroded and swept away.

In this study, I used tubeworm densities of 7.5% RD, which are common naturally. However, as, to my knowledge, no studies have examined the self-organisation of *L. conchilega* beds, I arranged the mimics in a homogenous pattern. This is certainly not the most natural formation of tubeworms (Reise 2001). For discussions of the benefits/drawbacks of using mimics over real tubeworms, please see Section 1.6.2. Future studies are needed to see what factors affect the distribution of tubeworm beds and to see whether organisms aggregate to particular densities of tube.

Finally, due to time constraints, each experiment was only run for seven days. I assumed that this was sufficient time to observe a change in biomass for the larger organisms. However, had the experiments been run for a longer period, I may have observed a biomass response as a result of decreased fitness caused by sediment erosion.

#### 6.6 Conclusion

Tubeworm mimics are capable of buffering stress associated with sediment erosion caused by increasing current speeds. In this study, benefits manifested at relatively low current speeds and only benefitted two small, surface-dwelling species: *Corophium spp.* and *Hydrobia ulvae*. In order to see whether tubeworms are able to facilitate larger organisms, studies are needed that look at interactions with mimics at much higher flow speeds. Such work would also allow us to observe tubeworm facilitation over a more complete stress gradient

### 7 General discussion

#### 7.1 Introduction

Species interactions are important drivers of ecosystem structure and function and their influence is expected to vary widely with their environs (Bruno & Bertness 2001). At present, my understanding of how and when these interactions switch from positive to negative and vice versa is not sufficient to be predictive and how this change influences other relationships (e.g. between species richness and ecosystem processes) is not well understood (Callaway et al. 2002, Maestre & Cortina 2004, Halpern et al. 2007). Currently, the broad framework of the stress-gradient hypothesis (SGH) is the best practicable predictor of how species interactions will shift with environmental forcers, although it is in a constant state of revision (Maestre et al. 2009). The basic SGH (bSGH hereafter) states that the relative frequency of facilitation and competition will vary inversely across abiotic stress gradients (Bertness & Callaway 1994). The bSGH is very broad and does not specify specific response variables or even at what level of organisation it refers to (Maestre et al. 2009). It does however provide a framework to test the importance of facilitation-stress relationships. Though the hypothesis is typically applied to pairwise interactions with a single benefactor (see Bertness & Leonard 1997), benefits can ultimately be seen in changes at a community level (Bruno & Bertness 2001).

In this study, I used the framework of the bSGH as a heuristic device to test whether interspecific interactions between two intertidal bioengineers and associated species conformed to its predictions. Intertidal soft-sediments are ideal systems within which to pose questions regarding facilitation as communities are often structured by ecosystem engineers, are regularly exposed to sharp gradients of environmental stress and positive effects of biomodification may be more common than negative effects in marine sediments (Schaffner 1990). In such systems, dominant ecosystem engineers can often be divided into those that stabilise the sediment and those that destabilise the sediment (Reise 2002). On the whole, I agree, but the magnitude of the de/stabilisation is largely determined by density of bioengineer and the dynamics of the system.

#### 7.2 Synopsis of experimental findings

Within this section of the general discussion, I synthesise the results and conclusions from the four experimental chapters of my thesis. I first discuss results pertaining to sediment stabilisation by tubeworms and then findings from my studies on bioturbation by lugworms. In the following section, I consider general conclusions that can be drawn from studies of these two model benefactor species and implications for broader SGH research.

#### 7.2.1 Facilitation by tubeworms

Results from fieldwork and laboratory studies suggest that tubeworms (*Lanice conchilega*) do conform to the bSGH as facilitation resulting from tubeworm engineering appears to increase in importance with current speed (though only for a single pairwise interaction). More precisely, species richness may be maintained and mortality of small species buffered by dense beds of tubeworm mimics as flow increases.

#### Facilitation by sediment stabilisers

Sediment erosion disturbs infauna in a number of different ways: most conspicuously by washing surface-dwelling benthos out of the sediment, but also by increasing suspended sediment, blocking filter-feeding apparatus, changing particle distribution and altering nutrient fluxes across the sediment-water interface (Thistle 1981, Hall 1994). This ultimately results in a fitness cost to organisms and/or, in extreme cases, mortality. Sediment erosion occurs when there is an imbalance between forces on the sediment-water interface and forces within the bed that resist erosion (Soulsby 1997). Bed shear stress (the force parallel to the bed face) is the most important factor controlling erosion of cohesive sediment (Araúju et al. 2008). Shear strength of a sediment (the resistance to the stress) is dictated by factors including sediment water content, sediment grain size, organic content and degree of compaction (Araúju 2004). Organisms influence all of these factors and are hence drivers of sediment stability and erodibility (Reise 2002). Sediment stabilisers cover a wide range of major functional groups, including mat-

forming phototrophs, rooted vascular plants, suspension feeders generating habitat, and dead hard parts of the benthos (after Reise 2002). Studies are needed to test whether, like tubeworms, the stabilising effect of these organisms increases in importance with current speed. Based on evidence of the mechanistic effects of epifauna structures in stabilising the sediment, stabilisation effect size can be calculated from simple organismal allometry (McCall & Tevesz 1982). If empirical findings can be generalised within facilitation mechanisms, then it would make application of theory to practical management of ecosystems easier.

#### Tubeworms maintain species richness as flow speed increases

In my flume studies, increasing densities of worm tube mimics increased sediment shear strength (from approximately 0.02 to 0.05 N.m<sup>-2</sup>) and species richness (from 10 to 15 spp.) at higher current speeds (21.49 cm.s<sup>-1</sup>  $\pm$  2.48) (Figure 7.1). At lower speeds (11.70 cm.s<sup>-1</sup>  $\pm$  1.86), there was also a similar increase in sediment shear strength (from 0.03 to 0.05 N.m<sup>-2</sup>), but this time, no influence on species richness. At higher current speeds, the number of species increased from fewer than to equivalent to those from lower speeds as tubeworm density increased. My field studies provide evidence to support the SGH and indicate that, if current speeds were to increase, then tubeworms may be able to buffer and maintain species richness. As tube density increases and approaches 100% RD (roughness density), then competition for space with other infauna species must begin to operate, something that was not observed here. Future work could test when the shift from facilitation to competitive exclusion occurs with increasing tube density.

#### Tubeworms benefit species with particular life history traits

To add to my field studies, my mesocosm studies aimed to identity the current speed at which sediment stabilisation by tubeworm mimics began to benefit associated species and how the relationship between benefits and competition changed along a flow speed gradient (Chapter 6). At low current speeds, I recorded no effects of tubeworm mimics or flow on live species biomass. However, I found that *Corophium volutator* - a small, surface-dwelling organism - that was easily washed free of the sediment, benefitted

from tubeworm presence at the highest generated current speed (*c*. 9 cm.s<sup>-1</sup>) where their deaths were prevented. Other small-bodied organisms (*Hydrobia ulvae*) also began to use tubeworms as an alternative surface for attachment at the highest current speeds. I suggest that, had the mesocosm walls not also acted as a similarly beneficial structure in controls, I would have observed positive effects of flow speed on *H. ulvae* behaviour here. Tubeworm mimics had no detectable effects on other, larger infauna species including *Cerastoderma edule*, *Hediste diversicolor* or *Macoma balthica*. This may have been because current speeds were not high enough to disturb these species or may have been because there was not adequate time for a change in biomass to be detected.

In my benthic chambers, I was unable to generate a sufficient stress gradient to stress all the species housed within and this would ultimately be necessary to identify the shape of the facilitation-stress relationship. The maximum generated flow speeds in the benthic chambers barely reached the lower current speeds from the flume study, though I was still able to observe some effects. The interpretation of a 'stress gradient' is a common discrepancy between SGH studies (e.g. Norkko et al. 2006) and ideally future work should ensure that the stressor increases to an upper limit where all beneficiaries are killed independent of benefactor (Grime 1977, Callaway 2007).

#### Density-dependency effects of tubeworms

In my studies, as with many facilitative relationships, benefactor density largely dictated effect size (Jones et al. 1997). Earlier work suggests that effects of tubes switch from destabilising to stabilising as density and diameter increase or as more of the sediment surface is covered (McCall & Tevesz 1982). Turbulence begins to interact over epibenthic structures when they reach a critical density and eventually forms a skimming flow (Friedrichs et al. 2000, Friedrichs 2004). Above this density, the effect on bed erosion should not change, though shear strength may continue to increase. In this study, an increase in sediment shear strength with increasing RD was seen regardless of current speed. This occurred because the shear strength of sediment is unaffected by current speed, which influences bed shear stress.

What ultimately determines the extent to which organisms are disturbed is the interaction between these two factors – i.e. bed shear strength and stress - that controls the critical bed stress and erodibility of the sediment (Araúju et al. 2008).

