

# **The role of genotype diversity within seagrass populations of the Baltic Sea**

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Der Dekan

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The best way to cope with surprise is resilience.....

Carpenter et al. 2001



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Eelgrass meadow near Maasholm / Schlei fjord.

## Summary

Seagrass communities belong to the ecologically most valuable ecosystems in the marine realm. As foundation species seagrasses build the basis for a highly diverse and productive associated floral and faunal community, including economically valuable species. Ironically, the importance of intact seagrass meadows for coastal ecosystem services was primarily realized after dramatic declines throughout the last century, as a result of habitat destruction, pollution and eutrophication. The impact of these 'traditional' anthropogenic pressures endangering all coastal ecosystems will be even exacerbated through predicted climate warming.

While positive effects of different components of diversity on ecosystem functioning and stability are relatively well studied in terrestrial ecosystems, experimental research on the role of biodiversity is lagging behind in marine ecosystems. This applies particularly to coastal ecosystems that often base on single dominant species like mussel beds, kelp forests, coral reefs, and seagrass meadows. These communities, in spite of lacking redundancy at the species level, persist in highly dynamic environments with steep abiotic gradients. My central tenet is that in these mono-dominant systems, within-species genetic and genotypic diversity may serve functions analogous to species diversity elsewhere.

In the present study, I evaluated the effects of genotypic diversity on ecosystem productivity and ecosystem stability. These proposed effects were tested within a *Zostera marina* L. (eelgrass) dominated macrophyte community in the south-western Baltic Sea. In indoor mesocosm experiments I exposed mixtures of eelgrass genotypes to different abiotic stressors, to test whether genotypic diversity could act as a functional substitute for species diversity. A strong eutrophication pulse (Experiment I) and increased water temperatures (Experiment II) as abiotic stress treatments were applied, as these two factors are known to affect eelgrass meadows negatively at present and even more in the future. In a third experiment (Experiment III) no stress was induced, yet the aim was to unravel the relative impacts of genotypic diversity and species diversity on productivity of a three species macrophyte community.

I found strong negative effects of eutrophication and warming on eelgrass in my first two studies. Genotypes in both studies differed in their individual reactions towards the respective abiotic stressors. A general positive effect of genotypic diversity on shoot density of eelgrass was only observable in the heat stress treatments. One genotype was found to perform better than other genotypes tested for both abiotic stressors. This multi-purpose genotype showed its advantageous performance only in monoculture and not in mixtures

with other genotypes, suggesting a trade-off between multiple stress tolerance and competitive ability.

The second experiment in which I used increased water temperature, corroborated observations from an eelgrass-field experiment during the European heat wave in 2003, as the negative impact of elevated temperatures and a positive effect of genotype diversity on eelgrass productivity could be demonstrated again.

Unravelling the relative impacts of genotype and species diversity on productivity, measured as shoot density in a three species macrophyte community showed a strong impact of species identity, whereas species richness had no effect. Final genotype richness showed a positive relationship with shoot density, driven by a strong selection effect due to *Zostera marina*, the largest of all three species.

In conclusion, my investigations emphasized the importance of the genotype diversity level within dominant foundation species. My results suggest genotype diversity to be the prerequisite to provide response diversity in the face of a changing environment. Conserving genotype diversity in different macrophyte foundation species should therefore be a major goal for coastal conservation in a future of global change.



## Zusammenfassung

Seegras Gemeinschaften gehören zu den ökologisch wertvollsten Ökosystemen im marinen Reich. Als sogenannte Gründerarten bilden Seegräser die Basis für eine hochdiverse und produktive assoziierte Pflanzen- und Tiergemeinschaft, der auch ökonomisch relevante Arten angehören. Ironischerweise wurde die Wichtigkeit intakter Seegraswiesen für Systemleistungen von Küstenökosystemen erst wahrgenommen, nachdem im vergangenen Jahrhundert dramatische Verluste auftraten, die auf Habitatzerstörung, Gewässerverschmutzung und Eutrophierung zurück zu führen sind. Eine zusätzliche Verstärkung dieser „traditionellen“ anthropogenen Belastungen, die alle Küstensysteme gleichermaßen gefährden, ist durch die vorhergesagte Klimaerwärmung zu erwarten.

Während positive Effekte verschiedener Diversitätskomponenten auf die Funktionen und die Stabilität von terrestrischen Ökosystemen bereits relativ gut untersucht sind, liegt die experimentelle Erforschung der Bedeutung von Biodiversität in marinen Ökosystemen noch zurück. Dies gilt besonders für Küstenökosysteme, die sich auf einzelne Arten gründen wie z.B. Muschelbänke, Tangwälder, Korallenriffe und Seegraswiesen. Trotz der fehlenden Artendiversität, vollbringen es diese Gemeinschaften dennoch in einer hochdynamischen Umwelt mit steilen abiotischen Gradienten zu bestehen. Meine zentrale These ist, dass in diesen Systemen innerartliche genetische und genotypische Diversität eine Funktion erfüllt, die der von Artendiversität in anderen Systemen vergleichbar ist.

In der vorliegenden Studie habe ich die Effekte genotypischer Diversität auf die Produktivität und Stabilität von Ökosystemen bewertet. Diese möglichen Effekte wurden einer von dem Großen Seegras (*Zostera marina*, L.) dominierten Makrophytengemeinschaft in der süd-westlichen Ostsee untersucht. In Mesokosmos-Experimenten habe ich Mischpflanzungen von Genotypen des Großen Seegrases verschiedenen abiotischen Stressfaktoren ausgesetzt, um zu überprüfen, ob genotypische Diversität als funktioneller Ersatz für Artendiversität dienen kann. Ein starker Eutrophierungspuls (Experiment I) sowie erhöhte Wassertemperaturen (Experiment II) wurden als abiotische Stressbehandlungen eingesetzt, da bekannt ist, dass diese beiden Faktoren Seegraswiesen bereits heutzutage negativ beeinflussen und dies verstärkt auch in der Zukunft der Fall sein wird. In einem dritten Experiment (Experiment III) wurde kein Stress eingesetzt, sondern das Ziel war die relativen Einflüsse von genotypischer und artlicher Diversität auf die Produktivität einer Gemeinschaft, zusammengesetzt aus drei Makrophytenarten, zu untersuchen.

Ich konnte in meinen ersten beiden Studien starke negative Effekte durch Eutrophierung und Erwärmung auf das Große Seegras feststellen. In beiden Studien unterschieden sich

die einzelnen Genotypen in ihren individuellen Reaktionen auf die jeweiligen abiotischen Einflüsse. Ein generell positiver Effekt genotypischer Diversität auf die Sprossdichte des Seegrases konnte nur in dem Erwärmungs-Experiment gezeigt werden. Einer der getesteten Genotypen schnitt unter beiden eingesetzten Stressfaktoren besser ab als die übrigen. Diese vorteilhafte Leistung zeigte dieser Multi-Funktions-Genotyp nur, wenn er in Monokultur kultiviert wurde, nicht jedoch in Mischungen mit anderen Genotypen, was auf einen *trade-off* zwischen multipler Stresstoleranz und innerartlicher Konkurrenzfähigkeit hindeuten könnte.

Das zweite Experiment, in dem ich eine Erhöhung der Wassertemperatur eingesetzt habe, bestätigte darüber hinaus die Beobachtungen eines Seegras-Feldexperimentes, welches während der europäischen Hitzewelle im Sommer 2003 durchgeführt wurde. Der negative Einfluss erhöhter Wassertemperaturen und der positive Effekt genotypischer Diversität auf die Produktivität des Seegrases konnte erneut gezeigt werden.

Die Untersuchung der relativen Einflüsse genotypischer und artlicher Diversität auf die Produktivität, gemessen als Sprossdichte in einer Makrophytengemeinschaft bestehend aus drei Arten, zeigte einen sehr starken Einfluss der Art-Identität, während die Artenanzahl keinerlei Effekt zeigte. Die finale Genotypen Anzahl zeigte eine positive Beziehung zur Sprossdichte, gesteuert durch einen starken Selektionseffekt, der von der größten der untersuchten Arten, *Zostera marina*, ausging.

Zusammenfassend betrachtet, unterstreichen meine Untersuchungen die Wichtigkeit der Ebene genotypischer Vielfalt innerhalb von dominanten Gründerarten. Meine Ergebnisse weisen darauf hin, dass genotypische Diversität die Voraussetzung ist für *response diversity* im Zuge einer veränderlichen Umwelt. Der Schutz genotypischer Diversität innerhalb verschiedener makrophytischer Gründerarten, sollte daher ein Hauptziel des Küstennaturschutzes in einer Zukunft des globalen Wandels sein.

## Introduction

### Causes and consequences of biodiversity

The term 'biodiversity' has been used in many ways but is clearly defined by the Convention on Biological Diversity as "the variability among living organisms from all sources including, inter alia, terrestrial, marine and other aquatic ecosystems and the ecological complexes of which they are part; this includes diversity within species, between species and of ecosystems (UN 1992). Unprecedented worldwide loss of biodiversity during the past few centuries is now well established across many ecosystems and taxonomic groups. Unfortunately, the current extinction crisis is predicted to even accelerate in the future (Pimm et al. 1995, Sala et al. 2000). Direct and indirect human impacts are the main causes for this trend (Vitousek et al. 1997b). The Millennium Ecosystem Assessment (<http://www.millenniumassessment.org>) identified habitat change, climate change, invasive alien species, overexploitation and pollution, as the five main direct drivers of biodiversity loss and ecosystem service changes (MEA 2005). Examples for loss through these five impacts or compounds of all are manifold (Tilman 1999b, Nystrom et al. 2000, Walther et al. 2002, Hughes et al. 2003, Myers & Worm 2003, Parmesan & Yohe 2003, Foley et al. 2005, Scheffer et al. 2005, Lotze et al. 2006). Such alteration of ecosystems will also deeply affect the flow of ecosystem services directly concerning the basis of human well-being is noticed with growing concern (MEA 2005, Díaz et al. 2006, Worm et al. 2006).

Although being part of the research agenda of basic ecology already for a long time, biodiversity research gained momentum through the alarming evidence of increasing diversity loss. The recent biodiversity-ecosystem functioning (BEF) debate focuses on the question how ecosystems will be affected in their properties, processes, goods and services by the decline of biodiversity (Loreau et al. 2002, Hooper et al. 2005). While former biodiversity research was concerned with patterns and causes of diversity (e.g. island biogeography, species coexistence), the perspective has changed to diversity being the independent (experimental) factor of interest. In the past 15 years an extremely successful research program focused on the relationships between diversity and productivity of ecosystems, and between diversity and ecosystem stability (summarized in Loreau et al. 2002, Hooper et al. 2005).

## **Diversity - productivity relationships**

Some of the first experimental studies in this field convincingly demonstrated positive relationships between species diversity and productivity, measured as biomass production in grassland plots (Naeem et al. 1994, 1995, Tilman et al. 1996, Tilman et al. 1997a, Hector et al. 1999) or in freshwater microcosm (McGrady-Steed et al. 1997, Naeem & Li 1997). Biomass production is the most commonly reported measure of ecosystem performance or functioning. This choice of ecosystem properties can be justified in most cases, as above-ground biomass production is often correlated with other desirable ecosystem properties, such as soil fertility, carbon storage and habitat provision for related communities.

The processes behind these relationships were first controversial. Reconciliation was reached through the work of Loreau & Hector (2001) who separated biodiversity effects into two groups: complementary effects and selection effects. The first process is based on the theory of niche complementarity and resource partitioning and posits that more species with differing functional traits will utilize a given pool of resources more completely, thereby yielding higher biomass, or another ecosystem function, than either species alone (Tilman et al. 1997b). Positive interactions (= facilitation; Bruno et al. 2003) and negative physical or chemical interference between species also belong to this first group of mechanisms. The second process can be reduced to a statistical sampling effect. Among several species, there is a higher likelihood of containing a dominant species with more than average productivity in the mixture (Huston 1997, Tilman et al. 1997b, Hector et al. 2002). Averaged over many combinatorial assemblages, one can show that, species-rich mixtures would perform better than species-poor ones. Note that both, complementarity and selection can also operate simultaneously or even antagonistically (Hooper et al. 2005).

Above studies on grassland and microcosms were important for unravelling the importance of species or functional group diversity within one trophic level. Subsequent experiments extended earlier approaches to manipulating species diversity in two or more functional groups, in different ecosystems and on different geographical scales (e.g. local, regional, global) yielded varying results (Hooper et al. 2005), while other levels of diversity (i.e. landscapes or genetic components) remained poorly tested or even un-tested. Still, incorporating trophic complexity is one of the most challenging goals, as multitrophic interactions produce a richer variety of diversity-functioning relationships than the monotonic changes predicted from single trophic levels (Duffy et al. 2007).

## **Diversity-stability relationships**

Another critical component of ecosystems is their stability. Although long being considered in ecological research (MacArthur 1955, May 1973, McCann 2000), the question of how diversity and the stability of ecosystems are related is one of the most difficult ones in ecology (Hooper et al. 2005). Problems start with definitions of different components of 'stability' (see below *Stability definitions*).

Two aquatic microcosm-experiments showed that ecosystems display smaller temporal variance, when being composed of several as opposed to few microbial species. Thus, it was deduced that ecosystem predictability (a term describing variability over time) is higher in more diverse species communities (McGrady-Steed et al. 1997, Naeem & Li 1997). These empirical results were later linked to theoretical considerations provided by competition theory (Tilman 1999a) and the insurance hypothesis model (Yachi & Loreau 1999), which predicted a reduction in the temporal variability of ecosystem properties (predominantly considered as productivity, i.e. biomass) with increased species diversity. Recently, Tilman and colleagues (2006) provided empirical support in a ten-year grassland field experiment, where a positive relationship between species diversity and the temporal variance of ecosystem functions was found. This relationship depends mostly on the individual species responses to environmental fluctuations, the asynchronicity of these responses, and their range of variation (Yachi & Loreau 1999, Elmqvist et al. 2003), and can be explained by statistical averaging (portfolio effect) over these three variability components (Doak et al. 1998, Tilman 1999a).

The evidence for a relationship between diversity and resistance or resilience of ecosystems is more contentious. When a functionally dominant species is lost from a community, other formerly 'redundant' species may provide insurance, i.e. compensate its function. The more species are included within a functional group with slightly differing traits, the higher the potential that they can buffer ecosystem functions when environmental conditions change ('response diversity' Elmqvist et al. 2003).

### *Stability definitions*

Stability can be divided into three components: resilience, resistance and variability. The last component is unproblematic and consistently defined as variance over time, whereas resilience and resistance have different definitions depending on the general view of ecosystems stability. Either ecosystems are viewed in equilibrium while another school holds that there are always multiple stable states. First, an equilibrium centred view, which is the more traditional one, defines resilience as the time to return to equilibrium following a perturbation and resistance as the degree to which a variable is changed, after a disturbance (Pimm 1984). These definitions concentrate on conditions near a single steady state where transient measurements of rate of return are made following small disturbances (Peterson et al. 1998).

The definition of resilience from a multiple stable state view focuses on the boundaries of the domain of attraction in dynamic ecosystems. This definition is consistent with 'ecosystem resilience' as proposed by Holling (1973) reflecting the capacity of a system to undergo disturbance and still maintain its functions, before it shifts from one stable state to another (Holling & al. 1995, Nystrom et al. 2000). Stability, on the other hand, is defined by Holling (1973), as "[...] the ability of a system to return to an equilibrium state after a temporary disturbance. The more rapidly it returns, and with the least fluctuation, the more stable it is. In this definition stability is the property of the system and the degree of fluctuation around specific states the result". The definitions related to stability given here, offer just a very broad overview. Therefore, for further reading the work of Grimm & Wissel (1997) is recommended, who list 163 definitions of 70 different stability concepts.

## **What lies beyond species diversity in terrestrial primary producers?**

Although the BEF research has better empirical support than many other ecological theories, many questions are still unresolved. One of the key extensions to be made is the inclusion of additional trophic levels. Models exist for the effects of biodiversity within foodwebs (Thébault & Loreau 2003, 2006), adding more complexity by including results from food-web ecology. Experiments in grassland plots (Mulder et al. 1999) and aquatic

microcosms (Naeem et al. 2000) showed that positive effects of plant species richness on productivity were erased when consumers were introduced. Duffy et al. (2003) found that varying consumer diversity invoked complex interactions that compensated positive plant diversity effects in a seagrass ecosystem. Moreover, relationships between species diversity and productivity, and species diversity and stability can be bidirectional (Worm & Duffy 2003), and studies showed that herbivores can modify, dampen or even reverse the directionality of biodiversity-productivity-stability linkages inferred from the plant level alone (Duffy et al. 2007). Conclusions gained from plant diversity experiments should therefore not be transferred to more complex trophic systems. Furthermore, models including abiotic factors demonstrate that diversity effects strongly depend on the ecological context, e.g. disturbance rates and spatial heterogeneity of the environment (Cardinale et al. 2000), which was supported by a study in stream ecosystems (Cardinale et al. 2005).

All processes involved in explaining diversity effects predict that diversity matters precisely because species differ in traits, causing composition to be as important as richness (Tilman et al. 2002). The BEF debate would benefit from the adoption of a view following the response diversity concept (Elmqvist et al. 2003, Naeem & Wright 2003) dealing with the number and variety of different characters, i.e. functional (effect and response) traits within an ecosystem, irrespective of taxonomic or functional group classification. At present, most experimental evidence for BEF-relationships result from terrestrial ecosystems while only few experiments were conducted in the marine realm (Emmerson & Huxham 2002, Giller et al. 2004, Duffy & Stachowicz 2006, Hendricks et al. 2006).