My field manipulation showed that shear strength and species richness both increased with tubeworm density under high current stress, but I did not have sufficient densities of mimics to be able to determine the shape of the density relationships i.e. whether they were linear, accelerating or asymptotic. Densities used in my flume study approached 10 % RD, which is much lower than the highest recorded concentrations of *c.* 80 % RD that can be found in nature (calculated from Ziegelmeier 1952). Inclusion of benefactor density into the broad framework of the SGH has already been proposed (Bruno & Bertness 2001). I recognise this as a key step in taking the basic concept of the SGH from pairwise interactions to modelling community-level effects. If benefactors are to be used in conservation and restoration, then the density at which they begin to benefit must be known (Bruno & Bertness 2001).

#### 7.2.2 Facilitation by lugworms

My studies on facilitation by lugworms (*Arenicola marina*) showed that their sediment-mediated interactions were more complex than anticipated and my results were inconclusive as to whether they conform or contradict the SGH.

#### Lugworm densities relate to the depth of the anoxic-oxic chemocline

Observational studies found that lugworm density related to both apparent redox potential discontinuity (aRPD) depth and species richness in hypoxic, but not normoxic sediments (Figure 7.1). I postulated that lugworms may be driving patterns these patterns in biodiversity by depressing the aRPD depth, thereby ameliorating hypoxic stress and increasing the space of benign environment for species to inhabit. My findings indicated that facilitation through hypoxic amelioration may be more important at higher stress, but did not prove causality because of the observational nature of the study. There were no effects on multivariate community structure here and I suggested

that an increase in benign habitat created by amelioration of sedimentary hypoxic stress was being filled stochastically by the larger species pool.

#### Common lugworm interactions are unaffected by sedimentary sulphide

Exclusion studies were intended to find out whether lugworms were driving patterns in biodiversity (Chapter 4). However, results were contradictory to my observational studies and there were no observable effects of lugworm on species richness.

If one assumes that the experimental design of the exclusion experiment was sufficient to detect effects, it means that the results were caused by either: 1) confounding factors similarly influencing lugworm density, aRPD depth and/or species richness; or 2) the aRPD depth forcing lugworm concentration. Another explanation is that there were shortcomings in the way the exclusion study was conducted. Limitations may have been caused by:

- An insufficient gap in hypoxic condition between sites (or too much variability within sites). Sedimentary H<sub>2</sub>S measurements indicated that my normoxic sites still had measurable quantities of sulphide at depth. This meant that there was not as great a disparity in hypoxic condition between sites as I my exposure model would have assumed. From the SGH, I was most likely to detect effects of lugworm facilitation in the most hypoxic sediment as this was where their stress-ameliorating role was predicted to be most important. At other points along a stress gradient there is no guarantee that effects of facilitation would be detectable as the benefactors role would not be essential for survival of associated species.
- Site-specific confounding effects. Due to the limited number of field sites, variability between individual sites could have had a large effect on my results. The most likely explanation is for confounding factors that covary with lugworm density. Experiments were started at an additional hypoxic and oxic site, but the field experiments were

disrupted in those locations during the course of the study and it was not possible to sample them.

- Too small an exclusion plot. This could have affected my results in two ways:
  - It may have been insufficient an area to markedly alter sediment chemistry. It is not known how far a lugworm's influence extends away from its burrow. It may be that 1 m<sup>2</sup> exclusion plots within a dense lugworm bed were insufficient to cause a big enough change in sediment chemistry to influence infauna.
  - 2) Due to the paucity of fauna in intertidal sediment, it could have been an insufficient size area to detect changes in the rarer species and thus changes in community organisation. From previous studies, it is often these rarer species (e.g. *Capitella capitata*) that are promoted by *A. marina* (Lackschewitz & Reise 1998).

Despite there being no correlation between lugworm presence and sulphide concentrations, there were significant pairwise interactions with both *Bathyporeia* spp. and *Corophium* spp. that were promoted and inhibited respectively by lugworm activity. Such interactions are well described in the literature and are thought to occur due to a beneficial association with the faecal cast (Hüttel et al. 1996, Lackschewitz & Reise 1998) and simple disturbance through sediment reworking (Flach 1992a, 1992b, 1993, Flach & Debruin 1993, 1994). These interactions did not vary with sedimentary hypoxia which is in disagreement with the SGH.

#### Application of SGH to bioturbators

One important question I asked in this study was: would bioturbators drive biodiversity patterns across a hypoxic gradient? Successional models of sedimentary systems indicate that biodiversity should peak at intermediate levels of hypoxia and abundance in more reduced sediments due to a shift in the life-history strategy of organisms (Nilsson & Rosenberg 2000). Many studies have already examined the interrelatedness of bioturbation, sediment chemistry and biodiversity in successional studies and, because these studies provide all the key parameters, it may be possible to try and fit the data to the bSGH.

Due to the inconsistency between my two lugworm studies, I believe that the complexity of an anoxic stress gradient is too great to fit community patterns to a simple model such as the bSGH.

#### Do species interactions with lugworms conform to the SGH?

Results from my studies are inconclusive as to the relevance of the bSGH to lugworm interactions. Compared to tubeworms, the bioengineering of lugworms could be considered to be more multi-faceted: *A. marina* constructs a complex burrow that modifies the sedimentary habitat in a number of ways. Besides oxygenation of the sediment, lugworm burrows create a cast of pseudo-faeces that modifies near-bed current (Brey 1991, Lackschewitz & Reise 1998), produces a funnel that provides refuge from desiccation and predation stress (Reise 1987, Flach 1992a, Retraubun et al. 1996a) and generates a feeding pocket with a unique chemistry (Reise 1981b, 1981a, 1985). This may be why net interactions with lugworms do not change with hypoxia and I suggest that interactions with other species such as *Bathyporeia* spp. would not either because they operate independently of sedimentary hypoxia.

In summary, effects of lugworm facilitation appear to be idiosyncratic. Though results from my experimental study would normally override those from my descriptive work, there were questions over the experimental design of the exclusion experiment. Overall, I suspect that although there is some evidence that pairwise interactions with lugworms conform to the SGH, the multiple modes of interaction associated with lugworms cannot be related to a single environmental stressor from my experiments, though this would need to be tested further.

#### 7.3 Synthesis of results

At present, whether the bSGH relates to natural systems is a highly contentious issue in community ecology (see arguments between Maestre et al. 2005, Lortie & Callaway 2006, Maestre et al. 2006). It has been proposed by different research groups that additional factors such as species traits (Choler et al. 2001), the nature of the stress gradient (Kawai & Tokeshi 2007), and the performance measure of beneficiaries (Maestre et al. 2005) will also affect any shift in interactions.

The bSGH was developed from Grime's (1977) definition of stressful environments i.e. "those in which producers are limited by the environment in their ability to convert energy to biomass" (Callaway 2007). Consequently, it was intended to relate specifically to a productivity-based stress gradient. Nobody has explicitly addressed how to relate this to different systems, though it is assumed that the upper limit of a stress gradient results in 100% mortality (Maestre et al. 2009). This is particularly difficult when conducting field studies, as it is difficult to know what factors are causing fluctuations in population density and each species within a community will have its own stress gradient limits (see Chapter 3).

In this study, results were inconsistent, but they indicate that the applicability of the bSGH is highly dependent upon the nature of the stress-ameliorating mechanism and the complexity of interactions with the benefactor species in question (Figure 7.1). Previous studies from plant communities have predominately focused on mechanisms of stress amelioration that relate to solar (Arriaga et al. 1993), salinity (Crain & Bertness 2005) or desiccation stress (Armas & Pugnaire 2005), where this mechanism is the dominant form of interaction. Most, but not all (e.g. Casper 1996), of these studies conform to the principles of the bSGH. For example, facilitation by fucoid algae is more important where desiccation stress is high, compared to low. In this study, tubeworm mimics isolated a single mechanism of *Lanice conchilega* habitat modification and appeared to support the theory of the bSGH. This could either have been because modes of interactions with tubeworms are relatively few compared to other infauna , or because by using imitation tubes

I effectively partitioned the single mechanism of facilitation I was interested in. In contrast, I believe that benefactors with complex webs of interactions are generally less likely to fit within the framework of the bSGH (e.g. Norkko My studies with lugworms found idiosyncratic effects of et al. 2006). lugworms on species richness and species density and I suggest that this was because the tranche of interactions resulting from bioturbation and burrow construction by A. marina was varying with multiple environmental drivers, not just sedimentary hypoxic stress. Also, that facilitation of hypoxia may not be the most important of these. For such organisms, it may only be possible to consider shifts in interspecific interactions with multiple abiotic factors. In the case of lugworms, one would either require the mechanism of sediment oxygenation to be partitioned in a similar way to my studies with L. conchilega or other engineering mechanisms to be considered alongside sediment oxygenation, such as pit and mound construction and formation of a feeding pocket. A major step in SGH research must be to consider multiple stressors simultaneously as this is far more realistic for natural systems and several benefactors.



Figure 7.1: Synthesis of relationships between benefactors, stressors, mechanisms of stress-amelioration and beneficiaries
#### 7.4 General limitations with empirical SGH studies

In order for the SGH to be used in conservation and management, it needs to be generally applicable to multiple systems or one must understand to which systems it can be applied. At present, it is difficult to compare results between SGH studies for a number of reasons.

#### 7.4.1 Selection of estimator of performance

The shape of the stress-gradient relationship changes with the response variable chosen to represent facilitation and this varies between studies. The most commonly used responses are fecundity, density, growth and survival of beneficiaries (Maestre et al. 2005). In this study, I considered population densities as the response variable for pairwise interactions when conducting field studies. This is because beneficiaries accumulate around benefactors when a stress is ameliorated and both response variables will increase. This was most suitable for the design of my studies as my manipulations were relatively small-scale and most species were mobile. There was therefore a lot of opportunity for immigration and emigration of species. In the future, studies could be directed to test the SGH in tubeworm and lugworm systems with alternative measures of performance, as beneficiaries may have been benefiting in ways other than those I measured e.g. reproductive success.

#### 7.4.2 At what scale to measure

In this study, I focused on macrofauna, but other studies have studied effects on much smaller organisms. Most conservation efforts are focused on these larger components of natural communities, but in terms of ecosystem processes, smaller species may have at least an equal part to play (Hakenkamp & Morin 2000). It is partly for this reason that the 'ecosystem approach' to conservation was developed. Lugworms are known to benefit many smaller species that may have been missed here by passing through the 500 µm sieve I used to sort the fauna. Meiofauna including nematoda, oligochaeta, and smaller copepoda are all facilitated through specific portions of the lugworm burrow (Reise & Ax 1979, Reichardt 1988, Wetzel et al. 1995). Furthermore, both lugworms and tubeworms alter near-bed currents

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and alter the composition of the surface sediment layer. This will have a knock-on effect on the diversity and function of microphytobenthos, a component of the benthos known to have an impact on sediment cohesion itself (Hakenkamp & Morin 2000, Lohrer et al. 2004).

#### 7.4.3 How to measure community facilitation

Effects of facilitation are not always consistent at a community level. Benefactors can facilitate richer communities, more productive communities, and/or more resilient communities, but these factors are rarely correlated and so may change independently. For example, when considering bioturbation, biodiversity, abundance and biomass all peak at different sedimentary successional stages.

Future research could refine the theory so that it is clearer in what context facilitation is important for which response variables. This would further improve the usefulness of the SGH to conservation projects by potentially identifying which components of a given community will benefit across multiple mechanisms of facilitation. Conceptual models predict that primary space-holding benefactors will predominately facilitate a richer community of secondary space holders that may continue to compete with larger organisms (Bruno & Bertness 2001). My results support this theory as species benefiting from both tubeworms and lugworms were typically smaller, but denser, species. In communities where species richness does not change, but the composition of the community does, it may be better to measure abundance or biomass of species in an attempt to better assess the shift in community organisation.

#### 7.5 Implications for biodiversity-ecosystem function studies

In a number of other areas of community ecology, species interactions are often cited as a driver of ecosystem function and resilience. What I have learnt from this study about the predictability of species interactions could inform other areas of community ecology, in particular, those relating to biodiversity and ecosystem functioning (B-EF) (Hooper et al. 2005).

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In B-EF research, the functional roles of species in isolation are often used to determine how function would respond if the community were disassembled, for example in the redundancy and insurance hypotheses (e.g. Worm et al. 2006, Thrush et al. 2008). Other studies predict that a relationship between functional importance and extinction proneness of species in turn shapes the B-EF relationship (Larsen et al. 2005). What the SGH shows, is that, for the same community of species, the functional roles of component species are driven in part by environmental forcers. Future studies could try and relate species interactions, biodiversity, ecosystem function and environmental parameters to one another. I believe that by understanding under what environmental conditions species become functionally important, one can deduce which B-EF hypotheses relate to which natural communities.

#### 7.6 Recommendation for future research

At present, many studies are simply testing *ad hoc* which benefactors and ecosystems the SGH applies to. Evidently, the SGH was initially developed as a starting block for facilitation research and we are now at a stage where new conceptual models, with increasing complexity, need to be developed. The bSGH considers systems with a single dominant benefactor and a single stressor, but this may be an unrealistic representation of natural systems. In this section, I identify areas that I believe should be priorities for future SGH studies in order to progress the field such that it could be useful to real management plans and inform.

#### 7.6.1 Is using a single stressor an oversimplification of natural systems?

A common problem with many conceptual models is that they are oversimplifications of complex, natural processes. The SGH may be unrealistic in that it only considers a single stress gradient. In reality, there are multiple environmental drivers that all interact with each other. For example, taken to extremes, the two abiotic gradients I considered here (hypoxia and current speed) could be considered to run in parallel but opposite directions, as very exposed sedimentary shores are extremely oxic and truly anoxic sites are very sheltered. It is my belief that the bSGH as it stands is too simple a construct to fit interactions resulting from bioturbation too, but provides a practical framework from which further conceptual models can be developed.

### 7.6.2 <u>How does the facilitation-stress relationship vary over a complete</u> stress gradient?

Many SGH studies simply test to see whether facilitation is relatively more important at high, compared to relatively lower, stress levels (e.g. Bertness & Leonard 1997). Comparatively fewer experiments have implicitly examined the shape of the facilitation-stress relationship (e.g. Maestre & Cortina 2004). In my mesocosm study, I attempted to identify how the importance of facilitation changed over a stress gradient, but the flow speeds I was able to generate within the benthic chambers were insufficient to test the shape of the relationship. It thus remains a critical part of SGH research. The shape of the facilitation-stress relationship will have a huge impact on how the SGH is applied to conservation as it will allow conservation managers to identify the environmental conditions under which benefits resulting from facilitation peak. Consequently, this would allow them not only to be able to identify the benefactor on which to focus conservation efforts, but also the environmental conditions under which levels is applied.

# 7.6.3 <u>How does the SGH relate to species that are currently being</u> protected?

My work considers how interactions with intertidal benefactors changes with stress, but many bioengineers protected under conservation legislation have a subtidal distribution e.g. biogenic reefs protected under the EC Habitats Directive: blue mussels *Mytilus edulis*, horse mussels *Modiolus modiolus*, ross worms *Sabellaria* spp., the serpulid worm *Serpula vermicularis*, and cold-water corals such as *Lophelia pertusa*. In order to make empirical studies most relevant to conservation objectives, studies need to examine how the influence of these species changes contextually, in order that we can prioritise examples for protection.

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#### 7.7 Management implications of SGH

The next logical step in SGH research is to try and apply what we already know to conservation strategies to see if the knowledge I have gained is truly valuable to conservation and restoration of real ecosystems. There are numerous published papers that talk about the theory of applying the SGH and differential effects of ecosystem engineers to conservation and natural systems, but few have empirically tested the practicality of this concept.

On a regional scale, knowledge of particular bioengineers could inform local conservation strategies. For example, lugworms are a commonly targeted species for bait diggers in the UK for use in recreational fishing. From what I have learnt here, lugworms have the potential to support a richer infauna community in certain habitats and at certain densities through facilitation. By using knowledge of the local effects of lugworms, bait-digging could be restricted to more oxic foreshores, where lugworm bioturbation has less of a beneficial effect. Furthermore, quantities could be potentially controlled so that levels were sufficient to support a richer associated community. If too many lugworms were removed, the sediment may become more hypoxic and species richness could drop as a result. If lugworm levels were found to be deficient at particular shores, then the shore could be closed to digging and lugworms maybe transplanted in to aid restoration and recovery of the biotope. However, more work would be required to investigate localised effects of lugworm activity on community structure and species richness before this could be applied.

The next major step in applying SGH theory to practical conservation concepts utilising facilitation and ecosystem engineers is to conduct field studies based on the knowledge currently available to test whether it is possible to predict when a benefactor's role is keystone. Detailed knowledge of specific systems is already being used in restoration projects in salt marshes (Halpern et al. 2007). Here knowledge of benefactors and how they interrelate in terms of density dependency, and spatial and temporal scale to community succession is utilised to create a customised management strategy (see Section 1.5 for details). This indicates that it is entirely possible

to use the bSGH to develop informed, bespoke conservation strategies that are more efficient and that have a higher degree of confidence associated with them.

Over the next century, the single largest challenge facing ecology will be whether it has developed into a sufficiently predictive science to be a valuable tool in conserving and restoring damaged ecosystems at local, regional, and global spatial scales. Ecosystem engineering can develop into a more rigorous, predictive concept in order to help meet this challenge.