## **The role of genetic and genotypic diversity**

Although genetic diversity is mentioned by the IUCN as one of the three forms of biodiversity deserving conservation, most experiments have equated biodiversity with the number of species and just few studies have explicitly dealt with intraspecific diversity so far. This certainly underestimates the problem of global diversity loss, as it ignores decrease on the population, genotypic and genetic diversity level (Hughes et al. 1997, Norberg et al. 2001, Luck et al. 2003).

Nevertheless there is already some experimental evidence for genetic or genotypic importance in the light of the BEF-discussion coming from different study-systems. In a five-year experiment with herbaceous clonal plant communities Booth & Grime (2003) showed that, genetic diversity within component species reduced the rate at which species diversity declined. Settling success in a marine invertebrate was enhanced by increased intraspecific diversity (Gamfeldt et al. 2005). Experiments in eelgrass study-systems found increased

resistance to geese grazing (Hughes & Stachowicz 2004) and higher biomass production, plant density, faunal abundance and faster recovery from heat stress in genotypically more diverse seagrass plots (Reusch et al. 2005). Other terrestrial studies found associated arthropod communities structured by host plant genetic diversity (Wimp et al. 2005, Johnson et al. 2006). It is therefore clear that in addition to species diversity, the level of genetic diversity may enhance population fitness and resilience (sensu Holling 1973) and therefore decrease extinction risk in the face of environmental change (Vellend & Geber 2005, Duffy 2006, Reusch & Hughes 2006).

Additional to this buffering-capacity for populations facing actual disturbances, genetic diversity is the prerequisite for adaptive evolution of populations as a response to environmental changes, e.g. climate warming (Lynch & Lande 1993, Lynch 1996, Frankham et al. 2002). The genetic level of diversity is particularly important in foundation species, as these represent whole functional groups with no redundancy at the species level (Dayton 1972, Jones et al. 1994, Micheli & Halpern 2005). It is presumed that genetic diversity may take over the functional role of species diversity in these special systems (Reusch & Hughes 2006). The effects of genetic diversity can extend to the community level according to the concept of the extended phenotype (Dawkins 1982). These kind of relations are tackled by 'community genetics', a research direction defined by (Antonovics 1976, 1992) as to investigate the "role of genetic variation in influencing species interactions and determining community structure" (see also Neuhauser et al. 2003, Witham et al. 2003). Such extended effects have been demonstrated for the composition of arthropod communities, which depended on the genotype of their host-plants (Wimp et al. 2005, Johnson et al. 2006) and the abundance of invertebrates in experimental eelgrass plots of different genotypic composition (Reusch et al. 2005). Clonal foundation species offer special advantages for experimental studies, as the measurement and manipulation of genotypic diversity is exactly analogous to that of species diversity, as the different genotypes within species may interact similar to different species within the same functional group (Vellend & Geber 2005).

### **Concept of foundation species and ecosystem engineers**

All organisms modify their environment (Vitousek et al. 1997b). In ecological theory there are many attempts to group species according to their importance for ecosystem structure and functions. Very famous, discussed, misused and redefined (Mills et al. 1993, Power et al. 1996) is the concept of keystone species introduced by Paine (1969).



Seagrasses belong to foundation species and ecosystem engineers. A foundation species as outlined by Dayton (1972) is 'a single species that defines much of the structure of a community by creating locally stable conditions for other species, and by modulating and stabilizing fundamental ecosystem processes.' Building the basis for diverse foodwebs, seagrasses positively interfere with nearly all organisms in this ecosystem, which has been interpreted as facilitation (Bruno et al. 2003). Seagrasses are also ecosystem engineers, defined as 'are organisms that directly or indirectly control the availability of resources to other organisms by causing physical state changes in biotic or abiotic materials. Physical engineering by organisms is the physical modification, maintenance, or creation of habitats', whereby their direct effects can last longer than the lifetime of the organism itself (Jones et al. 1994, 1997, Hastings et al. 2007).

In the light of the biodiversity–ecosystem functioning debate these foundation or engineering species take a special role. They may have pervasive impacts on the functioning of ecosystems (Grime 1998) and can override the potentially buffering effects of diversity in some situations (Duffy 2006). Secondly, as the results gained from experimental species diversity manipulations and models, cannot directly be applied on these mostly dominant species, which often occur in wide mono-specific stands and represent a whole functional group through one single species. In such cases we may expect, by analogy with effects of species richness, that genetic and phenotypic diversity within foundation species will be important (Reusch & Hughes 2006).



## Aim of the study

The aim of this study was to evaluate the role of genotypic diversity within populations of a temperate seagrass community. Positive relationships between species diversity and ecosystem functions are documented for many terrestrial systems, but mainly in grasslands. Experimental work has been mostly done using experimental sub-sets of these species-rich pasture communities. How can these findings be translated to communities consisting of few dominant species, like it is the case for temperate seagrass meadows? Can we find relationships between genotype diversity and productivity or stability of communities, similar to the relationships known from species diversity experiments? May genotypes in species poor systems take over the role of species, regarding their importance for BEF relationships?

In this thesis I present results of three independent experimental studies investigating the role of genotype diversity within populations of an eelgrass dominated macrophyte system. Each chapter reporting an experiment is organized in introduction, methods, results and discussion, whereas the study species and the part of material and methods used in all experiments are presented in the general chapter Material & Methods beforehand. All experiments were conducted in an indoor mesocosm facility to control environmental factors.

The first two experiments were designed to test, whether or not genotypic diversity, differentiated in richness and identity of genotypes, enhances resilience in *Zostera marina* L. (eelgrass) to environmental stress. In the first study a strong fertilization pulse was combined with three levels of genotypic diversity in experimental eelgrass plots (Experiment I). Six eelgrass clones were used for this experiment, having the unique opportunity to compare the results to a parallel field experiment using the same set of genotypes (Reusch et al. 2005). The second experiment was established to examine, whether summer heat waves have negative effects on eelgrass, resembling the 2003 European heat wave conditions (Experiment II). Again this treatment was combined with three levels of eelgrass genotype diversity. Twelve eelgrass genotypes were used for this experiment, six of them replicated in monocultures. These two experiments specifically tested (1) whether eutrophication or increased water temperature respectively have negative impacts on eelgrass performance, (2) the impact of genotypic richness on eelgrass plot performance, (3) the potential role of genotypic diversity buffering for negative effects due to abiotic stress (interaction), and (4) whether individual genotypes will react differently to the abiotic stress they are exposed to.

The third experiment was established to directly compare the effects of genotype versus species diversity within representatives of three naturally co-occurring coastal angiosperm species. The focus was to evaluate the potential ability of the genotype level in foundations species taking over the functional role of the species level, concerning community processes. I hypothesized that differences in the response variables due to genotype diversity should be in the same order of magnitude as those due to species diversity, i.e. explain a comparable or even higher amount of variance in the statistical model.



Indoor mesocosm facility, IfM-Geomar, Kiel

## Material & Methods

### Study species

All three species used in this study are clonal marine flowering plants, which naturally coexist and belong to the typical macrophyte community in temperate estuaries. As ecosystem engineers and foundation species, they stabilize sediments and add a complex three-dimensional structure to the otherwise bare sandy bottoms. These physical structures provide nursery grounds for many different species and also habitat for specialized fauna, e.g. pipefish. Above all, they are important primary producers and provide organic material to decomposing food webs, and build some of the most widespread and productive coastal ecosystem types (Constanza et al. 1997, Williams & Heck 2001, Duffy 2006). Therefore a lot of specific literature about seagrasses exists including a number of important books or review chapters (Den Hartog 1970, Phillips & McRoy 1980, Hemminga & Duarte 2000, Short & Coles 2001, Green & Short 2003, Larkum et al. 2006). The ISI-Web of knowledge counts approximately 3500 papers of original research in seagrasses since the 1960s (Procaccini et al. in press). Experimental studies in seagrass ecosystems have contributed substantially to marine community ecology (Williams & Heck 2001). Seagrass ecosystems decline worldwide, through direct and indirect human impacts (Short & Wyllie-Echeverria 1996, Lotze et al. 2006). This has caused concern, because seagrasses, as foundation species, provide irreplaceable ecological services to the coastal marine environment (Constanza et al. 1997, Orth et al. 2006). The responses of seagrasses to biotic and abiotic environmental factors have been investigated, in order to reveal the basic mechanisms maintaining an existing meadow or enabling a newly growing seagrass patch to establish (for a recent synthesis see Larkum et al. 2006).

Eelgrass *Zostera marina* L. (1753) is the species predominantly employed to study the effects of genotypic diversity on ecosystem functions in this thesis. Eelgrass is widely distributed in northern temperate coastal waters of both sides of the northern Atlantic and Pacific (Olsen et al. 2004) and occurs in annual or perennial populations. Although intertidal populations occur, populations are primarily subtidal, with light limited depth extension. Their vegetative shoots consist of bundles of linear leaves that reach up to 2m in length, normally with a width of 3-9mm and build the physical basis for a highly productive epiphytic community, which contributes to the high primary productivity as the basis of diverse food-webs (Den Hartog 1970, Williams & Heck 2001, Duffy & Stachowicz 2006). Generative shoots are upright and ramified with several monoecious inflorescences. Seagrasses have

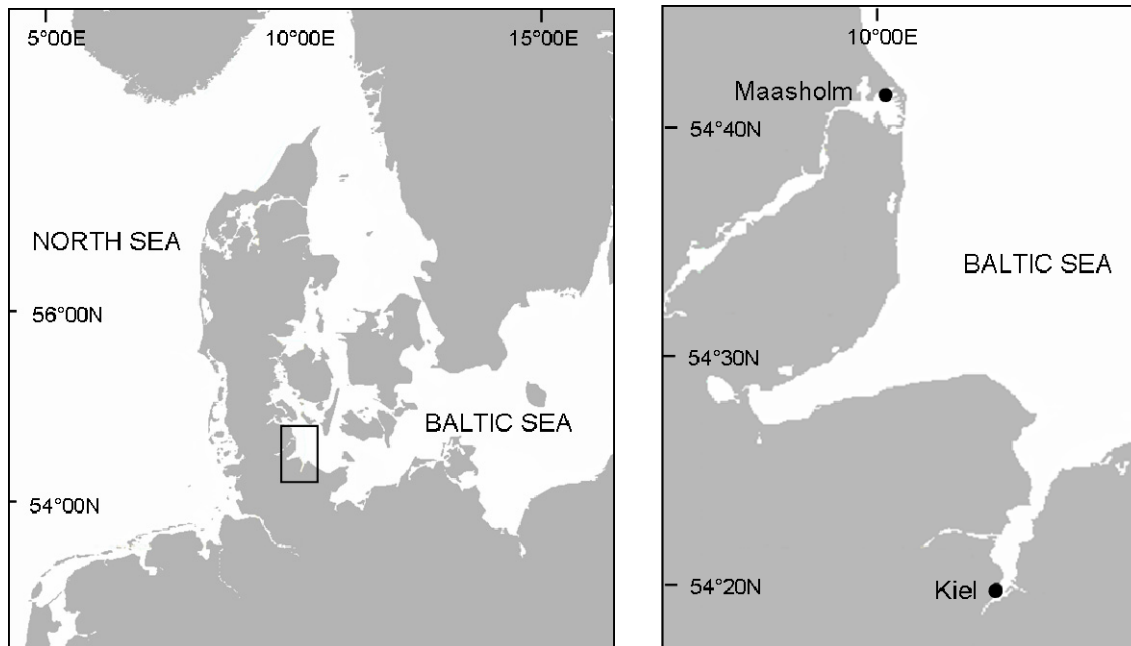
developed real underwater pollination mechanisms (hydrophily), with specialised filiform pollen and release ovoid fruits. Additionally they propagate via extension and branching of lateral rhizomes. This clonal growth allows particularly successful genotypes to cover wide areas within meadows or sites (Reusch et al. 1999).

*Zostera noltii* (Hornemann) belongs to the same family as *Z. marina*. It is smaller in size with leaf shoots reaching a height of 30 cm having a width of 2-3mm but otherwise exhibits most of the features described for *Z. marina*. Their generative shoots are differently structured, as they consist of only one bottom-standing spike, releasing ellipsoid fruits. *Z. noltii* also grows in a clonal mode via rhizome elongation and occupies mostly intertidal or very shallow water to a depth of maximal 4m. This species is abundant at the western Atlantic coast from Mauritania to southern Norway, but also occurs in the Black Sea, Caspian and Aral Sea.

Widgeon grass *Ruppia maritima* L. (1753) belongs to the family Ruppiacea. Seagrasses are defined as monocotyledons that have returned secondarily to the sea and as such, *Ruppia* qualifies for a seagrass. Molecular studies revealed their close relationship to the Posidoniaceae (Waycott et al. 2006) and therefore recent consensus has led to include them into the polyphyletic group of seagrasses. Yet, the genus is in need of revision, as the great intraspecific and morphological diversity has led to some confusion (den Hartog & Kuo 2006). *R. maritima* has a wide distribution in temperate and tropical regions all over the world and grows in a variety of shallow coastal waters and estuaries with a broad tolerance of salinities. Their weakly differentiated rhizomes bear no root bundles at the nodes and the richly branched stems with leaves of 0.5mm width can reach a height of more than 1m.

## **Donor site**

Plants were taken from macrophyte beds in the outer Schlei (54°41'N, 10°00'E) near Maasholm, Germany in the western Baltic Sea (Fig. 1). The Schlei is a 42 km long shallow glacial fjord with a mean depth of 2.4 m, multiply partitioned in distinct embayments with decreasing salinity towards the inland parts. In this non-tidal area water exchange and water level are wind driven. Depending on wind direction nutrient, phytoplankton and seston loads vary strongly, with high loads from the inner waters and comparatively low concentrations from the water masses of the adjacent Kiel Bight. Water levels change with an amplitude of  $\pm 0.5$ m around mean water level. Salinity fluctuates with season between 12-18 PSU in summer and 15-20 PSU in winter (Schramm et al. 1996). Maximum temperatures are reached normally during July or August exceeding 20°C, whereas



**Figure 1:** Location of the donor site Maasholm in the outer part of the Schlei fjord at the German Baltic Coast

generally water temperatures in the Schlei fjord are  $\sim 1^{\circ}\text{C}$  higher than in the neighbouring waters of the Kiel Bight (Dries & Adelung 1982, Lotze 1998).

The sampling site is located in the outermost bay at the mouth of the Schlei fjord, which is a vast shallow area of mostly less than 2m depth. Sediment consists of coarse sand with scattered rocks and boulders, with interspersed peat layers just few centimetres below a layer of sandy sediment. The bay is enclosed by typical shallow barrier beaches of boulder clay of glacial origin and neighbored by a nature protected area, which guarantees relatively undisturbed conditions. Large parts of this bay are covered by dense subtidal macrophyte beds with angiosperms distributed along a depth gradient and different kinds of macroalgae using the rocks and boulders as substrate. Whereas the eelgrass population seems to be stable, in different years a great variability was observed in the shallower part where different angiosperm species co-exist in a patchy distribution (A. Ehlers, personal observation). The eelgrass population in this bight has been subject to intense investigation already for years therefore estimates for general population genetic parameters, like average clone sizes, inbreeding coefficients, and overall heterozygosity exist (Reusch et al. 1999, 2000, Hämmerli & Reusch 2002, Reusch 2006).

## **Sampling scheme**

Before start of the main vegetation period, marked grids were set up in the eelgrass meadow by the use of SCUBA. A 10 m x 9.3 m marked grid was established in mid-April 2003, a 10 x10 m grid in 2004 and a 20 x 5 m grid in April 2005. At 33.3 cm intervals leaf tissue (1 cm length) of potential donor genotypes was taken from the shoot nearest to every grid point, resulting in 900 individual shoot samples. Out of the water, leaf samples were dried and stored in individual tubes on silica gel until laboratory analysis.

## **Genotyping and Clone map**

DNA was extracted by use of Qiagen DNeasy Plant Kit (Qiagen GmbH, Hilden). A multiplex PCR amplification using four microsatellite markers (GenBank Accession Numbers: AJ249307, AJ249305, AJ009900, AJ009898) was carried out according to published protocols (Reusch 2002, Hämmerli & Reusch 2003a, b, Reusch et al. 2005). Samples ran on an ABI 3100 capillary sequencer and alleles were scored by the use of GenScan Analysis 3.1 and Genotyper 2.0 Software (Applied Biosystems 2001). Clone assignment and preparation of a clone map was done using MATLAB 6.12. codes (MathWorks, 2000), see Hämmerli (2002) for details. Based on the clone map eelgrass clones with sufficient numbers of shoots (ramets), were targeted for sampling.

## **Mesocosm facility**

The experiment was conducted in an indoor mesocosm facility at the Leibniz-Institute for Marine Science in Kiel/Germany. The facility consists of 12 tanks (blocks) containing 4 sub-containers each. The experimental mesocosm tanks had a volume of 600 L each and contained ambient Baltic Sea water with a flow rate of 100 L/hour/tank. Additionally, the water within the tanks was circulated by air bubbles released by a perforated hose at the bottom of each tank, to prevent microalgal blooms at the water surface. Individual plots consisted of four 48 x 41 x 16 cm polyethylene compartments embedded within each tank. These compartments were filled with sandy sediment to a height of 6 cm in 2003 and 12 cm in 2004 and 2005. Each tank was equipped with a light panel (2x150W, HQI), 50cm above the water surface with a diurnal rhythm of 15:9 hours light:dark, according to latitudinal light regime during summer month. Average light levels were  $144 \mu\text{mol m}^{-2} \text{s}^{-1}$  (just below water surface) and  $65 \mu\text{mol m}^{-2} \text{s}^{-1}$  (20 cm below surface).