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## References

- Aarssen LW (1997) High productivity in grassland ecosystems: effected by species diversity or productive species? Oikos 80:183-184
- Araúju MAVC (2004) Erosion mechanisms in marine sediments. University of Minho
- Araúju MAVC, Teixeira JCF, Teixeira SFCF (2008) Application of laser anemometry for measuring critical bed shear stress of sediment core samples. Cont Shelf Res 28:2718-2724
- Armas C, Ordiales R, Pugnaire FI (2004) Measuring plant interactions: A new comparative index. Ecology 85:2682-2686
- Armas C, Pugnaire FI (2005) Plant interactions govern population dynamics in a semi-arid plant community. J Ecol 93:978-989
- Aronson RB, Precht WF (2001) White-band disease and the changing face of Caribbean coral reefs. Hydrobiologia 460:25-38
- Arriaga L, Maya Y, Diaz S, Cancino J (1993) Association between Cacti and Nurse Perennials in a Heterogeneous Tropical Dry Forest in Northwestern Mexico. J Veg Sci 4:349-356
- Arroyo MTK, Cavieres LA, Penaloza A, Arroyo-Kalin MA (2003) Positive associations between the cushion plant *Azorella monantha* and alpine plant species in the Chilean Patagonian Andes. Plant Ecol 169: 121-129
- Bagarinao T (1992) Sulfide as an environmental factor and toxicant: tolerance and adaptations in aquatic organisms. Aquat Toxicol 24:21-62
- Baumfalk YA (1979) Heterogeneous Grain-Size Distribution in Tidal Flat Sediment Caused by Bioturbation Activity of Arenicola Marina (Polychaeta). Neth J Sea Res 13:428-440
- Berkenbusch K, Rowden AA, Myers TE (2007) Interactions between seagrasses and burrowing ghost shrimps and their influence on infaunal assemblages. J Exp Mar Biol Ecol 341:70-84
- Berkenbusch K, Rowden AA, Probert PK (2000) Temporal and spatial variation in macrofauna community composition imposed by ghost shrimp Callianassa filholi bioturbation. Mar Ecol-Prog Ser 192:249-257
- Bertness MD (1989) Intraspecific competition and facilitation in a northern acorn barnacle population. Ecology 70:257-268
- Bertness MD, Callaway R (1994) Positive interactions in communities. Trends Ecol Evol 9:191-193
- Bertness MD, Hacker SD (1994) Physical stress and positive associations among marsh plants. Am Nat 144:363-372
- Bertness MD, Leonard GH (1997) The role of positive interactions in communities: lessons from intertidal habitats. Ecology 78:1976-1989
- Bertness MD, Leonard GH, Levine JM, Bruno JF (1999a) Climate-driven interactions among rocky intertidal organisms caught between a rock and a hot place. Oecologia 120:446-450
- Bertness MD, Leonard GH, Levine JM, Schmidt PR, Ingraham AO (1999b) Testing the relative contribution of positive and negative interactions in rocky intertidal communities. Ecology 80:2711-2726

- Beukema JJ (1979) Biomass and species richness of the macrobenthic animals living on a tidal flat area in the Dutch Wadden Sea - effects of a severe winter. Neth J Sea Res 13:203-223
- Beukema JJ, Debruin W, Jansen JJM (1978) Biomass and Species Richness of Macrobenthic Animals Living on Tidal Flats of Dutch Wadden Sea - Long-Term Changes During a Period with Mild Winters. Neth J Sea Res 12:58-77
- Biles C (2002) Marine benthic system function and biodiversity. University of St Andrews
- Biles CL, Solan M, Isaksson I, Paterson DM, Emes C, Raffaelli DG (2003) Flow modifies the effect of biodiversity on ecosystem functioning: an in situ study of estuarine sediments. J Exp Mar Biol Ecol 285:165-177
- Bolam SG (2004) Population structure and reproductive biology of Pygospio elegans (Polychaeta : Spionidae) on an interfidal sandflat, Firth of Forth, Scotland. Invertebr Biol 123:260-268
- Bolam SG, Fernandes TF (2002) Dense aggregations of tube-building polychaetes: response to small-scale disturbances. J Exp Mar Biol Ecol 269:197-222
- Bologna PAX, Heck KL (2002) Impact of habitat edges on density and secondary production of seagrass-associated fauna. Estuaries 25:1033-1044
- Bond EM, Chase JM (2002) Biodiversity and ecosystem functioning at local and regional spatial scales. Ecol Lett 5:467-470
- Boogert NJ, Paterson DM, Laland KN (2006) The implications of niche construction and ecosystem engineering for conservation biology. Bioscience 56:570-578
- Boyer KE, Zedler JB (1998) Effects of nitrogen additions on the vertical structure of a constructed cordgrass marsh. Ecol Appl 8:692-705
- Bray JR, Curtis JT (1957) An ordination of the upland forest communities of southern Wisconsin. Ecol Monogr 27:325-349
- Brenchley GA (1981) Disturbance and community structure an experimental study of bioturbation in marine soft bottom environments. J Mar Res 39:767-790
- Brey T (1991) The relative significance of biological and physical disturbance an example from intertidal and subtidal sandy bottom communities. Estuar Coast Shelf S 33:339-360
- Bronstein JL (1994) Our current understanding of mutualism. Q Rev Biol 69:31-51
- Brooker RW, Callaghan TV (1998) The balance between positive and negative plant interactions and its relationship to environmental gradients: a model. Oikos 81:196-207
- Brooker RW, Maestre FT, Callaway RM, Lortie CL, Cavieres LA, Kunstler G, Liancourt P, Tielborger K, Travis JMJ, Anthelme F, Armas C, Coll L, Corcket E, Delzon S, Forey E, Kikvidze Z, Olofsson J, Pugnaire F, Quiroz CL, Saccone P, Schiffers K, Seifan M, Touzard B, Michalet R (2008) Facilitation in plant communities: the past, the present, and the future. J Ecol 96:18-34
- Bruno JF (2000) Facilitation of cobble beach plant communities through habitat modification by Spartina alterniflora. Ecology 81:1179-1192
- Bruno JF, Bertness MD (2001) Habitat Modification and Facilitation in Benthic Marine Communities. In: Bertness MD, Gaines SD, Hay ME (eds) Marine Community Ecology. Sinauer Associates, Sunderland, Mass., p 550

- Bruno JF, Kennedy CW (2000) Patch-size dependent habitat modification and facilitation on New England cobble beaches by Spartina alterniflora. Oecologia 122:98-108
- Bruno JF, Stachowicz JJ, Bertness MD (2003) Inclusion of facilitation into ecological theory. Trends Ecol Evol 18:119-125
- Buhr KJ (1976) Suspension-Feeding and Assimilation Efficiency in Lanice-Conchilega (Polychaeta). Mar Biol 38:373-383
- Byers JE, Cuddington K, Jones CG, Talley TS, Hastings A, Lambrinos JG, Crooks JA, Wilson WG (2006) Using ecosystem engineers to restore ecological systems. Trends Ecol Evol 21:493-500
- Cadée GC (1976) Sediment reworking by Arenicola marina on tidal flats in Dutch Wadden Sea. Neth J Sea Res 10:440-460
- Callaway R (2003a) Juveniles stick to adults: recruitment of the tube-dwelling polychaete Lanice conchilega (Pallas, 1766). Hydrobiologia 503:121-130
- Callaway R (2003b) Long-term effects of imitation polychaete tubes on benthic fauna: they anchor Mytilus edulis (L.) banks. J Exp Mar Biol Ecol 283:115-132
- Callaway R (2006) Tube worms promote community change. Mar Ecol-Prog Ser 308:49-60
- Callaway RM (2007) Positive Interactions and Interdependence in Plant Communities, Vol. Springer, Dordrecth, The Netherlands
- Callaway RM, Brooker RW, Choler P, Kikvidze Z, Lortie CJ, Michalet R, Paolini L, Pugnaire FI, Newingham B, Aschehoug ET, Armas C, Kikodze D, Cook BJ
  - (2002) Positive interactions among alpine plants increase with stress. Nature 417:844-848
- Callaway RM, Nadkarni NM, Mahall BE (1991) Facilitation and interference of *Quercus douglasii* on understory productivity in Central California. Ecology 72:1484-1499
- Callaway RM, Walker LR (1997) Competition and facilitation: A synthetic approach to interactions in plant communities. Ecology 78:1958-1965
- Cardinale BJ, Palmer MA, Collins SL (2002) Species diversity enhances ecosystem functioning through interspecific facilitation. Nature 415:426-429
- Casper BB (1996) Demographic consequences of drought in the herbaceous perennial Cryptantha flava: Effects of density, associations with shrubs, and plant size. Oecologia 106:144-152
- Castro J, Zamora R, Hodar JA, Gomez JM, Gomez-Aparicio L (2004) Benefits of using shrubs as nurse plants for reforestation in Mediterranean mountains: A 4-year study. Restor Ecol 12:352-358
- Caswell H, Etter R (1999) Cellular automaton models for competition in patchy environments: Facilitation, inhibition, and tolerance. B Math Biol 61:625-649
- Chapin FS, Walker BH, Hobbs RJ, Hooper DU, Lawton JH, Sala OE, Tilman D (1997) Biotic control over the functioning of ecosystems. Science 277:500-504
- Choler P, Michalet R, Callaway RM (2001) Facilitation and competition on gradients in alpine plant communities. Ecology 82:3295-3308