Sediment was taken from a nearby shoreline and enriched with sediment from the donor seagrass-bed, to provide original microfaunal and microbial components as an inoculum. To control growth of epiphytic algae we added 250 individuals of the grazing snail *Littorina littorea* L. to each tank. Isopods *Idothea baltica* L. were added as alternative grazers with 12 individuals per tank. In addition, natural settlement and growth of larvae from Kiel fjord was allowed, resulting in recruitment of mostly mussels (*Mytilus edulis*, *Mya arenaria*) and polychaetes (Nereidae, Pygospionidae) within the mesocosms.

## Response variables

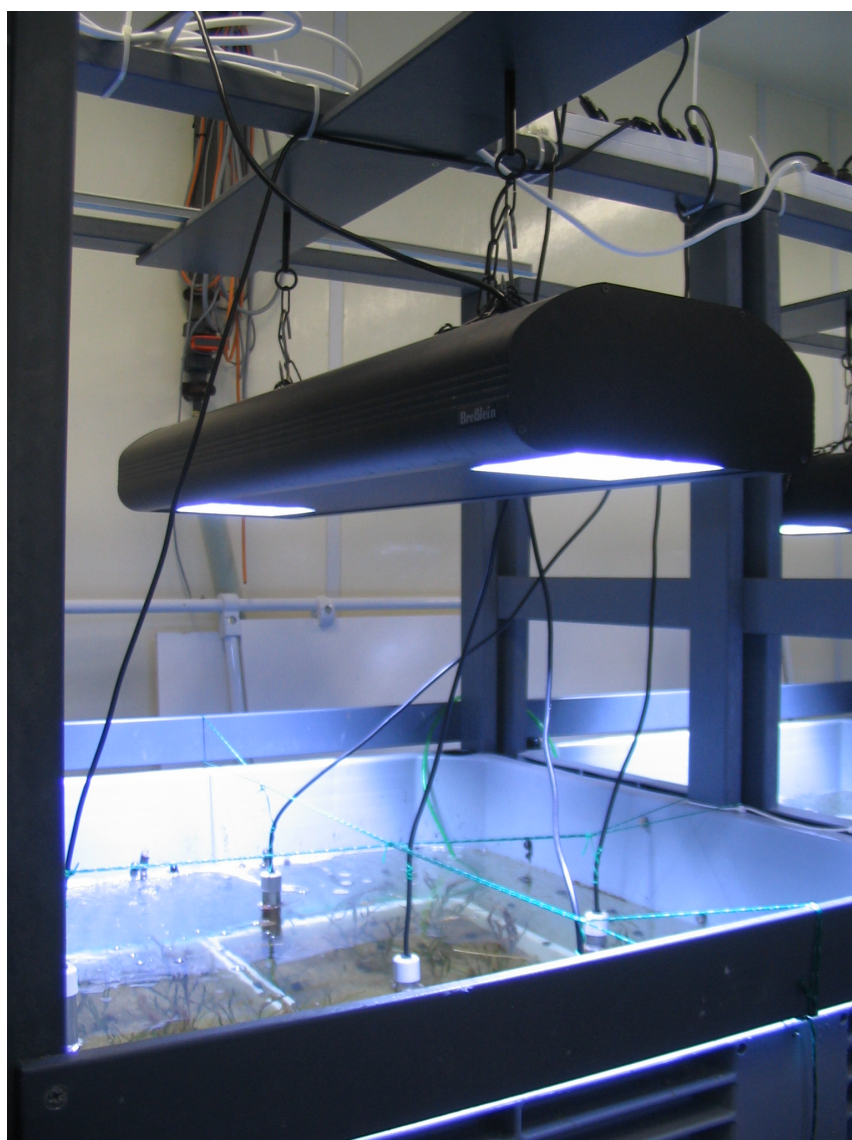
After the vegetation period in October the experiments were destructively harvested. Shoot densities per treatment were counted, and plant biomass was separated in above and belowground parts. Epiphytes were wiped off from shoots, and rhizomes were cleaned from adherent sediment particles before drying for >24 hours at 60°C and weighed to the closest 0.1 mg. Biomasses were then summed up per genotype per treatment.

Plant biomass is an important community property and commonly used as a response variable in studies on biodiversity-ecosystem functioning research (Loreau et al. 2001). This is especially appropriate for seagrasses, as they are mostly not directly consumed by grazers, by contrast macroalgal standing biomass depends heavily upon grazer abundance (Cyr & Face 1993). In short-term experiments also shoot density, which includes newly initiated lateral shoots is an important parameter of productivity, as these new shoots are not immediately transferred into biomass. Additionally, shoot density is an ecologically important community property in seagrass systems, as shoots build up the three-dimensional structure of a seagrass meadow, which attracts many associated organisms (Boström & Bonsdorff 2000).

## Statistical analysis

The first two experiments (Experiment I and II) were laid out as factorial split plot designs. A general linear mixed effects model (GLMM), with 'genotypic diversity' as a continuous and 'fertilization' or 'warming' respectively, as a categorical fixed factor was used. 'Block' was treated as a random factor. As the calculation of F-ratios for split-plot designs need special error terms, the denominators for every variable are included in the respective result table. The third experiment (Experiment III) was analysed using 'genotype diversity' as a continuous variable and including 'block' again as a random factor (GLMM).

The response variables were eelgrass biomass and shoot number per plot at the end of the experiment at final harvest. Homogeneity of variance and normality of the data were examined by exploratory graphical methods. When necessary, data were transformed to meet model assumptions. Where this was not possible I used a non-parametric test (e.g. for epiphyte cover in Experiment II). Other minor analyses used are specified in the respective chapters.



Mesocosm tank

## Experiment I

### Genotypic identity mediates response of eelgrass to eutrophication

#### Introduction

Eutrophication continues to be a major problem in the Baltic Sea, as is in most coastal seas worldwide (HELCOM 2004). Eelgrass meadows as one of the most common and important ecosystems in coastal zones are immediately affected by anthropogenic nutrient inputs from coastal point-sources or through accumulated riverine nutrient loads in estuaries (Orth et al. 2006). Like filters, they prevent nutrients from entering more offshore regions of the marine ecosystem, but suffer severely. Due to this sensitivity, seagrasses were chosen as bioindicators for monitoring purposes, e.g. within the European Water Framework Directive (Devlin et al 2007, Foden & Brazier 2007). High nutrient levels may directly harm eelgrass, as observed for nitrogen in form of nitrate (Burkholder et al. 1994) and ammonium with toxic effects on eelgrass (van Katwijk et al. 1997, Bintz et al. 2003).

Yet, nutrient inputs more often hit seagrass beds indirectly. Massive occurrence of epiphytes, composed of either unicellular or multicellular opportunistic algae, which compete for light, carbon and nutrients may suffocate seagrass beds. Decline in eelgrass was observed, to be a consequence of shading by increased algal growth (Neckles et al. 1993, Williams & Ruckelshaus 1993, Short et al. 1995), a process which can be mediated by mesograzers (Hughes et al. 2004). Furthermore, epiphytes can lead to strong anoxic conditions after mass-mortality. Positive effects were also reported, with nitrate enrichment that increases eelgrass growth (Short 1987, Williams & Ruckelshaus 1993, van Lent et al. 1995). However, in some studies no significant growth response to nitrogen enrichment was observed (Dennison et al. 1987, Murray et al. 1992).

Despite general reductions in nitrogen and phosphorous runoff due to improvements in waste water treatment, eutrophication still presents an essential threat to ecosystems (Vitousek et al. 1997a, Tilman 1999b), especially in coastal estuarine systems, like the Baltic Sea (Cloern 2001, Rönnerberg & Bonsdorff 2004). Nutrient conditions for German Baltic coastal waters, including the Schlei fjord show ranges of  $<0.2 \mu\text{mol L}^{-1}$  and  $46 \mu\text{mol L}^{-1}$  for Ammonium,  $<0.2 \mu\text{mol L}^{-1}$  and  $320 \mu\text{mol L}^{-1}$  for Nitrate and  $<0.04 \mu\text{mol L}^{-1}$  and  $4 \mu\text{mol L}^{-1}$  for Nitrite in the surface waters over a whole year (period 1999-2002; BLMP 2005). In general, high nutrient levels are mostly depleted to near zero during the vegetation period in the summer months. For the German Baltic coastal zone it is generally assumed that

more than 60% of total soluble anorganic nitrogen originates from diffuse agricultural sources (BLMP 2005). Especially in inner coastal waters nutrient levels may show extreme variation. They are influenced by atmospheric and predominantly direct land-based nutrient inputs, which depend on factors like hydrology and coastal morphology and are highly influenced by meteorological conditions. In dry summers land-based nutrient inputs are low, while wet years with high precipitation during the summer month may lead to locally extremely high nutrient levels. Maximum summer values for a sampling station in a more inward part of the Schlei fjord (Lindholm) reached  $335.7 \mu\text{mol L}^{-1}$  of total Nitrogen and  $16.4 \mu\text{mol L}^{-1}$  of  $\text{NH}_4$  in July, 1980 and  $264.3 \mu\text{mol L}^{-1}$  of total Nitrogen and  $0.87 \mu\text{mol L}^{-1}$  of  $\text{NH}_4$  in July, 1993 (unpublished data from monitoring of coastal waters by the State Agency for Nature and Environment, LANU).

Based on these background data, I decided to use a strong nutrient pulse as an adequate stressor combined with the manipulation of genotypic diversity. Specifically the experiment tested (1) whether eutrophication has a negative impact on eelgrass performance, (2) if genotypic richness has an impact on eelgrass plot performance, (3) if genotypic diversity effects could buffer for negative fertilization effects (interaction), and (4) if individual genotypes will react differently to fertilization stress.

## Methods

In this experiment, six eelgrass genotypes, denoted as *A-F*, which were identical to those used in a parallel field experiment (Reusch et al. 2005). Under field conditions, these genotypes experienced a different stressor, namely elevated water temperatures at the lethal level during the European heat wave in summer 2003. Hence, I took the opportunity to check for similar patterns in the reaction of these individual genotypes to two different stressors (eutrophication, elevated water temperatures).

### *Experimental set-up*

The mesocosm facility (see general Material & Methods section) was used for this experiment. Experimental plots were filled with sandy sediment to a height of 6 cm. To control growth of epiphytic algae we added between 90 to 110 individuals of the isopod *Idothea baltica* L. to each tank. Grazing snails *Littorina littorea* L. were added as alternative grazers with 13 individuals per tank. The walls of the mesocosms were scrubbed twice a week to keep attached macroalgae growth from accumulating.

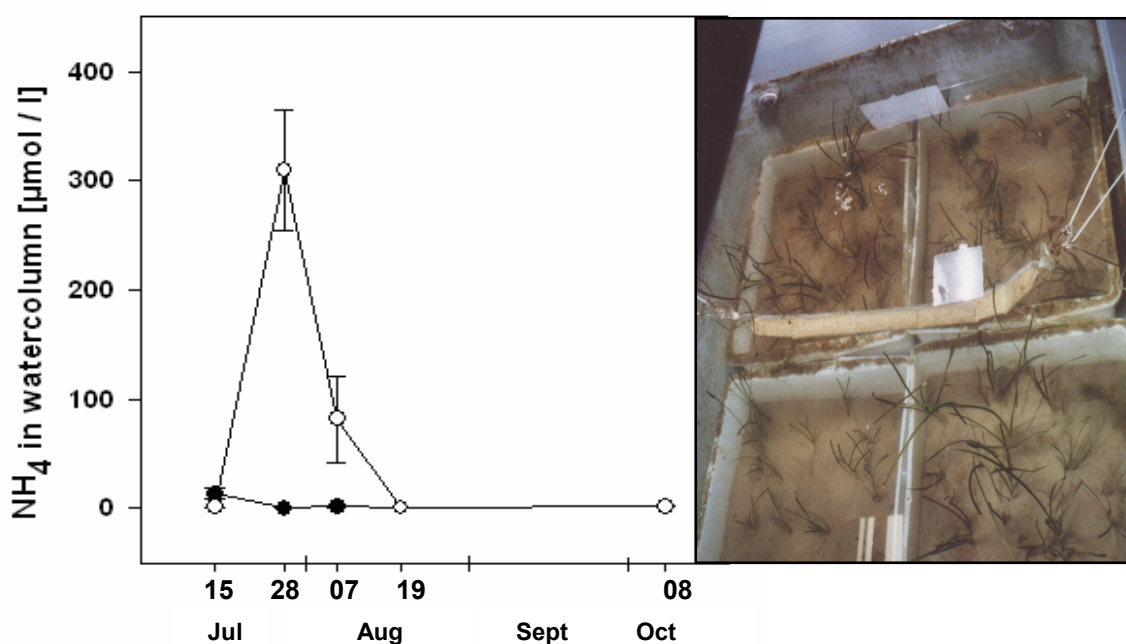
The experiment was designed as a 2x3 factorial with fertilization (fertilized / non-fertilized) and genotypic diversity of eelgrass (1, 3, 6 genotypes) as independent variables. In each tank, two compartments contained one-genotype treatments (monocultures), 1 three-genotype and 1 six-genotype treatment, randomly assigned to a position within the tank. In total, I assembled two replicates of monocultures of six distinct clones for each fertilization treatment (N=24) and six replicates of three- and six-genotype treatments (N=12) respectively. Mesocosms were planted with 18 eelgrass ramets per plot. After the set-up on 27 June 2003, plants were allowed to acclimatize for 3 weeks before start of the fertilizer treatment. For this purpose mesh-bags filled with 500g slow release N/P/K – fertilizer with 14% N, 9% P and 15% K (Plantacote plus 6M; AGLUKON GmbH & Co KG) were placed in the water column of the selected tanks. The amount of fertilizer per tank was estimated from former field experiments (Worm et al. 2000). The six non-fertilized tanks experienced water conditions of Kiel fjord. After 2 ½ weeks the fertilizer treatment was removed and all mesocosms were allowed to develop under ambient flow-through conditions for 10 weeks, until they were destructively sampled from 25-30 October 2003.

#### *Response variables*

Water samples were taken from mesocosms before the onset of the treatment, and after 1, 2 ½, 4 and 8 weeks as a treatment control. On each occasion, two water column samples were taken out of each tank with a syringe and a filter (Sterile Acrodisc, 5 µm Syringe filters; Gelman Sciences). A new filter was used for each tank. Pore water samples were taken twice, before onset of treatment and in October before termination of the experiment, 8 weeks after cessation of the fertilization treatment. Sediment cores (4 ml, 4 cm depth) were sampled in triplicate in each plot and pore water was obtained by centrifugation (3000 x g). The concentrations of NH<sub>4</sub>, and for water column samples additionally PO<sub>4</sub>, SiO<sub>2</sub>, NO<sub>3</sub> and NO<sub>2</sub>-concentrations as µmol L<sup>-1</sup>, were determined in a nutrient autoanalyzer according to standard protocols. We made two shoot counts during the run of the experiment, one at the day of fertilizer bag removal (7 August 2003) and another one 4 weeks later in September. Upon harvesting the experiment in October, in each compartment shoots per plot were counted and plant biomass was determined as dry mass. From a sub-sample of dried shoots and rhizomes, plant material was ground with a ceramic mortar and Carbon (C) and Nitrogen (N) contents were determined with an autoanalyzer according to standard protocols. The response variables for the main analyses were eelgrass biomass and shoot number per plot in October. To meet model assumptions data had to be transformed, using 'square root' or 'log10' - transformation, as indicated in Table I-1.

## Results

The fertilization treatment significantly reduced shoot number and final biomass in the eelgrass plots, while none of the response variables was affected by genotypic diversity (Table I-1, Fig. I-1). The set-up of the experiment in twelve tanks, which developed substantial differences over time, accounted for a significant block effect in October (Table I-1), that was substantially larger than the desired treatment effects. The reactions of genotypes to the abiotic stress showed clear differences, as most were negatively affected and lost shoots and biomass, while one genotype showed a slight increase in these parameters (Fig. I-2). Ammonia concentrations in the water column were significantly different between non-fertilized and fertilized tanks only on the sampling date one week after fertilizer deployment (July 28, 2003; t-test;  $P < 0.001$ ). Mean total concentrations for  $\text{NH}_4$  in the water column of fertilized tanks at that time were increased 300-fold relative to non-fertilized tanks. The amount of fertilizer used for the eutrophication treatment represents the high end of possible local short-time nutrient inputs into coastal waters. Fertilization therefore had a very strong negative effect on eelgrass performance, i.e. shoot numbers and biomass (GLMM,  $P$  for all  $< 0.01$ ) as it is shown in Table I-1.