- Clarke KR (1993) Nonparametric multivariate analyses of changes in community structure. Aust J Ecol 18:117-143
- Clarke KR, Gorley RN (2006) PRIMER v6: User manual/tutorial, Vol. PRIMER-E, Plymouth UK
- Cline JD (1969) Spectrophotometric determination of hydrogen sulfide in natural waters. Limnol Oceanogr 13:454-458
- Coleman FC, Williams SL (2002) Overexploiting marine ecosystem engineers: potential consequences for biodiversity. Trends Ecol Evol 17:40-44
- Connell JH (1978) Diversity in Tropical Rain Forests and Coral Reefs. Science 199:1302-1310
- Cordoso PG, Brandao A, Pardal MA, Raffaelli D, Marques JC (2005) Resilience of *Hydrobia ulvae* populations to anthropogenic and natural disturbances. Mar Ecol-Prog Ser 289:191-199
- Costanza R, dArge R, deGroot R, Farber S, Grasso M, Hannon B, Limburg K, Naeem S, ONeill RV, Paruelo J, Raskin RG, Sutton P, vandenBelt M (1997) The value of the world's ecosystem services and natural capital. Nature 387:253-260
- Costanza R, Farber SC, Maxwell J (1989) Valuation and management of wetland ecosystems. Ecol Econ 1:335-361
- Crain CM (2008) Interactions between marsh plant species vary in direction and strength depending on environmental and consumer context. J Ecol 96:166-173
- Crain CM, Bertness MD (2006) Ecosystem engineering across environmental gradients: implications for conservation and management. Bioscience 56:211-218
- Crain CM, Bertness ND (2005) Community impacts of a tussock sedge: Is ecosystem engineering important in benign habitats? Ecology 86:2695-2704
- Crooks JA (2002) Characterizing ecosystem-level consequences of biological invasions: the role of ecosystem engineers. Oikos 97:153-166
- Dame R, Bushek D, Prins T (2001) Benthic suspension feeders as determinants of ecosystem structure and function in shallow coastal waters. In: Reise K (ed) Ecological Comparisons of Sedimentary Shores. Springer, Berlin, p 11-37
- Darwin C (1859) The origin of species by means of natural selection, or, The preservation of favoured races in the struggle for life, Vol. Unit Library, London; New York
- Dayton PK (1971) Competition, Disturbance, and Community Organization -Provision and Subsequent Utilization of Space in a Rocky Intertidal Community. Ecol Monogr 41:351-&
- Dayton PK, Hessler RR (1972) Role of Biological Disturbance in Maintaining Diversity in Deep Sea. Deep-Sea Research 19:199-&
- de Groot RS, Wilson MA, Boumans RMJ (2002) A typology for the classification, description and valuation of ecosystem functions, goods and services. Ecol Econ 41:393-408
- Dean RL, Connell JH (1987) Marine-Invertebrates in an Algal Succession .3. Mechanisms Linking Habitat Complexity with Diversity. Journal of Experimental Marine Biology and Ecology 109:249-273

- Dean TA, Haldorson L, Laur DR, Jewett SC, Blanchard A (2000) The distribution of nearshore fishes in kelp and eelgrass communities in Prince William Sound, Alaska: associations with vegetation and physical habitat characteristics. Environ Biol Fish 57:271-287
- Degraer S, Moerkerke G, Rabaut M, Van Hoey G, Du Four I, Vincx M, Henriet JP, Van Lancker V (2008) Very-high resolution side-scan sonar mapping of biogenic reefs of the tube-worm Lanice conchilega. Remote Sens Environ 112:3323-3328
- Doak DF, Bigger D, Harding EK, Marvier MA, O'Malley RE, Thomson D (1998) The statistical inevitability of stability-diversity relationships in community ecology. Am Nat 151:264-276
- Duffy JE, Stachowicz JJ (2006) Why biodiversity is important to oceanography: potential roles of genetic, species, and trophic diversity in pelagic ecosystem processes. Mar Ecol-Prog Ser 311:179-189
- Eckman JE (1983) Hydrodynamic Processes Affecting Benthic Recruitment. Limnol Oceanogr 28:241-257
- Eckman JE (1985) Flow Disruption by an Animal-Tube Mimic Affects Sediment Bacterial-Colonization. J Mar Res 43:419-435
- Eckman JE, Duggins DO (1991) Life and Death beneath Macrophyte Canopies -Effects of Understory Kelps on Growth-Rates and Survival of Marine, Benthic Suspension Feeders. Oecologia 87:473-487
- Eckman JE, Nowell ARM, Jumars PA (1981) Sediment destabilization by animal tubes. J Mar Res 39:361-374
- Edgar GJ (1999a) Experimental analysis of structural versus trophic importance of seagrass beds. I. Effects on macrofaunal and meiofaunal invertebrates. Vie Milieu 49:239-248
- Edgar GJ (1999b) Experimental analysis of structural versus trophic importance of seagrass beds. II. Effects on fishes, decapods and cephalopods. Vie Milieu 49:249-260
- Ehrlich PR, Wilson EO (1991) Biodiversity studies science and policy. Science 253:758-762
- Estes JA, Palmisan.Jf (1974) Sea Otters Their Role in Structuring Nearshore Communities. Science 185:1058-1060
- Flach EC (1992a) Disturbance of benthic infauna by sediment-reworking activities of the lugworm *Arenicola marina*. Neth J Sea Res 30:81-89
- Flach EC (1992b) The influence of 4 macrozoobenthic species on the abundance of the amphipod *Corophium volutator* on tidal flats of the Wadden Sea. Neth J Sea Res 29:379-394
- Flach EC (1993) The distribution of the amphipod *Corophium arenarium* in the Dutch Wadden Sea relationships with sediment composition and the presence of cockles and lugworms. Neth J Sea Res 31:281-290
- Flach EC, Debruin W (1993) Effects of *Arenicola marina* and *Cerastoderma edule* on distribution, abundance and population structure of *Corophium volutator* in Gullmarsfjorden Western Sweden. Sarsia 78:105-118
- Flach EC, Debruin W (1994) Does the activity of cockles, Cerastoderma edule, and lugworms, Arenicola marina, make Corophium volutator more vulnerable to

epibenthic predators - a case of interaction modification. J Exp Mar Biol Ecol 182:265-285

- Ford RB, Paterson D (2001) Behaviour of *Corophium volutator* in still versus flowing water. Estuar Coast Shelf S 52:357-362
- Forster S, Graf G (1995) Impact of Irrigation on Oxygen Flux into the Sediment -Intermittent Pumping by Callianassa-Subterranea and Piston-Pumping by Lanice-Conchilega. Mar Biol 123:335-346
- Friedrichs M (2004) Flow-induced effects of macrozoobenthic structures on the near-bed sediment transport. Rostock University
- Friedrichs M, Graf G, Springer B (2000) Skimming flow induced over a simulated polychaete tube lawn at low population densities. Mar Ecol-Prog Ser 192:219-228
- Fuentes ER, Hoffmann AJ, Poiani A, Alliende MC (1986) Vegetation change in large clearings patterns in the Chilean Matorral. Oecologia 68:358-366
- Funk JL, Cleland EE, Suding KN, Zavaleta ES (2008) Restoration through reassembly: plant traits and invasion resistance. Trends Ecol Evol 23:695-703
- Giere O (1992) Benthic life in sulfidic zones of the sea: ecological and structural adaptations to a toxic environment. Verhandlungen Der Deutschen Zoologischen Gesellschaft 85:77-93
- Goldberg DE, Rajaniemi T, Gurevitch J, Stewart-Oaten A (1999) Empirical approaches to quantifying interaction intensity: Competition and facilitation along productivity gradients. Ecology 80:1118-1131
- Gómez-Aparicio L, Zamora R, Gomez JM, Hodar JA, Castro J, Baraza E (2004) Applying plant facilitation to forest restoration: A meta-analysis of the use of shrubs as nurse plants. Ecol Appl 14:1128-1138
- Graf G, Rosenberg R (1997) Bioresuspension and biodeposition: A review. J Marine Syst 11:269-278
- Grime JP (1977) Evidence for existence of 3 primary strategies in plants and its relevance to ecological and evolutionary theory. Am Nat 111:1169-1194
- Grimm NB (1995) Why Link Species and Ecosystems a Perspective from Ecosystem Ecology. Linking Species & Ecosystems:5-15
- Groenendaal M (1979) On sulfide and the distribution of *Arenicola marina* in a tidal mud flat in the Dutch Wadden Sea. Neth J Sea Res 13:562-570
- Groenendaal M (1980) Tolerance of the lugworm (*Arenicola marina*) to sulfide. Neth J Sea Res 14:200-207
- Groenendaal M (1981) The adaptation of *Arenicola marina* to sulfide solutions. Neth J Sea Res 15:65-77
- Hakenkamp CC, Morin A (2000) The importance of meiofauna to lotic ecosystem functioning. Freshwater Biology 44:165-175
- Hall SJ (1994) Physical disturbance and marine benthic communities life in unconsolidated sediments. Oceanography and Marine Biology 32:179-239
- Halpern BS, Silliman BR, Olden JD, Bruno JP, Bertness MD (2007) Incorporating positive interactions in aquatic restoration and conservation. Front Ecol Environ 5:153-160