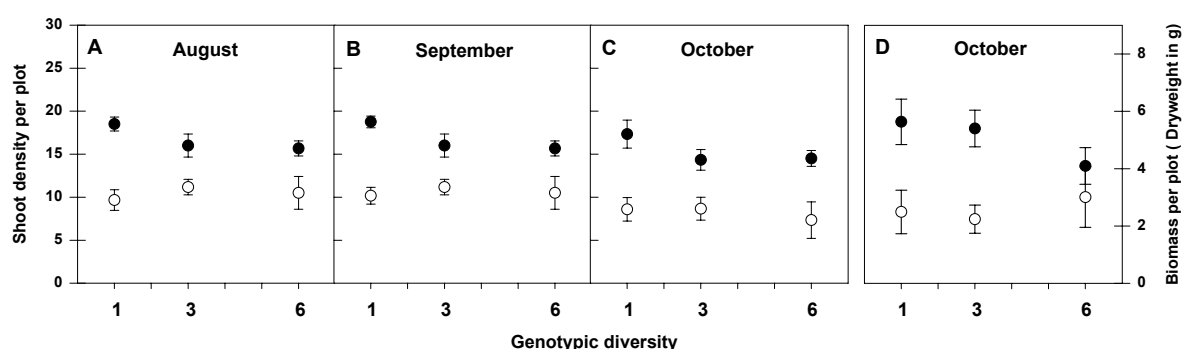
**Figure I-1:** Ammonium-concentration in the water column. Means  $\pm$  SE are given for non-fertilized ( $\bullet$ ) and fertilized ( $\circ$ ) tanks. Fertilizer bags were deployed from July 21 until August 07, 2003. The photograph shows a tank with a fertilizer bag hanging in the water column above the four experimental units, planted with eelgrass shoots.

**Table I-1:** General linear mixed model of the effects of experimental fertilization and genotypic diversity on eelgrass shoot density during the experimental period and biomass in October. Transformation of data to meet model assumptions is indicated (Sqrt, Log10), otherwise original data were used.

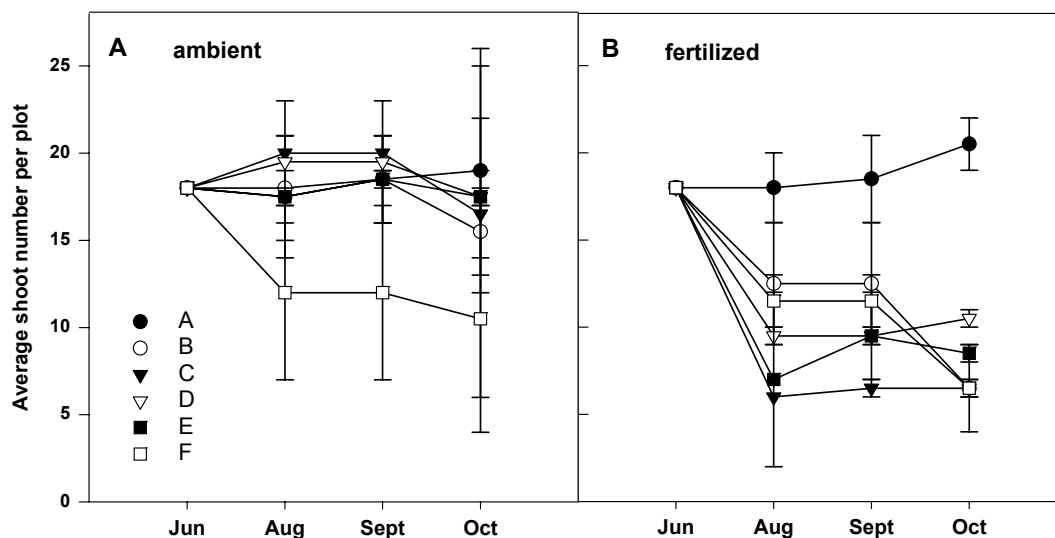
Source	df	Shoots						Biomass		Denominator
		August		September		October Sqrt		October Log10		
		<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	
Fertilization	1	28.79	< 0.001	39.39	< 0.001	10.85	0.008	19.20	0.001	Block(fert)
Block(fert)	10	1.49	0.201	1.30	0.283	2.39	0.039	1.08	0.413	Residual
Diversity	1	1.25	0.289	2.67	0.133	2.91	0.119	0.20	0.660	Block(fert) x Div
Fert X Div	1	4.59	0.058	4.41	0.062	0.00	0.995	0.49	0.501	Block(fert) x Div
Block(fert) x Div	10	0.80	0.627	0.92	0.532	1.26	0.304	1.55	0.184	Residual
Residual	24									

Despite the fact that shoot numbers in mesocosms never reached natural densities during the experimental period, non-fertilized plots nearly kept their initial planting densities, while shoot numbers in fertilized plots decreased on average by 47% from July until October (see Fig. I-2C). In October non-fertilized experimental units contained on average 5.5g dry eelgrass biomass, whereas fertilized plots held 3.2g (Fig. I-2D).

There was no effect of genotypic diversity on shoot number or biomass, i.e. plots composed of three or six eelgrass genotypes on average did not differ in the amount of shoots or biomass from clone monocultures. In August, a marginally significant interaction between fertilization and genotypic diversity occurred for shoot density (Table I-1). This may be based on the first two diversity levels which showed opposed patterns between fertilization treatments (Fig. I-2 A), i.e. that fertilized plots showed positive diversity trends, whereas unfertilized plots showed a negative one.)



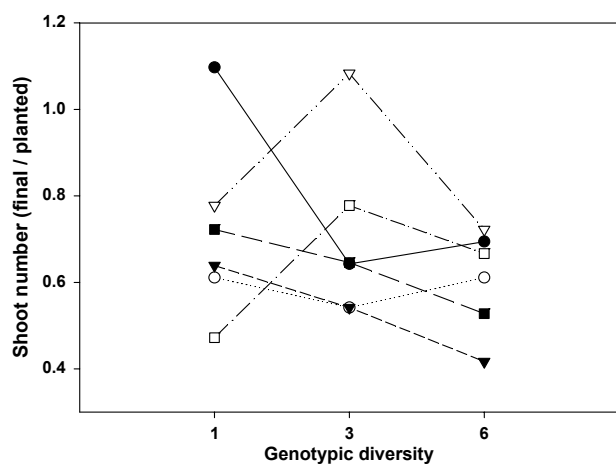
**Figure I-2:** Response of eelgrass to strong fertilization. Shown are means ( $\pm$ SE) for non-fertilized (●) and fertilized (○) experimental mesocosms for the three genotypic diversity levels (one-, three- and six-genotypes). (A) Shoot densities in August (directly after termination of fertilization treatment), (B) Shoot densities in September (1 months after fertilization treatment), (C) Shoot densities in October 2003 (2 months after fertilization treatment), (D) Dryweight biomass in October. Sample sizes are  $n=24$  for one-genotype treatments, and  $n=12$  for others.



**Figure I-3:** Individual genotype responses to fertilization. Shoot numbers in monocultures of six eelgrass clones (A-F) are shown for non-fertilized (A) and fertilized (B) experimental mesocosms in June (before fertilization treatment), August (directly after termination of fertilization treatment), September (4 weeks after) and October 2003 (2 months after fertilization). For each data point n= 2.

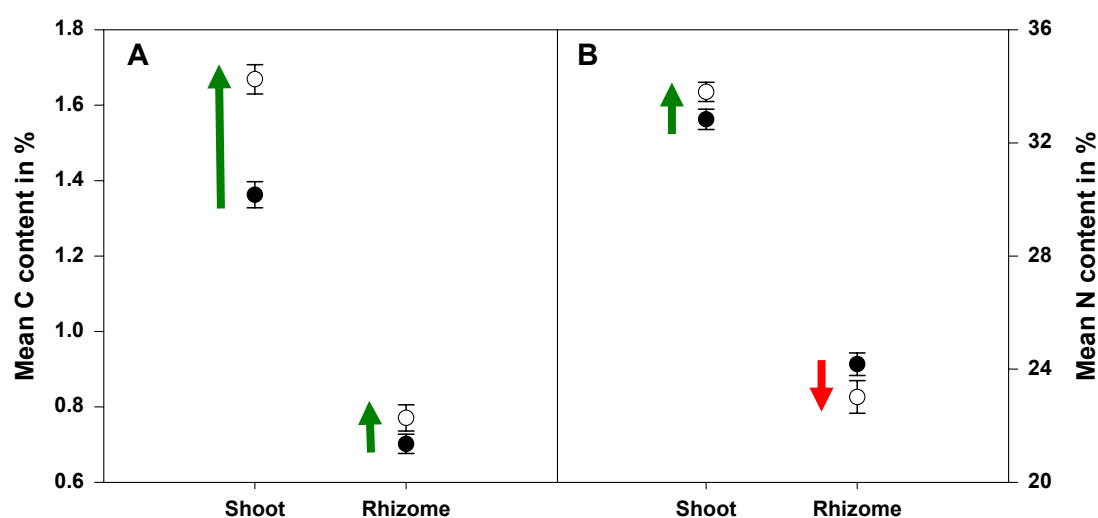
Having planted replicated monocultures we were able to compare the individual reactions of genotypes. The genotypes A-F showed significantly different responses to the fertilization treatment (one-way ANOVA; response variable final shoot density in fertilized plots,  $F [5,11] = 18.9, P < 0.01$ ).

Genotype A and F seemed to be ‘immune’ against the factor of strong fertilization, and kept their pattern when comparing the graphs A and B in Fig. I-3. The other four genotypes (B-E) showed a decline in shoot numbers in fertilized treatments already in the month of the fertilization treatment, while genotype A slightly increased shoot densities (Fig. I-3B). In contrast, control monocultures were not significantly different throughout the experiment. A closer look at the performance of



**Figure I-4:** Ratio of final / planted shoots, as a measure of the performance of eelgrass genotypes A-F in monocultures and in 3- and 6-genotype mixtures. Symbol-legend as indicated in Fig. I-3. Data were pooled without regard to fertilization treatment.





**Figure I-5:** Percent contents of carbon (A) and nitrogen (B) in ambient (●) and fertilized (○) eelgrass shoots and rhizomes. Note the general increase of C and N contents with fertilization (green arrows), despite for N content of rhizomes (red arrow). For non-fertilized shoots N=107, fertilized shoots N=56, non-fertilized rhizomes N=121 and fertilized rhizomes N=61.

genotypes at the final shoot count in October revealed that genotypes behaved differently in monocultures and multi-genotype mixtures (Fig. I-4). Genotype A which was capable of shoot increase in monoculture did not maintain initial shoot densities in mixtures of three or six genotypes, where it performed just as average.

An analysis of a sub-sample of dried shoots and rhizomes for carbon (C) and nitrogen (N) contents demonstrated differences between the fertilization treatments. In contrast to expectations, carbon contents in fertilized eelgrass shoots and rhizomes increased compared to the non-fertilized controls (Fig. I-5). While nitrogen contents were also higher in shoots from fertilized compared to non-fertilized plots, fertilized rhizomes showed an opposite pattern, with higher nitrogen contents in non-fertilized rhizomes.

The experimental set-up in twelve tanks (=blocks), which developed differently over the experimental period, accounted for a significant effect on eelgrass shoots in October, explaining > 26% of the variance in our statistical model.

## Discussion

Eelgrass meadows are endangered by terrestrial nutrient inputs, as they stand in the first row at the terrestrial-marine interface. In my experiment, eutrophication had a strong negative effect on eelgrass performance, but not all eelgrass genotypes were equally vulnerable to this deleterious impact.

As expected, the simulated strong eutrophication was shown to have pronounced negative effects on eelgrass (accepting hypothesis 1), by significantly reducing shoot number and final biomass. These harmful effects of nutrient over-enrichment on eelgrass are well documented (for a recent overview see Ralph et al. 2006). My aim was not to repeat the various studies, but to use a strong nutrient pulse as a severe stress for assessing potential interactions with genotypic identity and richness. The nutrient pulse in the present study was relatively high, suggesting that mainly direct, toxic effects on eelgrass resulted in shoot declines and mortality. Such effects have been described in eelgrass mesocosm experiments for nitrate (Burkholder et al. 1992) and ammonium concentrations as low as  $25\mu\text{mol L}^{-1}$  (van Katwijk et al. 1997), which are considerably lower concentrations than employed here.

Both roots and leaves of *Z. marina* are capable of nitrogen absorption and leaves are known to have a greater affinity for ammonium than the roots (Thursby & Harlin 1982, Short & McRoy 1984, Pedersen & Borum 1992, Hemminga et al. 1994). Surprisingly, rhizomes showed no increased nitrogen content under fertilized conditions contrary to all other analyzed plant parts (Fig. I-5), possibly due to the capability of rhizomes to reduce root hairs to regulate nitrogen uptake (Short 1983, Marschner 1995). It is thus speculated that the organism started to actively regulate against the nutrient situation, which probably involves costs subtracted from biomass production.

Individual eelgrass genotypes performed differently when exposed to eutrophication stress (accepting hypothesis 4). Genotype A was the only one able to keep its shoot densities compared to non-fertilized conditions and even performed slightly better under eutrophied conditions. Although any assignment of the processes explaining these different patterns remains speculative, these patterns are especially remarkable. It was also genotype A coping best with high water temperature conditions in the parallel field experiment (Reusch et al. 2005). Pooled over both fertilization treatments, genotype A showed its superior performance to other genotypes just when cultured alone (Fig. I-4), similar to the patterns in the field experiment (Reusch et al. 2005). Genotype F which was lowest performing in monoculture showed the reversed pattern and yielded above average in polycultures. I may

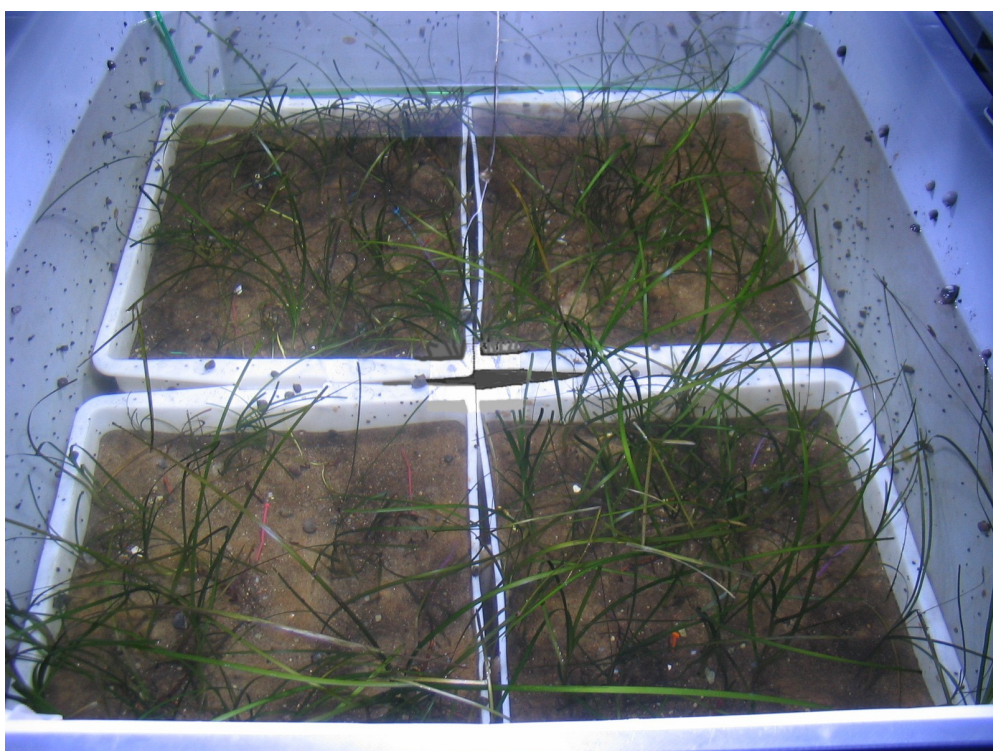
just hypothesize that *A* is a relatively bad competitor. This character may be of importance, as genotype *A* may not play an important role in a recent eelgrass meadow, as competition with other genotypes keeps it small. In case of strong, multifactorial disturbance the majority of available genotypes could perish and a survivor genotype, like *A*, would be able to use the opportunity to grow rapidly, released from intraspecific competition.

Evidence supporting my stated hypothesis (3), that genotypic diversity would buffer for negative fertilization effects are weak. There is a marginal significant interaction of genotype diversity and the fertilization treatment in August, directly after termination of the fertilization treatment, indicating that genotypic diversity can be beneficial under stressed, but not under ambient conditions. These trends vanished completely over the experimental period.

None of the response variables were affected by the richness of genotypes (refuting hypothesis 2). Reversely, for the same set of eelgrass genotypes *A – F*, a parallel field experiment showed a significant positive effect of genotypic richness on eelgrass shoot density and biomass under sub-lethal water temperatures (Reusch et al. 2005). It cannot be ruled out that my eutrophication treatment was confounded by unusually hot summer temperatures in 2003 which influenced our mesocosms through the direct water-pipe from the Kiel fjord. This should have affected all tanks, but particularly the fertilized as cumulative negative interactions of nutrient enrichment and increased water temperatures on eelgrass have been experimentally shown (Burkholder et al. 1992, Bintz et al. 2003). This is probably also the reason why I found poor vegetative reproduction in our tanks and natural densities were not reached. Several additional factors, like sediment height and epiphyte cover were checked, but no correlations explaining the weak performance of eelgrass have been found.

As already shown for species diversity in different ecosystems, composition and identity of genotypes play a similarly decisive role for those ecosystems that are based on single or a few species. My experimental eelgrass genotypes originated from a single meadow, thus came from one interbreeding pool. Nevertheless they showed differentiated responses to abiotic stress. This response diversity may remain undetectable under average ambient conditions, but may provide insurance against environmental fluctuations thus providing a critical response diversity (Elmqvist et al. 2003) for adapting and coping with coastal eutrophication. Although the number of genotypes alone had no effect on the evaluated productivity parameters, conserving genotype richness should increase the probability of including different traits, which are fundamental for maintaining and achieving response diversity.

The unique opportunity to not only compare genotypes *A-F* in the mesocosm experiment, but also in the field under different conditions, revealed one superior genotype *A*, which almost looks like a general purpose genotype (Lynch 1984), but only alone and not in competition with others. This response diversity may be critical for eelgrass populations adapting to and coping with coastal eutrophication and keeping up their function as a filter system for coastal water bodies and nursery grounds for dependent faunal communities.



Experimental plots planted with *Zostera marina*

## Experiment II

### Climate warming and the resilience of a dominant marine plant

#### Introduction

Recent climate change already affects structure and function of many ecosystems (Gitay et al. 2002, Parmesan & Yohe 2003, Schroter et al. 2005, Diaz-Almela et al. 2007). Coastal ecosystems are particularly vulnerable because human impacts such as overharvesting, pollution, eutrophication, and habitat destruction, have already undermined their resilience and capacity to buffer additional environmental stresses (Holling 1973, Jackson 2001, Hughes et al. 2003). Moreover, many disturbances are acting at the terrestrial-marine interface such as increased land run-off after floods, higher wave energies (due to increased storm frequency) and increasing turbidity. Negative effects of ocean warming on coastal biota are already evident (Harley et al. 2006 and references therein).

Global and regional climate models not only forecast a rise of mean air and water temperatures, but an even stronger increase in frequency and intensity of extreme climatic events (IPCC 2007). Heat waves, like the 2003 summer heat wave in Europe, are likely to occur more frequently (Meehl & Tebaldi 2004, Schär et al. 2004, Beniston et al. 2007). In addition, a regional climate model for the Baltic Sea area predicts an increase in mean summer temperatures of 3-5°C within the next century, which lies above the predicted global warming average (BACC 2006). In order to predict the fate of coastal communities and ecosystems, an assessment of vulnerability of coastal key species to climate change is highly warranted (Short & Neckles 1999, Hofmann et al. 2005, Harley et al. 2006). Here, I tested the effects of climate warming on a Baltic population of *Zostera marina* L. (eelgrass), a dominant coastal seagrass species that, as an ecosystem engineer (sensu Jones et al. 1994), contributes largely to habitat provision, nutrient cycling and primary productivity in temperate coastal ecosystems. Sustained water temperatures exceeding 25°C have been repeatedly reported to cause decline or die-off of eelgrass, both in the field (Penhale 1977, Evans et al. 1986, Moore et al. 1996) and under experimental conditions (Zimmermann et al. 1989, Williams 2001, Bintz et al. 2003). Most recently die-back was observed for different seagrass species following the heat wave in summer 2003 (Mayot et al. 2005, Reusch et al. 2005).

In addition to temperature effects, we also intended to test for effects of genotypic diversity of eelgrass on the performance of experimental communities. In marine ecosystems that are structured by foundation species such as seagrasses, kelps, mussels, oysters, or

rockweeds, species diversity at the level of structuring species is low (Bruno et al. 2003, Micheli & Halpern 2005). Recent evidence suggests that different functional traits displayed by distinct genotypes play an analogous role to species diversity in these environments, i.e. more genotypically diverse populations showed enhanced settling success or resilience to different disturbances compared to less diverse communities (Williams 2001, Hughes & Stachowicz 2004, Gamfeldt et al. 2005, Reusch et al. 2005, Reusch & Hughes 2006). Evidence for genetic or genotypic diversity buffering negative effects of global warming is only observational thus far.

In the present study I used temperature-controlled indoor mesocosms, to test the hypothesis that a simulated summer heat wave has a negative effect on eelgrass performance. In a factorial design I also manipulated genotypic diversity of eelgrass to test its ability to buffer losses caused by warming.

## Methods

### *Experimental design*

Based on the clone map (see general Material & Methods and Appendix II-2), twelve eelgrass clones with sufficient numbers of shoots (ramets), were targeted for sampling in June 2004, six of which covered an area of > 2 m<sup>2</sup>. The experiment was designed as a 2x3 factorial design with warming (heated / ambient) and genotypic diversity of eelgrass (1, 3, 6 genotypes) as independent variables (Appendix Fig. II-1). Half of the tanks were heated (see below), half were held at ambient temperatures. Each tank was considered as one block and contained 4 plots of which 2 were one-genotype treatments (monocultures), 1 was a three-genotype and 1 was a six-genotype treatment, randomly assigned to a position within the block. In total we had two replicates of one-genotype treatments of six clones (n=24) and six treatments of three- and six-genotype treatments (n=12) respectively.

While half of the six-genotype treatments were composed only of the six genotypes used for one- and three-genotype plots (see Fig. A-II-1; all 6), another six treatments were composed of a random assortment of six genotypes drawn from a total of twelve sampled genotypes (all 12). This was done to assure compositional replication at the highest diversity level, as former diversity experiments were criticised for the fact that higher diversity plots were more similar to one another in composition than lower diversity plots (Wardle 1998, Fukami et al. 2001).

The experiment was planted in mid-June 2004. After an initial six weeks of acclimatisation, the water temperature in half of the tanks was raised up to 25°C, which replicated observed conditions in 2003 (Reusch et al. 2005), when a heat wave caused increased warming across northern Europe (Luterbacher et al. 2004, Schär & Jendritzky 2004). The warming treatment used stainless steel heating rods (5 x 300 W / tank) placed into the water columns that were maintained for four weeks (4 August – 1 September 2004). The remaining six tanks experienced ambient water temperatures of Kiel fjord (see results; Fig. II-1). After the heating period the experiment was allowed to develop under ambient flow-through conditions for 6 weeks, until it was destructively sampled from 10-15 October 2004. The water within the tanks was circulated by air bubbles released by a perforated hose at the bottom of each tank to prevent microalgal blooms at the water surface.

### *Response variables*

Water temperatures were recorded daily during the heating period. When destructively harvesting the experiment in October, shoots per treatment were counted, and plant biomass was separated in above and belowground parts. Epiphytes were wiped off from shoots and rhizomes were cleaned from adherent sediment particles before drying for >24 hours at 60°C and weighed to the closest 0.1 mg. Biomasses were then summed up per genotype per treatment. We made two additional shoot counts during the experiment, one before (July) and one after experimental warming (September). Epiphyte cover, grazer counts and distribution records were taken on 1 September 2004.

### *Data analysis*

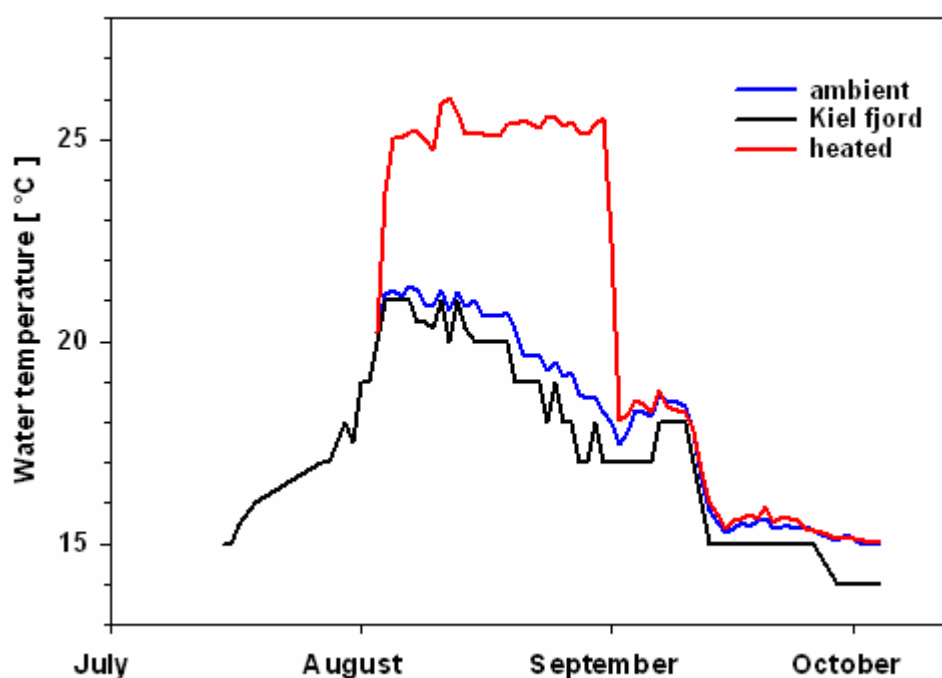
The experiment was laid out as a factorial split plot design. For the main analysis a general linear mixed effects model (GLMM), with 'genotypic diversity' as a continuous and 'warming' as a categorical fixed factor, whereas 'block' was treated as a random factor was used. The response variables were eelgrass biomass and shoot number per plot in October.

Additionally, I analysed shoot counts from July and September. When necessary, data were transformed (log<sub>10</sub>) to meet model assumptions. Where this was not possible I used a non-parametric test (e.g. for epiphyte cover).

## Results

The warming treatment was maintained at a mean water temperature ( $\pm$ S.E.) of 25.3°C ( $\pm$ 0.1°C) during the experimental warming period (Fig. II-1). This was on average 5.0°C ( $\pm$ 0.2°C) above the ambient temperature in the control tanks. The timing of the warming treatment corresponded to the warmest summer temperatures measured in Kiel fjord in 2004 (Fig. II-1), and closely simulated observed field conditions during the 2003 heat wave.

Warming resulted in a decrease of shoot numbers in October (Fig. II-2C). Both earlier shoot counts revealed, that one month after planting, prior to warming, shoot numbers were not statistically different between warmed and ambient tanks (Fig. II-2A). Whereas six weeks later, directly after termination of the heat treatment (2 September 2004), shoot density in all plots was generally high. There was a tendency, though not statistically significant, of reduced shoot numbers in the heated tanks (Fig. II-2B). In the following four weeks up to the final sampling in October, we observed increased detachment of shoots in all tanks, unrelated to former warming treatment (t-test,  $P=0.24$ ). However, final shoot count in October showed on average 44% reduction in shoot numbers of warmed treatments compared to treatments with ambient water temperatures (see Table II-1 and Fig. II-2C).



**Figure II-1:** Water temperatures during the experimental period in summer 2004. The black line gives the background temperature from Kiel fjord, measured in a depth of 6 m. The blue and red lines show daily mean temperatures of ambient and heated treatments respectively.

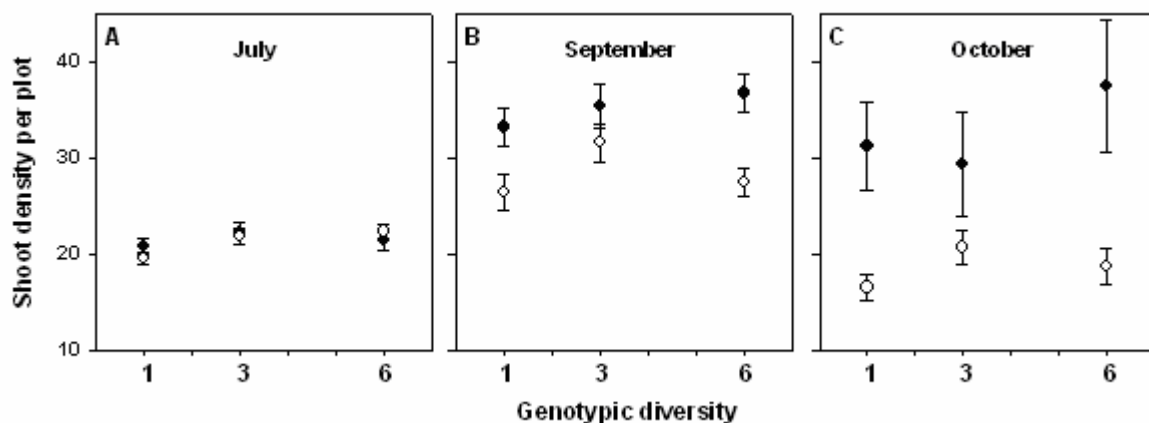


**Table II-1:** General linear model of the effects of experimental warming and genotypic diversity on eelgrass shoot density. Significant values ( $P < 0.05$ ) are shown in bold.

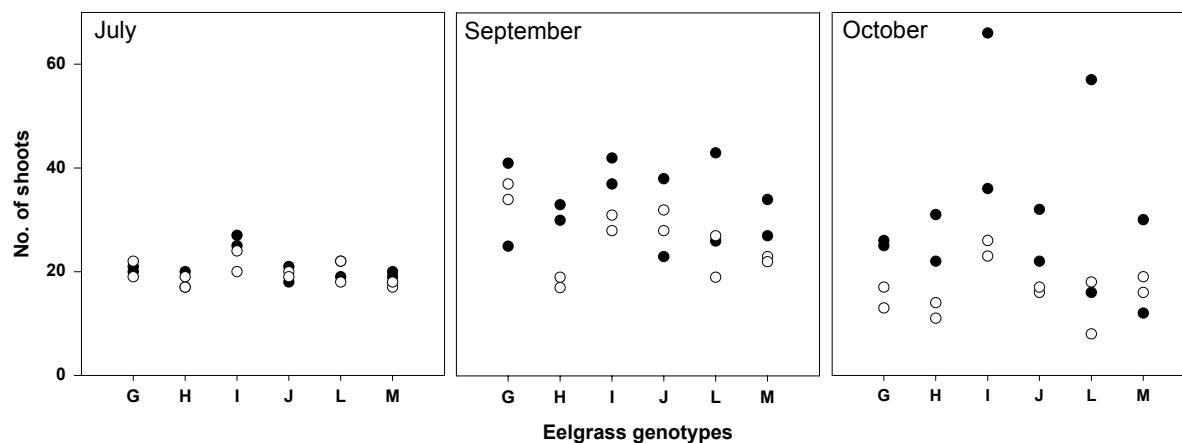
Source	Shoots							
	df	July		September		October		Denominator
		<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	
Heat	1	1.65	0.227	4.95	<b>0.050</b>	6.49	<b>0.029</b>	Block (Heat)
Block (Heat)	10	1.34	0.267	0.68	0.729	1.48	0.206	Residual
Diversity	1	4.68	0.056	7.41	<b>0.021</b>	8.79	<b>0.014</b>	Block (H) x Div
Heat x Diversity	1	1.63	0.230	0.38	0.550	0.24	0.635	Block (H) x Div
Block (H) x Div	10	1.17	0.355	0.21	0.993	0.28	0.979	Residual
Residual	24							

Plants in ambient water tanks had developed more lateral shoots compared to formerly warmed treatments, where mainly terminal shoots had been maintained.

A significant effect of eelgrass genotypic diversity was obtained on shoot densities, increasing over the experimental period (Table II-1). A positive diversity effect was also observed between the two variants of the highest diversity treatments in July. Those six-genotype mixtures assembled from a pool of 12 genotypes had developed more shoots than those composed out of a pool of 6 genotypes (t-test,  $P = 0.006$ ). During September and October no differences appeared in shoot densities between six-genotype treatments assembled out of six or twelve genotypes (t-tests,  $P > 0.5$ ). For all censuses of shoot numbers we found no interaction for ‘warming’ and ‘genotypic diversity’ (Table II-1; all  $P > 0.1$ ). We found neither temperature nor diversity effects on eelgrass above- or belowground biomass (data not shown;  $P > 0.1$ ).



**Figure II-2:** Response to warming. Shown are means ( $\pm$ SE) of eelgrass shoot numbers for ambient (●) and warmed (○) experimental mesocosms for the three genotypic diversity levels (one-, three- and six-genotypes) in July (before heat treatment), September (termination of heat treatment) and October 2004 (6 weeks after heat treatment). Sample sizes are  $n=24$  for one-genotype treatments, and  $n=12$  for others.



**Figure II-3:** Individual genotype responses to warming. Shoot numbers in replicates of six eelgrass clones (G, H, I, J, L, M) are shown for ambient (●) and warmed (○) experimental mesocosms in July (before heat treatment), September (termination of heat treatment) and October 2004 (6 weeks after heat treatment).

I found an effect of warming on the distribution of the main grazer *Littorina littorea*. In heated tanks 16% of snails attached themselves to the inner tank wall above the water surface as compared to 7 % in the ambient tanks (one-way ANOVA  $F [1,10] = 20.6$ ,  $P = 0.001$ ). Despite this behavioural effect, the final abundance of snails, counted in October, was not affected by warming (one-way ANOVA  $P > 0.5$ ). In all tanks, additional snails had recruited over the experimental period.

The analysis of the performance of individual genotypes suffered from limited power due to the small sample size of  $n=2$  for each genotype under both temperature conditions. Nevertheless, there is some statistical support for differential performance among clones. Individual genotypes yielded significantly different shoot numbers in July, prior to experimental warming (one-way ANOVA,  $F [5,18] = 5.18$ ,  $P = 0.004$ ). Post-hoc comparisons between genotypes showed that this difference was due to genotype *I* ( $P < 0.05$ ; see Fig. II-3). In September and October, no significant differences between shoot numbers of individual monocultures and their reaction to warming were detectable, while an increase of variance in individual genotype response was obvious (Fig. II-3). In ambient treatments, all six genotypes increased their shoot densities until September, when four of them began to lose shoots. Genotype *I* showed a nearly linear increase of shoots until harvest in October (Fig. II-3). In the heated plots, monocultures composed of three genotypes (*H*, *L*, *M*) were strongly affected by raised temperatures and were not able to produce numerous new shoots. The other three genotypes (*G*, *I*, *J*) reached shoot densities comparable with those in ambient tanks until September. During the last weeks of the experiment, shoot densities in all but one genotype (*I*) declined even below initial densities (Fig. II-3).