- Harper JL, Hawksworth DL (1994) Biodiversity Measurement and Estimation -Preface. Philos T Roy Soc B 345:5-12
- Hastings A, Byers JE, Crooks JA, Cuddington K, Jones CG, Lambrinos JG, Talley TS, Wilson WG (2007) Ecosystem engineering in space and time. Ecol Lett 10:153-164
- Heck KL, Wetstone GS (1977) Habitat Complexity and Invertebrate Species Richness and Abundance in Tropical Seagrass Meadows. J Biogeogr 4:135-142
- Holthe T (1978) Zoogeography of Terebellomorpha (Polychaeta) of Northern European Waters. Sarsia 63:191-198
- Hooper DU, Chapin FS, Ewel JJ, Hector A, Inchausti P, Lavorel S, Lawton JH, Lodge DM, Loreau M, Naeem S, Schmid B, Setala H, Symstad AJ, Vandermeer J, Wardle DA (2005) Effects of biodiversity on ecosystem functioning: a consensus of current knowledge. Ecol Monogr 75:3-35
- Hughes RG (1988) Dispersal by Benthic Invertebrates the Insitu Swimming Behavior of the Amphipod Corophium-Volutator. J Mar Biol Assoc Uk 68:565-579
- Huston MA (1997) Hidden treatments in ecological experiments: Re-evaluating the ecosystem function of biodiversity. Oecologia 110:449-460
- Hüttel M (1990) Influence of the lugworm Arenicola marina on porewater nutrient profiles of sand flat sediments. Mar Ecol-Prog Ser 62:241-248
- Hüttel M, Ziebis W, Forster S (1996) Flow-induced uptake of particulate matter in permeable sediments. Limnol Oceanogr 41:309-322
- Ives AR, Carpenter SR (2007) Stability and diversity of ecosystems. Science 317:58-62
- Jensen KT (1985) The presence of the bivalve *Cerastoderma edule* affects migration, survival and reproduction of the amphipod *Corophium volutator*. Mar Ecol-Prog Ser 25:269-277
- Jones CG, Lawton JH, Shachak M (1994) Organisms as ecosystem engineers. Oikos 69:373-386
- Jones CG, Lawton JH, Shachak M (1997) Positive and negative effects of organisms as physical ecosystem engineers. Ecology 78:1946-1957
- Jones GP, Syms C (1998) Disturbance, habitat structure and the ecology of fishes on coral reefs. Aust J Ecol 23:287-297
- Jones SE, Jago CF (1993) Insitu assessment of modification of sediment properties by burrowing invertebrates. Mar Biol 115:133-142
- Judge ML, Coen LD, Heck KL (1992) The effect of long-term alteration of *in situ* currents on the growth of *Mercenaria mercenaria* in the Northern Gulf of Mexico. Limnol Oceanogr 37:1550-1559
- Kappner I, Al-Moghrabi SM, Richter C (2000) Mucus-net feeding by the vermetid gastropod *Dendropoma maxima* in coral reefs. Mar Ecol-Prog Ser 204:309-313
- Karlson K Rosenberg R Bonsdorff E (2002) Temporal and spatial large-scale effects of eutrophication and oxygen deficiency on benthic fauna in Scandinavian and Baltic waters - a review. Oceanography and Marine Biology 40: 427-489

- Kawai T, Tokeshi M (2007) Testing the facilitation-competition paradigm under the stress-gradient hypothesis: decoupling multiple stress factors. P R Soc B 274:2503-2508
- Kondoh M (2001) Unifying the relationships of species richness to productivity and disturbance. P Roy Soc Lond B Bio 268:269-271
- Kravitz JH (1970) Repeatability of 3 Instruments Used to Determine Undrained Shear Strength of Extremely Weak, Saturated, Cohesive Sediments. J Sediment Petrol 40:1026-&
- Kruskall JB, Wish M (1978) Multidimensional Scaling, Vol 07-011. SAGE, London
- le Roux PC, McGeoch MA (2008) Spatial variation in plant interactions across a severity gradient in the sub-Antarctic. Oecologia 155: 831-844
- Lackschewitz D, Reise K (1998) Macrofauna on flood delta shoals in the Wadden Sea with an underground association between the lugworm *Arenicola marina* and the amphipod Urothoe poseidonis. Helgolander Meeresun 52:147-158
- Larsen TH, Williams NM, Kremen C (2005) Extinction order and altered community structure rapidly disrupt ecosystem functioning. Ecol Lett 8:538-547
- Lee JSF, Berejikian BA (2009) Structural complexity in relation to the habitat preferences, territoriality, and hatchery rearing of juvenile China rockfish (Sebastes nebulosus). Environ Biol Fish 84:411-419
- Levinton JS, Lopez GR (1977) Model of Renewable Resources and Limitation of Deposit-Feeding Benthic Populations. Oecologia 31:177-190
- Lohrer AM, Thrush SF, Gibbs MM (2004) Bioturbators enhance ecosystem function through complex biogeochemical interactions. Nature 431:1092-1095
- Loreau M, Naeem S, Inchausti P, Bengtsson J, Grime JP, Hector A, Hooper DU, Huston MA, Raffaelli D, Schmid B, Tilman D, Wardle DA (2001) Biodiversity and ecosystem functioning: Current knowledge and future challenges. Science 294:804-808
- Lortie CJ, Callaway RM (2006) Re-analysis of meta-analysis: support for the stressgradient hypothesis. J Ecol 94:7-16
- Luckenbach MW (1986) Sediment Stability around Animal Tubes the Roles of Hydrodynamic Processes and Biotic Activity. Limnol Oceanogr 31:779-787
- Luckhurst BE, Luckhurst K (1978) Analysis of Influence of Substrate Variables on Coral-Reef Fish Communities. Mar Biol 49:317-323
- Mackey RL, Currie DJ (2001) The diversity-disturbance relationship: Is it generally strong and peaked? Ecology 82:3479-3492
- Maestre FT, Bautista S, Cortina J, Bellot J (2001) Potential for using facilitation by grasses to establish shrubs on a semiarid degraded steppe. Ecol Appl 11:1641-1655
- Maestre FT, Callaway RM, Valladares F, Lortie CJ (2009) Refining the stressgradient hypothesis for competition and facilitation in plant communities. J Ecol 97:199-205
- Maestre FT, Cortina J (2004) Do positive interactions increase with abiotic stress? -A test from a semi-arid steppe. P Roy Soc Lond B Bio 271:S331-S333
- Maestre FT, Valladares F, Reynolds JF (2005) Is the change of plant-plant interactions with abiotic stress predictable? A meta-analysis of field results in arid environments. J Ecol 93:748-757

- Maestre FT, Valladares F, Reynolds JF (2006) The stress-gradient hypothesis does not fit all relationships between plant-plant interactions and abiotic stress: further insights from arid environments. J Ecol 94:17-22
- McCall PL, Tevesz MJS (1982) Animal-sediment relations: the biogenic alteration of sediments, Vol. Plenum
- McIlroy D, Worden RH, Needham SJ (2003) Faeces, clay minerals and reservoir potential. J Geol Soc 160: 489-493
- Meadows PS, Tait J (1989) Modification of Sediment Permeability and Shear-Strength by 2 Burrowing Invertebrates. Mar Biol 101:75-82
- Menge BA (1976) Organization of New-England Rocky Intertidal Community Role of Predation, Competition, and Environmental Heterogeneity. Ecol Monogr 46:355-393
- Menge BA (1978) Predation Intensity in a Rocky Inter-Tidal Community Relation between Predator Foraging Activity and Environmental Harshness. Oecologia 34:1-16
- Menge BA (2000) Testing the relative importance of positive and negative effects on community structure. Trends Ecol Evol 15:46-47
- Menge BA, Olson AM (1990) Role of Scale and Environmental-Factors in Regulation of Community Structure. Trends Ecol Evol 5:52-57
- Menge BA, Sutherland JP (1987) Community Regulation Variation in Disturbance, Competition, and Predation in Relation to Environmental-Stress and Recruitment. Am Nat 130:730-757
- Mermillod-Blondin F, Marie S, Desrosiers G, Long B, de Montety L, Michaud E, Stora G (2003) Assessment of the spatial variability of intertidal benthic communities by axial tomodensitometry: importance of fine-scale heterogeneity. J Exp Mar Biol Ecol 287:193-208
- Mermillod-Blondin F, Rosenberg R (2006) Ecosystem engineering: the impact of bioturbation on biogeochemical processes in marine and freshwater benthic habitats. Aquat Sci 68:434-442
- Meysman FJR, Galaktionov ES, Gribsholt B, Middelburg JJ (2006) Bioirrigation in permeable sediments: Advective pore-water transport induced by burrow ventilation. Limnol Oceanogr 51:142-156
- Mills LS, Soule ME, Doak DF (1993) The keystone species concept in ecology and conservation. Bioscience 43:219-224
- Morris HM (1955) A new concept of flow in rough conduits. Transactions of the American Society of Civil Engineers 120
- Munksby N, Benthien M, Glud RN (2002) Flow-induced flushing of relict tube structures in the central Skagerrak (Norway). Mar Biol 141:939-945
- Naeem S (1998) Species redundancy and ecosystem reliability. Conserv Biol 12:39-45
- Naeem S, Thompson LJ, Lawler SP, Lawton JH, Woodfin RM (1994) Declining biodiversity can alter the performance of ecosystems. Nature 368:734-737
- Nilsson HC, Rosenberg R (2000) Succession in marine benthic habitats and fauna in response to oxygen deficiency: analysed by sediment profile-imaging and by grab samples. Mar Ecol-Prog Ser 197:139-149