## Discussion

Although there is a growing body of literature on coastal ecosystems and possible effects of climate change (Short & Neckles 1999, Duarte 2002, Gitay et al. 2002, Hughes et al. 2003, Oviatt 2004, Harley et al. 2006), only few experiments simulated realistic increases in water temperature in coastal communities (Williams 2001, Bintz et al. 2003, Allison 2004). To my knowledge, my experimental study is the first that directly manipulated water temperatures as a component of global change combined with different genotypic diversities in any plant community. My experiment demonstrated a negative effect of temperature conditions, simulating the 2003 European heat wave, on a temperate population of eelgrass *Zostera marina*. The percentage loss of shoot numbers in our warmed tanks relative to the control tanks (44%) approached the magnitude of shoot loss observed in the field during the 2003 heat wave compared to former years (48-52%), which already suggested a strong negative response of eelgrass shoot density to warming at a critical threshold temperature of 25°C (Reusch et al. 2005).

Furthermore I observed a persistent and increasing positive effect of increasing genotypic diversity on eelgrass shoot densities. This outcome was comparable to the effects observed in the field in 2003 (Reusch et al. 2005). Contrary to our expectations, we were unable to find a significant 'genotypic diversity' x 'warming' interaction.

The negative effect of prolonged water temperature maxima on shoot densities increased over time (see Table II-1 and Fig. II-2). The difference in final shoot densities between ambient and warmed treatments was due to a shift in the growth strategy of eelgrass under warmed conditions. Ramets in warmed treatments showed fewer vegetative recruits (as new lateral shoots) in October than those in ambient treatments. This observation is in accordance with previous studies where eelgrass in combination with other macrophytes was exposed to increased water temperatures (Bintz et al. 2003). Because we did not find any effects on the above or belowground biomass allocation, we are not able to make any predictions concerning how and if at all these effects will proceed to the next vegetation period.

Considering the major heat waves already occurring during the last years over Europe an increase of such events is predicted (Beniston et al. 2007). My temperature treatment of 25°C water temperature for four weeks represents a highly probable scenario. However, it is artificial in that it showed continuously high temperatures during day and night times, which could exacerbate the negative effects, as it is known that the internal oxygen levels can be critically low during night times. This effect should be fostered due to higher respiration rates at elevated temperatures (Greve et al. 2003, Borum et al. 2006).

Increased genotypic diversity was advantageous for shoot development in newly planted mesocosm plots six weeks after set-up of the experiment in July and six weeks after termination of the warming treatment in October. These patterns suggest that buffering by genotypic diversity occurred independently to both temporarily separated disturbance events, i.e. the planting of mesocosms, and the subsequent warming treatment. It is unknown whether positive diversity effects will also appear after the establishment of a permanent stressor or when multiple stressors will act synergistically on an eelgrass system.

Taken together, field observations and mesocosm results jointly support the hypothesis that genotypic diversity can be important in providing different responses to environmental variation (Witham et al. 2003, Gamfeldt et al. 2005, Hughes et al. 2005). Although we found statistical support for among-genotype differentiation in shoot density only for July, field data from 2003 showed highly differentiated responses of individual eelgrass genotypes to prolonged summer temperature maxima (Reusch et al. 2005). This genetic variation may be critical in maintaining the resilience of ecosystems that are based on single habitat-forming species (Duffy 2006, Reusch & Hughes 2006) and may provide the potential for selection towards heat-tolerant genotypes and hence adaptation to changing environmental conditions.

Without doubt, my planted mesocosm 'community' did not capture the full complexity of natural seagrass communities. For example, other grazer species such as isopods and amphipods were underrepresented in the experiment and may react differently to ocean warming than littorinid snails. Nevertheless, the magnitude of negative temperature effects was nearly identical in previous field observations and in my mesocosm experiment. The observed appearance of cyanobacterial mats and the reduced grazing activity of gastropods, may be an artefact of our mesocosms, nonetheless highlighting the need to study warming effects on communities as complete as possible. This way, both direct ecophysiological effects upon seagrass genotypes, and indirect effects by altered interactions on seagrass plants can be examined.

Losses of seagrass over the last decades are dramatic and may accelerate with increasing climate change (Short & Wyllie-Echeverria 1996, Short & Neckles 1999, Oviatt 2004 and references therein). As seagrass meadows represent important habitats on marine soft-bottom sediments in higher latitudes, regional decline or extinction of seagrasses could lead to regime shifts in coastal areas, with unpredictable ecosystem effects (Harley et al. 2006).

As in previous field observations, genotypic diversity enhanced eelgrass performance. The results of my experiment and the field data from 2003 clearly show that there is a heritable component in coping with temperature stress among genotypes, while phenotypic plasticity of eelgrass alone is not sufficient to compensate the effects of increased climatic variation associated with ocean warming. My results present a stark warning that eelgrass meadows and their associated ecosystems may not be able to keep pace with rapid climate warming. The protection of coastal habitats may, through conservation of genotypic diversity, offer a potential for evolutionary adaptation of eelgrass systems to global climate change (Reusch & Wood 2007).



*Zostera marina* and *Ruppia maritima* in a mixed stand in the Schlei fjord

## Experiment III

### Relative effects of genotype versus species diversity on processes in an experimental community of three dominant macrophytes

#### Introduction

Communities and populations are simultaneously controlled by abiotic and biotic interactions, both of which may be under anthropogenic influence. Biotic interactions can operate at different levels, in particular between species, between genotypes within a species and also between individual genotypes of different species. In plants, which are sessile and have to cope with a close and dense floral neighbourhood, biotic interactions within the same trophic level play an important role (Aarssen & Turkington 1985).

Research on positive plant species interactions has a long tradition rooting in agricultural interests. Different species mixtures were cultivated to yield high productivity, a procedure called intercropping. Some of the already 'classical' studies of biodiversity- ecosystem function relationships were carried out in grassland systems and found, besides different responses of distinct species to their abiotic environment, manifold interactions between the species involved (Tilman et al. 2002). Also in seagrass systems differentiated responses on the inter-specific level have been found. For example, in tropical multi-species seagrass meadows, distinct responses on the species level were observed following experimental burial (Duarte et al 2002). In another tropical meadow Williams (1990) found two species of the seagrass genera *Syringodium* and *Thalassia* differing in rooting depth, suggesting complementary resource use. Although temperate seagrass species such as *Zostera marina* and *Zostera noltii* often form mono-specific stands, they also occur interspersed with other taxa. Mixed stands are common in peripheral parts of meadows, where abiotic conditions are more variable. For the species used in this work, different optima and maxima for temperature, light, salinity, sediment conditions, nutrient enrichment and wave exposition have been reported (Verhoeven 1979, Evans et al. 1986, Larkum et al. 2006). For example, *Zostera marina* and *Ruppia maritima* coexist in the Chesapeake Bay by showing temporarily separated biomass peaks, mainly controlled by water temperature and light requirements of the two species (Orth & Moore 1988). Furthermore Johnsen et al. (2003) reported a dominance shift from *Zostera* to *Ruppia* during the 1997/1998 ENSO event in southern California. Furthermore, experimental studies revealed higher temperature and eutrophication tolerance for *Ruppia maritima* compared to *Zostera marina*.

To summarize in the context of this study, different traits in multi-species seagrass beds may affect community composition and competition, and also may provide response diversity at the species level. The experiments reported in the preceding Chapters (Experiment I-II) revealed strong differentiation of individual eelgrass genotypes with respect to their abiotic stress tolerance and productivity related traits. Additional evidence comes from field studies, which showed important genotypic diversity effects for eelgrass resilience after disturbance (Hughes & Stachowicz 2004, Reusch et al. 2005). The relative importance of these genotypic diversity effects compared to the role of species diversity in plant communities, is unresolved. Experimental studies that investigate the relative contributions of intra- and interspecific diversity on community- and ecosystem processes are therefore highly warranted (Hooper et al. 2005, Vellend 2005, Crutsinger et al. 2006). I am aware of only one empirical study that explicitly manipulated genotypic diversity within competing plant species in a grassland community (Booth & Grime 2003). Here species diversity level was held constant, while the genotypic composition within experimental plots varied. Genetic diversity within component species reduced the rate at which species diversity declined. This is in accordance to model predictions, which show mostly positive relationships between species and genotype diversity (Vellend 2005).

My experiment was designed to directly compare the effects of genotype versus species diversity within representatives of a naturally co-occurring coastal macrophyte community. The three marine angiosperms or seagrasses *Zostera marina*, *Zostera noltii* and *Ruppia maritima* were used. The core hypothesis of the present study was that different genotypes in foundation species functionally replace the role of biodiversity at the species level, in particular for community processes such as shoot or biomass production. I therefore predicted, that differences in the response variables due to genotype diversity would be in the same order of magnitude as those due to species diversity, i.e. explain a comparable or even higher amount of variance in the statistical model. The simultaneous manipulation of genotypic and species diversity further allows for multiple levels of comparison, i.e. (1) between species ignoring the level of genotypic diversity (2) within species, comparing genotype monocultures with 3-genotype mixtures, (3) between 3-species-mixtures of different (high or low) genotypic diversity. Specifically I will test for differences between species-monocultures composed of three distinct genotypes (1-high) and treatments consisting of three species each represented by one genotype (3-low). These two treatments have the same number of genotypes, yet with different levels of species diversity. The specific comparison could contribute in revealing the proportions of genotypic and species diversity to shoot numbers and biomass production.



## Methods

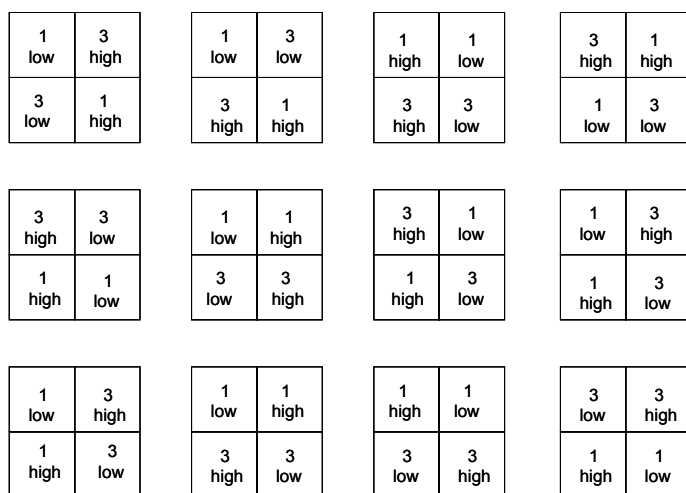
### *Donor site and clonal mapping*

Similar to the sampling and genotyping procedures of the preceding years we established a sampling grid in April 2005 within a part of the Maasholm *Zostera marina* meadow. Additionally patches of *Zostera noltii* and *Ruppia maritima* adjacent to the eelgrass meadow in the shallower part towards the coastline were marked and sampled for genotyping. In this shallow part of the bight with a depth between 0.6-1.5m, there exists no closed vegetation, probably due to regular grazing by mute swans (*Cygnus olor*), which easily reach these depths. All three macrophyte species occur here in a patchy distribution, not as dense as in closed monospecific stands. To gain enough individuals for *R. maritima* we also marked and sampled an area of 5x5m in an adjacent embayment 5 km away from the other Maasholm macrophyte stands.

With the resulting clone maps we set up a field-experiment in June dedicated to address the same questions outlined above in a field context, with high replication levels. I decided to standardize the experiment on the level of shoot numbers. One week after establishment I had to abandon the experiment due to devastation by green crabs (*Carcinus meanas*) which destroyed 85% of the experimental plots, excavating the sediment and thereby removing the genotyped and transplanted shoots. I had to re-design the experiment and established a reduced version in the indoor mesocosm facility to avoid further negative interactions with crabs. Because of the insufficient remaining number of mapped donor genotypes and the limited time due to the ongoing vegetation period, additional small isolated patches of each species had to be exploited, assuming they were consisting of one genotype only.

### *Experimental design*

The experiment was laid out to allow for analyses on multiple levels of diversity. The overall experimental set-up (highest level of comparisons) was an ANOVA design with 'species richness' (2 level) and 'genotype richness' (2 level), both as independent categorical factors. 'Species richness' comprised monocultures and mixtures of all three species and 'genotype richness' incorporated the two levels 'low', i.e. one genotype per species and 'high', i.e. 3 genotypes per species. This entails, that a mixture of all three species with a high level of genotype richness (3-high) represents a total genotype richness of 9 different genotypes per experimental community. The experiment was conducted in the indoor mesocosm facility at



**Figure III-1:** Experimental set-up in the mesocosm facility, as established in August 2005. The figure shows the twelve tanks (=blocks) with four compartments each. **1-low** labels species monocultures with low genotypic diversity, i.e. consisting of only one genotype, **1-high** denotes species monocultures composed out of three genotypes, **3-low** denotes mixtures of all 3 species each species represented by only one genotype, **3-high** names mixtures of all 3 species each present with 3 different genotypes.

the Leibniz-Institute for Marine Sciences, Kiel, having 12 tanks with 4 experimental plots each resulting in 48 experimental units.

Each block contained one replicate of each treatment (see general Material & Methods). Treatments were randomly distributed to a position within the block (Fig. III-1). After final harvest in October a sample of every plant was analysed again using microsatellite markers (see Material & Methods). This was done, because I had to use additional genetically not determined shoots for the set-up

of this mesocosm experiment, after the green crabs had wasted much of the previously genotyped clones. Therefore post-genotyping was performed to achieve certainty regarding the realised genotype richness in the experimental plant communities. Unfortunately, some irregularities appeared referring to the planned experimental design, which got unbalanced as genotype levels were more variable than the planned two categories of low (= 1 genotypes per species) and high (=3 genotypes per species) genotype diversity per species.

*Response variables and Statistical methods*

Shoot numbers and aboveground biomass as dry weight per plot were used as dependent variables. Plants were harvested in October 2005, counted and weighed as described in the general Material & Method section.

In the overall ANOVA, genotype-richness and species-richness were treated as categorical fixed factors. Due to the discrepancy between the planned and actual set-up of the experiment I had to rearrange some of mixtures into the fixed group categories (see below in results). The block-factor was left out, because the factual set-up did not contain every genotype treatment in each block. Albeit unbalanced, the planned GLM model was used for this data set, as Quinn & Keough (2002) recommend this as uncritical, yet they plead for

caution, when in these cases p-values are close to the critical levels. The outcome of our analyses was unambiguously far away from the defined significance level of 0.05. A specific contrast was tested between the 1-high and the 3-low treatments.

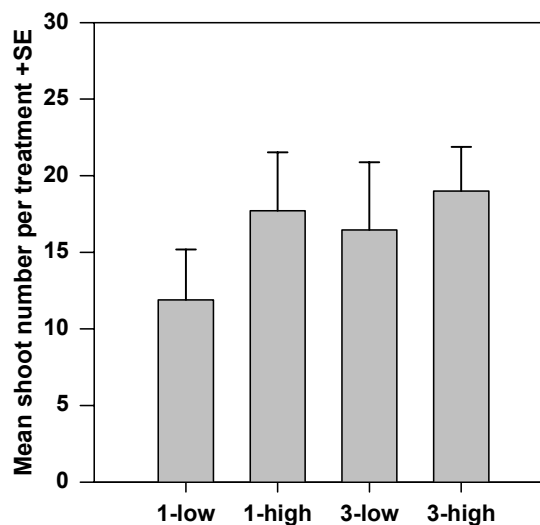
For a second analysis the species richness factor was divided into the three individual species and the 3-species mixtures, treated as a categorical variable representing 'species identity'. The categorical approach was abandoned as the genotype treatment had far more realized levels, than the planned two of 'low' and 'high' genotype richness per species. Accordingly, the final realized number of genotypes per treatment was considered as a continuous independent variable. In this analysis 'block' was included as a random factor, resulting in a mixed effects general linear model. Data were sqrt-transformed to achieve homogeneity of variances.

I compared genotype monocultures (low) to the 3-genotype-mixtures (high) within individual species and differences between three-species mixtures of high or low genotypic diversity using T-tests. Additionally the standardized effect sizes (SES) for species richness (1 species communities versus 3 species communities), genotype richness within species (1-low versus 1-high) and genotype richness within 3-species mixtures (3-low versus 3-high) were calculated. Therefore the difference, measured as number of standard deviations between the genotypically 'low' and 'high' diverse groups was calculated, as the unbiased standardized mean difference ( $d$ , according to Scheiner & Gurevitch, 2001, S. 356).

## Results

After post-experimental genotyping it became apparent, that in some cases realized genotype mixtures corresponded poorly to designed plot mixtures. Therefore, some experimental communities had to be assigned to other categories, so that replicate number got unequal (shown in Table A-III-1 in the Appendix). Also the block structure with each block containing each treatment was broken up on the genotype treatment level. For example, at the time of harvest none of the high-diverse communities, i.e. the 3-species mixture with 3 genotypes each (3-high), contained the intended level of 9 genotypes in total, yet showed lower genotype richness.

The analysis of variance revealed no significant effects of genotype richness and species richness on aboveground biomass and shoot numbers (Fig. III-2) and no interaction between treatments ( $F [1,44] = 0.041$ ,  $P > 0.05$ ) were observable. The planned comparison between the 1-high versus 3-low treatments, including the same total number of genotypes but with different species diversity, revealed no differences between these two groups ( $F [1,44]$ ,  $P > 0.1$  for both response variables), which points to an equivalent role of species and genotype diversity. The standardized effect sizes for species richness (1 species communities versus 3 species communities) were  $SES=0.291$ , for genotype richness within species (1-low versus 1-high)  $SES=0.371$  and for genotype richness within 3-species mixtures (3-low versus 3-high)  $SES=0.326$ .

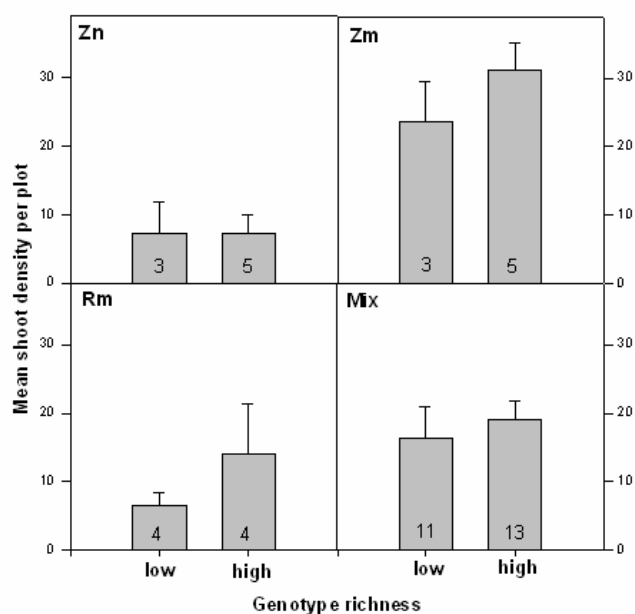


**Figure III-2:** Means of shoot numbers for diversity treatments with bars indicating 1SE. The number given for the treatment combination on the x-axis refers to species richness, **low** and **high** determine levels of genotypic diversity. For treatment 1-low  $n=10$ ; 1-high  $n=11$ ; 3-low  $n=14$ ; 3-high  $n=13$ .

**Table III-1:** Results of General linear model for aboveground biomass as dryweight and for shoot numbers per plot. 'Species identity' was used as a fixed categorical, 'Realized genotype richness' as fixed continuous and 'Block' as a random categorical factor.

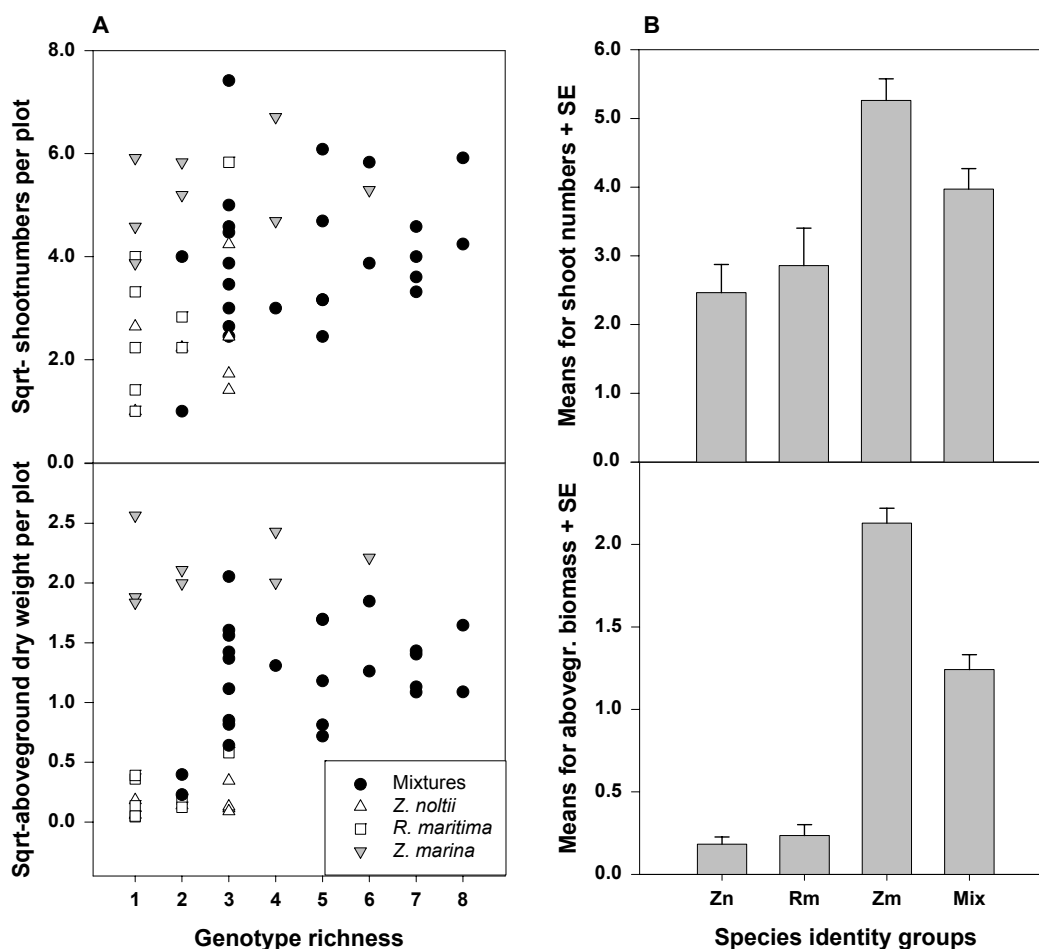
Source	df	Aboveground dry weight		Shoot number	
		F	P	F	P
Block	11	4.64	< 0.001	9.63	< 0.001
Spec_id	3	21.54	< 0.001	6.86	0.001
Gtyp_rich	1	0.61	0.440	6.55	0.016
Gtyp_rich * Spec_id	3	0.52	0.671	1.12	0.356
Residual	28				

The general linear model with 'species identity' as a categorical factor with levels 'Zm', 'Zn', Rm' and 'Mix' and 'genotype richness' as a continuous variable showed strong effects of species identity groups on both response variables ( $P \leq 0.001$ , see Table III-4, Fig. III-3).



**Figure III-3:** Mean shoot numbers for the three species Zn = *Zostera noltii*, Zm= *Zostera marina*, Rm= *Ruppia maritima* and the 3-species mixtures = Mix; with low and high genotype diversity. Numbers in the bars indicate sample size, error bars indicate 1 SE.

This effect was stronger for aboveground biomass. For shoot numbers the species identity effect was accompanied by a significant effect of genotype richness ( $P = 0.016$ ), but I found no interactions. Furthermore both analyses found highly significant influences of the 'block'-factor, i.e. evident differences between the distinct experimental tanks. Species identity groups were compared with post-hoc tests. While no significant differences in shoot numbers



**Fig. III-4 :** (A) Relationship between realized genotype richness and shoot numbers (top) and aboveground biomass (bottom), (B) Group means  $\pm$  1SE of the sqrt- transformed data of the 'species identity' treatments for shoot number (top) and aboveground biomass (bottom). Treatments on the x-axis are Zn= *Z. noltii*, Rm= *Ruppia maritima*, Zm=*Zostera marina*, Mi x= three-species-mixtures. n for (Zn, Rm, Zm)= 8, n (Mix)= 24.

and aboveground biomass were detectable between experimental communities of *Ruppia maritima* and *Zostera noltii*, all other 'species identity' groups were significantly different from each other (Tukey HSD,  $P < 0.005$ ).

Comparisons of low and high genotypic richness within individual species and the 3-species mixtures showed no significant statistical differences in shoot numbers and aboveground biomass (t-tests, all  $P > 0.1$ ), which is not surprising when regarding the low power of tests due to the low replicate number of groups (Fig. III-3). But graphs for all but *Zostera noltii*, show a tendency that within species and the 3-species-mixtures an increase of genotype richness may be positively correlated with shoot numbers.

## Discussion

Species identity explained most of the ecosystem functioning variables of this study, differences in shoot density and aboveground biomass. All three study species form dominant stands, are foundation species (sensu Dayton 1972) and are clearly distinguished in their optimal growth conditions for different abiotic factors. These features are different in well-studied terrestrial grassland communities, where a few dominants are accompanied by a set of minor species which seem to be redundant under normal conditions, and show their potential functional roles when environmental parameters shift (for example Walker et al. 1999). The species used here coexist in the donor habitat, but in a zone where probably for none of them optimal conditions are fulfilled. There is some degree of niche overlap, but species borders and their dominance patterns seem to be more influenced by the steep abiotic gradients of the coastal environment.

Genotype richness when considered as a continuous variable, showed a positive relationship with shoot numbers. This is in accordance to the theoretical predictions we intended to test (Duffy 2006, Reusch & Hughes 2006). A closer look reveals that this relationship is driven by the overwhelming dominance of *Zostera marina* (see Fig. III-4). In the experimental plots containing genotype monocultures the average values for the three species are clearly highest for *Zostera marina* that also displays higher shoot numbers than the other two species. Within the 3-species, 3-genotype treatments (3-high) none showed the intended level of 9 total genotypes per plot at the time of harvest. These reductions were mostly due to loss of genotypes from *Zostera noltii* and *Ruppia maritima* species, while most *Zostera marina* survived with the intended richness of three genotypes. In other words, a strong selection effect sensu Loreau and Hector (2001) contributed by *Z. marina* is responsible for the results observed. Such strong selection effects are especially prominent in experiments with relatively short duration, where strong species sorting by competition can produce dominances and hence strong selection effects on the local scale (Hector et al. 2002, Ives et al. 2005, Fargione et al. 2007).

A comparison of the standardized effect sizes for species richness ( $d=0.29$ ), genotype diversity (high vs. low) within species ( $SES=0.37$ ) and genotype diversity (high vs. low) within species mixtures ( $SES=0.33$ ), shows that richness shows a higher standardized effect size on the genotype level, than on the species level. The effects sizes found in the present study are generally low, compared to results for species diversity in Cedar Creek Biodiversity experiments ( $SES$  for species diversity on Aboveground Net Primary Production = 1.35) and genotypic diversity in an experiment with *Solidago altissima* ( $SES$  for

genotypic diversity on ANPP = 1.33; Crutsinger et al. 2006). This may be due to the short duration of my study, but can be also caused by the specific setting in a community of foundation species. As Crutsinger et al. (2006) found similar values for genotypic diversity in their experiment compared to the Cedar Creek outcome for species diversity they concluded that both diversity levels have comparable impacts on the BEF relationship. As species richness had no significant influence on the response variables in this study, I will focus on species identity. Compared to the final genotype richness taken as a continuous variable in the second analysis, species identity explained the fourfold of variance in the statistical model. This emphasizes, that the identity of dominant species is the main driver of the community processes concerned.

These patterns should be related to the fact, that this study in dealing with dominant species only, whereas studies in grassland ecosystems normally include sets of 'sub-dominant', redundant species. It was shown, that in the case that one of the dominant grassland species gets lost, one of the sub-dominants of the same functional group takes over the role of the lost dominant species (Walker et al. 1999). In the underlying study system, each species is characterized by distinct optima and maxima, and there is no redundancy on the species level. It would be interesting to repeat this experiment adding an abiotic stress factor. A dominance shift from *Z. marina* to *R. maritima* was described following elevated water temperature during an ENSO event (Johnson et al. 2003). Another experiment manipulating water-column nitrate enrichment found *R. maritima* growth stimulated under elevated nitrate conditions, whereas *Z. marina* declined. It would be interesting to know, how relative contributions of genotype versus species diversity will vary during these times of transition and if the positive effects of genotype diversity within species would moderate such processes. Additionally, these examples from the literature point towards the fact, that there is indeed also an insurance effect given, by the co-occurrence of already three species, to maintain the function of a macrophyte meadow under changing environmental conditions.

In conclusion, I was able to show that in dominant co-existing macrophytes species identity was the main factor determining shoot number and aboveground biomass of experimental plots, whereas the number of species alone had no influence. In this system characterised through low species diversity, the diversity level underneath, namely genotype richness showed a positive relationship with shoot numbers of experimental plots, driven by a strong selection effect. Maintaining diversity on the genotypic and species level should provide the best "insurance" to cope with surprises.



## General conclusion

Overall, the results of my studies demonstrate the importance of genotype diversity within species for stability and functioning of plant communities. I found clearly distinguished responses of eelgrass genotypes to different abiotic stressors, albeit genotypes were taken from relatively small, spatially restricted donor sites within a wider meadow. In that respect the conclusions are conservative, and much more pronounced genotype differentiation can be expected at larger spatial scales (see also Joshi et al 2001).

An unexpected finding was the apparent existence of multi-purpose genotypes that can cope with different stressors, here nutrient peaks and high temperatures. Interestingly, this advantage seems to be traded-off by weaker competitive ability, as the superior genotype found in two parallel experiments in 2003 (Experiment I and Reusch et al. 2003) performed only average in mixtures with other genotypes. Such trade-offs clearly deserve more attention, because if true, ecophysiological experiments should always contain treatments with competing genotypes.

In the first two mesocosm experiments (Experiment I & II), I found different responses of individual eelgrass genotypes to both stressors. These distinct reactions may be linked to different traits of the individual genotypes, which are fundamental to response diversity in the face of a changing environment (Elmqvist et al. 2003). Genotype identity may therefore show analogous effects, as those found for species identity on the species level in classical biodiversity research. To my knowledge, both experiments are one of the first demonstrating response diversity on the genotype level in coastal macrophyte communities. So far, conservation agencies have stressed the importance of the genetic level regarding the protection of coastal habitats and elaboration of practical conservation guidelines, but recommendations are based on little experimental data (Usher 2005, EEA 2006).

I found a general positive effect of genotypic diversity on shoot density in the second experiment, supporting results from our experiment in 2003 in the field (Reusch et al. 03). Contrary to my expectations the effect of genotypic diversity was not more pronounced under heat stress. This, however, may simply be a problem of not having enough statistical power to detect an interaction among 'diversity \* stress' in the mesocosm setting. Nevertheless, any interpretation of having sufficient response diversity on the genotype level within the diversity hot-spot of the western Baltic eelgrass populations is premature. The effects observed may provide a basis for prediction, but far longer experimental durations would be needed. Moreover, to assess whether or not response diversity allows

population persistence would need an inclusion of compounded perturbations, which are typical for anthropogenic influences (Paine et al. 1998).

The underlying genetic basis to the diverging traits of individual genotypes was beyond the scope of my experiments, but may be further investigated by the emerging field of ecological genomics. One of the first studies in eelgrass was carried out by Ransbotyn & Reusch (2006) on eelgrass individuals from the Maasholm site. This study reports reference genes as a baseline to search for temperature tolerance and stress gene induction in eelgrass, to unravel the processes for the performance patterns we found under water temperature stress (Experiment II and Reusch et al. 2005). Procaccini et al. (2007) predict an increasing role of genetic and genomic tools for seagrass ecology and conservation and also present seagrasses as highly suitable organisms to progress in detecting the genetic basis for adaptation to biotic and abiotic change.

In the third experiment including genotypes of three angiosperm macrophyte species, the key question was to assess how the factor of species diversity additionally influences ecosystem functioning compared to the already known effects of genotypic diversity. Again I was able to validate a positive effect of genotype diversity, this time pooled over different species and their mixtures on shoot numbers in my experimental plots. Due to experimental constraints I had no replicated genotype monocultures in this experiment to compare directly to the former results, but the relative contribution of the species components could be evaluated. My results support the observational and theoretical findings, that the genotype level in foundation species plays a crucial role, in that it shows a positive BEF relationship for productivity and stability, which cannot be achieved on the species level in these communities due to lack of redundancy, i.e. lack of species diversity (Reusch & Hughes 2006, Duffy 2006).

For each of my experiments I sampled genotypes from unique 10 x 10m sampling sites, located within a large undisturbed eelgrass bed, which cover approximately 1/2km<sup>2</sup> in the respective Maasholm embayment. The donor sites, for which clonal maps were established, displayed high genotypic diversity compared to a worldwide set of *Z. marina* populations, comprising more than 50 populations (Olsen et al. 2004). Eelgrass grows too slow to propagate clones of a size needed to establish experiments with high replication on the genotype level in the lab. Therefore, I took advantage of clones growing in nature. The harvested modules (ramets) of these clones naturally grow contiguous to one another in the field. While I cannot principally exclude that previous environmental conditions influenced

clone performance, which means that different responses of the clones to different experimental conditions may have been a result of phenotypic plasticity, I consider this unlikely. The eelgrass habitat at the sampling site is homogeneous, including sediment type and water depth. Moreover, distinct clones did not show any obvious signs of plasticity in the course of the experiment, i.e. the clones their morphological patterns displayed in the field, i.e. small plants remained small, throughout the experiment.

It may be evaluated critically to derive conclusions for real populations from results gained in indoor mesocosm approaches. However, the presented results are in line with those from a parallel field-experiment (Reusch et al. 2005) and further studies in seagrass systems (Williams 2001, Hughes & Stachowicz 2004). For example, experiments by Burkholder et al. (1994) and Bintz et al. (2003) showed strong interactions between temperature and nutrients in their effects on different species, on interactions between species and on relative abundances of species in coastal macrophyte communities. Although testing the mentioned factors separately, I was able to expand this view to the genotype level where I found distinct reactions within species, also affecting the competitive ability of individual genotypes.