- Norkko A, Hewitt JE, Thrush SF, Funnell GA (2006) Conditional outcomes of facilitation by a habitat-modifying subtidal bivalve. Ecology 87:226-234
- Odling-Smee FJ (1988) Niche-constructing phenotypes. In: Plotkin HC (ed) The Role of Behavior in Evolution. MIT Press, Cambridge, p 73-132
- Odling-Smee FJ, Laland KN, Feldman MW (2003) Niche construction : the neglected process in evolution, Vol. Princeton University Press, Princeton, N.J.; Oxford

Odling-Smee L (2005) Dollars and sense. Nature 437:614-616

- Paine RT (1966) Food web complexity and species diversity. Am Nat 100:65-75
- Paine RT (1969) The *Pisaster–Tegula* interaction: Prey patches, predator food preference, and intertidal community structure. Ecology 50:950-961
- Paine RT (1974) Intertidal community structure: experimental studies on relationship between a dominant competitor and its principal predator. Oecologia 15:93-120
- Pearson TH (2001) Functional group ecology in soft-sediment marine benthos: The role of bioturbation. Oceanogr Mar Biol 39:233-267
- Petchey OL, Gaston KJ (2002) Extinction and the loss of functional diversity. P Roy Soc Lond B Bio 269:1721-1727
- Peterson CH, Andre SV (1980) An Experimental-Analysis of Interspecific Competition among Marine Filter Feeders in a Soft-Sediment Environment. Ecology 61:129-139
- Philippart CJM (1994) Interactions between Arenicola-Marina and Zostera-Noltii on a Tidal Flat in the Wadden Sea. Mar Ecol-Prog Ser 111:251-257
- Powers SP, Peterson CH (2000) Conditional density dependence: the flow trigger to expression of density-dependent emigration in bay scallops. Limnol Oceanogr 45:727-732
- Rabaut M, Guilini K, Van Hoey G, Magda V, Degraer S (2007) A bio-engineered soft-bottom environment: The impact of Lanice conchilega on the benthic species-specific densities and community structure. Estuar Coast Shelf S 75:525-536
- Ragnarsson SA, Raffaelli D (1999) Effects of the mussel Mytilus edulis L. on the invertebrate fauna of sediments. J Exp Mar Biol Ecol 241:31-43
- Rees EIS, Bergmann M, Galanidi M, Hinz H, Shucksmith R, Kaiser MJ (2005) An enriched Chaetopterus tube mat biotope in the eastern English Channel. J Mar Biol Assoc Uk 85:323-326
- Reichardt W (1988) Impact of bioturbation by *Arenicola marina* on microbiological parameters in intertidal sediments. Mar Ecol-Prog Ser 44:149-158
- Reise K (1981a) Gnathostomulida abundant alongside polychaete burrows. Mar Ecol-Prog Ser 6:329-333
- Reise K (1981b) High abundance of small zoobenthos around biogenic structures in tidal sediments of the Wadden Sea. Helgolander Meeresun 34:413-425
- Reise K (1985) Tidal Flat Ecology, Vol. Springer, Berlin
- Reise K (1987) Spatial niches and long-term performance in meiobenthic plathelminthes of an intertidal lugworm flat. Mar Ecol-Prog Ser 38:1-11

- Reise K (2002) Sediment mediated species interactions in coastal waters. J Sea Res 48:127-141
- Reise K, Ax P (1979) Meiofaunal Thiobios Limited to the Anaerobic Sulfide System of Marine Sand Does Not Exist. Mar Biol 54:225-237
- Reise K, Volkenborn N (2004) Large worms as ecosystem engineers in intertidal sediments. In: Tamaki A (ed) Proceedings of the Symposium on "Ecology of Large Bioturbators in Tidal Flats and Shallow Sublittoral Sediments—from Individual Behavior to their Role as Ecosystem Engineers", Nagasaki University, Japan
- Retraubun ASW, Dawson M, Evans SM (1996a) The role of the burrow funnel in feeding processes in the lugworm *Arenicola marina* (L). J Exp Mar Biol Ecol 202:107-118
- Retraubun ASW, Dawson M, Evans SM (1996b) Spatial and temporal factors affecting sediment turnover by the lugworm *Arenicola marina* (L). J Exp Mar Biol Ecol 201:23-35
- Rhoads DC, Mccall PL, Yingst JY (1978) Disturbance and production on estuarine seafloor. Am Sci 66:577-586
- Rhoads DC, Young DK (1970) Influence of deposit-feeding organisms on sediment stability and community trophic structure. J Mar Res 28:150-&
- Riisgård HU, Banta GT (1998) Irrigation and deposit feeding by the lugworm Arenicola marina, characteristics and secondary effects on the environment. A review of current knowledge. Vie Milieu 48:243-257
- Rodriguez LF (2006) Can invasive species facilitate native species? Evidence of how, when, and why these impacts occur. Biol Invasions 8:927-939
- Ropert M, Dauvin JC (2000) Renewal and accumulation of a Lanice conchilega (Pallas) population in the bale des Veys, western Bay of Seine. Oceanol Acta 23:529-546
- Rosenberg R (2001) Marine benthic faunal successional stages and related sedimentary activity. Sci Mar 65:107-119
- Rosenberg R, Elmgren R, Fleischer S, Jonsson P, Persson G, Dahlin H (1990) Marine eutrophication case studies in Sweden. Ambio 19: 102-108
- Schaffner LC (1990) Small-scale organism distributions and patterns of speciesdiversity - evidence for positive interactions in an estuarine benthic community. Mar Ecol-Prog Ser 61:107-117
- Schanz A, Polte P, Asmus H (2002) Cascading effects of hydrodynamics on an epiphyte-grazer system in intertidal seagrass beds of the Wadden Sea. Marine Biology 141:287-297
- Schlapfer F, Schmid B (1999) Ecosystem effects of biodiversity: A classification of hypotheses and exploration of empirical results. Ecol Appl 9:893-912
- Schlapfer F, Schmid B, Seidl L (1999) Expert estimates about effects of biodiversity on ecosystem processes and services. Oikos 84:346-352
- Schulze ED, Mooney HA (1993) Biodiversity and ecosystem function, Vol. Springer-Verlag, Berlin ; London
- Schwartz MW, Brigham CA, Hoeksema JD, Lyons KG, Mills MH, van Mantgem PJ (2000) Linking biodiversity to ecosystem function: implications for conservation ecology. Oecologia 122:297-305