The underlying study has not touched associated faunal communities, which constitute a major part of the good reputation seagrass ecosystems have referring to the ecosystems services they provide. Arthropod communities in terrestrial systems have been shown to depend severely on host-plant genetic compositions and diversity (Crutsinger et al. 2006). In the field-study, manipulating the same set of genotypes which have been used for one experiment presented in this thesis (Reusch et al. 2005), increasing genotype diversity was related to higher abundances of epifaunal species. Although this effect was mainly explained through increased shoot numbers and therefore higher leaf area in more diverse plots, this is very likely to be an important link connecting the genotype level with higher community and ecosystem processes. Although current conservation strategies target mostly rare species, it may be just as important to conserve the genetic variation in dominant, foundation or keystone species, because their extended phenotypes may affect the rest of the community (Whitham et al. 2003).

Grime (2002) stated, “The planet is losing plant species and populations at a rate too fast to allow a case by case evaluation of the consequences for ecosystem functioning and human need.” Although there is a continuing need for controlled experiments to identify mechanisms of diversity effects on ecosystem functioning, implications for conservation should be already clear. One strategy to conserve genetic diversity is the conservation of

species over as wide a geographical range as possible, to protect a wide range of different genotypes (Usher 2005). Another approach is the conservation of habitats and the connectivity between them, which could be done through the establishment of marine protected areas and no-take reserves. Allowing natural gene-flow between adjacent populations may be particularly important in keeping pace with e.g. climate change (Reusch & Wood 2007).



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Photograph on page 16 by Rüdiger Stöhr, all others by A. Ehlers.

Clone maps in the Appendix by August Hämmerli.





## Appendix

all	B	E	all	ADF	A	C	CBE
CBE	A	AEF	C	all	D	D	all
ADF	E	F	BAE	A	BAE	C	ABC
all	D	B	all	B	all	all	A
F	BEF	E	all	F	BEF	all	F
D	all	AEF	C	all	E	ABC	B

**Figure A-I-1:** Set-up for the 'eutrophication'-experiment. 12 blocks with 4 plots each. Grey shades represent fertilized blocks (tanks). Individual genotype composition of each monoculture and three-genotype mixture is indicated by letters A-F. Plots marked with 'all' indicate a six-genotype mixture containing ramets from all six genotypes A-F.

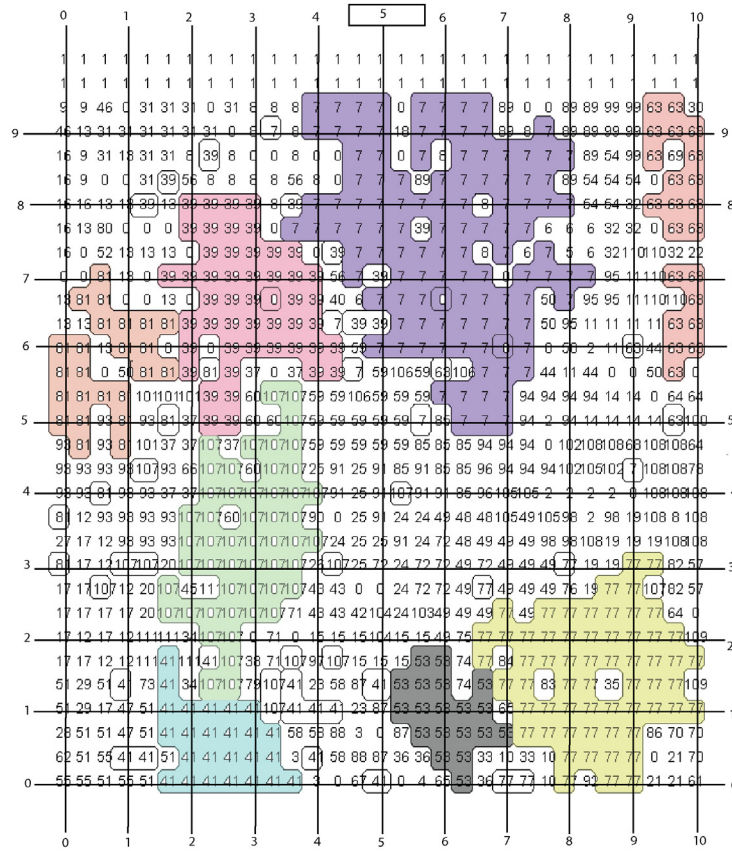
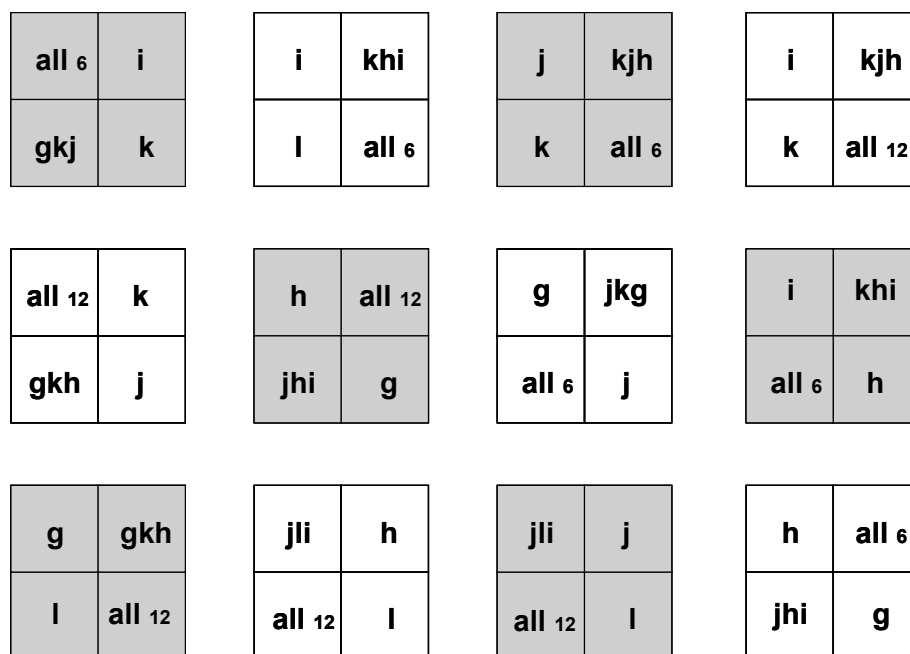


Figure A-I-2: Clonal map for a 10 x 9.3 m part of the eelgrass bed in Maasholm / Schlei, 2003



**Figure A-II-1:** Set-up for the 'heat wave'-experiment. 12 blocks with 4 plots each. Grey shades represent warmed blocks (tanks). Individual genotype composition of each monoculture and three-genotype mixture is indicated by letters G-L. Plots with a 6(6) indicate a six-genotype mixture containing ramets from all six genotypes G-L, plots with 6(12) are randomly composed out of a wider pool of 12 genotypes, containing G-L genotypes and six others that were not cultured as monocultures.

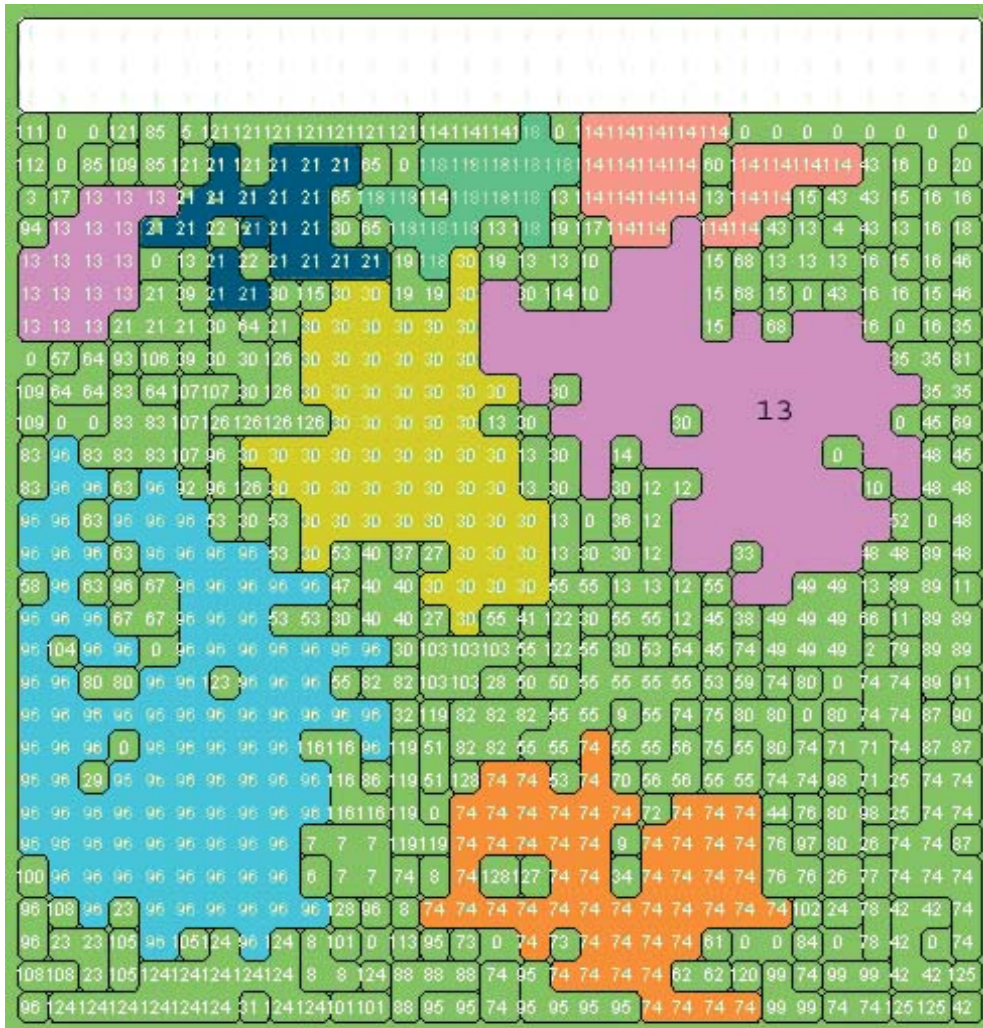


Figure A-II-2: Clonal map for a 10 x 10 m grid in the eelgrass bed in Maasholm /Schlei 2004

**Table A-III-1:** Regrouped treatments. Treatments are named according to the species identity groups, where Mix 1-1-1 corresponds to the 3.low treatment, Mix 3-3-3 to the 3.high, and the six mono-species-treatments belong to the 1.low and 1.high treatments.

	<b>3.low</b>	<b>3.high</b>	<b>1.low</b>	<b>1.high</b>	<b>1.low</b>	<b>1.high</b>	<b>1.low</b>	<b>1.high</b>
	<b>Mix1-1-1</b>	<b>Mix3-3-3</b>	<b>Zm 1</b>	<b>Zm 3</b>	<b>Zn 1</b>	<b>Zn 3</b>	<b>R 1</b>	<b>R 3</b>
	1-1-1	3-3-1	Zm 1	Zm 6	Zn 1	Zn 3	R 1	R 3
	1-1-1	3-1-1	Zm 1	Zm 4	Zn 1	Zn 3	R 1	R 1
	1-1-1	3-3-2	Zm 1	Zm 3	Zn 2	Zn 3	R 1	R 1
	1-1-1	3-2-3		Zm 3		Zn 3	R 2	R 1
	1-1-1	2-2-2		Zm 4		Zn 1		
	1-1-1	3-1-1						
	1-2-2	3-1-0						
	1-1-1	4-2-1						
	1-1-1	3-1-1						
	1-1-1	3-2-2						
	1-1-1	3-2-1						
		3-2-0						
		2-3-2						
planned	n= 12	12	4	4	4	4	4	4
real	n= 11	13	3	5	3	5	4	4

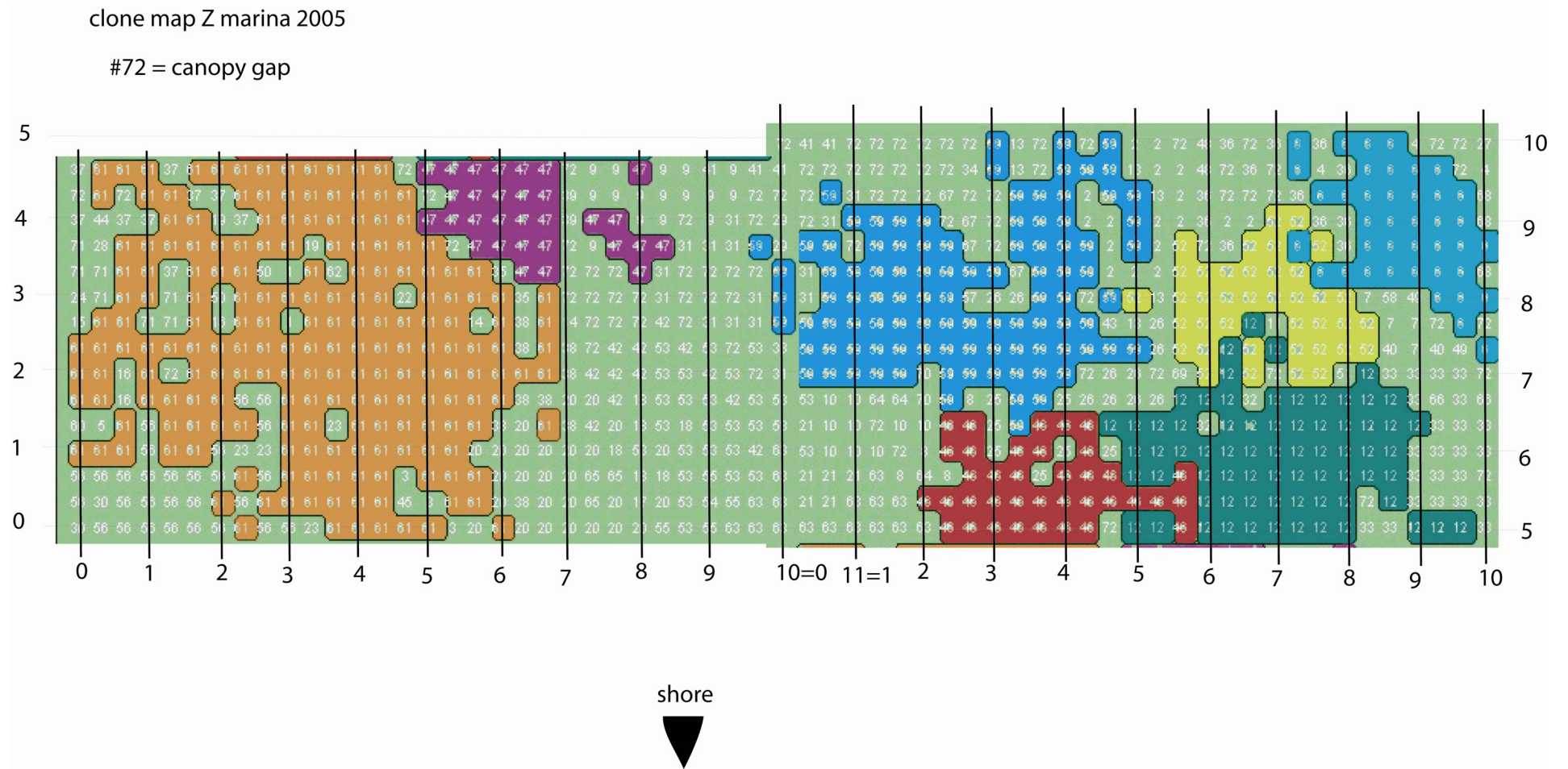


Figure A-III-1: Clonal map for a 20 x 5 m grid in the eelgrass bed in Maasholm / Schlei

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## Curriculum vitae

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## Ausbildung

Juli 1994	Allgemeine Hochschulreife, Kaiser-Karl-Schule in Itzehoe
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Okt 1996	Vordiplom Biologie
März-Aug 2000	Forschungstaucherausbildung an der Universität Kiel
April 2001	Diplomprüfungen in den Fächern Zoologie, Biologische Meereskunde, Geologie und Botanik
2001-2002	Diplomarbeit "Positive und negative Effekte von Wasservögeln auf submerse Vegetation" betreut durch Prof. Dr. H. Brendelberger, Universität Kiel und Dr. T. Reusch am Max-Planck-Institut für Limnologie, Plön; dabei Forschungsaufenthalte in der AWI Wattenmeer Station List auf Sylt, sowie Versuchsreihe am NIOO / Nieuwersluis in den Niederlanden
seit Mai 2003	Promotion in der Abteilung 'Experimentelle Ökologie' am Leibniz-Institut für Meereswissenschaften in Kiel.



## Erklärung

Hiermit erkläre ich, dass diese Arbeit – abgesehen von der Beratung durch meine akademischen Lehrer – nach Inhalt und Form meine eigene ist und keine weiteren als die angegebenen Hilfsmittel und Quellen verwendet wurden. Ich habe bisher keinen anderen Promotionsversuch unternommen, und diese Arbeit hat weder im Ganzen noch zum Teil an anderer Stelle im Rahmen eines Prüfungsverfahrens vorgelegen. Bei der Erstellung dieser Abhandlung habe ich mich an die Regeln guter wissenschaftlicher Praxis gehalten.

Kiel, den 29.05.2007

Anneli Ehlers