- Schwindt E, De Francesco CG, Iribarne OO (2004a) Individual and reef growth of the invasive reef-building polychaete Ficopomatus enigmaticus in a southwestern Atlantic coastal lagoon. J Mar Biol Assoc Uk 84:987-993
- Schwindt E, Iribarne OO (2000) Settlement sites, survival and effects on benthos of an introduced reef-building polychaete in a SW Atlantic coastal lagoon. B Mar Sci 67:73-82
- Schwindt E, Iribarne OO, Isla FI (2004b) Physical effects of an invading reef-building polychaete on an Argentinean estuarine environment. Estuar Coast Shelf S 59:109-120
- Shashar N, Kinane S, Jokiel PL, Patterson MR (1996) Hydromechanical boundary layers over a coral reef. J Exp Mar Biol Ecol 199:17-28
- Shea K, Roxburgh SH, Rauschert ESJ (2004) Moving from pattern to process: coexistence mechanisms under intermediate disturbance regimes. Ecol Lett 7:491-508
- Smith ST (1967a) Development of *Retusa obtusa* (Montagu) (Gastropoda Opisthobranchia). Can J Zoolog 45:737-&
- Smith ST (1967b) Ecology and life history of *Retusa obtusa* (Montagu) (Gastropoda Opisthobranchia). Can J Zoolog 45:397-&
- Snelgrove PVR, Butman CA (1994) Animal Sediment Relationships Revisited -Cause Versus Effect. Oceanography and Marine Biology, Vol 32 32:111-177
- Solan M, Cardinale BJ, Downing AL, Engelhardt KAM, Ruesink JL, Srivastava DS (2004) Extinction and ecosystem function in the marine benthos. Science 306:1177-1180
- Soulé ME, Estes JA, Miller B, Honnold DL (2005) Strongly interacting species: conservation policy, management, and ethics. Bioscience 55:168-176
- Soulsby R (1997) Dynamics of marine sands : a manual for practical applications, Vol. Telford, London
- Sousa WP (1979) Disturbance in marine intertidal boulder fields: the nonequilibrium maintenance of species diversity. Ecology 60:1225-1239
- Stachowicz JJ (2001) Mutualism, facilitation, and the structure of ecological communities. Bioscience 51:235-246
- Stachowicz JJ, Fried H, Osman RW, Whitlatch RB (2002) Biodiversity, invasion resistance, and marine ecosystem function: Reconciling pattern and process. Ecology 83:2575-2590
- Sthultz CM, Gehring CA, Whitham TG (2007) Shifts from competition to facilitation between a foundation tree and a pioneer shrub across spatial and temporal scales in a semiarid woodland. New Phytol 173:135-145
- Suchanek TH (1994) Temperate Coastal Marine Communities Biodiversity and Threats. Am Zool 34:100-114
- Syms C, Jones GP (2000) Disturbance, habitat structure, and the dynamics of a coral-reef fish community. Ecology 81:2714-2729
- Syms C, Jones GP (2004) Habitat structure, disturbance and the composition of sand-dwelling goby assemblages in a coral reef lagoon. Mar Ecol-Prog Ser 268:221-230
- Symstad AJ, Chapin FS, Wall DH, Gross KL, Huenneke LF, Mittelbach GG, Peters DPC, Tilman D (2003) Long-term and large-scale perspectives on the

relationship between biodiversity and ecosystem functioning. Bioscience 53:89-98

- Thistle D (1981) Natural physical disturbances and communities of marine soft bottoms. Mar Ecol-Prog Ser 6:223-228
- Thompson RC, Wilson BJ, Tobin ML, Hill AS, Hawkins SJ (1996) Biologically generated habitat provision and diversity of rocky shore organisms at a hierarchy of spatial scales. J Exp Mar Biol Ecol 202:73-84
- Thrush SF, Coco G, Hewitt JE (2008) Complex positive connections between functional groups are revealed by neural network analysis of ecological time series. Am Nat 171:669-677
- Tilman D (1999) The ecological consequences of changes in biodiversity: a search for general principles. Ecology 80:1455-1474
- Tilman D, Lehman CL, Thomson KT (1997) Plant diversity and ecosystem productivity: Theoretical considerations. P Natl Acad Sci USA 94:1857-1861
- Tolley SG, Volety AK (2005) The role of oysters in habitat use of oyster reefs by resident fishes and decapod crustaceans. J Shellfish Res 24:1007-1012
- Travis JMJ, Brooker RW, Clark EJ, Dytham C (2006) The distribution of positive and negative species interactions across environmental gradients on a duallattice model. J Theor Biol 241:896-902
- Valentine JF, Heck KL, Harper P, Beck M (1994) Effects of Bioturbation in Controlling Turtlegrass (Thalassia-Testudinum Banks Ex Konig) Abundance - Evidence from Field Enclosures and Observations in the Northern Gulf-of-Mexico. J Exp Mar Biol Ecol 178:181-192
- Van Hoey G, Guilini K, Rabaut M, Vincx M, Degraer S (2008) Ecological implications of the presence of the tube-building polychaete Lanice conchilega on softbottom benthic ecosystems. Mar Biol 154:1009-1019
- van Wesenbeeck BK, Crain CM, Altieri AH, Bertness MD (2007a) Distinct habitat types arise along a continuous hydrodynamic stress gradient due to interplay of competition and facilitation. Mar Ecol-Prog Ser 349:63-71
- van Wesenbeeck BK, van de Koppel J, Herman PMJ, Bakker JP, Bouma TJ (2007b) Biomechanical warfare in ecology; negative interactions between species by habitat modification. Oikos 116:742-750
- Vismann B (1991) Sulfide tolerance: physiological mechanisms and ecological implications. Ophelia 34:1-27
- Vitousek PM, Hooper DU (1993) Biological diversity and terrestrial ecosystem biogeochemistry. In: Schulze E-D, Mooney HA (eds) Biodiversity and Ecosystem Function. Springer-Verlag, Berlin, Germany
- Vitousek PM, Mooney HA, Lubchenco J, Melillo JM (1997) Human domination of Earth's ecosystems. Science 277:494-499
- Volkenborn N, Hedtkamp SIC, van Beusekom JEE, Reise K (2007a) Effects of bioturbation and bioirrigation by lugworms (*Arenicola marina*) on physical and chemical sediment properties and implications for intertidal habitat succession. Estuar Coast Shelf S 74:331-343
- Volkenborn N, Polerecky L, Hedtkamp SIC, van Beusekom JEE, de Beer D (2007b) Bioturbation and bioirrigation extend the open exchange regions in permeable sediments. Limnol Oceanogr 52:1898-1909

- Volkenborn N, Reise K (2006) Lugworm exclusion experiment: responses by deposit feeding worms to biogenic habitat transformations. J Exp Mar Biol Ecol 330:169-179
- Volkenborn N, Reise K (2007) Effects of *Arenicola marina* on polychaete functional diversity revealed by large-scale experimental lugworm exclusion. J Sea Res 57:78-88
- Volkenborn N, Robertson DM, Reise K (2009) Sediment destabilizing and stabilizing bio-engineers on tidal flats: cascading effects of experimental exclusion. Helgol Wiss Meeresunters 63:27-35
- Walker BH (1992) Biodiversity and ecological redundancy. Conserv Biol 6:18-23
- Wall CC, Peterson BJ, Gobler CJ (2008) Facilitation of seagrass Zostera marina productivity by suspension-feeding bivalves. Mar Ecol-Prog Ser 357:165-174
- Wetzel MA, Jensen P, Giere O (1995) Oxygen/sulfide rexime and nematode fauna associated with *Arenicola marina* burrows: new insights in the thiobios case. Mar Biol 124:301-312
- Wilson WH (1981) Sediment-Mediated Interactions in a Densely Populated Infaunal Assemblage - the Effects of the Polychaete Abarenicola-Pacifica. J Mar Res 39:735-748
- Witman JD, Grange KR (1998) Links between rain, salinity, and predation in a rocky subtidal community. Ecology 79:2429-2447
- Wittebolle L, Marzorati M, Clement L, Balloi A, Daffonchio D, Heylen K, De Vos P, Verstraete W, Boon N (2009) Initial community evenness favours functionality under selective stress. Nature 458:623-626
- Wojdak JM, Mittelbach GG (2007) Consequences of niche overlap for ecosystem functioning: An experimental test with pond grazers. Ecology 88:2072-2083
- Woodin SA (1974) Polychaete Abundance Patterns in a Marine Soft-Sediment Environment - Importance of Biological Interactions. Ecol Monogr 44:171-187
- Woodin SA (1978) Refuges, Disturbance, and Community Structure Marine Soft-Bottom Example. Ecology 59:274-284
- Worcester SE (1995) Effects of Eelgrass Beds on Advection and Turbulent Mixing in Low Current and Low Shoot Density Environments. Mar Ecol-Prog Ser 126:223-232
- Worm B, Barbier EB, Beaumont N, Duffy JE, Folke C, Halpern BS, Jackson JBC, Lotze HK, Micheli F, Palumbi SR, Sala E, Selkoe KA, Stachowicz JJ, Watson R (2006) Impacts of biodiversity loss on ocean ecosystem services. Science 314:787-790
- Yachi S, Loreau M (1999) Biodiversity and ecosystem productivity in a fluctuating environment: The insurance hypothesis. P Natl Acad Sci USA 96:1463-1468
- Zebe E, Schiedek D (1996) The lugworm *Arenicola marina*: a model of physiological adaptation to life in intertidal sediments. Helgolander Meeresun 50:37-68
- Ziegelmeier E (1952) Beobachtungen über den Röhrenbau von Lanice conchilega (Pallas) im Experiment und am natürlichen Standort. Helgol Wiss Meeresunters 4:107-129
- Ziegelmeier E (1969) Neuere Untersuchungen über die Wohnröhren-Bauweise von Lanice conchilega (Polychaeta, Sedentaria). Helgol Wiss Meeresunters 19:216-229

.

- Zühlke R (2001) Polychaete tubes create ephemeral community patterns: *Lanice conchilega* (Pallas, 1766) associations studied over six years. J Sea Res 46:261-272
- Zühlke R, Reise K (1994) Response of macrofauna to drifting tidal sediments. Helgolander Meeresun 48:277-289